NUTRACEUTICALS

Nanotechnology in the Agri-Food Industry, Volume 4

Edited by

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SERIES FOREWORD

The emergence of nanotechnology has reached impressive heights in recent years and the development of special nanodevices and nanomaterials has found intriguing applications in agriculture and food sector. Most of the investigated nanotechnological approaches initially aimed to solve evolving problems in the agri-food industry in order to impact on the economic potential. Soon after the implementation of new technologies and approaches that were using nanostructured materials, the worldwide concern was rapidly extended to numerous applications that could be developed by using the science of nanosized materials. Smart materials, biosensors, packaging materials, nutraceuticals, and nanodevices have been designed to address numerous agri-food related issues with direct impact in health, economy, ecology, and industry. As the engineering of nanostructures has constantly progressed and extended its applications, there is virtually unlimited potential in this sector. However, the widely differing opinions on the applicability and usefulness of nanotechnology between both specialists and the general public has hampered progress. The main concern manifested by people is related to the potential risk for health and the environmental impact of the recently developed nanoengineered materials and devices. Therefore, current approaches are strictly considering these concerns when designing nanotechnological solutions for agriculture and food sectors.

This multivolume series was developed by the constant need to discover current inquiries and approaches on the field of agri-food science and also to learn about the most recent progress, approaches, and applications that have emerged through nanotechnology.

As agriculture is the backbone of most developing countries, nanotechnology has the potential to revolutionize the agriculture and food sector by promoting productivity through genetic improvement of plant and animal foods. It can also ensure the delivery of drugs, genes, and pesticides to specific sites at cellular levels in targeted plants and animals, by limiting side effects. Nanotechnology can be used to evaluate gene expression under different stress condition for both plant and animal foods through the development of nanoarray-based gene-technologies. Additionally, this technology can detect fertilizers, pesticides with high precision by smart nanosenors for an adequate management of the natural resources. Moreover, numerous industrial-related applications with direct impact on economy have emerged. For example,

nano- and micro-structured arrays can detect the early presence of pathogens, contaminants, and food spoilage factors. Other applications for this technology are smart integration systems for food processing and packaging, as well as nanoemulsion-based decontaminants for food equipment and storage compartments, and nanoparticles that facilitate the bioavailability and delivery of nutrients directly to cells.

The potential benefits of nanotechnology for agriculture, food, fisheries, and aquaculture were identified and supported by many countries, which invested a significant amount of money in the development of applications. Also, numerous campaigns are currently trying to increase awareness on the developing process and recent technologies in order to influence the acceptance of customers. Although nanoagri-food industrialized concept could help to find a sustainable solution for the current global food crisis, the offered advantages should balance the concerns regarding soil, water, environment, and health related issues that such approach could bring.

The series entitled *Nanotechnology in the Agri-Food Industry* brings comprehensive and recent knowledge regarding the impact of the science of nanometer-sized materials on the field of agriculture and food industry, but also discuss the current inquiries regarding risks of these applications in all relevant fields such as environment and health, aiming to increase awareness to a wider amount of readers.

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SERIES PREFACE

About the Series (Volumes I–X)

In a permanently changing society, health and well being remain the key drivers for the food industry. Despite the technological progress made in the agri-food industry, a true food crisis emerges in several areas of the globe. This can be explained by insufficient food but mostly by inadequate food for a very distinct range of consumers. In this context, innovative technologies represent the core throughout the whole food chain from raw materials/ingredient sourcing, food processing, quality control of finished products, and packaging. Nanotechnology, coupled with novel interdisciplinary approaches and processing methods, has enabled some important advances recently flourishing in many of these areas. The science of nanosized materials can improve and even resolve the huge challenges faced by the food and bioprocessing industries for developing and implementing systems that can produce qualitative and quantitative foods that are safe, sustainable, environment friendly, and efficient. This emerging tool finds its applications in various fields and represents an endless approach for the development of innovative strategies in food development, processing, and packaging.

This multivolume set aims to bring together the most recent and innovative applications of nanotechnology in the agri-food industry, but also to present the future perspectives in the design of new or alternative foods.

The series contains 200 chapters organized in 10 volumes, prepared by outstanding research groups that made significant impacts on the field of nanotechnology and food-related research sectors. This comprehensive set represents an updated and highly structured material for undergraduate and postgraduate students in food science, biotechnological, engineering fields, but also a valuable resource of recent scientific progress, along with most known applications of nanomaterials on the food industry to be used by researchers, engineers, and academia. Moreover, novel opportunities and ideas for developing or improving technologies in the agri-food industry by innovative companies, biotechnological industries, and other economical structures are highlighted and their potential is widely dissected. This series may be also valuable for the wide audience interested in recent nanotechnological progress in the agri-food field worldwide.

These 10 volumes cover almost all aspects related to the applications of *Nanotechnology in the Agri-Food Industry* and are named as:

Volume I Novel Approaches
Volume II Encapsulations
Volume III Emulsions
Volume IV Nutraceuticals
Volume V Nutrient Delivery
Volume VI Food Preservation
Volume VII Food Packaging
Volume VIII NanoBioSensors
Volume IX Water Purification
Volume X New Pesticides and Soil Sensors

Each volume contains 20 chapters, which were carefully composed and illustrated to highlight the most innovative and intensively investigated applications of nanotechnology on particular wide interest domains of the agri-food industry field.

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VOLUME PREFACE

Nutraceuticals are gaining significant attention due to their apparent safety, nutritional, and therapeutic perspectives. Scientific indications have reinforced dietary interposition as an effective implement for a healthy life style. Various bioactive components have been reported to exhibit antioxidant, antiinflammatory, antimicrobial, hypocholesterolemic, hypoglycemic, antimutagenic, and anticarcinogenic roles in the living systems. Regardless of the effectiveness of bioactive components, their applications in the food and pharmaceutical industry are limited due to poor bioavailability, storage, heat instability, and insolubility in aqueous medium. During storage, essential bioactive components are lost quickly as a result of volatilization, chemical degradation, and certain other physical and chemical reactions. Nanotechnology is one of the most interesting areas concerned with consumer products including electronics, cosmetics, household appliances, textiles, and food production as well as in various medical products. Although the applications of nanoscale particles in the development of therapeutic systems have been well documented and various systems have been designed for intelligent, modulated, and selective delivery of drugs to specific areas in the body in order to maximize drug action and minimize side effects, nanotechniques are relatively new in the food industry. Various natural or synthetic polymer-based nanoparticulate systems and their conjugates are potentially available to the food industry; it includes proteins, lipids, carbohydrates, or other biopolymers. Furthermore, direct nanoparticle uptake is controlled by the size and surface chemistry of the nano system. The use of this direct nanoparticle uptake, in particular for soluble but poorly absorbed ingredients, is one of the areas that needs to be explored in the future, as well as the potential side effects of these nanoparticle carriers. To address this challenge, it is necessary to understand the chemical structure and properties of different nutraceuticals. Based on the scope of nanotechnology in the development of nutraceuticals, this book describes the potential role and impact of nutraceutical delivery systems in food industry.

Volume IV contains 20 chapters, prepared by outstanding international researchers from Bulgaria, Egypt, Estonia, India, Italy, Japan, Oman, Pakistan, Poland, Portugal, Serbia, Spain, and Turkey.

In Chapter 1, *Mineral and Vitamin Fortification*, Biljana Arsic et al. present an up-to-date review regarding mineral and vitamin

fortification used for removing deficiency in poor people, pregnant women, and young children. Fortifying of meat products, products of wheat and refined fat are widespread, and numerous research groups are developing healthier methods to include these foods into wide consumption behavior of population. However, sometimes these products contain more vitamins or minerals due to technological omissions than recommended values by WHO and national agencies, and they are believed to be involved in the development of several metabolic diseases.

Khushwinder Kaur et al., in Chapter 2, *Functional Nutraceuticals: Past, Present, and Future*, present an interesting overview about different formulation strategies that have been reported in literature to improve the drug dosage form and delivery of the bioactive compounds to the desired part of the body. These include liposomal carrier systems, electrospun fiber mats, cyclodextrin complexation, hydrogels, nanoemulsions, nanosuspensions, nanomicelles, nanoparticles, microparticles, microspheres, self-emulsifying drug delivery systems, nanostructured lipid carriers, and many more. Numerous methodologies that help in food-quality control, authenticity, impurities, and provenance are also discussed with in this chapter.

Chapter 3, *Nutraceuticals: A Proactive Approach For Health Care*, prepared by Monika Sharma et al., details the various important phytochemicals as nutraceuticals, highlighting recent advances and importance of quality, purity, safety, and efficacy for nutraceuticals.

Ibrahim M. El-Sherbiny et al., in Chapter 4, *Potential of Nanotechnology in Nutraceuticals Delivery for the Prevention and Treatment of Cancer*, outline the medical importance with emphasis on anticancer activity of different nutraceuticals. The potential of nanotechnology and nanomaterials in the controlled delivery of nutraceuticals for prevention and treatment of cancer is also discussed.

In Chapter 5, *Adulteration and Safety Issues in Nutraceuticals and Dietary Supplements: Innocent or Risky?*, Ilkay Erdogan Orhan et al. present an updated overview on regulatory issues in connection with adulteration and contamination cases reported in nutraceuticals and dietary supplements are presented using reported market analyses.

Federico Benetti et al., in Chapter 6, *Regulatory Perspectives on Nanotechnology in Nutraceuticals*, present current international and European regulatory aspects. Special attention is given to the European Union regulatory framework, with the upcoming new definition of nanomaterials and the subsequent modification of existing regulations and declaration of new laws.

To comply with existing directives and regulations for marketing of new nanotechnology-based products, European Food Safety Authority guidelines for assessing potential risks from nanotechnologies in food sector is described and discussed.

Chapter 7, prepared by Ali Asghar et al., *Elucidating the Therapeutic Potential of Nutraceuticals*, discusses how microencapsulation system promotes the bioavailability and bioactivity of essential bioactive components by improving entrapment capacity, retention time, and controlled release at targeted sites. Various bioactive components have been reported to exhibit antioxidant, antiinflammatory, antimicrobial, hypocholesterolemic, hypoglycemic, antimutagenic, and anticarcinogenic roles in the living system.

Surashree Sen Gupta et al., in Chapter 8, *Advanced Nanocarriers for Nutraceuticals Based on Structured Lipid and Nonlipid Components*, give an overview on the recent developments in the field of nutraceuticals and their fabrication to develop nanocarriers rich in these bioactive compounds. Investigations on the fundamentals of formulations, methodologies, characterization techniques of the diverse nanosized compounds are also discussed here.

Chapter 9, *Encapsulation of Nutraceuticals in Novel Delivery Systems*, prepared by Joana F. Fangueiro et al., presents the main approaches utilized for the micro- and nanoencapsulation of nutraceuticals that provide a variety of advantages regarding stability, in vitro and in vitro performance, enhancing bioavailability and biological activity. Nanocarriers, as nanoemulsions, liposomes, lipid and polymeric nanoparticles, micelles, and cyclodextrins are described along with their application in nutraceuticals delivery.

Chapter 10, *Novel Paradigm of Design and Delivery of Nutraceuticals with Nanoscience and Technology*, prepared by Aswathy Ravindran Girija et al., discusses the role of nanoscience and technology in delivering nutraceuticals. The authors focus on the development of various nanoparticles for the encapsulation of nutraceuticals and the nanoscale delivery agents that are being used.

Asif Ahmad et al., Chapter 11, *Nutraceutical Aspects of β-Glucan with Application in Food Products*, present novel approaches related to β-glucan and its various health benefits. A wide range of industrial applications from food to cosmetics and medicine and, most importantly, availability from distended sources including plant (cereals), fungus, and bacteria, make these compounds interesting for intense investigation. Health benefits of β-glucan have a long list, including antidiabetic, anticonstipation, hypolipidemic, and anticancer. In food its application expands from bakery to dairy and meat industry. The authors conclude that β-glucan is a new dawn of food for mankind

and its many applicative attributes will be explored by scientists in the near future.

Chapter 12, *Nanotechnological Approach to Improve the Bioavailability of Dietary Flavonoids with Chemopreventive and Anticancer Properties*, by Katrin Sak et al., presents novel trends regarding the encapsulation of flavonoids into various nanosized drug delivery systems that substantially improve the bioavailability of these plant secondary metabolites and increase their therapeutic efficacy. Therefore, it is feasible that advances in nanotechnology may bring a step closer the inclusion of flavonoids in future anticancer treatment schemes.

Swati Pund et al., in the Chapter 13, *Improving Bioavailability of Nutraceuticals by Nanoemulsification*, describe the concept of nutraceuticals, nanoemulsion fabrication, with special emphasis on nanoemulsification of curcumin, resveratrol, and lutein for enhancing the therapeutic efficacy of the foods that contain these compounds.

Canan Ece Tamer et al., Chapter 14, *Bioavailability and Delivery of Nutraceuticals by Nanoparticles*, discuss some design and applications of nanonutraceutical products. Designing and developing functional food ingredients with improved bioavailability, solubility, thermal stability, organoleptic attributes, and physiological performance are important applications of nanotechnology in food technology and nutrition.

Chapter 15, *Bioavailability Enhancement of Curcumin Nutraceutical Through Nanodelivery Systems*, by Tapan Kumar Giri et al., focuses on the chemistry, pharmacology, and pharmacokinetics of curcumin. Authors also summarize the development of various nanoformulations of curcumin for sustained and efficient delivery.

Chapter 16, *Microencapsulation of Probiotic Cells: Applications in Nutraceutic and Food Industry*, by María Encarnación Morales et al., describes the techniques for probiotic microencapsulation, the compounds used in the microencapsulation process, as well as applications and impact in the food industry.

Edite Teixeira-Lemos et al., in Chapter 17, entitled *New Trends in Food Science: The Use of Nutraceuticals as an Antiinflammatory Therapeutic Tool in Exercise*, start with a brief review of the effects of exercise on immunity, followed by an analysis on how nutraceuticals, such as omega-3 fatty acids, glutamine, BCAAs, or phytochemicals, can counteract the negative effects of strenuous exercise in athletes. Finally, how nanostructured delivery systems can constitute a new trend in enhancing bioavailability and optimizing the action of nutraceuticals is discussed, using the example of food beverages.

Celile Aylin Oluk et al., in the Chapter 18, *Functional Food Ingredients and Nutraceuticals, Milk Proteins as Nutraceuticals NanoScience and Food Industry*, describe functional food ingredients and milk proteins as nutraceuticals, and their usage in nanotechnologic applications. The chapter is focused on: nanodelivery systems; milk proteins with nanotechnologic applications; and nanoemulsion-encapsulated phytochemicals.

In Chapter 19, *Protein-Based Dietary Supplements as Nutraceuticals*, Semih Ötleş et al. discuss various protein-based dietary supplements, which are used for human well-being against a diversity of health problems. These supplements have lots of beneficial effects with and/or without nanotechnology applications. As a result, if the dosage can be controlled, there are lots of beneficial effects on health, especially for exceptions.

Mahendran Botlagunta et al., in the Chapter 20, *Nutraceuticals Loaded Chitosan Nanoparticles for Chemoprevention and Cancer Fatigue*, address the controlled release of chemotherapeutic drugs along with vitamins through microencapsulation. Also, they provide novel insights to elicit the importance of nutraceuticals against cancer therapy to reduce cancer fatigue.

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MINERAL AND VITAMIN FORTIFICATION

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1 Introduction

The lives of prehistoric men and women living in caves were very simple and without stresses. They used natural food without the fear of poisoning with heavy metals, pesticides, and insecticides. Food preparation was quite different. They mainly used fresh fruits and vegetables, and did not thermally process the food. Thermal processing is the cause of the destruction of thermally sensitive vitamins. Also, they did not use refined sugar, which can cause obesity and, in more severe cases, diabetes. Fats and oils were natural, and not refined. Humans in the Stone Age did not suffer from many "modern" diseases (Williams, 1941). Due to all these changes in diets and life, in order to balance mineral and vitamin intakes, food fortification must be conducted.

In this chapter we briefly point out the role of minerals in vitamins in biochemical processes taking place in the body, their sources, and the diseases caused due to their deficiencies. Also, we discuss the current state of the fortification of food in the world.

2 Minerals

The term "heavy metals" is used for metals with specific density higher than 5 g/cm^3. Some heavy metals are in trace amounts as elements that are essential for numerous functions in the human body, and their shortage leads to the appearance of serious disease symptoms. Increased concentration in the organism is unwanted and dangerous. Usually the question about the toxicity is actually the question about the quantity, and this range varies considerably for each particular element. The accumulation of these elements in fat cells, bones, endocrine glands, brain, hairs, or central nervous

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system often results in harmful health consequences, and not often in serious diseases. The World Health Organization (WHO) develops detailed studies on dangerous effects of heavy metals on human health.

For example, normal iron quantities defend the body from anemia, while zinc is cofactor in more than 100 enzyme reactions. These metals appear in low concentrations and very often they are called rare metals. In high quantities, they can be toxic or can cause the deficiency of some other metals.

There are more reasons why the exact amount necessary for health is still unknown for identified essential metals. One of the reasons is analytical difficulties in the determination of low concentrations, precisely the very low levels in which these elements are usually present. Also, the manner of their classification represents the problem during the determination of the number of essential elements (Nicholas, 1961).

Many authors proposed a large number of different definitions about the necessity of elements. One of the simplest is that an essential element is a "metabolic or functional nutrient" (Mertz, 1981). One of the more complex definitions is that an essential element is the element which is necessary for maintaining life, and its shortage can lead to the violations of functions from optimal to suboptimal values (Mertz, 1981). Violation of functions can lead to the development of diseases, metabolic anomalies, or development of particular abnormalities (Da Silva and Wiliams, 2001). In order to be taken as an essential element,

1. must be present in healthy tissue;
2. its concentration must be relatively constant in different organisms;
3. its lack must cause precise, particular biochemical changes;
4. the change leads to similar abnormalities in different species;
5. its intake leads to the correction of caused abnormalities (Cotzias, 1967).

When we are talking about properties and the biological role of essential elements in traces, it is important to say that they represent less than 0.1% of the total composition of the human body. Four nonmetals—hydrogen, oxygen, carbon, and nitrogen—represent 99% of the content of not only humans, but also all biological systems. Seven elements, which are called the main elements—sodium, potassium, calcium, magnesium, phosphorus, sulfur, and chlorine—represent the rest of 0.9%, so essential metals in traces share the remaining 0.1% with all other elements, metals, and nonmetals (Szefer and Nriagu, 2006).

Different circumstances can lead to different problems in the organism: competitive inhibition of toxic elements, bad absorptions,

bad digestions, and so forth. Establishment of optimal digestive functions can be a very important aspect in basic nutrition.

Heavy metals are very stable and bioaccumulative, which means that the organism cannot transform them, and as such they are accumulated in the body. Some heavy metals do not have any function in the organism and can be very toxic, for example, mercury, lead, nickel, arsenic, and cadmium.

The quantity of metals that plants can take from the soil does not depend only on their total content in the soil, it depends also on the availability of these elements to the plant alone. Actually, its availability for the adoption (mobility), as well as the accumulation from the plant alone determines if the particular plant species will contribute significantly as a source of particular metals necessary in nutrition. Availability of the metal to the plant was determined on the basis of their properties such as: chemical form of the metal, and solubility and capability to complex with organic matter.

A plant on its own can increase the capability to absorb metals such as changing the chemistry of the soil on the site of plantation, by releasing hydrogen ions and organic chelating agents.

Plants are the premier source of the transport of inorganic nutritional matters for humans from the soil while animal products (meat, milk, eggs) are secondary sources of these elements. By the combination of these two ways metals are reaching human organisms.

For example, iron in plants is present in very small amounts, and in the form not suitable for absorption, so the human body takes in this element primarily via meat, where iron is present in high concentrations (particularly in organs and muscles). This metal is translating the animal body into organic form, which is significantly easier for absorption in the digestive tract of the organs. Also, animals are a much better source of zinc than plants.

Soil is the main source of the metals that are nourishing the body via plants and animals. However, there are numerous other ways that metals can be included in food. Industrial production, which leads to the pollution of the environment, is the main source of toxic metals such as cadmium and lead. On the other hand, technological process can lead to the increase of particular nutritional metals in food. For example, the use of dishes made of iron leads to the increase of the content of this metal in food in a very satisfactory way (Borigato and Martinez, 1998). There is evidence that confirms that an important quantity of nickel and chromium is incorporated in food as a result of the release of these metals from stainless steel, which is used for the production of kitchen cutlery and dishes for the storage of food (Smart and Sherlock, 1985).

Copper, similar to nickel and chromium, can be released from the dishes made from the alloys of this metal. Although copper, nickel, and chromium are essential metals increased quantities of these elements in food prepared in dishes from their alloys can lead to liver damage, particularly in children (Taner et al., 1983).

If heavy metals are accumulated in the body faster than an organism can perform detoxification, it leads to the formation of toxins. Exposure to a high concentration of any heavy metal does not mean that it will result in the poisoning of the organism itself. Accumulation in the organism will happen.

The exposure to heavy metals is not a new phenomenon. In history, there have been different cases of poisons affecting populations; for example, during the Roman Empire the entire city of Rome was poisoned by lead leaching from water pipes.

2.1 Copper

Copper is a reddish metal that occurs in nature in rocks, soil, water, sediments, and the air. It also occurs in plants and animals. It is an essential element for humans. The average concentration in the Earth's crust is around 50 ppm, in the air it goes from several ng/m^3 to 200 ng/m^3, in the soil between 2 and 250 ppm, and in drinking water from 20 to 75 ppb. Very often many households have increased copper concentration because of the use of copper pipes and brass taps.

Copper is an important component of many enzymes in the body that have important roles in the energy production within the cell. Activity of these enzymes is the highest in the heart, brain, liver, and kidneys. Besides, enzymes responsible for the formation of binding proteins of tissues (collagen and elastin) contain copper. Therefore, it can be said that copper is necessary for the development and maintenance of blood vessels, skin, bones, and joints. It helps in maintaining the cells of the central nervous system. It is suitable for osteoporosis—a condition in which bones become brittle because of calcium deficiency. It is involved in the release of iron from storage in the cells, as well as the formation of bone marrow and the maturation of red blood cells. Copper is also necessary for the synthesis of phospholipids of cell membrane and it supports myelin (the sheathing of nerves) in this way and regulates the level of neurotransmitters (Stern 2007; Solioz et al. 2007).

It is part of the enzyme copper-zinc superoxide dismutase (Cu/Zn SOD), which serves as an antioxidant important for protecting an organism from the damage caused by free radicals. Maintenance of the proper ratio of copper and zinc is very important for the normal functioning of an organism (Ashish et al., 2013).

Copper is very important for the immune response of the organism to the infections. During the inflammation process (infection) the mobilization of two compounds occurs, included in their structures copper ion: superoxide-dismutase and ceruloplasmin. Also, it is necessary for the maturation and function of T cells. It plays an important role in the contractions of heart muscle, as well as normal function of small blood vessels controlling the circulation of blood, nutrients, and wastes. Copper affects the normal function of blood vessels and muscles and is involved in the lining of blood vessels. Creation of melanin (natural pigment in hair and skin) includes enzymes containing copper. The enzyme histaminase which metabolizes amino acid histamine also contains copper (Ashish et al., 2013).

Copper is also involved in the metabolism of fat and cholesterol, as well as the normal function of insulin (which regulates sugar metabolism). It is involved in the synthesis of prostaglandins—compounds that regulate numerous functions such as heartbeat, blood pressure, and wound healing (Eck and Wilson, 1989; Stern, 2007; Solioz et al., 2007).

2.2 Iron

Iron is the compositional part of hemoglobin in erythrocytes and myoglobin in tissues. The role of iron is binding of oxygen and transport to the tissues, as well as elimination of carbon dioxide from the body. The second molecule of protein which in its structure contains iron is myoglobin which transports oxygen to the muscles and therefore it is essential for the cell activity in all tissues (Gurzau et al., 2003).

Iron is incorporated in many enzymes and hormones involved in metabolic processes, and it is necessary for the division of cells, cell growth, and DNA synthesis. An important role of iron is in the transport of oxygen in cytochromes and enhancement of the immune system.

Iron deficiency in the body is one of the most frequent nutrition disorders in the developed world. Iron deficiency and anemia are still frequent in girls in adolescence and in pregnant women. In the event that a pregnant woman has an iron deficiency, fetus infection after delivery can happen, and it can also lead to miscarriage and preterm labor. Iron deficiency can cause low body weight in newborns and an increased risk of child anemia. In drastic cases the death of the child can happen at birth (Lieu et al., 2001).

Children younger than 2 years of age are in danger of iron deficiency because of their fast growth, low iron reserves, and the low content of iron in milk. It can also occur in elders because they

suffer from a decrease of hydrochloric acid in the stomach. Iron deficiency is connected to increased mortality of elders because a low level of iron increases the risk from heart disease. In the event of operations or injuries, the appearance of anemia is a natural consequence.

Symptoms of iron deficiency include anemia, fatigue, increased heart work, gasping, decreased concentration, dizziness, disturbed sleep, hard menstrual pains and bleeding, chapped lips, eye infection, mouth ulcers and, hair loss. Iron deficiency can cause sleeping problems, headaches, rheumatoid arthritis, sand syndrome, seized legs, sweating, unconsciousness, decreased efficacy and decreased productivity at work, and daytime sleepiness. Iron deficiency causes the difficulties during physical and brain work, decreases the immune response, and causes anemia, muscle pain, headache, and hair loss (Lieu et al., 2001).

Low level of iron in blood plasma can cause skin itching, particularly in elders. Nails become soft, brittle, and white.

Iron in food can be found in the form of Fe^{3+} ions, and it is tightly bound to organic molecules. Good iron sources are meat, particularly liver, beans, walnuts, dry fruits, chicken, fish, and shellfish. Cooking in iron dishes can increase the iron quantity as much as 20%, but the iron in this form is difficult to metabolize. Long cooking in this kind of pan causes more iron to be embedded into food. Replacement of iron pots with aluminum, stainless steel, and plastic pots decreases iron intake.

Persons who do not eat red flesh, the best source of iron, as part of their diet must increase the content of plant food with dark green leaves, beans, and wheat. Vegetarians should take an increased quantity of vitamin C, which assists in iron absorption.

2.3 Zinc

Zinc is involved in enzymatic reactions in humans. Its role is mainly in the synthesis and stabilization of genetic material. It is also necessary for cell division, and synthesis and degradation of hydrocarbons, lipids, and proteins (Osredkar and Sustar, 2011). Being a part of the enzyme copper-zinc superoxide dismutase, it helps protect cells and some compounds from harmful effects caused by free radicals (Plum et al., 2010).

Zinc is necessary for the structure and normal function of cell membranes. It takes part in the formation of binding tissue, teeth, bones, nails, and skin. It plays an important role in the incorporation of calcium into the bones and influences the action of growing hormones (Nriagu, 2007).

It can be considered as one of the most important nutrients of the immune system because it is necessary for the formation of antibodies, leucocytes, thyroid glands, and the function of hormones. Therefore, it is important in keeping the resistance of the body toward infections, as well as wound healing. One of the important roles of zinc is taking part in secretion, synthesis, and use of insulin. Also, it protects pancreatic β-cells (cells which produce insulin) from decomposition. Zinc is involved in the metabolic processes of thyroid and adrenaline glands, ovaries, and testicles. It is important for the normal development of male sexual hormones and prostate work.

Normal function of skin requires the presence of zinc. It is necessary for the proper work of sweat glands, activation of local hormones, control of inflammatory processes, formation of proteins binding vitamin A, and tissue regeneration.

Zinc is essential for the normal growth and fetus development and milk production during lactation period. Its level during pregnancy is connected to the normal development of palate and mouth, brain, eyes, bones, lungs, heart, and the urogenital system in infants. Appropriate zinc intake is necessary for the formation of neurotransmitters in the brain. Normal function and release of vitamin A from the liver requires the presence of zinc. Zinc is necessary for the maintenance of eyesight and sense of taste and smell. It is the most abundant microelement in the eye, and is involved in the formation of hydrochloric acid in the stomach and the transformation of fatty acids into prostaglandins that regulate body processes, such as heartbeat and blood pressure. Zinc is necessary for muscle contractions and maintenance of acid-base equilibrium in the body. Also, it helps in detoxification from alcohol.

Until now, more than 20 metal enzymes are known in which zinc is present: carbonic anhydrase, lactate dehydrogenase, glutamate dehydrogenase, alkaline phosphatase, thymidine kinase, and so forth. It is not surprising that zinc deficiency is followed by systematic dysfunction.

Important sources of zinc are animal proteins, while processed foods, lemons, and vegetables without leaves contain it in small quantities. Rich in zinc are shellfish, oysters, meat (particularly liver), fish, cheddar cheese, hazelnut, eggs, and seeds.

On average, it is desorbed at a rate of around 20–40% of zinc taken by food. However, desorption depends on zinc quantity in the body—small quantities are reabsorbed faster. Utilization of zinc is affected by other nutrients, as well as the type of food. Zinc is easier to accept from animal food (and fish), taking into consideration that high protein foods contain amino acids that bind zinc and make it soluble. Zinc from plants, fruits, and cereals are hard to

accept because this food contains compounds such as phytates and oxalates—compounds that bind zinc and reduce the zinc quantity that can be reabsorbed. Additives added to the food also can decrease its reabsorption. The presence of EDTA and high quantity of plant proteins decrease the reabsorption of zinc. Zinc reabsorption decreases with age, so people of age 65 or more can reabsorb only half the amount of zinc than can those who are of age 25–30.

Particular diseases, such as liver failure due to alcoholism, burns, recovery state after operation, stress, body weight loss, chronic infections, virus hepatitis, diabetes, and some kidney diseases require increased zinc intake. People who practice intensive sports activities must take increased quantities of zinc into the body.

2.4 Manganese

Only 3–5% of manganese can be reabsorbed from food. After reabsorption manganese is transported to liver. Its daily intake should be in the quantity of more than 1 mg. In high quantities manganese salts can be toxic (http://www.osha.gov/SLTC/metalsheavy/index.html).

Manganese is an essential microelement for the human. An average adult body contains between 12 and 20 mg, and it is mostly present in bones, liver, kidneys, and heart.

Manganese takes part in many enzymatic systems, although its role is not completely understood. It acts as a cofactor in enzymes necessary for energy production, and it is involved in the glucose metabolism, keeping glycogen in liver and aiding in the digestion of proteins and synthesis of cholesterol and fatty acids. It is also necessary for the synthesis of DNA and RNA.

Manganese is necessary for the growth and the maintenance of the nervous system, development and maintenance of bones and joints, the function of women's sexual hormones, and thyroid hormones (Santamaria, 2008).

Superoxide dismutase (SOD, MnSOD) is an antioxidant enzyme with a structure that contains manganese. By regular function, this enzyme provides protection from free radicals and the damage that can be caused by them. In addition, this protein protects brain cells from the damage caused by stroke and Alzheimer's disease. MnSOD protects the liver from damage. In alcoholics, it is observed that a higher quantity of MnSOD probably protects the body from the oxidative damages caused by alcohol.

Symptoms of manganese deficiency are very rare and they were recorded for the first time in 1972. Low manganese intake increases the loss of calcium from bones and increases the possibility of osteoporosis appearance.

Persons with diabetes have low manganese levels, which contributes to weak regulation of glucose and decreased function of pancreatic cells.

Manganese deficiency plays an important role in epilepsy and infertility. It leads to artery damage. Damage of arterial walls leads to the binding of harmful LDL cholesterol and the formation of atherosclerotic tiles.

Other symptoms of manganese deficiency are: unconsciousness, bone problems, slow growth of hair and nails, whole weakness of the organism, hearing problems, body weight loss, irregular walk, and skin problems. It can cause paralysis and blindness in children (Manganese, US EPA).

Harmful effects appearing with high manganese intake are very rare and include the appearance of lethargy, unwilling moves, change in tonus and attitude, and in more difficult cases even a coma. Toxic effect is known as "manganese madness," because it was recorded first in miners working in manganese mines. Symptoms include uncontrolled laughing, impulsivity, insomnia, bullying, and hallucinations.

Good sources of manganese are cereals, spinach, bread from integral wheat, walnut, fruit, vegetables with dark green leaves (cauliflower, broccoli), celery, aronia, fig, tea, liver, wheat bran, unpolished rice, almond, buckwheat, lentil, green beans, carrots, dried grapes, and dried plums.

2.5 Nickel

The importance of nickel was first discovered at the end of 1960s, although until then it was regarded only as a toxic metal. Since then, nickel is regarded as a "potential" essential microelement for human being, but its role in the body is still little known. The highest number of the investigations related to the role of nickel in the body was performed on chickens and rats.

It is thought that there are around 10 mg of nickel in human body. Nickel is a microelement present in numerous enzymes. Daily it should be minimally taken in the quantity of 0.3 mg (Cempel and Nikel, 2006).

The exposure to metallic nickel and soluble nickel compounds should not cross 0.05 mg/cm^3 for 40 h per week. Fume and dust of nickel sulfide are cancerous, and it is assumed that many nickel compounds are cancerous, too. Nickel carbonyl is an extremely toxic gas.

The biological role of nickel is still unknown. Although nickel is mainly equally distributed in the body, a somewhat higher quantity is present in nucleic acids, particularly ribonucleic acid (RNA

and DNA), and it is thought that somehow it affects the structure or function of proteins connected to nucleic acids.

Besides, the role of nickel is connected to enzymes with the influence on the degradation and use of glucose, and the formation of prolactins.

Enzymes that use nickel were revealed although nickel activates and inhibits the enzymes containing other metals. Besides of its role in enzymes, nickel is involved in the production and action of some hormones (Cempel and Nikel, 2006).

Nickel influences optimal growth, and structure of the bones. It is involved in the iron metabolism (because it affects the absorption of iron from the food) and plays the role in the formation of red blood cells—erythrocytes. It is necessary in the metabolism of sugars, fats, hormones, and cell membranes (Das et al., 2008).

The highest number of the investigations of the role of nickel is now performed in animals, so its relevancy on human cannot be confirmed.

Nickel causes allergies to the skin, dermatitis. These allergies may come from jewelry (Clarkson, 1988; Kasprzak et al., 2003).

Plants are major sources of nickel. Plants growing on soil polluted with nickel can contain higher nickel quantities. The foods that are rich with nickel are walnuts, hazelnuts, beans, chocolate, soybean, lentil, oats, buckwheat, barley, and corn. Among fruits, nickel is present in bananas and pears. Food of animal origin is poor in nickel, but nickel can be found in drinking water.

Besides its presence in food, nickel can be found in nonfood products, such as coins, jewelry, eyeglass frames, various household appliances, and so forth. A particular quantity of nickel can enter the body through the skin.

2.6 Daily Needs for the Essential Metals

Needs for the particular essential metal are the smallest quantities necessary for the individual to preserve good health. It is different among individuals, even among persons of the same age, sex, body size, and level of physical activity.

Recommended daily allowances (RDA) of any nutrient are the levels for which it is considered as enough to satisfy the needs of all people in a group with similar characteristics (such as age, sex, and body size and level of physical activity). RDA value is defined for vitamins and the majority of minerals and proteins and satisfies the highest levels of the population.

Daily intake (DI) does depend a lot on external and internal factors such as chemical forms of minerals, their presence and the level in the consumed food, percentage of the absorption in

gastrointestinal tract, and also habits during nutrition, weight, years, sex, and economical status of the individual. It can be said that DI represents the quantity of nutrients necessary to avoid their deficit and provides for necessary metabolic processes in human body.

Recommended daily allowances (RDA) of essential minerals represent standards in nutrition set by the US National Academy of Science, and the value is expressed in milligrams per bird. RDA value defines the level of essential nutrients necessary in order to reach the nutritional needs of a normal, healthy person.

In Table 1.1, daily intakes (DI) of some microelements, the percentage of the absorption (PA) in gastrointestinal tract, as well as recommended daily allowances (RDA) of these elements necessary to achieve optimal positive effects to the health of adults are provided. Results in Table 1.2 represent data from three relevant world organizations in this area (Expert Group on Vitamins and Minerals (EVM), England; Food and Nutrition Board: Institute of Medicine (FNB), United States; Food and Agriculture

Table 1.1 DI, PA in Gastrointestinal Tract and RDA of Macro- and Micrometals Expressed in Milligrams for Adults (Randjelovic, 2015)

Elements	DI	PA	RDA
Iron	15	10–40	10–15
Zinc	12–18	30–70	12–15
Manganese	5.6–8	40	2–3
Copper	2.4	25–60	1.5–3
Molybdenum	>0.15	70–90	0.075–0.250
Chromium	<0.15	10–25	0.05–0.20
Nickel	0.16–0.20	30–50	0.05–0.3
Cobalt	0.003–0.012	30–50	0.002
Vanadium	0.012–0.030	<1	0.01–0.025
Selenium	0.06–0.22	~70	0.055–0.07
Silicon	21–200	3–40	21–46
Boron	1–3	>40	1–2
Lithium	<0.001–0.99	60–100	—

Table 1.2 Recommended Daily Doses for Babies and Children under the Age of 9 (http://www.vision-srbija.com/vitamini-minerali/vitamini/131-preporuene-dnevne-doze-vitamina-i-minerala--rda#RDA1)

Mineral	0–6 Months	7–12 Months	1–3 Years	4–8 Years
Calcium	210 mg	270 mg	500 mg	800 mg
Chromium	0.2 μg	5.5 μg	11 μg	15 μg
Copper	200 μg	220 μg	340 μg	440 μg
Fluorine	0.01 mg	0.5 mg	0.7 mg	1 mg
Iodine	110 μg	130 μg	90 μg	90 μg
Iron	0.27 mg	11 mg	7 mg	10 mg
Magnesium	30 mg	75 mg	80 mg	130 mg
Manganese	0.003 mg	0.6 mg	1.2 mg	1.5 mg
Molybdenum	2 μg	3 μg	17 μg	22 μg
Phosphorus	100 mg	275 mg	460 mg	500 mg
Selenium	15 μg	20 μg	20 μg	30 μg
Zinc	2 mg	3 mg	3 mg	5 mg
Potassium	0.4 g	0.7 g	3.0 g	3.8 g
Sodium	0.12 g	0.37 g	1.0 g	1.2 g

Organization (FAO); World Health Organization (WHO) (Hodgson and Levi, 2004; Sparks, 2003; McBride, 1994).

The medicinal faculty of Academy of Sciences of the United States published the recommended daily doses of minerals depending on age and sex (Tables 1.2, 1.3, and 1.4).

From these data on heavy metals, the classification of diseases and their possible causes can be derived. Classification of diseases was performed by World Health Organization (Table 1.5) (http://www.who.int/classifications/icd/en/).

3 Vitamins

Vitamins are organic compounds of low molecular weight of different chemical structures. They are mainly of plant and rarely of microbiological origin. Vitamins can be produced in vertebrates

Table 1.3 Recommended Daily Doses for Older Children (9–18 Years) (http://www.vision-srbija.com/vitamini-minerali/vitamini/131-preporuene-dnevne-doze-vitamina-i-minerala--rda#RDA1)

Mineral	Men 9–13 Years	Men 14–18 Years	Women 9–13 Years	Women 14–18 Years
Calcium	1300 mg	1300 mg	1300 mg	1300 mg
Chromium	25 μg	35 μg	21 μg	24 μg
Copper	700 μg	890 μg	700 μg	890 μg
Fluorine	2 mg	3 mg	2 mg	3 mg
Iodine	120 μg	150 μg	120 μg	150 μg
Iron	8 mg	11 mg	8 mg	15 mg
Magnesium	240 mg	410 mg	240 mg	360 mg
Manganese	1.9 mg	2.2 mg	1.6 mg	1.6 mg
Molybdenum	34 μg	43 μg	34 μg	43 μg
Phosphorus	1250 mg	1250 mg	1250 mg	1250 mg
Selenium	40 μg	55 μg	40 μg	55 μg
Zinc	8 mg	11 mg	8 mg	9 mg
Potassium	4.5 g	4.7 g	4.5 g	4.7 g
Sodium	1.5 g	1.5 g	1.5 g	1.5 g

from the corresponding precursors. Some vitamins are synthesized in colons of animals, but in insufficient quantities, so they must be taken with food. The roles in catalysis in biochemical reactions are known for the majority of vitamins. Vitamins of B group are like coenzymes, built in prosthetic groups of enzymes playing important roles in biochemical processes. Characteristic problems caused by vitamin deficiencies are known as deficiency diseases. These phenomena are usually manifested in the form of deadlock in growth or changes on skin, which is particularly sensitive in some vitamin deficiencies.

On the basis of solubility, vitamins can be divided into two groups: vitamins soluble in water and vitamins soluble in fats.

Table 1.4 Recommended Daily Doses for Pregnant Women and Wet Nurses (http://www.vision-srbija.com/vitamini-minerali/vitamini/131-preporuene-dnevne-doze-vitamina-i-minerala--rda#RDA1)

Mineral	Pregnant Women 14–18 Years	Pregnant Women 19–50 Years	Wet Nurses 14–18 Years	Wet Nurses 19–50 Years
Calcium	1300 mg	1000 mg	1300 mg	1000 mg
Chromium	29 μg	30 μg	44 μg	45 μg
Copper	1000 μg	1000 μg	1300 μg	1300 μg
Fluorine	3 mg	3 mg	3 mg	3 mg
Iodine	220 μg	220 μg	290 μg	290 μg
Iron	27 mg	27 mg	10 mg	9 mg
Magnesium	400 mg	360 mg	360 mg	320 mg
Manganese	2.0 mg	2.0 mg	2.6 mg	2.6 mg
Molybdenum	50 μg	50 μg	50 μg	50 μg
Phosphorus	1250 mg	700 mg	1250 mg	700 mg
Selenium	60 μg	60 μg	70 μg	70 μg
Zinc	12 mg	11 mg	13 mg	12 mg
Potassium	4.7 g	4.7 g	5.1 g	5.1 g
Sodium	1.5 g	1.5 g	1.5 g	1.5 g

3.1 Vitamins Soluble in Fats (Oils)

These vitamins can be found in food rich in lipids, such as liver, milk fat, eggs, and oils. They are extracted by urine, so very often in vitamin therapy they are accumulated and cause very unpleasant effects. Vitamins A, D, E, and K belong to this group (Živanović and Kostić, 2008).

3.1.1 Vitamin A

Vitamin A includes several structurally different vitamins; among them the most important and active are retinol (vitamin A_1) (Fig. 1.1a) and 3-dehydroretinol (vitamin A_2).

β-Carotene shows high importance for human organism. It is one unusual type of lipid (fat) antioxidants. It can supplement

Table 1.5 Tabular Representation of Diseases and Their Potential Causes (http://www.who.int/classifications/icd/en/)

		Potential Causes	
	Group of Diseases	**Deficiency**	**Excess**
1	2nd group: Tumors	Zn	Zn, Fe
2	3rd group: Blood diseases and diseases of forming organs and disorders of immunity	Cu, Zn, Fe	Zn
3	4th group: Diseases of endocrine glands, nutrition and metabolism	Zn, Mn	
4	5th group: Mental and behavior disorders	Cu, Zn, Fe	Cu, Mn, Fe
5	6th group: Disorders of the nervous system	Fe, Mn, Cu	Cu
6	7th group: Eye diseases	Zn, Mn, Fe	
7	8th group: Ear diseases and diseases of mastoid resume	Cu	
8	9th group: Circulatory system diseases	Cu, Zn, Mn, Fe	Fe
9	10th group: Respiratory diseases		
10	11th group: Diseases of the digestive system	Zn, Fe, Mn	Fe, Cu, Zn
11	12th group: Skin and subcutaneous tissue diseases	Cu, Mn, Zn, Fe	Cu, Fe
12	13th group: Diseases of muscle-bone system and connective tissue	Fe, Cu, Mn, Zn	Fe, Cu, Mn
13	14th group: Diseases of urinary tract	Zn	Cu, Fe
14	15th group: Pregnancy, delivery, and confinement	Zn, Fe, Mn	
15	18th group: Symptoms, signs, and pathological clinical and laboratory reports	Zn, Fe, Cu, Mn	

antioxidant properties of vitamin E which is effective in high oxygen concentrations, and β-carotene is effective in low oxygen concentrations. Absorption of vitamin A in small intestine is being performed using bile acids. When β-carotene is used, in mucous membrane of the intestine and liver, carotene is partially transformed into vitamin A.

The role of vitamin A is:

- normal growth and health;
- eyesight-intensive investigation on vitamin A enabled researchers to confirm its role in the process of eyesight. In sticks

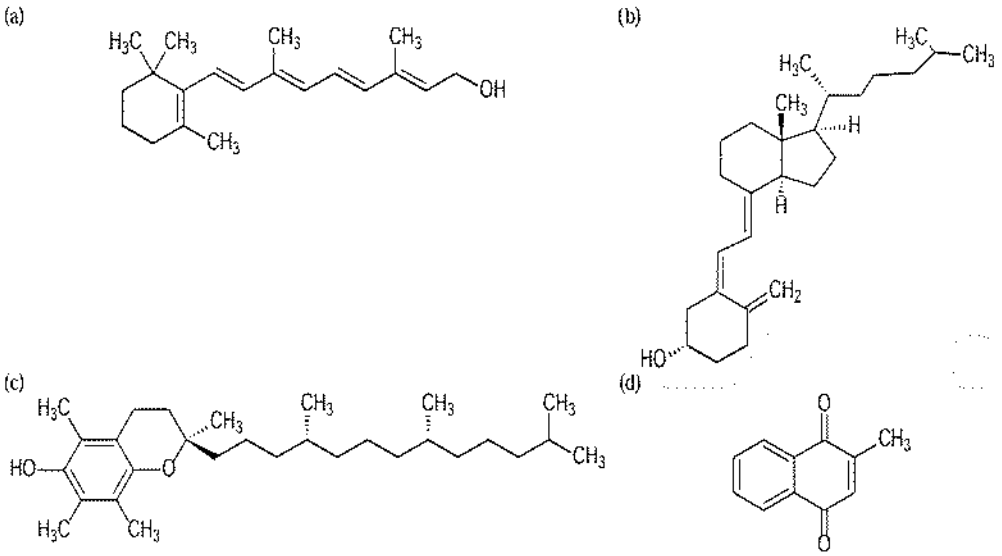

Figure 1.1. Structure of vitamins soluble in fats (oils). (a) *trans*-Retinol; (b) vitamin D_3; (c) vitamin E; (d) vitamin K_3.

of retina the visual pigment rhodopsin is situated. These cells react on the light signals of small intensity, but they are not sensitive to light. Active ingredient in the eyesight process is the oxidized form of retinol–retinal or the aldehyde of vitamin A-connected with the protein opsin giving rhodopsin which acts against night blindness and low vision.

- reproduction,
- secretion of mucus,
- maintenance of differentiated epithelium,
- development of cells,
- increase of immunity, and
- antioxidant—It seems that β-carotene plays a role as an antioxidant, and therefore reduces the possibility of cancers. It was observed that in people taking for a longer period of time insufficient quantities of vitamin A, the possibility of getting lung cancer is considerably higher compared to persons taking this vitamin in sufficient quantities. This is particularly expressed in smokers (in case if they are taking insufficient quantity of vitamin A). Different studies showed that this is not only related to lung cancer, it is valid also for throat cancer, bladder, stomach, colon, and prostate (http://www.stetoskop.info).

3.1.2 Vitamin D

Vitamin D was wrongly named a vitamin, although it is a prohormone which is transformed in the organism into steroid hormone-vitamin D hormone (calcitriol). For human, the most important is vitamin D_3 which develops in the skin from provitamin D_3 (Fig. 1.1b).

Provitamin D_3 from the food is absorbed in small intestine using bile salts, although it can be synthesized from the skin using 7-dehydrocholesterol under the influence of ultraviolet lights. Provitamin D_3 bound to protein carrier via blood firstly is transported into the liver where by enzymatic hydroxylation it is undergone transformation into 25-hydroxycholecalciferol, and then into kidneys where by the second hydroxylation into the active form of vitamin D hormone-1,25-dihydroxycholecalciferol (calcitriol) (http://www.stetoskop.info).

The most abundant form of this vitamin is vitamin D_3 or cholecalciferol. It is usually formed in the skin in humans and animals under the influence of sun radiation or ultraviolet light. In plants, vitamin D is formed from the provitamin ergosterol. Irradiation of ergosterol leads to ergocalciferol (vitamin D_2), while in animals by skin irradiation is made cholecalciferol (vitamin D_3) from the precursor 7-dehydrocholesterol.

It was found that vitamin D enables adoption of calcium and phosphorus in small intestine. Vitamin D enhances repeatedly adoption of phosphorus in kidneys. It enhances retention of calcium in bone marrow. It was determined that in animals taking insufficient quantity of calcium or small amount of phosphorus, rachitis is developing faster.

As some other vitamins, vitamin D has a protective role in various tumors (particularly tumor of colon and breast). Experimentally it was determined that vitamin D inhibits metastasis of colon tumor cells in humans. It is proposed that this vitamin has a particular positive effect in retinoblastoma and human leukemia.

Vitamin D increases the immunity of the organism. More than 10 years ago (before the antibiotic era) tuberculosis patients facilitated their difficulties by going to sanatoria in mountains, where they were exposed to fresh air, sunlight, and good nutrition. Later it was supposed that vitamin D probably influenced on the improvement of conditions of tuberculosis patients. The most abundant claim is that the active form of vitamin D stimulates the human macrophages (cells in blood for the "removal" of foreign and dead cells). These macrophages slow down or completely disable reproduction of bacteria which cause the disease. In this case, vitamin D is immunomodulator (http://www.stetoskop.info).

3.1.3 Vitamin E

Vitamin E (Fig. 1.1c) represents the common name for the group of several (probably 8) liposoluble compounds of alcoholic origin. For the human population the most important is α-tocopherol. It is found in the form of yellowish oil, resistant to the action of acids, insoluble in water and thermostable. Particularly, it is important that it undergoes slow oxidation which enables the exposure of its antioxidant properties and prevention of the oxidation of unsaturated fatty acids.

α-Tocopherol represents the strongest natural liposoluble antioxidant agent. Particularly, it is important for the prevention of oxidative stress in multi unsaturated fatty acids present in membrane structures of cells (particularly cells of immune systems, red blood cells, motor neurons, and retina). Antioxidant properties of vitamin E are enhanced when it is utilized with enzymes containing selenium. These enzymes also show antioxidant influence, common application enables the decrease of individual doses of both preparations (possible protective role in the prevention of atherosclerosis process and slowdown of the development of several forms of dementia).

Antioxidant effect of vitamin E is shown at high oxygen concentrations so it is most often concentrated in those regions with the highest content of oxygen (such as erythrocyte membrane and membranes of the respiratory tract).

Recently it was determined its role in mental disorders. It was shown that vitamin E plays a key role in normal mental functioning in humans, and animals as well. Different neurological disorders in human derive because of deficiency of this vitamin, and can be cured or prevented timely by the addition of vitamin E.

It is supposed that vitamin E positively affects the duration of life, so it is called the "youth vitamin" (http://www.stetoskop.info).

3.1.4 Vitamin K

Vitamin K represents group of compounds including liposoluble K_1 (phylloquinone) and K_2 (menaquinone) as well as hydrosoluble K_3 (menadion) (Fig. 1.1d). They are responsible for blood coagulation. For the discovery of vitamin K, Danish scientist Henrick Dam from University of Copenhagen received the Nobel Prize in Physiology and Medicine. Vitamin K_1 (phylloquinone) is the most often form of vitamin K found in food, while vitamin K_2 (menaquinone) is synthesized in colons (by colon bacteries) and represents the inner source of vitamin K.

Biologically the most active vitamin is vitamin K_1. Biochemical function of this vitamin is in blood coagulation. It was determined

that vitamin K is necessary for the normal formation of proteins in blood plasma-prothrombin, which is inactive precursor of thrombin. Thrombin transforms fibrinogen of blood into fibrin, making a clot. In this process calcium ions are also necessary. Vitamin K is necessary for the maintenance of normal concentrations of blood coagulation factors II, VII, IX, and X (they are synthesized in the liver). All these factors in liver are synthesized in inactive form, and their transformation into active form depends on vitamin K.

Because of the fact that vitamin K is produced by numerous microorganisms and majority of plants, and it was discovered in tissues of all organisms, the question arose if there is another activity except coagulation. Some data showed that it could be the coenzyme in the specialized path of electron transfer in animal tissue.

Important role of vitamin K is in bones mineralization. It is very important therefore to take vitamin K because of better healing of injured bones, prevention and treatment of osteoporosis. In persons with osteoporosis calcium is leaving bones, and it was observed that the level of vitamin K is decreased. However, if sufficient quantity of vitamin K is taken, this loss is decreasing and osteoporosis is slowing. It can be said that vitamin K plays a role in bone calcification.

In experimental conditions it was determined that vitamin K_3 can inhibit various tumors (particularly breast tumor, ovary, colon, stomach, kidneys, and lungs). The effect of this vitamin can be compared with some chemotherapeutic agents (http://www.stetoskop.info).

3.2 Vitamins Soluble in Water

Vitamins soluble in water includes vitamins from so-called B-complex and vitamins C, F, and H. The majority of vitamins from this group becomes part of coenzymes or presents prosthetic groups of enzymes. Because they are soluble in water, they are excreted from the body easily, so there is no danger of agglomeration and harmful influence (excessiveness) (Živanović and Kostić, 2008; Petrovic and Velimirovic, 2002).

3.2.1 Vitamin C

The first data on vitamin C are connected to British sailors, who observed that on long trips the intake of lemon juice can prevent the appearance of scurvy, a disease that manifests with heavy bleedings and which appears because of poor nutrition. The chemical name for vitamin C (Fig. 1.2a) is ascorbic acid and its structure is very similar to glucose. But, different from animals,

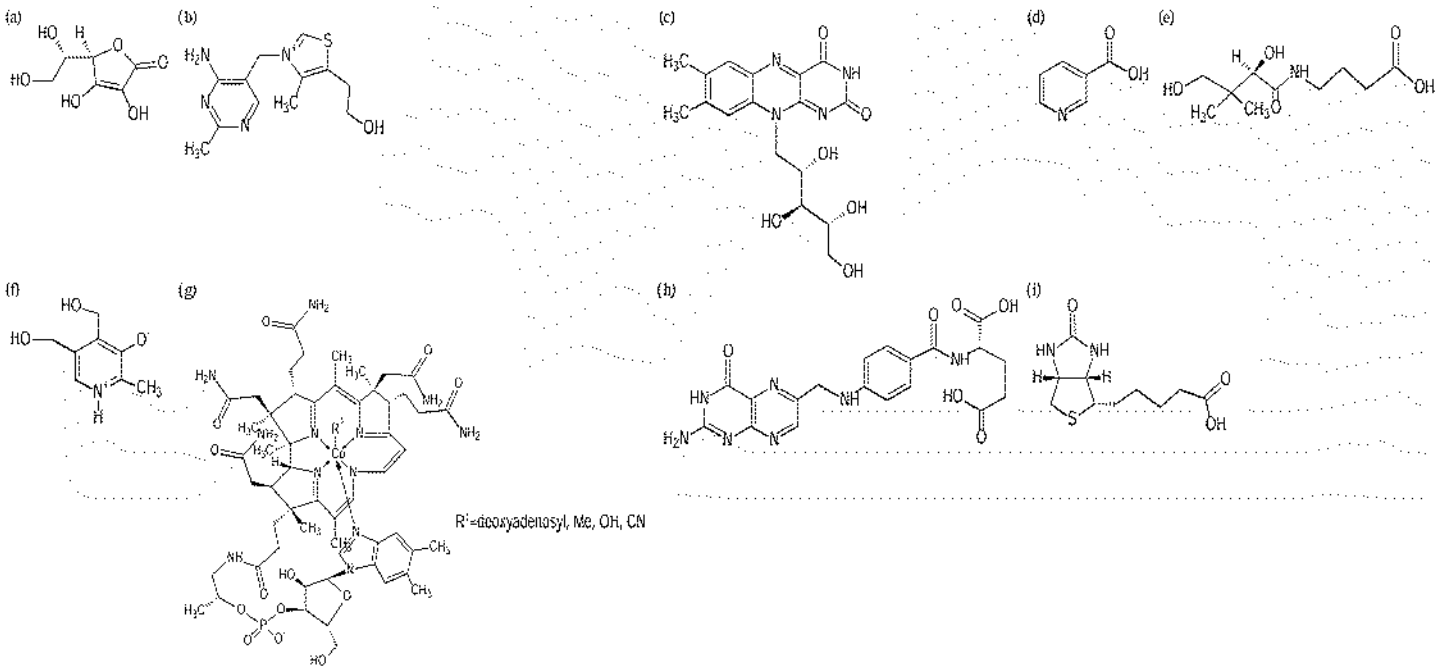

Figure 1.2. Structure of vitamins soluble in water. (a) Vitamin C; (b) vitamin B_1; (c) vitamin B_2; (d) vitamin B_3; (e) vitamin B_5; (f) vitamin B_6; (g) vitamin B_{12}; (h) folic acid; (i) vitamin H.

people cannot synthesize vitamin C alone because of the lack of a specific enzyme that transforms glucose into ascorbic acid.

The human body is not capable of synthesizing vitamin C alone, so it must be taken by food. The highest quantity of vitamin C is present in fresh fruits and vegetables.

This vitamin can be transformed into oxalate in the human body, which is excreted by urine. However, calcium salt of ascorbic acid, which is easily made from oxalate, is completely insoluble in water, so it can cause the appearance of kidney stones.

The biological role of ascorbic acid is connected to the participation in oxidation-reduction processes, blood coagulation, tissue regeneration, formation of steroidal hormones, and transformation of folic into tetrahydrofolic acid, and activation of many enzymes.

Vitamin C has an influence on the function of the central nervous system, stimulates the function of endocrine glands, enhances liver functions, enables absorption of iron in the colon and participates in blood formation (taking into account that it takes part in the synthesis of procollagen and collagen, as well as normalization of permeability of capillaries).

One of its very important roles is its antioxidant activity. This vitamin binds free radicals and it can play an important role in the prevention of various heavy diseases, such as tumors. This is particularly related to stomach tumor, tumor of esophagus and colon, and in alcoholics and smokers tumor of larynx. There are data that vitamin C prevents the growth of leukemia cells in humans. However, vitamin C has a role only in the prevention of tumors, but when the tumor is already developed, its role becomes negligible.

Vitamin C has a role in the protection of other vitamins (vitamin A and E) from the harmful effects of oxidation. It slows down aging in general and aging of wrinkles. It removes toxic metals from the body, provides protection from stress and strengthens general physical condition. Vitamin C maintains eyesight, thus preventing the formation of cataract, and can be useful in the treatment of glaucoma.

Vitamin C eases the consequences of asthma. Perennial investigations showed that in asthmatics the level of vitamin C decreases in the body, so it is necessary to take additional doses of this vitamin.

Vitamin C enhances the immune system of the body and prevents sniffles. It increases the resistance against infectious diseases (particularly against flu, and perhaps assists in avoidance of AIDS). The function of white blood cells partially depends on vitamin C. The low level of this vitamin in various infections and during exposure to radiation, drugs, alcohol, and cigarettes decreases white blood levels and thus decreases their activities.

In men, it can help in curing infertility caused by agglutination of spermatozoids. Vitamin C increases the adoption of zinc, magnesium, copper, and potassium, which are necessary for the normal function of spermatozoids (http://www.stetoskop.info).

3.2.2 *Vitamin B_1: Thiamine*

Thiamine (Fig. 1.2b) is a stable water-soluble vitamin, reactive to the effect of alkali. It contains thiazole ring.

The main role of thiamine is in the control of energy metabolism. Around 90% of the total quantity of thiamine is present in the form of thiamine pyrophosphate (TPP), a coenzyme in key reactions of glucose degradation and transformation of glucose into fats. It takes part in some key metabolic paths in nervous tissue, the heart, and formation of red blood cells (erythrocytes). It has an important role in smooth and striated muscles.

In tissues of humans and other animals, vitamin B_1 is present in the form of thiamine-pyrophosphate, which serves as a coenzyme in numerous enzymatic reactions, enabling the process of the transfer of aldehyde group.

Vitamin B_1 is absorbed in the colon, and the majority of the products of the metabolism of this vitamin is excreted by urine. There is no proof on the toxicity of this vitamin (http://www.stetoskop.info).

3.2.3 *Vitamin B_2: Riboflavin*

Riboflavin (Fig. 1.2c) is relatively thermostable and photosensitive water-soluble vitamin. It contains sugar ribose and yellow-green pigment-flavin.

Vitamin B_2 is adopted in the mucosa of the small intestine in the form of phosphate esters, and partly in free state. It makes deposits in liver, kidneys, and other organs. In small quantities it is present in blood, also.

It is involved in the activity of the enzyme glutathione reductase, which is taking part in the maintenance of the level of glutathione, the molecule protecting the organism from free radicals. This vitamin possesses antioxidant properties as well.

It was determined that biological activity of vitamin B_2 is higher in substrates, containing yellow pigments-flavins. Riboflavin is part of two important coenzymes: flavin-mono-nucleotide (FMN) and flavinadenin dinucleotides (FAD).

Flavin nucleotides act as prostetic groups of flavo-enzymes or flavoproteins. These enzymes participate in oxidative degradation of pyruvates, fatty acids, amino acids, and in the transfer of electrons (http://www.stetoskop.info).

3.2.4 Vitamin B_3: Niacin

Niacin is present in food in two forms: niacin (nicotinic acid) (Fig. 1.2d) and nicotinamide, which is water-soluble, thermostable. When it crystallizes it forms white powder. It is particularly important that niacin can be formed in the human body from the essential amino acid tryptophan, and in the ratio: 60 mg of tryptophan makes 1 mg of niacin. This quantitative ratio represents niacin equivalent (NE).

This vitamin has a very important role in the lowering of cholesterol level, as well as in the protection from various cardiovascular diseases. This characteristic was observed even at the beginning of the 1950s, when it was noticed that nicotinic acid lowers the level of cholesterol and triglycerides in the blood.

Vitamin B also plays a role in controlling diabetes, and it reduces migraine. It is assumed, although it is not still scientifically proven, that it helps in arthritis and decreases blood pressure. It is important for the synthesis of sexual hormones (estrogens, progesterone, testosterone), as well as cortisone, thyroxine, and insulin. The vitamin is necessary for the healthy state of the nervous system and the function of the brain (http://www.stetoskop.info).

3.2.5 Vitamin B_5: Pantenonic Acid

Pantenonic acid is an amide of pantoic acid and β-alanine (Fig. 1.2e). It is taken into the body as a food, and an important part is synthesized by colon bacteria. With phosphorus it forms coenzyme A (CoA).

Vitamin B_5 is absorbed in the small intestine via simple diffusion, and later it is phosphorylated using ATP. Vitamin B_5 is transformed into active coenzyme A.

The biological role of vitamin B_5 was determined by F. Limmann and H. Kaplan in 1950. Vitamin B_5 becomes part of coenzyme A. Coenzyme A is necessary for the activity of numerous enzymes, taking part in many reactions where acetyl and acyl groups are involved. Transformation of carbohydrates, lipids, and in substantial measure amino acids is found for the coenzyme A.

Vitamin B_5 plays an important metabolic role in human body. One of the important roles of vitamin B_5 is related to the production of hormones of the adrenal glands and production of energy.

Vitamin B_5 lowers the cholesterol levels in blood and protects the organism from cardiovascular diseases. This is among other benefits related to the derivative of pantothenic acid-pantethine. It helps in faster wound healing. It seems that this vitamin stimulates cell growth and the healing process. This claim

is not completely confirmed, but there are numerous indications (http://www.stetoskop.info).

3.2.6 Vitamin B_6: Pyridoxin

Pyridoxin takes its name from the pyridine ring representing the basis of its chemical structure. In nature, there are three forms for the group of compounds with similar function, which is commonly called vitamin B_6: pyridoxin, pyridoxal, and pyridoxamine (Fig. 1.2f). In the human body all three forms make pyridoxal-phosphate (PLP).

All three forms of vitamin B_6 are equally active and act as precursors of coenzyme pyridoxal-phosphate. Pyridoxal-phosphate represents the prosthetic group of numerous enzymes and participates in the metabolism of amino acids (in processes of phosphorylation, decarboxylation, and racemization) and metabolism of fats and carbohydrates.

It is absorbed in the small intestine mainly in the form of pyridoxal which is then transformed into pyridoxamine. All three compounds, covered with the name vitamin B_6 serve as the substrate for the enzyme pyridoxal-kinase, which uses ATP and phosphorylated them into corresponding phosphate esters. Besides, this vitamin is necessary for the work of more than six enzymes and smooths the synthesis of proteins and nucleic acids.

Important role of this vitamin is present in its role in the reproduction of all cells and formation of white blood cells and cells of the immune system.

It can play a role in the protection of the organism from the intake of oral contraceptive agents. Namely, women using contraceptives very often experience various disorders in tryptophan metabolism, and this can be regulated by the intake of vitamin B_6.

The positive role of vitamin B_6 in the prevention of cataracts was also observed (http://www.stetoskop.info).

3.2.7 Vitamin B_{12}: Cobalamine

Cobalamine (vitamin B_{12}) (Fig. 1.2g) is a complex crystalline compound of red color. Its intake into the body is mainly through food of animal origin, although it is partly synthesized by human colon bacteria.

Vitamin B_{12} is an important energy source for humans. There is no doubt that individuals with B_{12} deficiency disease benefit from taking this vitamin supplement. Even in persons suffering from megaloblastic anemia, significant signs of improvement occur with intake of this vitamin. The effect of the increase of energy

is particularly noticed when vitamin B_{12} is taken during periods of greater stress, fatigue, and as a protection against different diseases.

This vitamin facilitates neuropsychiatric problems. Deficiency of vitamin B_{12} is tightly bound to deterioration of mental functioning, that is, the lack of B_{12} causes neurologic and numerous physiological disorders.

Vitamin B_{12} has a protective role against tumors, particularly those forms caused by smoking. Since smokers have very low levels of vitamin B_{12} and low levels of vitamin B_9, researchers decided to investigate the effect of the addition of these vitamins in smokers. They found that smoking decreases the level of these vitamins, particularly in lung cells, and that the addition of the combination of these two vitamins considerably decreases the possibility of getting lung or bronchial cancers.

Vitamin B_{12} is a part of many biochemical processes, but the complete mechanism of its activity is not clear. Part of the coenzyme capable of reduction is cobalt. The function of vitamin B_{12} is tightly bound to vitamin B_9. It takes part in the formation of creatine, adrenaline, nitrogen bases, nucleic acids, proteins, and other biologically active substances (http://www.stetoskop.info).

3.2.8 Vitamin B_9: Folic Acid

Folic acid (Fig. 1.2h) consists of pteridine ring, a pair of aminobenzoic acid, and glutamic acid. Depending on the number of molecules of glutamic acid, there are pteroyl, di, and hepta derivatives of glutamic acid.

Vitamin B_9 in tissues is transformed into tetrahydrofolic acid (THFA, FH4), which can be called a coenzyme. This vitamin is taking part in the biosynthesis of nitrogen basis, nucleic acids, creatine, methionine, and in the formation of amino acid serine. Vitamin B_9 has a role (together with vitamin B_{12}) in the protection of different tumors, particularly lung tumors. It is necessary for the formation of red blood cells, and it has an important role in the prevention of innate defects.

In the organism of men and animals, vitamin B_9 is deposited in the liver and is excreted from the body in urine (http://www.stetoskop.info).

3.2.9 Vitamin H: Biotin

Biotin (Fig. 1.2i) belongs to sulfur compounds, and it is important for the whole metabolism in the body. Protein avidin situated in eggs can be bound to biotin and prevent its absorption in the colon.

Vitamin H is produced in the small intestine in humans, and it is produced by bacteria. One part of this vitamin comes from food. It functions as a component (that is, coenzyme) of specific enzymes, and catalyzes the reactions of carbon dioxide transfers (carboxylation). In this way, vitamin H is taking part in the synthesis of fatty acids, purine nucleotides, and the metabolism of branched amino acids. Vitamin H also contributes to healthier hair and protects from its loss (http://www.stetoskop.info).

4 Fortification of Food With Vitamins and Minerals

According to the Global Progress Report on Vitamin and Mineral deficiency, it seems that half of the population in Africa does not take large enough quantities of critical vitamins and minerals (UNICEF, 2004). Around 350 million women and children in Africa suffer from deficiencies in iron, vitamin A, and folic acid (World Health Organization, 2002). Interestingly, there are no clinical symptoms on deficiencies (hidden hunger) (Burchi et al. 2011). It is interesting to point out that vitamins in food are susceptible to losses or destruction due to technological/cooking processes. Some vitamins are more sensitive to temperature and light, and some fat-soluble vitamins are affected by oxygen (de Lourdes Samaniego-Vaesken et al., 2012).

According to Nilson and Piza, food fortification is the most efficient and viable solution; it is available to the poor, pregnant women, young children, and the population in general. Food fortification is also available to the elderly, the sick and other groups that are not able to maintain a balanced diet.

According to the Codex Alimentarius, food with nutritional equivalence is food to which a minimum of 5% of the RDA was added to the portion, reconstituted food is that to which 10–30% of the RDA was added and fortified food is the responsibility of the authorities of each country (de Lourdes Samaniego-Vaesken et al., 2012). In this moment, food fortification is present in several countries (main food is margarine, milk and its derivatives, cereal flours, and sugar).

Interestingly, one of the earliest reports of food fortification is from around 4000 BC, the Persian physician, Melampus, added iron filings to sweet wine to strength sailors “resistance to spears and arrows and to enhance their sexual potency” (Richardson, 1990; Panda et al., 2011). In 1833, the French chemist, Boussingault, recommended the addition of iodide to salt to prevent goiter in South America (Cowgill, 1964). In the 1920s, medical researchers

proclaimed that iodine can prevent goiter (Backstrand, 2002), and salt ionization began in Switzerland (Das et al., 2013). It was concluded that it was a successful fortification program. In 1932, milk was fortified with vitamin D, and this action was supported in public because of the prevalence of rickets in children (Backstrand, 2002). This successful fortification was followed by the 1941 fortification of flour and bread with B vitamins (Bishai and Nalubola, 2002).

WHO made the categorization of food fortification strategies into three possible approaches: (1) mass, (2) targeted, and (3) market driven (Allen et al., 2006). Mass fortification involves foods that are widely consumed (wheat, salt, sugar); targeted foods consumed by specific age groups, such as infant complementary foods; and specific brands for particular consumer niches, such as in a market-driven approach (Das et al., 2013). Food groups usually considered for fortification can be grouped into three broad categories: (1) staples (wheat, rice, oils); (2) condiments (salt, soy sauce, sugar); and (3) processed commercial foods (noodles, infant complementary foods, dairy products) (Das et al., 2013).

Disadvantages of the fortification strategy are: (1) the dose is a function of food quality consumed; (2) lower specificity; (3) varying standards legislated for each country and quality control; and (4) regulatory challenges pertaining to fortification levels with the manufacturers of fortified foods (Ritu and Gupta, 2014).

The best and basic fortification practices are summarized in Table 1.6 (Johnson and Wesley, 2010).

Table 1.7 describes the national practices of mandatory and voluntary fortification before 2006 (Flynn et al., 2009).

Fortification of food with vitamin A is necessary in case of risk groups because of its loss during cooking and storage (Cort et al., 1976). The most frequent vitamin A fortificant for cereals is vitamin A palmitate (a dry compound that is stable in flour) (UN, 2010). In case of dry products, such as cereal flours, it is required to convert vitamin A into a water-soluble compound and dry it. Meat products must be fortified because of the loss of useful compounds during the heat treatment. Until now, there are two efficient trials on the fortification of wheat flour with vitamin A (Table 1.8). In the Philippines, a popular bun–*pandesal*—was fortified with vitamin A (4.5 mg/kg) (Solon et al. 2000). Since 1969, cereal flour–based food aid commodities (particularly wheat-soy and corn-soy blends) have been fortified with 7 and 10 mg vitamin A/kg, respectively (Table 1.9).

Iron deficiency in developing countries affects at least half of all pregnant women and young children (WFP, 2006; Merx et al., 1996). The fortification of maize meal or flour with iron and folate is

Table 1.6 Best and Basic Fortification Processes (Johnson and Wesley, 2010)

Fortification Component	Basic Practice	Indicator	Best/Enhanced Practice	Indicator
Quality system	GMPs	GMP manual	HACCP and/or ISO 9001 or 9002	Manuals Third party audits certificates
Premix				
Premix specifications	On file in GMPs	Documents	Quality system manuals	Documents
Premix ordering	SOPs	Documents	Quality system manuals	Documents
Packaging, storage and handling	SOPs, dry, out of sunlight, boxes closed, premix lot numbers recorded	Inspection and documents	SOPs, dry, out of sunlight, boxes closed, premix lot numbers recorded	Inspection documents
Handling practices	SOPs, feeder hoppers covered, employee protection masks and gloves	Inspection	SOPs, feeder hoppers covered, employee protection masks and gloves	Inspection
Mill preblends	SOPs, production records	Inspection and documents	SOPs, production records	Inspection documents
Feeders				
Type	Volumetric, screw, disk, or drum	Inspection	Gravimetric, loss in weight	Inspection
Feeder calibration	SOPs	Documents	SOPs	Documents
Feeder maintenance	SOPs	Documents	SOPs	Documents
Fortification component	Basic practice	Indicator	Best/enhanced practice	Indicator
Fortification process				
Feeder location or addition point	At least 3 m from discharge of flour collection conveyor OR additional blending system, pneumatic or conveyor	Inspection	At least 3 m from discharge of flour collection conveyor OR additional blending system, pneumatic or conveyor	Inspection
Feeder control	Electrical interlock system	Inspection	Electrical interlock system	Inspection

Table 1.6 Best and Basic Fortification Processes (Johnson and Wesley, 2010) (*cont.*)

Fortification Component	Basic Practice	Indicator	Best/Enhanced Practice	Indicator
Continuous monitoring			Feeder controls tied in with flour scale and computer or microprocessor controlled	Inspection
Production rate determination	SOPs calculations	Inspection and documents	SOPs calculations	Inspection and documents
Premix feed rate determination	SOPs calculations	Inspection and documents	SOPs Calculations	Inspection and documents
Routine check weighing	SOPs feeder discharge Check weighing every 8 h or once per shift by miller	Documents	SOPs, feeder discharge Check weighing every 4 h Or once per shift by miller	Documents
Mill QC				
Sampling schedule	SOPs, iron spot test every 4 h	QC records and documents	SOPs, iron spot test every 2 h	QC records and documents
Analytical testing qualitative	SOPs, QC methods iron spot test	QC records and documents	SOPs, QC methods Iron spot test compared with standard sample	QC records and documents
Analytical testing quantitative	Composite samples, monthly basis using external lab-iron only	QC records and documents	Composite samples, monthly basis using external lab—all added micronutrients	QC records and documents
Usage and inventory control	SOPs, calculations on monthly basis	QC records and documents	SOPs, calculations on weekly basis	QC records and documents

GMP, Good manufacturing practices; *HACCP*, hazard analysis and critical control points; *SOPs*, standard operating procedures; *QC*, quality control.

Table 1.7 Fortification Practices in Europe Prior to 2006 (Flynn et al., 2009)

Country	Fortification Practice
Denmark	Mandatory fortification of household salt and salt used in bread—practiced since 2000 Permission needed for voluntary fortification Optional fortification permitted for vitamin A and β-carotene to margarine and fat spreads; calcium, phosphorous, iron, vitamin B_1, B_2, and niacin to certain flours and breakfast cereals and vitamin C to juices
Finland	No mandatory fortification Common practice (consensus between authorities and food industry) fortification: iodine in salt, vitamin D in milk and margarines, vitamin A in margarines Large voluntary fortification (permission needed): for example, vitamins A, E, and C and calcium in fruit juices, calcium in milk and margarine, group B vitamins in energy drinks, juices, and ready-to-eat breakfast cereals Permission granted on safety aspects only
Germany	No mandatory fortification Voluntary fortification permitted for water-soluble vitamins and vitamin E. Vitamin A and vitamin D are allowed in certain foods (milk products and margarine) For minerals and fat soluble vitamins (in other products) permission is needed for fortification: relatively difficult to obtain, especially for products that are not already sold in EU Iodization of salt is encouraged
Ireland	Mandatory food fortification: vitamins A and D to margarine; Common practice to add vitamins A and D to fat spreads Voluntary fortification: no statutory controls on the levels of nutrients added to food. Voluntary fortification practiced mainly for ready-to-eat breakfast cereals and drinks
Italy	No mandatory fortification Permission needed for voluntary fortification A list of factories authorized to produce special dietary foods, fortified food and food supplements was published in May 2007 Iodization of salt is encouraged. Outlets selling salt must have also iodized salt (50%)
The Netherlands	No mandatory fortification Iodine in salt, iodized salt in bread, vitamins A and D in margarine common Fortification with vitamin A (as retinoid), vitamin D, folic acid, selenium, copper, and zinc is prohibited, since 2004 exemption is possible Since 2004: only on the basis of harm to public health fortification can be prohibited Voluntary fortification of other micronutrients, in particular in beverages, dairy products, and breakfast cereals
Poland	Mandatory fortification: vitamins A and D in margarine, iodine in salt Voluntary fortification encouraged: minimum portion or 100 g contains 15% of recommended daily intake (RDI), maximum portion or 100 g contains 50% RDI (vitamin C and folate: 100% RDI)

Table 1.7 Fortification Practices in Europe Prior to 2006 (Flynn et al., 2009) (*cont.*)

Country	Fortification Practice
Spain	No mandatory food fortification Voluntary fortification encouraged with respect to iodization of salt. Fortification level: minimum 15% and maximum 100% of RDI Voluntary fortification of dairy products, especially liquid ones
United Kingdom	Mandatory fortification of white and brown flour (calcium, iron, thiamin, and niacin), margarine (vitamins A and D), infant formulas, and foods intended for use in energy restricted diets Voluntary fortification: no statutory controls on the levels of nutrients added to food

feasible, inexpensive, safe, and likely to be beneficial (Ingram et al., 1999). Iron fortification was improved when NaFeEDTA was used for this purpose in Thailand and Vietnam (Garby and Areekul, 1974; Thuy et al., 2003), soy sauce in China (Mannar and Gallego, 2002), sugar in Guatemala (Viteri et al., 1995), and curry powder in South Africa (Ballot et al., 1989). The addition of ferrous bisglycinate to flavored milk in Saudi Arabia (Osman and Al-Othaimeen, 2002) and Brazil (Miglioranza et al., 2003) drastically reduced anemia in children and adolescents after 3 and 12 months, respectively. Ascorbic acid is the most commonly added compound for the enhancement of iron absorption from iron-fortified foods (attributed to its reducing and chelating properties during digestion of the food) (Conrad and Schade, 1968). Encapsulated ferrous sulfate and encapsulated ferrous fumarate are in use for the fortification of infant formulas and cereals (Hurrell et al., 2004).

Zinc fortification of cereal food (wheat flour, maize flour, or rice) is not widely practiced (Table 1.10). At this moment, four countries (Indonesia, Mexico, Jordan, and South Africa) require zinc fortification of wheat flour. Voluntarily thirteen countries include zinc in their fortification programs, and five recently proposed their own zinc programs. The recommended levels of zinc fortification range from 14 to 33 mg Zn/kg flour. Mexico, South Africa, Uganda, and Zambia also include zinc in maize fortification programs—levels of zinc fortification range from 10 to 25 ppm. Several zinc compounds are considered safe for humans. Zinc oxide is the cheapest chemical form, although there were concerns regarding its bioavailability because it is insoluble at neutral pH.

Table 1.8 Summary of Efficacy Trials on the Impact of Vitamin A–Fortified Wheat Flour (Klemm et al., 2010)

Country	Design and Study Subjects	Sample Size: Fortified Flour	Sample Size: Unfortified Flour	Food Vehicle	Nutrient Level	Target Daily Vitamin A Intake From Fortified Flour Product	Results
Philippines (Solon et al., 2000)	Individually randomized, controlled trial Schoolchildren 6–13 year	396	439	Pandesal	6000 μg RAE/kg	133 μg (33% RDA)	Mean (±SD) serum retinol (μmol/L) fortified vs. nonfortified: Baseline: 1.17 ± 0.33 vs. 1.18 ± 0.30 30 weeks: 1.32 ± 0.37 vs. 1.31 ± 0.40 % low liver stores based on MRDR[a] ≥ 0.06 among subsample of children with lowest baseline serum retinol concentration ($n = 72$ and $n = 77$ for vitamin A and no vitamin A) at 30 weeks: 29 vs. 15[b]
Bangladesh (Rahman et al., 2003)	Cluster-randomized, controlled trial Schoolchildren 6–15 year	191	143	Chapatti	3000 μg RAE/kg plus other nutrients	212 μg (35–55% RDA)	Mean (±SD) serum retinol (μmol/L) fortified vs. nonfortified: Baseline: 0.96 ± 0.26 vs. 0.98 ± 0.29 3 months: 1.07 vs. 1.04 6 months: 1.05 vs. 0.94[b] % < 0.70 μmol/L: vitamin A vs. no vitamin A Baseline: 13.6 vs. 15.4 3 months: 7.9 vs. 16.2[b] 6 months: 7.4 vs. 22.5[b]

MRDR, modified relative dose response; *RAE*, retinol activity equivalent; *RDA*, recommended dietary allowance.
[a] MRDR is an indirect assessment of liver stores that tests the relative responsiveness of serum retinol following receipt of a standard, small dose of vitamin A.
[b] Between-group differences are statistically significant at $p < .05$.

Table 1.9 Countries with Voluntary or Mandatory Vitamin A Fortification of Wheat Flour (Klemm et al., 2010)

Country	Product	Mandated level-μg RAE/g (IU/g)
Nigeria	Wheat flour	9.0 (30)
South Africa	Wheat flour (white)	(5.36)
	Wheat flour (brown)	1.414 (4.712)
	Wheat bread (white)	0.8 (2.664)
	Wheat bread (brown)	0.700 (2.331)
Lesotho	Wheat flour	1.784 (5.947)
Indonesia	Noodles	
Palestine	Wheat flour	1.0 (3.333)
Philippines	Enriched wheat flour	3.0–6.5 (10.0–21.7)
Afghanistan[a]	Wheat Flour	7.078 (23.594)
Bangladesh[a]	Wheat flour	3.3 (11.0)
Venezuela	Wheat flour	2.85 (9.5)
Jordan	Wheat flour	1.5 (5.0)
Ghana	Wheat flour	2.0 (6.666)
Uganda[b]	Wheat flour	2.52 (8.4)

RAE, retinol activity equivalent.
[a] Managed by the World Food Programme.
[b] Voluntary except for World Food Program–purchased flour.

Currently, sodium ethylenediaminetetraacetate is considered for a possible role as the enhancer of zinc absorption.

According to regulations from March 1996 proposed by the United States Food and Drug Administration (FDA), all products made from cereal grain flours from January 1, 1998, must be fortified with folic acid (140 μg/100 g of flour) in order to prevent NTDs (Food and Drug Administration 1996). By 2009, 59 countries proposed national regulations for mandatory wheat flour fortification and 51 required folic acid supplementation (http://www.sph.emory.edu/wheatflour/coutrydata.php). From 2005, systematic reviews on the potential beneficial and adverse effects associated with folic acid were conducted by Food Standards Australia New Zealand (FSANZ) (http://www.foodstandards.gov.au/_srcfiles/FAR_P295_Folic_Acid_Fortification_%20Attachs_1_6), the Food

Table 1.10 Current Levels of Zinc Fortification Recommended in National Flour Fortification Programs (Brown et al., 2010)

Country	Type of Flour	Type of Program	Fortification Level (mg Zinc/kg Flour)
Azerbaijan	Wheat	Voluntary	18
Bangladesh	Wheat	Voluntary	33
China	Wheat	Voluntary	25
Fiji	Wheat	Voluntary	30
Ghana	Wheat	Voluntary	20
Guinea	Wheat	Voluntary	14
Indonesia	Wheat	Mandatory	30
Jordan	Wheat	Mandatory	20
Kazakhstan	Wheat	Voluntary	18
Kenya	Wheat	Voluntary	30
Kyrgyzstan	Wheat	Voluntary	18
Lesotho	Wheat	Voluntary	15
Mexico	Wheat and maize	Mandatory	16
Mongolia	Wheat	Voluntary	18
Palestine	Wheat	Voluntary	15
South Africa	Wheat and maize	Mandatory	15
Tajikistan	Wheat	Voluntary	18
Tanzania	Wheat	Voluntary	30
Uganda	Wheat and maize	Voluntary	30
Uzbekistan	Wheat	Voluntary	18
Vietnam	Wheat	Voluntary	30
Zambia	Wheat and maize	Voluntary	15

Safety Authority of Ireland (http://www.fsai.ie/uploadedFiles/folic_acid.pdf), the Scientific Advisory Committee on Nutrition (SACN) for the UK Food Standards Agency (Scientific Advisory Committee on Nutrition, 2006), and the Health Council of The Netherlands (Health Council of The Netherlands, 2008).

Interesting investigations were conducted in Sweden and Hungary, and it was connected to the maternal use of folic acid and the increase of twinning (Czeizel et al., 1994; Ericson et al., 2001; Kallen, 2004; Czeizel and Vargha, 2004). However, this can happen because of assisted reproductive technologies in these countries (Vollset et al., 2005; Berry et al., 2005).

In the Optimal Fortification with Vitamin D (OPTIFORD) European Project, a relative contribution of sun exposure during summer and diet to vitamin D status were analyzed in five European countries (adolescents versus elderly women). During the assessment of vitamin D, it was found that elderly females consumed 3.9 ± 5.0 μg/day, and adolescent girls 2.8 ± 2.7 μg/day with fish and eggs as the main food sources. In contrast, sun exposure, measured with an adhesive skin dosimeter (J/m^2) revealed that Spanish elderly women received less than half the sun exposure compared to youngsters. This situation was not observed in the rest of participating countries (eg, Finland) (Food and Agriculture Organization/World Health Organization, 1995). Vitamin D is a fat-soluble vitamin, so it can be added to "fat-rich" products (whole milk, cheese) and "fat-poor" foods (skim milk, fat-free yogurt, orange juice, etc.). Several formulations of vitamin D are used to suit all matrices (World Health Organization, 2006; Grossmann and Tangpricha, 2010).

5 Determination of Vitamins and Minerals in Fortified Food

A rapid and reliable method for the simultaneous determination of vitamins B_1, B_6, and B_{12} in homogenized boiled ham and in burgers was performed (Riccio et al., 2006).

However, serious mistakes are being made in the vitamin doses used for the fortification during food processing. In Massachusetts (United States), the concentration of vitamin D_3 in milk was found to be 70–600 times higher than the RDA (10 μg/L) (Scanlon et al., 1995; Rasmussen et al., 2000). In another study, it was found that 80% of the fortified milk samples presented a variation in vitamin content of 20% in relation to the amount printed on the label. One sample found 914% more vitamin D than specified (Chen et al., 1993). In Honduras, although sugar fortification is mandatory, vitamin A was not detected in 34%, and 21% of the sugar consumed in rural and urban regions, respectively (Nestel et al., 1999). Mills (Mills, 2000a,b) wrote that in the United States, fortified cereals, consumed in large quantities by children, contained 200% or more folate than stated on the label.

6 Improvements of Awareness and Health Culture on Fortified Food

The health benefits of successful fortifications must be advertised on TV programs for children, and for people of all ages, and become part of school curricula. Examples of successful fortifications are the best ambassadors of this campaign.

Here, we will mention only a few effective fortifications:

1. In Guatemala, mandatory sugar fortification was introduced in 1988 and caused the reduction of vitamin A deficiency from 22% to 5% in 1 year (Mora et al., 2009).
2. Addition of folic acid in South Africa to wheat and maize flour in 2003 decreased neural tube defects by 30% (GAIN, 2012).
3. In Chile, the addition of folic acid to wheat flour led to a three- to fourfold increases in blood folate levels in women of reproductive age and caused a decrease in neural tube defects from 51 to 46% (Gottlieb, 2012).
4. In China, surveys in 21 health centers showed a decrease in anemia by approximately one third following the fortification of soy sauce with iron in 2003 (GAIN, 2010).

7 Conclusions

Fast-paced lifestyles and inappropriate diets are frequent causes of numerous diseases in the modern world. Fast food is very often poor in minerals and vitamins. Fortification of common foods in the world is a prerequisite for a balanced diet, decreases in diseases, and prevention of their appearances. Numerous fortifications are successful in many countries of the developed and developing world. However, there are occasional overfortifications, with very negative effects. Proper control, and unique standards recommended by world agencies for the control of food quality, can prevent such phenomena.

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2

FUNCTIONAL NUTRACEUTICALS: PAST, PRESENT, AND FUTURE

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1 Introduction

Diet and food plays a vital role in human health. This belief has been held since time immemorial in human history. But what is new now? It is its logical confirmation through "scientific processes" and the new "parlance." Scientific process is a way of structuring knowledge and making predictions that can be tested. The term "nutraceutical" was first revealed some 20 years ago to describe the union between nutrition and pharmaceutics, both key contributors to human wellness. Nutraceuticals are food ingredients or sourced from food products, as the case may be, that provide extra health benefits, in addition to the basic nutritional value found in foods. They are considered to prevent chronic diseases and help improving health. As a natural corollary thereof they help in improving the quality of life, delay aging, and increase life expectancy. A plethora of evidence supports the health benefits and value of nutraceuticals in the treatment and prevention of diseases and the important role these compounds play in physiological functions.

Numerous health benefits of nutraceuticals, however, still remain a mystery. The extent up to which they provide health benefits continues to be a subject matter of research. But what we do know so far is that they work in harmony with other vitamins and nutrients present in fruits and vegetables to keep our bodies well and healthy. In the past 20 years, many research publications have been devoted to so-called "functional foods" and "nutraceuticals." Research into functional ingredients has been showing promise for the use of such ingredients in food products, thereby creating added value for manufacturers and health benefits for consumers (Lipi et al., 2012; Sinéad et al., 2011). The growing interest in this

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field can be gauged by the number of publications on food and nutraceuicals, which has prominently risen from year to year.

2 Naturally Derived Bioactive Compounds

Keeping the aforementioned in mind the main focus of this chapter is on "nutraceuticals." Bioactive natural products, that is, nutraceuticals are the main source of new drugs, functional foods, and food additives. They are secondary metabolites of plants and those get generated through various biological pathways in secondary metabolism processes. Typical features of these bioactive natural products include: (1) diverse structures, that is, flavonoids, alkaloids, sterols, terpenes, quinones, phenyl propanoid, and so forth; (2) molecular mass between 200 and 1000, usually with complex structures containing a skeleton of aromatic rings or multirings and a number of functional groups; (3) their various physiological activities; and (4) their boiling points, which are mostly above 200°C, some of which are also heat-sensitive.

1.1 Subtlety of Nutraceuticals

For many years there has remained confusion about the definition of "nutraceuticals." The European Nutraceutical Association defines nutraceutical as products having nutritional value concerning human well-being (www.enaonline.org). But as compared to pharmaceuticals, which are artificial or chemical compounds for definite indications, nutraceuticals are food ingredients or sourced from food products. For this reason the definition coined by the European Nutraceutical Association does not include dietary nonnutrient compounds commonly marketed as "Nutraceuticals" and which include polyphenols or some carotenoids. American Nutraceutical Association in this context is steadfast. It uses the definition coined by De Felice (1989). To quote De Felice, he described nutraceuticals as "a food (or a part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease." (Brower, 1998). There may be an array of products containing nutrients that may have little in common with nutritional supplements and definite food regimes, diet planned to suit genetic requirements, products derived from medicinal plants, brews, soups, and processed and preserved nutritious grains. However, a direct reference to food (cereals, soups, and beverages) reveals that current concepts of "functional food" and "nutraceutical" are overlapping each other and their definitions are not well established.

In the United States, a product is classified and regulated as food, food supplement, diet constituent, or nutritional supplement on the basis of its components and the tag with which the same is marketed (http://www.fda.gov/Food/DietarySupplements/QADietarySupplements/default.htm#top; http://www.nap.edu/openbook.php?record_id=10882). Food and Drug Administration (FDA), under Dietary Supplement Health and Education Act of 1994 (DSHEA) has laid down specific guidelines that protect the consumer for any misstatement by the manufacturer. DSHEA places the onus on the manufacturer to ensure, before it markets its product, that a dietary supplement or component is safe for human consumption (http://www.fda.gov/Food/DietarySupplements/default.htm).

Under the Canadian law, a "nutraceutical" or "functional food" has no legal distinction. Both of these can either be marketed as a food or as a drug. Drug and food products must comply with all the quality and safety requirements of the Food and Drug Regulations. In addition to quality and safety requirements, drugs may be approved for sale if they meet the regulatory requirements for efficacy. The law describes *nutraceutical* as a product derived or refined from nutrients that is marketed in medicinal form but not typically connected with food and is validated to provide functional benefit or shield against protracted illness. *Functional food* for the said law is visually comparable with or may be traditional food taken as customary diet and is revealed to provide bodily health in addition to basic nutritional functions. Besides it may also aid in reducing the threat of chronic ailment.

There exists deficient information in the literature about the definition of nutraceuticals and functional foods. Some ambiguity is also occasioned by the want of uniformity in the method of their intake. It is, therefore, important that we differentiate perceptions about functional foods, dietary supplements, and nutraceuticals so as to segregate them from each other. Functional foods are always consumed in ordinary food format. Nutraceuticals are usually consumed as pills or capsules while dietary supplements are those that supplement food and may contain one or more nutritive components including minerals; amino acids, herbs; vitamins; and other substances. Therefore, when a phytochemical is added to food it is considered as a functional food. However, when the same is consumed as a pill it is called a nutraceutical. This confusion, as it exists in literature, has necessitated the documentation of some protocol to differentiate between functional foods, dietary supplements, and nutraceuticals

This confusion in the literature has been clarified by González-Sarrías et al. (2013) by differentiating functional foods, nutraceuticals, medical foods, and botanicals. Therefore, their

heterogeneous nature/arrangement can be explained as in the following outline.

1. *Functional foods* are those that provide a scientifically proven specific health benefit (health claim) beyond their nutritional format. These include processed foods or foods with health-promoting additives such as the addition of iodine to table salt or vitamin D to milk.
2. *Dietary (or food) supplements* are products that are consumed to complement dietary (or food) supplements. They are not intended to treat, diagnose, mitigate, prevent, or cure diseases. Besides other substances, they are believed to include fatty acids, vitamins, minerals, amino acids, or fiber. It is also believed that more than 50,000 dietary supplements are available, mostly multivitamins. They are consumed in a pharmaceutical format, that is, pill, tablet, powder, and so forth, but not in the form of beverages (soups, juices, etc.) or conventional foods (this feature is common for all dietary supplements). Dietary (food) supplements can be divided into:
   a. *Medical foods* are supplements with formulations for dietary management of diseases and are intended to provide specific nutritional requirements, commonly in patients, and those under medical supervision. For example, nutrient-modified products for patients suffering from cancer, metabolic disorders, and so forth.

A *botanical* is a plant or a plant part valued for its medicinal or therapeutic properties. Botanicalsare dietary supplements from nonfood origin (plants, algae, fungi, or lichens). A compendium of 900 botanicals has been reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements (http://www.fda.gov/Food/DietarySupplements/default.htm). They are not essential for human life. They can be also be found in food preparations. For example: Ginseng, gingko extracts, and so forth. Except for the appro priate use of vitamins, dietary supplements should not be used for prevention of disease or to treat the same.

3 Indicative Matrix of Phytochemicals (With Nutraceutical Properties) and Possible Health Benefits

Plants need strong antioxidants to protect them against cellular damage that may be caused by a large number of free radicals that are produced during photosynthesis. Antioxidant plant pigments, primarily carotenoids and flavonoids, also called

phytochemicals, provide most of the protection to the plants. Research has shown that human ingestion of these compounds results in similar protection.

Phytochemicals is a broad name for a variety of compounds produced by plants. Allyl sulfides, anthocyanidins, catechins, carotenoids, flavonoids, flavones, isoflavones, isothiocyanates, phytonotrients, and polyphenols are all common names for phytochemicals. It is believed by researchers that there are some 4000 phytochemicals; some have been identified by the researchers but all have not been studied closely. However, they all are found in vegetables, fruit, beans, and grains (González-Sarrías et al., 2013).

A balanced intake of vegetables, fruit, beans, and grains can provide a variety of beneficial compounds. New studies are emerging to demonstrate that there are multiple effects of vegetables and fruits. They are low in fat, salt, and sugar. They are a good source of dietary fiber. They contain many vitamins and minerals that are good for health. These include vitamin A (beta-carotene), C and E, magnesium, phosphorous, folic acid, and zinc. It is now believed that these may have a greater role to play in human health than is already understood by researchers (http://www.fruitsandveggiesmorematters.org/). Phytochemicals, which are concentrated in the skin, are found in all edible portions of a fruit or vegetable, though some have a higher content compared to others.

"Anthocyanins" (Fig. 2.1) are powerful antioxidants and are found in blue/purple fruits and vegetables such as purple grapes, figs, blueberries, and blackberries. Besides being rich in flavonoids, there is plenty of anthocyanin in veggies and fruits that are red or blue in color. A belief is also held that the deeper the hue of the said color the higher will be the concentration of phytochemicals in such vegetables and fruits. "Anthocyanins" is believed to support healthy blood pressure, reduces risk of heart disease, improves memory function, and lowers risk of cancer, and helps prevent bacteria from sticking to cells and improves motor skills.

Natural plant pigment (phytochemical) "Chlorophyll" imparts green color to fruits and vegetables (Fig. 2.2). Some of the green fruits and vegetables such as dark leafy greens, pistachios, peas, and cucumber also contain the phytochemical "lutein." Many green plant foods are also rich in "isothiocyanates." Spinach and broccoli are excellent sources of vitamin B.

"Lycopene" (Fig. 2.3) is a powerful antioxidant. It is found in plants such as tomatoes, watermelon, and pink grapefruit. Red/pink fruits and vegetables are also rich in anthocyanins. Certain flavonoids are also contained in red fruits and vegetables. Such fruits and vegetables also host higher levels of vitamins and folate. Cranberries contains anthocyanins and are a good source of tannins.

Figure 2.1. Natural Fruits and vegetables containing Anthocyanins.

Figure 2.2. Natural vegetables containing Chlorophyll.

Figure 2.3. Natural Fruits and vegetables containing Lycopene.

Figure 2.4. Natural Fruits and vegetables containing carotenoids.

The natural plant pigments called "carotenoids" (Fig. 2.4) make fruits and vegetables orange/yellow. Orange-friendly carotenoids are beta-cryptoxanthin, beta-carotene, and alpha-carotene. These can get converted to vitamin A in the body. Orange/yellow fruits are also a superb source of omega-3 fatty acids and vitamin C besides folate. The later compound is vitamin B and has the potential to reduce possibility of occurrence of birth defects. The chemical "allicin" (Fig. 2.5) is also contained in many white fruits and vegetables such as potatoes, banana, garlic, turnip, and onion. Allicin also helps in lowing cholesterol and blood pressure levels besides reducing the risk of heart disease and stomach cancer.

Notwithstanding the colors formed by natural plant pigments and as discussed earlier, there are many more beneficial

Figure 2.5. Natural Fruits and vegetables containing allicin.

phytochemicals that are also colorless. A good number of useful phytochemical are colorless. They not only act as powerful antioxidants but also help to neutralize the formation of free radicals (http://gentleworld.org/phytochemicals-eating-from-the-rainbow/).

3.1 Therapeutic Properties of Phytochemicals

In modern times we can use sound diagnosis to substantiate the beliefs we held in historical times. The literature is replete with references to well-known plants with several promising prospects. Some of the potential therapeutic properties are shown in Fig. 2.6. A good number of those are being explored for their phytochemical and therapeutic potentials. The availability and relatively cheaper cost of medicinal plants makes them more attractive as therapeutic agents vis-à-vis modern medicines. Some of the important phytochemicals and their possible health benefits are listed in Table 2.1.

4 Chronic Disease Management with Nutraceuticals

Nutraceuticals possess numerous therapeutic benefits. They are now being recognized more and more for their antiobesity effects, cardiovascular effects, antiaging effects, antidiabetic effects, immune enhancement, natural antioxidant activity, anticancer, antiosteoarthritis, and antiinflammatory effects (Gerson, 1978; Holt, 1999; Kowaltowski et al., 2009). They also provide protection against

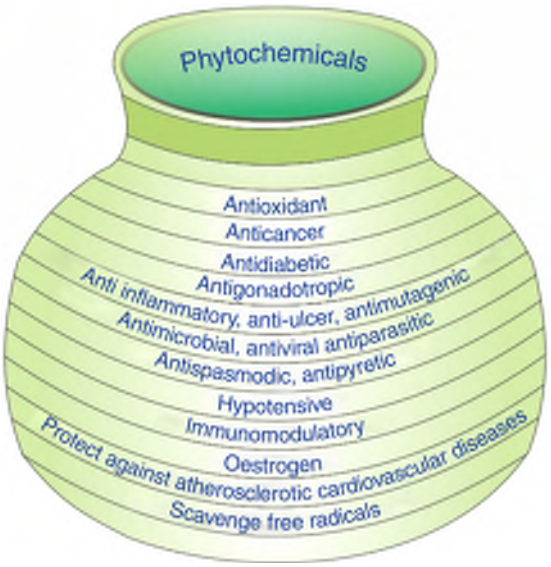


Figure 2.6. Potential Therapeutic properties of nutraceuticals.

Table 2.1 List of Some Important Phytochemicals and Their Possible Health Benefits

Phytochemicals	Matrix	Possible health effect
Terpenoids (isoprenoids)		
Carotenes (α,β,γ,δ,Lycopene	Sweet potato, orange, leafy greens and red, orange, and yellow fruits and vegetables	Antioxidant, anticancer, fat metabolism, antimicrobial
Xanthophylls (zeaxanthin, leutin, astaxanthin, canthaxanthin)	Mango, tangerine, orange, spinach, turnip, greens, romaine, lettuce, eggs, microalgae, yeast	Antioxidants, antiinflammatory agents, and regulators of development, reproduction, cellular differentiation, and vision protection
Triterpenoid (saponins, oleanolic acid, ursolic acid	Soybeans, beans, other legumes, honey, cranberries, lavender, oregano,	Antihypertensive, antidiabetic, liver protective
Phenolic compounds		
Natural monophenols		
Carvacrol	Oregano, thyme, pepperwort, wild bergamot	Antibacterial, antifungal, antioxidant, anticancer
Carnosol	Rosemary, sage	Arthritis, sore muscles, and other joint and muscle pains, anticancer, neuroprotectives

Table 2.1 List of Some Important Phytochemicals and Their Possible Health Benefits (*cont.*)

Phytochemicals	Matrix	Possible health effect
Dillapiole	Dill, fennel root	Chemopreventive, antiinflammatory, antibacterial, antidiabetic
Rosemarinol	Rosemary	Hepatoprotective, antimicrobial, antithrombotic, diuretic, antidiabetic, antiinflammatory, antioxidant and anticancer
Ferulic acid	Oats, rice, orange, pineapple, ground nut	Protects against cancer, bone degradation, and menopausal system (hot flushes)
Polyphenols		
Curcuminoids	Curcumin, turmeric	Antihypertensive, antiinflammatory, antioxidants, and cancer preventive
Quercetin	Onions, buckwheat, French beans, apples, apricots, cherries, grapes, wine, and tea, both green and black	Strong antioxidant reduces LDL oxidation, vasodilator, and blood thinner.
Resveratrol	Blueberry, groundnut, grapes, and red wine	Antioxidant: prevents aging, cancer, diabetes, and heart disease. Potential to block the activation of NF-kappaB, a protein complex that makes many cancers more aggressive and resistant to chemotherapy
Anthocyanins	Blackberry, cherry, orange, purple maize, raspberry, red grapes	Antiallergic, antiinflammatory, antioxidants and pigments
Catechins	Tea	Antioxidant, CNS stimulant, and diuretic
Apigenin	Apple, artichoke, basil, celery, cherry, grapes, nuts, parsley	Antiinflammatory, antioxidants, antispasmodic, chemopreventive, induce apoptosis and inhibits breast and ovarian cancers
Piperine	Pepper	Aerometic, analgesic, hepatoprotective, and stomachic
Caffeic acid	Artichoke, pear, basil, oregano, carrots, leafy greens, and red, orange, and yellow vegetables, pumpkin	Antiinflammatory, antifatigue, and antistress properties, anticarcinogenic, enhances release of immunogenic cytokines IL-1 and TNF-alpha, provide cornea protection against UV-light, stimulates DNA repair enzymes

(*Continued*)

Table 2.1 List of Some Important Phytochemicals and Their Possible Health Benefits (*cont.*)

Phytochemicals	Matrix	Possible health effect
Ellagic Acid	Cranberry, grapes, pecan, pomegranate, raspberry, strawberry, walnut	Anticancer and antioxidants
Galtic Acid	Tea, mango, strawberry, soy	Cytotoxic, antioxidative activities, antileukemic, antioxidant, anticancer, antineoplastic, antiinflammatory, antidiabetic.
Steroids		
Diosgenin	Fenugreek seeds	Hypolipidaemic
Stigmasterol	Soybean, calabar bean, and rapeseed	Fractures, mastitis, jaundice, bloody dysentery, diarrhea, glossitis, haematuria, miscarriage, indigestion, hernia

ultraviolet radiation or aggression by pathogens. Different polyphenols are absorbed in different places in the human body. Some of them get absorbed in the intestines while some get absorbed in the gastrointestinal tract. The orally ingested nutraceuticals are subjected to diverse physiological and physiochemical barriers, which reduce the doses reaching the systemic circulation. However, some of them get absorbed in other parts of the digestive system.

For the treatment of various diseases the most commonly used nutraceuticals available in nature are discussed in detail in the subsequent pages. However, there is a caveat. There exists no evidence in research publications that may substantiate that taking phytochemicals is as good for long-term health benefits as consuming the beans, fruits, grains, and vegetables from which they are drawn. However, scientific evidence exists to suggest that regular intake of lots fruit and vegetables lowers the risk of type 2 diabetes, stroke, heart (cardiovascular) disease, cancer—some forms of cancer, later in life—and high blood pressure (hypertension), and so forth. Therefore, authoritative organizations recommend getting phytochemicals from whole foods, such as fruits and vegetables, rather than from supplements.

4.1 Alzheimer's Disease (AD)

Nutraceuticals such as curcumin, resveratrol, quericitin, and so forth have been long known for the cure and prevention of

neurodegenerative disorders such Alzheimer's disease (AD). AD is an age-related disorder and it sometimes get coupled with metabolic disorders such as type 2 diabetes and obesity. However, the mechanism underlying this disease is still a subject matter of debate for the scientific community. On most of the occasions its origin has been discovered in oxidative stress, decreased levels of plasma antioxidants, and total plasma antioxidant activity. Increased levels of reactive oxygen species (ROS) can result in significant damage to cell structures. This also leads to amyloid deposition and neurofibrillary tangles. Therefore, one of the early steps involved in AD pathogenesis is oxidative stress. Also celluar mitrochondrial dysfunction resulting in decreased production of ROS has been reported to exacerbate the disease (Choi et al., 2012; Dumont & Beal, 2011).

Polyphenols such as resveratrol, curcumin, catechins, and so forth have particularly been known to be "rescue" nutraceuticals. Catechins exert an antioxidative action by chelating metal ions, such as iron (Fe^{2+}) and copper (Cu^{2+}). This prevents the generation of hydroxyl radical via Fenton reaction. Polyphenols also transfer an electron to ROS-induced radical sites on DNA and thereby prevent oxidative DNA modifications (Singh et al., 2008). Another polyphenol curcumin has been reported to suppress oxidative damage, inflammation, cognitive deficits, and amyloid accumulation by preventing the formation of fibrillar Abeta. The destabilization of fibrilla Abeta in the central nervous system thus has a preventive effect on AD as fibrilla Abeta produces neurotoxins that are thought to play a role in Alzheimer's pathogenesis. Similarly, resveratrol has also been reported to stabilize the levels of amyloid-beta 40 (Aβ40) in cerebrospinal fluid. It also prevents the formation of fibrilla Abeta. The destabilization caused by it also has a preventive effect on AD, thus providing a preliminary shield against AD (Irvine et al., 2008).

4.2 Cardiovascular Diseases (CVD)

Nutraceuticals have also been reported to have a protective effect against cardiovascular diseases (CVD). It has been found that increased levels of ROS and malondialdehyde (MDA), a metabolite which forms when ROS and oxidized Low Density Lipoproteins (LDL) attack fatty acids in cell membranes, result in cardiac arrest (Ayala et al., 2014; Bianca and Michael, 2001; Perez-Vizcaino et al., 2009). Flavonoids protect LDL from oxidation and attenuate atherosclerosis. Most of the flavanoids such as Quercetin act by directly capturing the unpaired ROS electrons, thus forming less reactive species. (Quiñones et al., 2013) They also have the capacity to complex with transition metals such as Fe^{2+} and Cu^{2+}, which

further avoids the formation of ROS (Krinsky, 1992). The incorporation of curcumin into food has been reported to reduce circulation of C-reactive protein levels. It also impedes proliferation of peripheral blood mononuclear cells and vascular smooth muscle cells. Besides, curcumin prevents the oxidation of LDLs. It also inhibits platelet aggregation and reduces the incidence of myocardial infarction (Yang et al., 2005). Other significant potentials attached to curcumin are that it inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo (Aggarwal and Shishodia1, 2004; Prasad et al., 2014).

Anti-hypertensive effect of resveratrol is believed to be facilitated by both endothelium-dependent and endothelium-independent mechanisms. In several animal models, resveratrol supplementation in endothelium-dependent effects has been shown to improve flow mediated vasodilation—a surrogate for endothelial function (Beshay et al., 2015). In addition to stimulating endothelium-dependent vasodilation, resveratrol has also been shown to have endothelium-independent vasodilatory mechanisms.

4.3 Carcinoma

It is also believed that the generation of ROS in human body can harm the normal cells and result in the formation of polyps which later on develop into different types of carcinomas. The generated free radicals can induce post transcriptional modification in cancer and over express their related proteins. The generated free radicals such as O_2^- and OH may increase normal human colonocyte activity and result in the formation of colon polyps. Natural antioxidants such as quercetin, curcumin, resvaretrol can limit the oxidative damage in colon cells. These are effective in inducing apoptosis in colon cancer cells.

Lentinan, β-1,3 beta-glucan with β-1,6 branching, is an edible mushroom that occurs naturally in *Lentinula edodes*. It is an adjuvant extracted from the fruit body of *L. edodes*. It has been reported to significantly reduce the formation of colon tumors in animal models. Orally active lentinan stimulates production of white blood cells in the human cell line (Sia and Candlish, 1999). Lentinex is a formulation featuring lentinan and is approved as a safe novel food in the EU (Carlo et al. 2010; Kuppusamy et al., 2014).

4.4 Renal Diseases

Oxidative stress aids a wide range of renal impairments, ranging from acute renal failure, obstructive nephropathy and glomerular damage to chronic renal failure associated with inflammation

(Bhandari and Galanello, 2012; Park et al., 2013). It may alter the structure and function of the glomerulus because of the effect of ROS on mesangial and endothelial cells (Yadav et al., 2013). Clear evidences are reported in literature apprising that nuts, fruits, vegetables, and oil seeds rich diet is the best source to fight against ROS. In a recent report by Al-Okbi et al. (2014) the authors studied the protective effect of extracts prepared from avocado, walnut, flaxseed, and Eruca sativa seeds in a rat model of kidney dysfunction induced by intraperitoneal cisplatin.

Curcumin has also been found to protective agent against autosomal dominant polycystic kidney disease. The disease is caused by massive enlargement of fluid-filled cysts of renal tubular origin that compromise normal renal parenchyma that eventually leads to renal failure. It has been reported by Gao et al. (2011) that curcumin inhibits the formation of cysts. The inhibitory pathway has been studied by Madin–Darby canine kidney (MDCK) cyst model and murine embryonic kidney cyst model. Curcumin has been found to significantly inhibit MDCK cyst development. At maximum dose of curcumin caused 62% inhibition of the cyst formation (IC50 was 0.12 μM). It has also been reported that Curcumin neither induced cytotoxicity nor apoptosis in MDCK cells at 100 μM. Also, renal tissue fibrogenesis has been reported to be prevented by resvaretrol (Bai et al., 2014).

4.5 Osteoarthritis

Osteoarthritis (OA) is a prevalent disease that causes pain and inflammation of joints. To date, there is no cure for OA. The only available treatments is aimed at reducing symptoms that is, pain and inflammation so as to maintain joint mobility and limit the loss of function. Nutraceuticals have come to the rescue of patients for the treatment of OA. n-3 PUFAs (linolenic acid and eicosapentenoic acid (EPA), essential fatty acids, are candidates for the reduction of inflammation as they can substitute arachidonic acid (main precursor of prostaglandins) in the synthetic pathway of inflammatory mediators. The reduction of inflammation can also have an impact on the catabolic pathways and by that way on disease progression. n-3 PUFAs have been extensively studied in various cell types, and their antiinflammatory or anti-OA effects in joint cell models. In vitro studies have been identified using bovine chondrocytes or human and bovine cartilage explants. These studies used n-3 PUFAs alpha-linolenic acid (ALA), EPA and docohexanoic acid (DHA) (Curtis et al., 2000). OA symptoms are effectively reduced by nutrients such omega 3 and omega 6 fatty acids, which decrease the need for nonsteroidal drugs and may reduce

adverse events. They (particularly EPA) exert antiinflammatory effect, inhibit catabolic processes, and stimulate the anabolic process in cartilage and joint. Many different evidences validate that omega 3 alleviates the progression of OA and has exciting therapeutic potential for preventing cartilage degradation associated with chronic inflammation of joints.

The anti-OA potential of curcumin has also been widely investigated in vitro. It downregulates catabolic and degradative effects observed in cartilage explants or chondrocytes stimulated with IL-1b, Lipopolysaccharide (LPS) or tumor necrosis factor (TNF). Curcumin inhibits the production of MMP-3, -9, and -13 and restored type II collagen and GAG synthesis (Henrotin et al., 2011), thus having a preventive effect on OA.

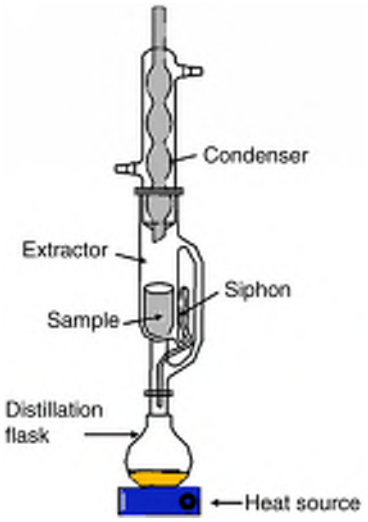
Quercetin is also known to show antiinflammatory and antioxidant properties. It demonstrates potential antiinflammatory effect by the inhibition of TNF-a mediated-IL-8 and monocyte chemoattractant protein-1 (MCP-1) expression. However, not many studies have been conducted on anti–OA effect of Quercetin (Sato et al., 1997).

5 Extraction of Nutraceuticals from Plants and Animals

5.1 Soxhlet Extraction

The extraction of the required nutraceutical from a plant/animal is the first and foremost step before their health and medical benefits can be exploited. Soxhlet extraction is one of the oldest and well-established techniques for the extraction of nutraceutical from plant matrix. Traditionally, Soxhlet extraction was the main method used for extraction of nutraceuticals. But this method has its own limitations as (1) it involves the use of a large quantity of solvent and (2) the process can be quite time consuming, taking from a few hours up to several weeks. The schematic diagram for Soxhlet extraction process is shown in Scheme 2.1.

These limitations prompted the development of more extraction techniques that along with shortened extraction time, increased efficiency of extraction, reduced organic solvent consumption, and increased pollution prevention. The efforts in this direction culminated in the development of modern extraction methods such as microwave-assisted extraction, supercritical fluid extraction, accelerated solvent extraction, and ultrasound-assisted extraction (Kaufmann et al., 2001a,b; Marr & Gamse, 2000; Lang and Wai, 2001; Vinatoru, 2001).



Scheme 2.1. Diagrammatic representation of Soxhlet extraction method.

Normally a solid material containing the desired compound is placed inside a thimble made from thick filter paper that is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapor travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor cools and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times over hours or days. During the extraction process the solvent is usually recovered by evaporation. The process is advantageous as (1) it repeatedly brings fresh solvent in contact with solid matrix, (2) it maintains high extraction temperature, and (3) no filtration is required after leaching (Castro and Priego-Capote, 2010; Castro and García-Ayuso, 1998).

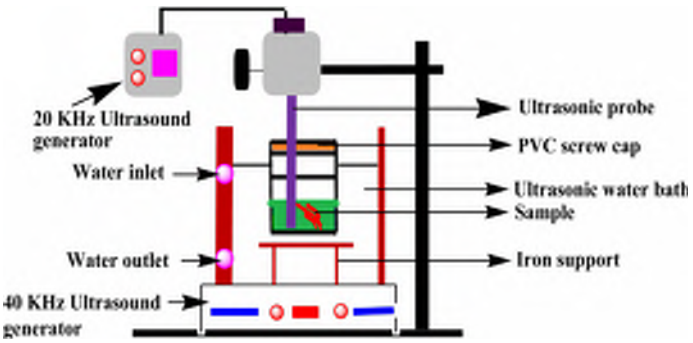
There are serious drawbacks of Soxhlet extraction technique when compared to other methods for solid sample preparation. The former requires longer period of time for extraction and the volume of the extracted waste is also large. It is not only costly to remove and get rid of waste but is also expensive at the same time besides causing environmental problems. For extracting samples with the Soxhlet extraction process, plant matrix is kept boiling

over an extended period of time. It can cause thermal decay of thermolabile target species. Soxhlet device has also certain inherent drawbacks. It does not have the mechanism to hasten the process of extraction. With this process, the amount of extractant obtained is large. It requires additional steps to obtain the required concentration. It follows that the Soxhlet extraction technique is difficult to automate and limited by extractant.

5.2 Sonication Assisted Extractions

Sonication assisted extractions process, as is being employed, replicates the process that bees use to shake pollen from flowers by vibrating their wing muscles. It is inexpensive in terms of human labour and is a simple and efficient alternative to conventional extraction techniques. Sonication assisted extraction generally involves the use of ultrasound assisted extractor or closed extractors fitted with ultrasonic horn transducer (Rupasinghe et al., 2011; Falleh et al., 2012). Schematic diagram for sonication assisted extraction is shown in Scheme 2.2. The mechanical effects of the ultrasound cause disruption of biological cell walls, facilitating the release of contents. Therefore, efficient cell disruption and effective mass transfer are cited as two major factors leading to increased extraction with ultrasonic power. Physical conditions of the plant, however, have a bearing on the efficiency of the extraction process.

This method involves the measurement of moisture content, particle size, and solvent used for the extraction process. Frequency, pressure, temperature, and sonication time also affects the optimum performance of the sonication process. Though the increased extraction yield and kinetics depends on the nature of the plant material to be extracted, it is also greatly dependent on the ultrasound frequency, power, time, and temperature. The maximum ultrasound power is observed in the vicinity of the radiating



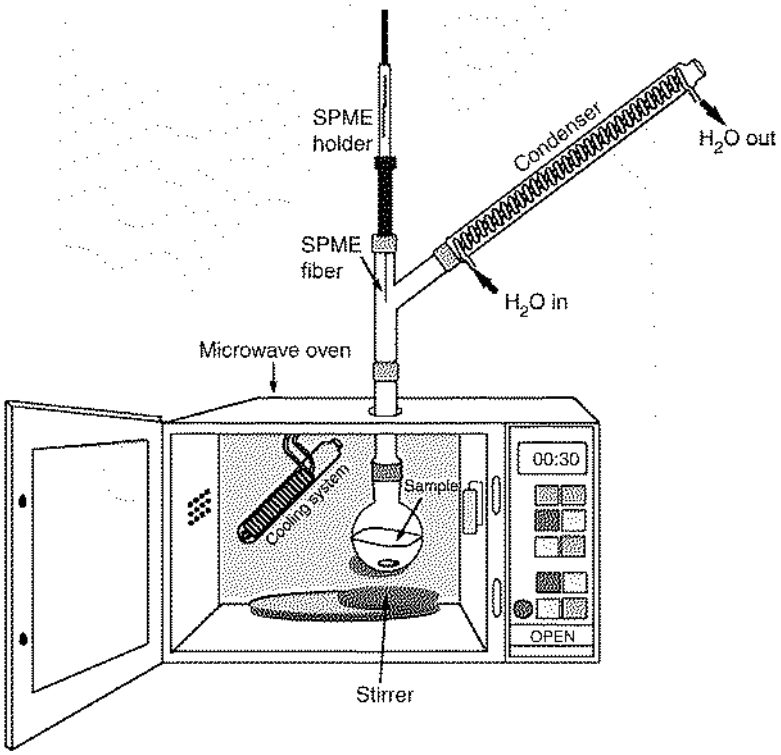
Scheme 2.2. Diagrammatic representation of Sonication assisted extraction.

surface of the ultrasonic horn. However, as the distance from the radiating surface increases, the ultrasonic intensity decreases rather abruptly. The sonication time control is absolutely necessary otherwise this may damage or change the quality of extract.

The use of sonication method not only results in increased extraction yield and faster kinetics but it also reduces the operating temperature allowing the extraction of thermolabile compounds. Any solvent can be used with this technique for extracting a wide variety of natural products. However, what is important is that due care is taken while designing the ultrasound assisted extraction, as ultrasound is known to have an effect on plant matrix.

5.3 Microwave Assisted Extraction (MAE)

Microwave extraction is a proven technique that is fast, uses significantly less solvent than traditional techniques, and is cost-effective. Schematic diagram for microwave assisted extraction is shown in Scheme 2.3. MAE is the process of heating solvents in



Scheme 2.3. Diagrammatic representation of Microwave assisted extraction process.

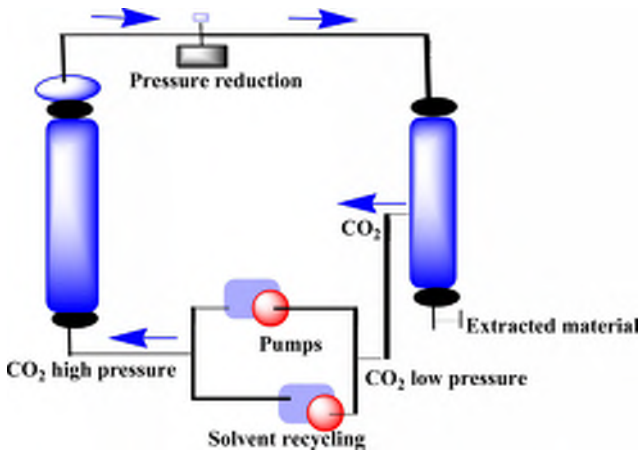
contact with a sample with microwave energy to strip compound of analytical interest from sample matrix into the solvent. There are two types of commercially available MAE systems: (1) closed extraction vessels under controlled pressure and temperature and (2) open microwave ovens at atmospheric pressure (Kaufmann and Christen, 2002). The closed MAE system is generally used for extraction under drastic conditions such as high extraction temperature. The pressure in the vessel essentially depends on the volume and the boiling point of the solvents. Heating the solvent above their atmospheric boiling points increases the efficiency of the process. The open MAE system works at atmospheric pressure, and the maximum temperature is determined by the boiling point of the solvent used (Renoe, 1994; Letellier & Budzinski, 1999). The solvent is heated and refluxed through the sample. In this case the microwaves are focused on the sample placed into the vessel allowing homogeneous and very efficient heating (Demesmay & Olle, 1993). The sample to be extracted can be placed into a Soxhlet-type cellulose cartridge in order to avoid filtration steps, or may be directly dipped into the solvent. Compared to closed vessel extractions systems that are subject to several shortcomings, atmospheric pressure (open-vessel) microwave sample preparation offer increased safety in sample handling and, furthermore, they allow larger samples to be extracted. Open-vessel operation is more suitable with thermolabile species as it uses low temperatures relative to closed-vessel systems. But closed-vessel extraction systems also have their advantages (Mandal et al., 2007).

The plant particle size and the status in which it is presented for microwave-assisted extraction can have a profound effect on the recoveries of the compounds. The particle sizes of the extracted materials are usually in the range of 100 μm–2 nm (Eskilsson and Bjorklund, 2000). Fine powder can increase extraction by providing larger surface area because it provides better contact between the plant matrix and the solvent. Moreover, finer particles can allow much deeper penetration of the microwave. For example, for MAE of cocaine, finely ground coca powder was more easily extracted than large particles (Brachet et al., 2002). Choice of the solvent to be used for the extraction process depends on the solubility of extracts of interest, interaction between the solvent and the extract, and the dielectric constant of the solvent. Generally polar solvents with high dielectric solvents are preferred. Temperature is a factor that controls the efficiency of the process. Generally, elevated temperature results in improved extraction efficiency. However, high temperature may cause degradation of themolabile compounds. So in that case chosen power for MAE has to be set carefully to prevent extreme temperature. MAE is a preferred

method over traditional techniques as it requires less extraction time, reduced solvent usage and improved extraction yield.

5.4 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but it can also be from liquids. The most commonly used supercritical fluid is CO_2, though it is sometimes modified with cosolvents such as methanol, ethanol (SFE system is shown in Scheme 2.4). The system must contain a pump for the CO_2, a pressure cell to contain the sample, a means of maintaining pressure in the system and a collecting vessel. During SFE raw plant material is loaded into an extraction vessel. The extraction conditions are controlled with temperature controllers and pressure valves at both inlet and outlet. The extraction vessel is pressurized with the fluid by a pump. The dissolved material is swept from the extraction cell into a separator at lower pressure, and the extracted material settles out. The CO_2 can then be cooled, recompressed, and recycled, or discharged in atmosphere. The fluid is further regenerated and cycled (Sihvonen et al., 1999). However, several dynamics of the system are relevant to this process. They include sample preparation, selection of supercritical fluid, and modifiers. The extraction conditions are also important for successful extraction process. CO_2 has emerged as the most commonly used SFE solvent. This is because of its nontoxic and nonflammable nature. Its extraction properties can be widely



Scheme 2.4. Diagrammatic representation of SFE extraction process.

and precisely manipulated with subtle changes in pressure and temperature. Many nutraceuticals such as phenolics, alkaloids, and glycosidic compounds cannot be extracted because they are poorly soluble in carbon dioxide. Therefore, techniques aimed at overcoming the limited solubility of polar substances in supercritical CO_2 have been explored. Addition of polar cosolvents (modifiers) to the supercritical CO_2 is known to significantly increase solubility of polar compounds. Among all the modifiers including methanol, ethanol, acetonitrile, acetone, water, ethyl ether, and dichloromethane; methanol is the most commonly used because it is an effective polar modifier and is up to 20% miscible with CO_2. However, ethanol may be a better choice in SFE of nutraceuticals because of its lower toxicity (Hamburger et al., 2004). The quality of the plant material and the moisture content is an important factor while preparing samples for SFE. Freshly cut plants are rich in moisture content. This may cause clogging due to ice formation. Therefore, chemicals like Na_2SO_4 and silica gel are added to the plant extract to retain the moisture content and also prevent clogging. Another factor that determines the efficiency of extraction process is the solubility of the target compound in the supercritical fluid. Low molecular weight and less polar compounds are more readily extracted because the extraction mechanism is generally controlled by internal diffusion. Therefore, the exact composition of the extracted compound varies with the extraction time.

6 Analytical Techniques Used for the Characterization of Nutraceuticals

It has long been known that nutraceuticals have potential health and medical benefits. They are considered to be safer and have less secondary side effects as compared to many drugs prescribed for the treatment of certain symptoms. However, while they are expensive, they may not work if they are not manufactured as per pharmaceutical level manufacturing standard controls (McAlindon, 2006). The quality of nutraceuticals and the contents of the active component therein are also impacted by the place and the season in which a source plant is grown/harvested. Therefore, factors such as their identification, collection, quantification, and standardization of manufacturing process to ensure uniformity in quality are of paramount importance.

Consumer awareness is rising by the day. Consumers, as such, are becoming more and more conscious about the quality of the food they eat. This has pushed the food industry to focus on more

analytical techniques that give quick and reliable information on the type, content, and percentage of nutraceuticals in food. This has increased the demand for quick, cheap, powerful, sensitive, and precise analytical procedures.

Time has progressed. Analytical sciences have also evolved with time. New techniques have been developed. They have come to the rescue of researchers/industry for the exact characterization of natural products. These include Mass Spectrometry (MS), Nuclear Magnetic Resonance (NMR), High Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE), HPLC-NMR, HPLC-MS, GC-MS, and CE-MS, Atomic spectroscopy. These techniques help us understand the complex natural matrix and health-promoting effects of the nutraceuticals. They also help us to know the body exposure and bioavailability after the consumption of nutraceuticals (Sener & Orhan, 2005; Rafi, 2003; Laporta et al., 2004). Presently, a large number of biological techniques are being used by scientists for developing novel methods of neutraceutical characterization. These include enzyme-linked immunosorbent assay (ELISA), immunoradiometric assay (IRMA), immunodotting, radioimmunoassay (RIA), fluorescence: fluorescence immunoassay, enzyme-linked fluorescent immunoassay, fluorescence polarization immunoassay (FPIA), time-resolved fluorescence immunoassay (TRFI), chemiluminescence immunoassay (CIA), and polymerase chain reaction (PCR).

Confocal laser microscopy and other imaging techniques are recently being used to get information on the nature of heterogeneous food products. These imaging techniques give information on the type of manufacturing process used, protein and enzyme network of the food product, structure of metabolites, and other biological properties of the food product.

The choice of an analytical technique depends on the physiochemical nature of the nutraceutical matrix. Apart from this, method of preparation of sample, separation mechanism, and the type of detector used, they all strongly influence the analytical process. Usually a combination of analytical techniques is used because the characterization of nutraceuticals is composed of a complex matrix and no single technique can provide exactly accurate results. The combination of techniques quickly allows the analyst to comment on the sample nature.

NMR is now being used for the characterization of complex organic metabolites present in fruit juices. The type of compound present in the fruit juice matrix can be determined from a single NMR spectrum. The obtained NMR spectra are highly complex with large number of signals. Since it is a quantitative technique it can be used for determining the concentration due to direct

proportionality between integral of NMR protons and the number of nuclei. Usually an external reference compound is used to determine the exact concentration. The absolute intensities of the external reference and the unknown compound are correlated by the measurement of precise 360° pulse. The mentioned procedure has been developed jointly by Bruker Biospin GmBH and SGF international e.V (Minoja & Napoli, 2014). This constitutes the preliminary step in analyzing juice matrix with NMR technique. The second step is Spin generated fingerprint (SGF) profiling. SGF techniques classify samples on normality models (normality model is spectral database of more than 3000 samples of different fruits obtained from >50 different countries). In SGF profiling every single peak obtained in the NMR spectra is considered regardless of its involvement in the targeted analysis. The obtained peaks are than compared with normality models to get the exact information.

Since a nutraceutical is a mixture of components, sensitivity and selectivity of the analytical method is very important. HPLC combined with mass spectrometry (HPLC MS) is now being used for nutraceutical analysis. It is one of the best analytical methods that can be used to characterize any nutraceutical/food item at molecular level. Various types of mass spectrometers are used in combination with liquid chromatography. The simplest ones are quadruple-type instruments. These are simple, sensitive, and relatively low-cost instruments used for quantification. Among advanced techniques tandem mass spectrometry (MS/MS) is most important and most widespread. It is a sensitive and selective technique that even allows identification of coeluting compounds. As far as ionization techniques are concerned electrospray ionization (ESI) is used in combination with HPLC–MS. Sensitivity depends significantly on molecular structure. Compounds of low polarity cannot be efficiently studied by ESI. For such compounds atmospheric pressure chemical- or photo-ionization (APCI and APPI, respectively) techniques may be used. The method can be useful for screening a large number of trace level analytes in various food matrices. The most crucial step in HPLC-MS analysis is the sample preparation step. In some compounds such as carbohydrates the groups to be identified are closely related structurally whereas in other cases such as vitamins, the groups are related by their biological role. So, different sample preparation methods are required for different analytes depending on the nature of the groups to be identified. Nowadays, HPLC-MS is being replaced by (U) HPLC-MS (ultra high performance liquid chromatography) for the characterization of a large number of vitamins and

caretenoids. This is because of their polar nature and the presence of large number of structural variants (De Quiros and Costa, 2006; Dachtler et al., 2001). Sample preparation is followed by analysis, usually carried out by HPLC-MS or UHPLC. For analysis C-18 column with small particle size is usually preferred. Sensitivity and selectivity is another issue that has to be dealt with carefully while doing such types of separation analysis. This is usually done by using HRMS coupled with UPHLC (Holčapek et al., 1990).

Another technique finding important place in nutraceutical/food analysis is FTIR. It is noninvasive, sensitive, and a rapid method of analysis. The technique is based on the simple principle of vibrations of the groups within the sample on absorbing infrared radiations. The vibrations are then correlated with the chemical species. This obtained spectra is then compared with the data available in literature (Dunn and Ellis, 2005; Ellis et al., 2007). However, one of the major limitations of using FTIR analysis is that the absorption of water is very high in the mid-IR region and this usually complicates the spectra. However, this can be overcome by dehydrating the sample, by reducing the path length, subtraction of water signal, and use of attenuated total reflectance (ATR) (Ellis et al., 2002; Nicolaou and Goodacre, 2008; Schmitt and Flemming, 1998; McClements and Li, 2010a).

7 Issues Related with the Incorporation of Nutraceuticals in Foods

As referred to earlier, nutraceuticals such as resveratrol, curcumin, quercitin, sulphoraphane, benzyl isothiocyanate and so forth have various health benefits for humans. Most of them are antioxidants, anticancer, antiinflammatory, and show heart and brain protective effects. However, utilization of these nutraceuticals in food and drug industry is currently limited because of poor water solubility, pH sensitivity, easy degradation, and low bioavailability. Further, nutraceutical formulations have high molecular weight as compared to pharmaceutical formulations. This restricts the choice of excipients that can be used. The fewer excipients and variety of actives in the same formulation make it difficult to achieve certain desired outcomes such as disintegration time, hardness, and friability. It also faces the constant challenge of adding the correct amount of each ingredient in to the tablet. Nonuniform content distribution can be more fatal than useful. Also the use of nutraceuticals is not regulated by any laws in most countries. Therefore, there is an increased risk of toxicity and overdose.

8 Delivery Systems for Enhancing the In Vivo Bioavailability and Stability of Nutraceuticals

It has been interpreted from the research results that if nutraceuticals are to be the part of diet, the absorption of nutraceuticals must maintain a meaningful therapeutic level. Research these days is gaining pace to develop delivery vehicles that not only prevent the degradation of nutraceuticals, but also increase their bioavailability. To put it differently efforts are afoot to ensure that the functional assembly of molecule is ingested to become available at the critical site (McClements and Li, 2010a,b). For absorption to occur the compound must be soluble or disperse in the aqueous intestinal lumen. Lipophilic compounds, due to their low aqueous solubility, are poorly absorbed by the intestines, whereas hydrophilic compounds have low transport coefficient across the intestinal lining. Further, these bioactives are subjected to various metabolic pathways once they are absorbed by the intestines, which may lead to the change in their chemical structure and activity.

For this, the formation of appropriate delivery vehicle is of utmost necessity. For this, the formation of appropriate delivery vehicle is of utmost necessity. Many effective delivery systems have been developed to enhance the therapeutic efficacy of nutaceuticals (Langer, 1980; Samad et al., 2007; Sastry et al., 2000; Torchilin, 2001). However, synthetic ingredient based delivery systems may not be used for pharmaceutics because of adverse consequences that they might produce in the cases of their prolonged intake. Therefore, Generally Recognized as Safe (GRAS) materials are preferred for their formulation to abate side effects.

8.1 Some of the Presently Focused Nutraceutical Delivery Vehicles

8.1.1 Phospholipid-Based Delivery Vehicles

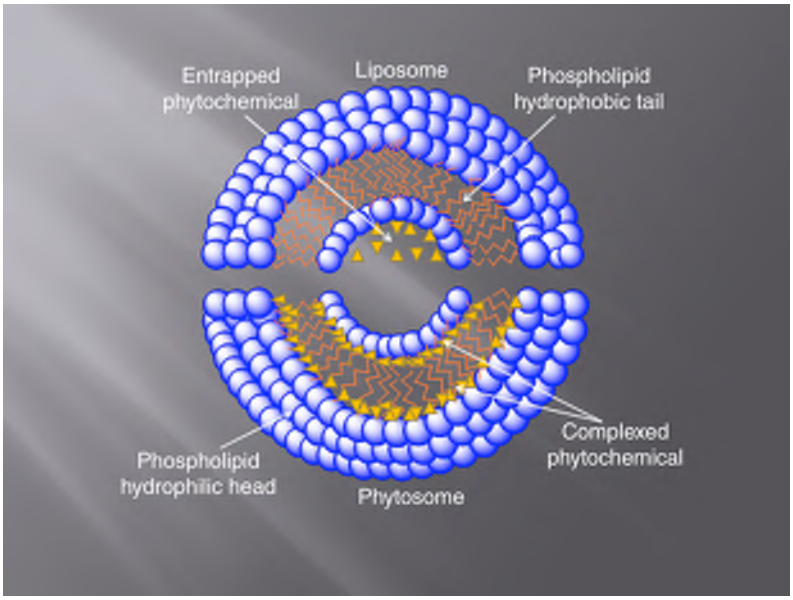
Phospholipids-based nanotherapeutics (Burgo et al., 2014) are gaining popularity these days as delivery vehicles because they are biocompatible, biodegradable and possess properties that can enhance their bioavailability. When engaged in the aqueous environment, phospholipids simultaneously adapt themselves into a bilayer structure with polar heads facing outward and nonpolar tails pointing to the inner region of the bilayer structure. They are amphiphillic in nature and can orient themselves into lipid

bilayers. Due to this unique bilayer structure, phospholipids have been utilized as carrier materials for enhancing the dose efficiency and the potency of bioactive compounds.

8.1.2 Liposomes as Carrier Systems

Liposomes (Scheme 2.5) are spherical microscopic lipid vesicles formed from phospholipids holding a small amount of solvent in which they exist. They serve as a delivery vehicle for both the hydrophilic and lipophilic compounds. When hydrophilic compounds are to be encapsulated, the aqueous center of liposome serves as a suitable pocket carrying the bioactive through the journey of GI tract digestion and absorption. Liposomes have been reported to enhance the oral bioavailability of variety of nutraceuticals such as curcumin and resveratrol by entrapping them in the phospholipid bilayers (Takahashi et al., 2009; El-Samaligy et al., 2006). The encapsulation results in improving the aqueous solubility, provides protection against unstable stimuli, modulating intestinal absorption, and facilitating lymphatic transport.

The incorporation of resveratrol into liposomal carrier systems has conferred improvements in stability, biological activity, and efficacy with a better side-effect profile, making possible oral

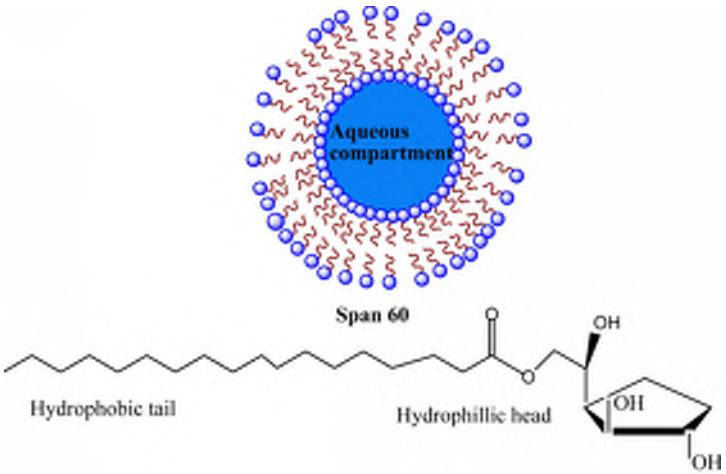


Scheme 2.5. Diagram showing Liposome as delivery vehicle.

and intravenous dosage formulations of the compound (Amri et al., 2012). (+)-Catechin, an isomer in tea, is known to have potent antioxidant and neuroprotective effects against age-related cognitive diseases, as discussed earlier. Meanwhile, the oral application of (+)-catechin generally requires a large administration dose since the absorption efficiency is low at 5%. The ability of liposome to provide protection from physiochemical degradation, increase membrane permeability, and resist metabolic activities makes it a suitable delivery system to augment the bioavailability and organ distribution of (+)-catechin (Huang et al., 2011). They have also proven to be wonderful delivery vehicles for cosmetic delivery systems and in the prevention of various skin diseases. Liposomal systems have also been employed in oromucosal sprays for sublingual absorption of some nutraceuticals like melatonin that showed improvements in bioavailability when compared to the conventional tablet formulations (Keller, 2001).

8.1.3 Niosomes as Carrier Systems

Niosomes (Scheme 2.6) here are composed mainly of hydrated nonionic surfactants in addition to, in many cases, cholesterol (CHOL) or its derivatives. The unique structures of niosomes make it capable of encapsulating both hydrophilic and lipophilic substances. This can be achieved by entrapping hydrophilic in vesicular aqueous core or adsorbed on the bilayer surfaces while the lipophilic substances are encapsulated by their partitioning into the lipophilic domain of the bilayers. Recently niosomes has been used as delivery vehicles for the encapsulation of antioxidants by



Scheme 2.6. Diagram showing Liposome as delivery vehicle.

Tavano et al. (2014). It has been suggested that niosomal encapsulation of these antioxidants protect them from degradation and control their release in the body.

8.1.4 Emulsion-Based Delivery Systems

Because of the small particle size—usually >100 nm—and their ability to be formulated with generally recognized as safe (GRAS) material, emulsions are being used extensively for the delivery of nutraceuticals. The class includes microemulsions, nanoemulsions, and double emulsions (representative structure has been depicted in Scheme 2.5). The nanoemulsion based oral formulations have been reported to significantly enhance the absorption of nutraceuticals such as curcumin, alpha tocopherol when compared to their nonencapsulated forms. In a study done by Lin et al. (2011), the reduction in emulsion particle sizes resulted in the improvement of dibenzoylmethane (DMB, 1,3-diphenylpropanedione) oral bioavailability. (DMB) is a potent suppressor of 7,12-dimethylbenz [a]anthracene (DMBA) and estradiol-induced tumor development. DMB encapsulated in nanoemulsion produced 3 times higher bioavailability than conventional emulsion. Due to the lipophilic chemical structure the oral bioavailability and efficacy of DMB were significantly attenuated by its low aqueous solubility.

The bioavailability of curcumin is mainly dependent upon two factors. One is the preingestion incubation temperature and the second the droplet size. It was noticed in a study (Zou et al., 2015) that the conversion of curcumin from the powdered form into the excipient emulsions was much higher when incubated at 100°C as compared to at 30°C. This was probably because of the increased water and oil solubility of curcumin with increasing temperature. The increase in droplet size of curcumin microemulsion was found to have intricate effects. This could be ascribed to the competing effects of droplet surface area on the chemical degradation and mass transfer rates. These properties have important effects in determining optimum processing conditions and emulsion microstructures for enhancing curcumin bioavailability.

The authors stressed that the conditions should be optimized not only to ensure a high transfer of curcumin into the excipient emulsions, but to also ensure a low rate of curcumin degradation. More emulsion based delivery systems that enhance the solubility of many other nutraceuticals such as Quericitin, curcumin, resvaretrol, Vitamin D_3, and so forth have also been reported in literature (Ozturk et al., 2015; Mukherjee et al., 2015).

8.1.5 Microemulsion/Nanoemulsions as Delivery Vehicles

Microemulsions (ME), with droplet diameters less than 100 nm, are spontaneous structures of water, oil, and surfactants. They offer the advantages of optical transparency, thermodynamic stability, long-term stability, and ease of preparation. Microemulsions have been employed over the past few years to improve the solubility of poorly water-soluble drugs/nutraceuticals in aqueous solution either for the penetration into or absorption by cells in the pharmaceutical and functional foods. Phospholipids-based microemulsions (composed of food-grade ingredients soybean oil and soybean lecithin) are being considered now as potential candidates for natural products because of their nanometer size, which enhances the trans membrane permeation as well as increases the loading capacity for lipophilic nutraceuticals. It is believed that the small size allows passage through organ filtering and cellular membrane systems, both of which are highly desirable characteristics. A study conducted by researchers has established the superiority of soybean oil over ethyl oleate as the oil phase in curcumin microemulsion by broadened microemulsion region in the phase diagram. The formulated microemulsion was found to have cytotoxic effect on hepatocellular HepG2 cell lines (Lin et al., 2014).

Nanoemulsions on the other hand are assemblies similar to microemulsions. The line of demarcation between the two nanoassemblies is very thin. The only difference is that microemulsions are formed spontaneously and are thermodynamically stable whereas nanoemulsions require energy for their formulation and are kinetically stable systems. Representative structures are shown in Scheme 2.6. Wang et al. (2008) successfully prepared high-speed and high-pressure homogenized O/W emulsions for delivery of curcumin using medium chain triacylglycerols (MCT) as oil and Tween 20 as emulsifier with mean droplet sizes ranging from 618.6 nm to 79.5 nm. The enhanced antiinflammation activity of curcumin encapsulated in O/W emulsions was evidenced by the mouse ear inflammation model. The authors observed 43 or 85% inhibition effect of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced edema of mouse ear for 618.6 and 79.5 nm 1% curcumin O/W emulsions, respectively, but a negligible effect was found for 1% curcumin in 10% Tween 20 water solution.

8.1.6 Ethosomes as Carrier Systems

Ethosomes are composed mainly of phospholipids (phosphatidyl choline, phosphatidyl serine, and phosphatitidic acid), ethanol (relatively high concentration, 40–45 %) and water. The higher content of ethanol makes ethosomes efficient permeation

enhancers and are usually added to vesicular systems to prepare elastic nanovesicles. The size of ethosomes varies between 30 nm to a few microns. However, ethosomes are low on stability and degrade once the ethanol evaporates. Apigenin (a plant flavanoid)-loaded ethosomes were prepared for local action at the skin. They showed higher skin deposition than liposomes or deformable liposomes both in vitro and in vivo. Ethosome-mediated apigenin delivery produced the strongest effect on UVB-induced skin inflammation by suppressing COX-2 levels (Shena et al., 2014).

A synthetic ligustrazine product, Ligustrazine Phosphate, easily penetrates the blood-brain barrier. But, ligustrazine phosphate has a short elimination life of less than 2 h, besides having low oral bioavailability (10–30%). Ligustrazine phosphate-loaded ethosomes have been designed by Shi et al. (2012) to achieve better therapeutic effects via transdermal ligustrazine administration. It was found that prepared ethosomes exhibited enhanced skin permeation in vitro and efficient pharmacodynamic responses in amnesic animal model. The antiamnesic efficiency of this ethosomal system not only contributed to behavioral improvement, but also slowed down the progression of Alzheimer's disease by mitigating the oxidative stress.

8.1.7 Self-Microemulsifying Drug Delivery Systems (SMEDDS)

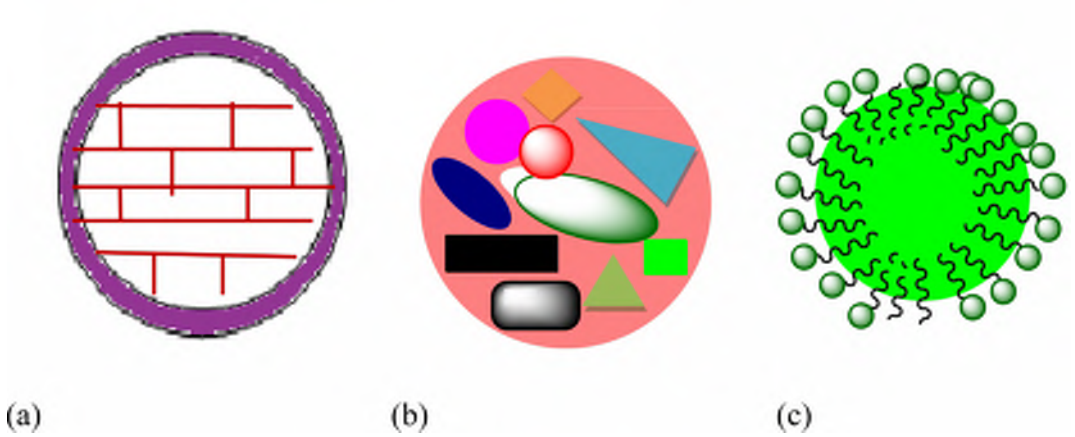
SMEDDS has been a unique method for formulation of microemulsions for a long time and are known to be excellent vehicles for delivery of drugs/nutraceuticals. The system achieves dilution to specific aqueous phase content because of its ability to form fine oil-in-water (O/W) microemulsions of oil, surfactant, and water under gentle agitation. Nutraceuticals are poorly soluble and/or poorly permeable. However, significant improvement in their reproducibility and bioavailability could be achieved with SMEDDS. SMEDDS themselves are considered incomplete emulsion systems because they retain their isotropic structure until they are in contact with aqueous solutions. SMEDDS are simultaneously emulsified into thermodynamically stable emulsions with particle sizes ranging from 100 to 300 nm when they are exposed to aqueous environment. When taken orally, gastrointestinal mortality is enough to induce the transformation of SMEDDS into emulsion. SMEDDS subsumes many advantages of most emulsion systems available. Therefore, they are a convenient and physically stable alternative for the delivery of nutraceuticals. For compounds like curcumin that are poorly water soluble, SMEDDS composed of only the isotropic organic phase have been established to be an exceptional carrier system (Chopra et al., 2011). Some nutraceuticals may appear to be crude extracts containing mixtures of multiple bioactive

compounds unlike other single isolated bioactive compounds. Crude extracts from Ginkgo biloba have been encapsulated into the SMEDDS and have been reported to show significant improvement in oral bioavailabilities (Tang et al., 2007; Shao et al., 2010). But unlike other single isolated bioactive compounds the encapsulation of extracts has been influenced by many factors such as differences in solubility, distribution, and potential interaction among compositional bioactives. Therefore, it can be said that SMEDDS formulations provide a physically stable environment for homogeneously containing the mixture of bioactives during storage while allowing rapid emulsification upon contact with water.

9 Nanoencapsulation-Based Delivery Vehicles

9.1 Solid Lipid Nanoparticles (SLNs)

Submicron emulsions, that is, solid lipid nanoparticles (SLNs), comprise a solid or semisolid lipid core structure and are considered an excellent controlled-release system capable of preventing burst release. They prolong the gastric retention time of bioactives. Representative structure is shown in Scheme 2.7. SLNs are a robust protective mechanism against GI tract degradation activities, such as coenzyme Q10, retinol, citral, and peptides because of the reduced mobility of lipid crystalline structure (Yoo et al., 2013; Müller et al., 2000; Maswal & Dar, 2013). SLNs have



Scheme 2.7. Representative diagram (a) Solid Lipid Nanoparticles (SLNs) (b) Nanostructured lipid carriers (NLCs) (c) Nanoemulsions.

been extensively used to enhance the bioavailability of lipophilic bioactive compounds. Their effectiveness has been established by pharmacokinetic studies of Camptothecin from Camptotheca acuminate (Yang et al., 1999), and quercetin (Li et al., 2009; Mei et al., 2005). Gastric enzyme activities find it difficult to break down SLNs as compared to emulsions with liquid disperse phase. Therefore, pharmacokinetic profiles of bioactives delivered in SLNs normally have a delayed peak time and have a more stable plasma concentration trend than liquid-lipid emulsion systems. Yu and Huang (Yu & Huang, 2012) developed an interesting SLN system that included a semisolid organogel formulation. Organogel is a nonpolar semisolid gel composed of gelator and nonpolar solvent (usually lipid in oral formulation). The gel structure allows better physical stability and loading capacity of entrapped compounds. Curcumin, incorporated in the organogel-derived emulsion, when compared to curcumin water suspension, showed 9.8-fold growth in the area-under-curve of the pharmaceutical profile. Notwithstanding the variation in core materials, SLN systems are effective carriers to enhance and control the bioavailability of lipophilic bioactive compounds.

9.2 Nanostructured Lipid Carriers (NLCs)

NLCs (Scheme 2.7) are formed by controlled mixing of solid lipids with spatially incompatible liquid lipids. This leads to the formation of complex nanostructures that increases nutraceutical loading, modulation of the release profile and stable nutraceutical incorporation during storage. Different methods such as hot/cold homogenization, microemulsion technique are used for the preparation of NLC. NLC are a modified SLN in which the lipidic phase contains both solid (fat) and liquid (oil) lipids at room temperature (Müller et al., 2002a,b). NLC's composed of cetyl palmitate and caprylic/capric triacylglycerols have been prepared to deliver the nutraceutical CoQ10 (Teeranachaideekul et al., 2007) to the skin more efficiently. They provide a fast release initially for skin saturation followed by a slow and prolonged release profile to maintain the skin concentration of Q10. The most suitable model to describe the release profile of Q10 from NLC has been found to be the Higuchi model.

10 Challenges Associated with Epidemiological Research on Nutraceuticals

The nutraceutical industry is huge and growing. Nutraceutical products are being sold on the basis of health claims, many times with inadequate or even completely without supporting

epidemiological research evidence. From several large-scale intervention studies around the world, there is clear evidence that dietary supplementation to adequately nourished populations do not provide additional health benefits as reductions in disease risk, or may actually increase disease risk. This scenario is biologically plausible for other nutraceuticals and extracted bioactive components to disease risk as well. There is no denying the value of proper nutrition derived from eating "good food" for health maintenance and disease prevention.

Numerous other issues are also associated with the epidemiological research on nutraceuticals. These include (1) quality control issues related to chemical composition (eg, plant selection, source, constituents, and contaminants); (2) standard protocols for formulation and testing (both chemical and pharmacological profiles); (3) safety testing, which is usually assumed, but not proven; (4) identification of mechanism-based biomarkers to evaluate beneficial/harmful effects; and (5) prospective clinical trials.

Well-designed further epidemiological studies are needed while some have already been conducted, for example, a one-year consumption of a grape nutraceutical containing resveratrol to generate human evidence for the benefits/ harms of using nutraceutials.

11 Future of Nutraceutials and Nutraceutials Research

The consumers are getting health conscious for the simple reason that a human being lives in his body and it is his body about which he is more concerned. On the contrary there are business interests of the commercial entities which have prompted them to expand their business interests to meet the consumer demand. This has led to increased commercial availability in the market of nutraceuticals products containing phytochemicals. However, the scientific evidence supporting their health benefits are still insufficient and what so ever is available is mostly based on in vitro or animal model assays. Clinical trials that may evaluate the actual physiological effects in humans are, however, scarce and where conclusions have been drawn, the findings are controversial. Disagreement of results is not unexpected because there are many challenging factors that may have an impact on the final outcome of the trials that is, the stability of the bioactive compounds in the different pharmacological forms available and (or) in the gastrointestinal tract. Any chemical alteration of the original bioactive compound that may take place during storage or digestion can severely impact the bioavailability and bioactivity of the compounds.

However, it needs no emphasis that interindividual variability for bioavailability and metabolism as well as for the biological response remains a crucial factor in the theory of "nutraceutials."

12 Conclusions

There is a true relationship between diet and health. Food indisputably affects humans but different foods affect each of us differently because everyone has a different genetic makeup. Around 400 BC Hippocrates said, "Let your food be your medicine, and medicine be your food." Nutritious and unhealthy diets are fundamentals of health and disease. There exists some scientific basis to support biological activity of phytochemicals but the task is far from completed and further research is needed. The scientists are widening their area of research to discover the true hidden potentials of phytonutrients/phytochemicals and to authenticate the original claims of the indigenous inhabitants as they appear in history about treatment of different chronic degenerative diseases with plants that have therapeutic properties. We need to look back in the knowledge bank of the entire world to find remedies for the crises created by expensive healthcare and unmanageable chronic diseases. Different nutraceuticals of plant origin may be revealed as essential disease-preventive dietary food components. However, more and better-designed clinical trials need to be carried out in order to prove the benefits of phytochemicals. The future is twinkling in the eyes of scientists—a future that will be better than the past.

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3

NUTRITION NUTRACEUTICALS: A PROACTIVE APPROACH FOR HEALTHCARE

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1 Introduction

Food is the basic requirement of life as it provides nutrients for proper functioning and nourishment of body. Recently, lifestyle disorders have caused nutritional deficiency and many diseases stressing various body organs from liver, kidney, and brain to genetic mutations in cancer. Obesity due to consumption of junk food is responsible for the increasing rate of heart diseases and related deaths. The expensive modern treatment for lifestyle disorders also makes these conditions frustrating. Owing to these issues emphasis on dieting for health has emerged as a popular resolution (Das et al., 2012; Deal and Moskowitz, 1999). "Let food be thy medicine and medicine be thy food," is the best solution, expressed in this quote from Hippocrates (Wildman et al., 2006). This purpose is well achieved by nutraceuticals: a range of natural products that serve the purpose of food and medicine together. Thus, this is an age-old concept with modern dimensions, using foods or plants for treatment of severe ailments and diseases. Though emerging from a traditional background, nutraceuticals are the future of the food industry and serve as an inspiration for healthy and novel food products. For ages, it was well explored that some foods are offer health benefits related to prevention and treatment of various diseases (Beecher, 1999). Traditionally, Indians and Chinese consume a large range of natural products with food viewed as having medicinal value; ancient Egyptians and Sumerians also valued food as medicine (Shibamoto et al., 2008). In the modern era, Japan was considered as the place of the genesis

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of the current nutraceutical market since 1980s. The expansion of modern foods has makeover the folk medicines used from centuries into nutraceuticals through new researches in food sciences and development of innovative techniques and technologies. Modern food science redefines food from product used as fuel for body to provide energy with nutritional value in terms of composition of food (carbohydrates, fats, vitamins, proteins, and minerals) to therapeutic agents. The medicinal benefits of foods attract high interest of customers for well-being using "essential nutrients" for prevention and treatment of diseases (Ahern et al., 2006). Currently, due to extensive research on health foods scientific evidences illuminates how diet is connected to disease and how food can be effectively used to manage disease (Dillard and German, 2000). The aim of this chapter is to detail the general concept of nutraceuticals and their functional application in current therapies.

2 Nutraceuticals

Nutraceutical is an amalgam of two terms, "nutrition" and "pharmaceutical," hybrid word introduced by Stephen L. DeFelice in 1989, founder and chairman of the Foundation of Innovation Medicine (Wildman et al., 2006). Nutraceuticals are food product of natural origin owing health benefits like the improvement of physiological performance and also effective various diseases.

The nutraceuticals includes a variety of products derived from isolated nutrients, dietary supplements, and genetically engineered designer foods, herbal products, processed foods, and beverages. Among them, "vitamin-enriched" health-promoting products and also fresh foods like vegetables, fruits, and fermented foods populated with live cultures can be considered to be functional foods with probiotic benefits. Thus, nutraceuticals provide a proactive healthcare approach with tremendous therapeutic impacts on human body (Das et al., 2012; Bagchi et al., 2015). Nutraceuticals are a leading trend in healthcare medicine in the market and connected to food science with pharmaceutical dosage forms hosting food bioactive compounds as active principles in formulations (pills, powders, capsules, parenterals, etc.) (Folkerts and Garssen, 2014; Martinez, 2013). These bioactive constituents are phytochemicals and are known to sustain or promote health. The nutraceutical industry exploited a broad class of phytochemicals described as phytoestrogens, terpenoids, limonoids, glucosinolates phytosterols, polyphenols, carotenoids, flavonoids, isoflavonoids, and anthocyanidins with specific pharmacological effects on human health as antioxidants, antiinflammatory, antibacterial, antiallergic, antifungal, chemopreventive,

hepatoprotective, hypolipidemic, neuroprotective, hypotensive, antiaging, diabetes, osteoporosis, carminative, antispasmodic, heart diseases, induce apoptosis, DNA damage, cancer, diuretic, CNS stimulant, analgesic, and immunomodulator (Gupta and Prakash, 2014; Karwande and Borade, 2015). Nutraceuticals are magic bullets holding a promising future for public health. Although nutraceuticals are not a new product nowadays, confusion still exists between foods with health claims and dietary supplements. We discuss the classification of nutraceuticals in the following section to explain the wide area of nutraceuticals.

3 Classifying Nutraceutical Factors

Nutraceuticals are products derived from food sources that provide additional health benefits, along with the basic nutritional value found in foods. Depending on the jurisdiction, products may or may not claim to prevent chronic diseases, improve health, delay the aging process, increase the span or quality of life or sustain the body for regular functioning. Nutraceuticals can be used as dietary supplements and functional foods as per its function and health benefits (Fardet and Rock, 2014). Owing to these wide-ranging functions of nutraceuticals, we design a systematic classification of nutraceuticals depending on their function, source, and bioactive components, as shown in Fig. 3.1.

3.1 Dietary Supplements

Dietary supplements are the nutrients found in food products and processed in suitable dosage forms depending on their mode of administration. According to the Dietary Supplement Health and Education Act (DSHEA), 1994 in USA, dietary supplements are defined as products comprised of "dietary constituents" and orally administered to supplement the nutritional requirement of diet. "Dietary constituents" refers to bioactive components comprising amino acids, vitamins, minerals, important metabolites, and certain enzymes. Dietary supplements also include herbs or extracts available in tablets, capsules, powders, liquids, and any other dosage form.

Besides fulfilling the nutrition requirement, dietary supplements also participated in therapies related to a variety of ailments. National health organizations created standards for a balanced diet to avoid the malnutrition chances and minimize the risk related with some severe diseases. Likewise, there are several examples of dietary supplements being redefined as medicine. Even cereals and grains high in calcium serve as supplementary

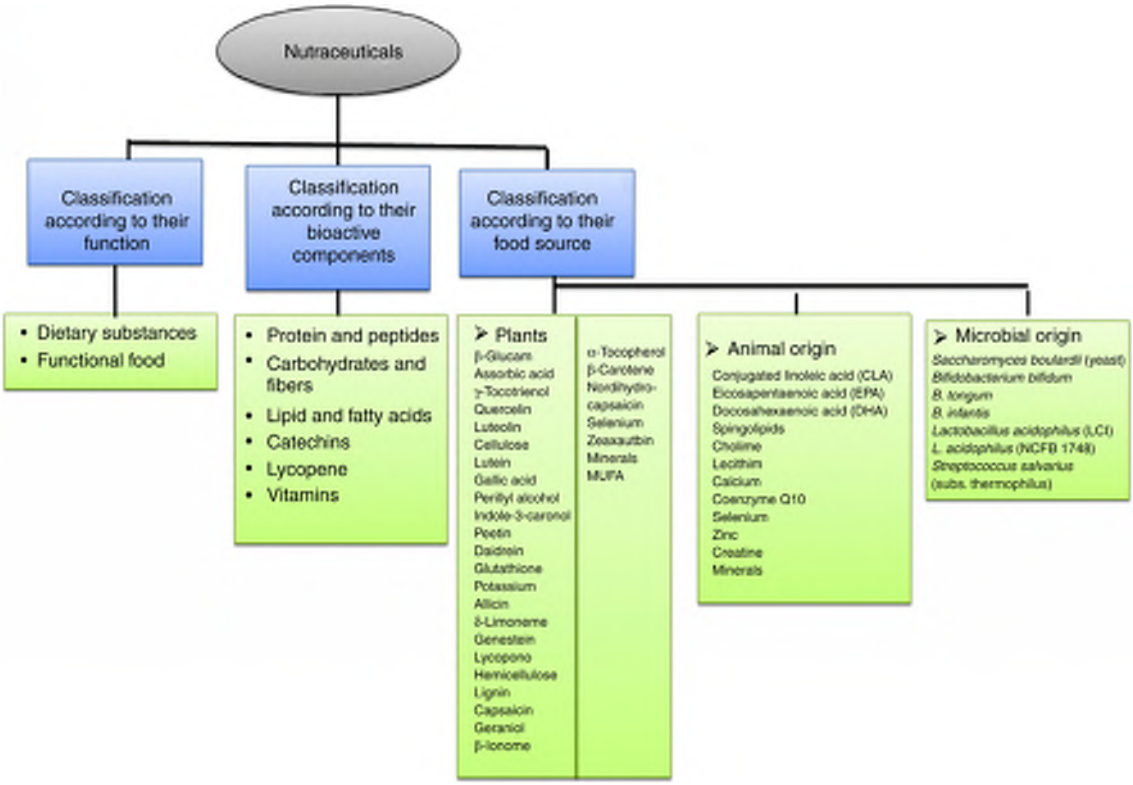

Figure 3.1. Schematic detailed classification of nutraceuticals on the basis of three broad aspects: function, bioactive component, and food source.

nutraceuticals. Currently, nutrigrain food and bars are among the popular products sold as dietary supplements. Even big brands such as Kellogg's offers multigrain food products in the market. A diet rich in ketogenic bodies is also reported to control diseases like seizures, whereas a diet rich in fat and low in protein and carbohydrates has some demerits as the disease may be aggravated. Dietary agents also combat the obesity issues and confront the insulin resistance. Fibrous foods like whole grains reduce coronary heart disease (CHD) risk and also manage type 2 diabetes mellitus. It was also emphasized from results obtained from metabolic experiments that intake of whole grains improves lipid profiles and controls type-2 diabetes in comparison to refined grains (Liu, 2002). Soy bread had become a preferred option of nutri-rich food with phytoestrogens: phytoestrogen are natural bioactive components with a structure similar to the hormone estrogen and supplements the requirement of estrogen to augment the level of estrogen in the body. Thus, it offers a natural

way to restore hormonal balance, prevent menopausal-related hot flashes, and also chemopreventive action implicated in breast cancer. Even edible mushrooms are highly rich in polyphenols with antioxidant, lipid lowering, immunomodulatory, and anticancer effects (Das et al., 2012). Furthermore, both chondroitin sulfate and glucosamine sulfate are also effective in osteoarthritis-related symptoms (Deal and Moskowitz, 1999). Another popular functional food is buckwheat protein with the therapeutic effect of lowering cholesterol, relieving antihypertension, and offering a laxative effect with its abundant fiber (Aluko, 2012; Mine and Shahidi, 2005). Dairy products are also developed as promising health-stimulating nutraceuticals. Among them the popular products contain probiotic bacterias that enhance gut health (Saraf et al., 2010). One such product is bio-yoghurt, introduced in Japan and containing *Bifidobacteria* and *Lactobacillus acidophilus*, while Yakult yoghurt (providing *L. casei Shirota*) and some other fermented products such as those offered by Culturelle (providing *Lactobacillus GG*) along with Nestlé's LC1 (providing *Lactobacillus johnsonii*) are also chief performers. Health drinks are exponentially increased in popularity as nutraceuticals (Saulnier et al., 2007; Kaushik et al., 2009; Wang et al., 2012). All these potential sources of nutraceuticals are rationally accepted throughout the health market and are also functionalized as an adjuvant therapy in conjunction with conventional therapies. Though dietary supplements do not need any regulatory requirement, it should manufactured in companies with high-quality manufacturing facilities approved by US-FDA or any other authority depending upon the country. Dietary supplements are actually preordained for maintenance of regular body functions and may not make claims for treatment of disease or ailment (Wildman et al., 2006).

3.2 Functional Foods

Functional foods are food derived from natural origin enriched in nutrients (Jones, 2002). Functional foods are processed to complement food with essential nutrients with nitrification process. Functional foods are defined by the Health Canada as "regular food with some components having specific therapeutic effect along with rich nutritional value to meet daily nutritional requirement" (Wildman et al., 2006). In Japan functional foods are assessed on the basis of three important standards: (1) functional foods must be derived from natural sources and consumed in their native state instead of processed in different dosage forms like tablet, capsule, or powder; (2) consumed regularly as a part of

daily diet; and (3) exert a dual role in prevention and management of disease and contribute in biological processes (Arai, 1996).

Herbs have always been known and used as food and medicine and they are readily used as remedies of acute and chronic diseases. Medicinal herbs have many active components responsible for their biological effects. Some commonly used herbs with medicinal benefits are listed in Table 3.1.

3.3 Nutraceuticals as Bioactives for Health Benefits

Nutraceuticals include compounds derived from nature, such as plant, animal, or marine sources, which are utilized for desired health benefits. The functional component of nutraceuticals are the well-characterized and standardized herbal preparations, fractions or extracts comprising bioactive compounds and served as active ingredients of food and pharmaceutical preparations along with cosmetics (Das et al., 2012). Recently, new investigations focusing on health-promoting foods have been highlighted and several experiments revealed neutraceuticals associated with various foods/food components for human health (Kretchmer, 1994). In this section, we closely discuss some important nutraceuticals shown to have potential health benefits and categories them in Fig. 3.2.

Probiotics and prebiotics: Our gastrointestinal lumen is inhabited by a large number of bacteria, and the consequences of these microbiota on gastrointestinal (GI) health are still under investigation. However, these same microbiota are equally important in GI health (Sikorski, 2002). Some of them also serve as nutraceuticals and act as friendly bacteria. These friendly bacteria protect the intestinal tract from contentious bacterias and yeast and also strengthen immune system and contribute in synthesis of vitamin K. Antibiotic therapy, stress, and poor dietary choices are a major cause of intestinal dysbiosis, causing bacterial imbalance. Overconsumption of antibiotics destroys the healthy bacteria of our digestive system (Fuller and Peridigón, 2008). Probiotics restore the balance of microbiota and helps to maintain the microbial balance by adding healthy bacteria in our intestinal tract. Bacteria commonly used as probiotics are *Lactobacillus* and *Bifidobacterium*, found in fermented foods such as sauerkraut and yoghurt. Prebiotics are dietary food stuffs that help in the growth and propagation of healthy bacteria. These nondigestible carbohydrate fibers generally come from oligosaccharides such as fruits, legumes, and whole grains. Several researches also demonstrated the effect of prebiotics on intestinal microbiota. Studies like these provide new

Table 3.1 Common Herbs and Their Phytochemical Products Available in the Market

Compound	Therapeutic Activity
Aloe vera gel (*Aloe vera* L. N.L. Burm.)	Dilates capillaries, antiinflammatory, emollient, wound healing properties
Chamomile (*Matricaria recutita* L.)	Antiinflammatory, spasmolytic, antimicrobial, wound healing
Echinacea (*Echinacea purpurea* L.)	Immunostimulant, treatment of cold and flu symptoms
Eleuthera (*Eleuthero coccussenticosus* Rupr. & Maxim., Maxim.)	Adaptogen
Ephedra *(Ephedra sinica* Stapf., *Ephedra intermedia* Schrank., *Ephedra equisetina* Bunge.)	Bronchodilator, vasoconstrictor, reduces bronchial edema, appetite suppressant
Evening primrose oil (*Oenothera biennis* L.)	Dietary supplement of linoleic acid, treatment of atopic eczema
Feverfew (*Tanacetum parthenium* L.)	Treatment of headache, fever, and menstrual problems; prophylactic to reduce frequency, severity, and duration of migraine headaches
Garlic (*Allium sativum* L.)	Antibacterial, antifungal, antithrombotic, hypotensive, fibrinolytic, antihyperlipidemic, antiinflammatory
Ginger (*Zingiber officinale* Rosc.)	Carminative, antiemetic, cholagogue, positive inotropic, treatment of dizziness
Ginseng (*Panax ginseng, Panax quinquefolius* L.)	Adaptogen
Ginkgo (*Ginkgo biloba* L.)	Vasodilation, increased peripheral blood flow, treatment of postthrombotic syndrome, chronic cerebral vascular insufficiency, short-term memory loss, cognitive disorders secondary to depression, dementia, tinnitus, vertigo
Goldenseal (*Hydrastis canadensis* L.)	Antimicrobial, astringent, antihemorrhagic, treatment of mucosal inflammation, dyspepsia, gastritis
Horehound (*Marrubium vulgare* L.)	Expectorant, antitussive, choleretic
Licorice *(Glycyrrhiza glabra* L., *G. uralensis* Fisch.)	Expectorant, secretolytic, treatment of peptic ulcer
Melissa (*Melissa officinalis* L.)	Topical antibacterial and antiviral
Plantago seed *(Plantago arenaria* Waldst., *P. arenaria* Kit., *Plantago ovata)*	Cathartic
Slippery elm (*Ulmus rubra* Muhl.)	Mucilaginous demulcent, emollient and nutrient, used to sooth irritated mucous membranes, ulcerations of the digestive tract
St. John's wort (*Hypericum perforatum* L.)	Anxiolytic, antiinflammatory, antidepressant, monoamine oxidase inhibitor
Valerian (*Valeriana officinalis* L.)	Spasmolytic, mild sedative, sleep aid

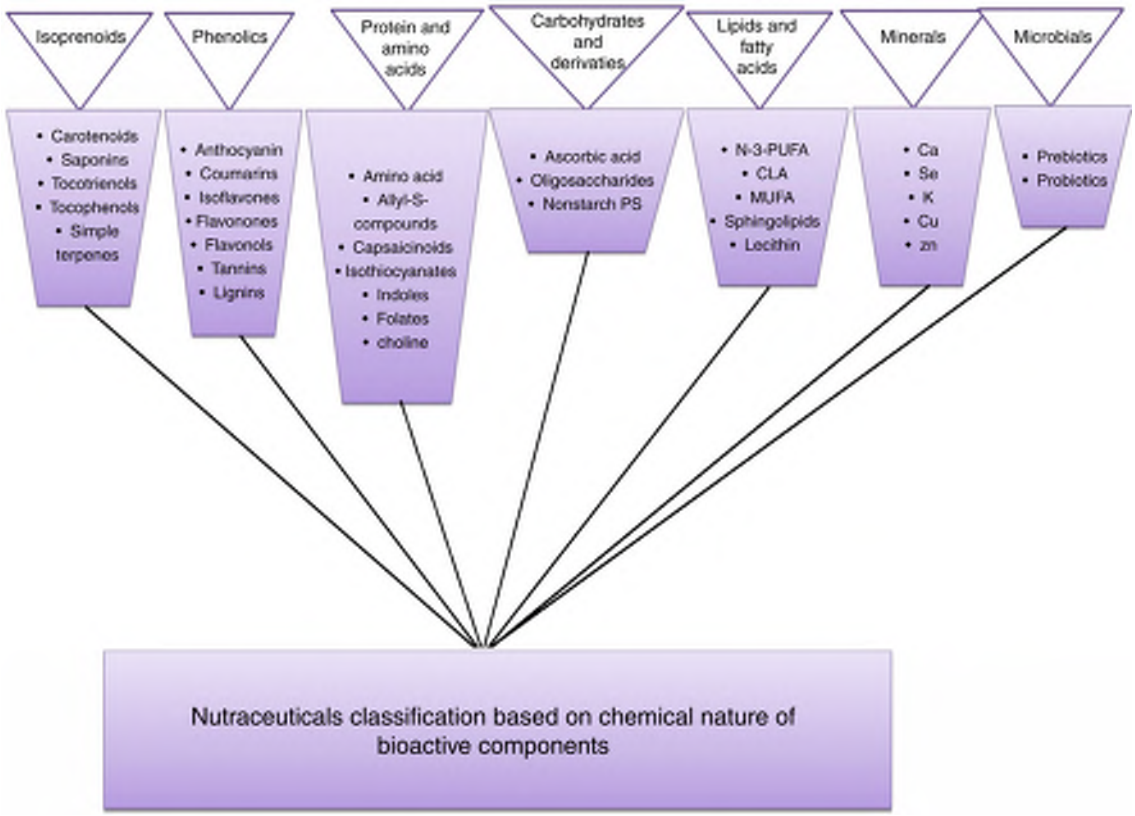

Figure 3.2. Detailed subdivision of bioactive components of nutraceuticals.

findings for the development of symbiotic combinations of probiotics (Saraf et al., 2010; Fuller and Peridigón, 2008).

Proteins and peptides: Proteins are long-chain amino acids while their shorter forms are peptides. They execute fundamental roles in health promotion. Proteins remain indigestible in gut and promote excretion of bile and toxins. They further convert to peptides and are absorbed into blood circulation. Buckwheat and soy protein are popularly used proteins that assist in the maintenance of a healthy gut (Beecher, 1999). Bioactive peptides from soy proteins are capable of lowering blood cholesterol level. Bioactive peptides are multifunctional units with large biological outcomes like antimicrobial, antifungal, blood cholesterol lowering, blood pressure lowering, antithrombotic, and immunomodulatory effects and localized effects in the gut (Cho, 2009; Paliyath et al., 2011).

Carbohydrates and fibers: Carbohydrates are major macronutrients constituting a large part of our diet in the form of sugars, oligosaccharides, starches, and fibers. Almost 55% of the calories in our diets are derived from carbohydrates. Dietary fibers are part of the function of carbohydrates in the maintenance of bowel movement. Carbohydrates can be obtained from diverse sources ranging from fruits to grains. The most popular marketed carbohydrate product is fortified infant formula, composed of fructose oligosaccharides and galactose oligosaccharides. They help in the development of the immune system of neonates (Paliyath et al., 2011).

Lipids and fatty acids: Fish oil is a common functional food and it is reported to reduce blood pressure and even lower the risk of cardiovascular diseases. Fish oil is rich in health-promoting omega fatty acids, especially omega-3 and omega-6. Major omega-3 fatty acids in fish oil are docashexaenoic (DHA) and eicosapentaenoic acids (EPA) (Misurcova et al., 2011). DHA is an important component contributing in memory functions of the brain. Recently, DHA is very popular in health drinks and baby foods and is incorporated to enhance the memory function of the brain. In elders DHA reduces the chances of Alzheimer's disease (Corsinovi et al., 2011). Other important omega fatty acids are linoleic acid and linolenic acid with cardiovascular benefits.

Lycopene: Lycopene is an abundantly available compound present in tomatoes and other colorful fruits and vegetables like papaya, watermelon, carrot, pink guava, and black grapes. Cooked tomatoes are served as a better source of lycopenes than raw tomatoes. Therefore, nowadays, concentrated tomato products like sauces, pastes, and ketchups are available in the market as rich sources of lycopene. Lycopene exhibits an antioxidant effect, neutralizing the harmful reactive oxygen species (ROS) in our body. ROS damages the cell DNA and even causes cancer. They cause lipid peroxidation and damage the heart and blood vessels leading to hypertension. Lycopene is reported to reduce blood pressure in hypertension patients (Waliszewski and Blasco, 2010).

Vitamins: Vitamins are important dietary supplements and nutritional components of nutraceuticals. They hold a diverse biological profile. Folic acid and vitamin B group components are important due to their chemopreventive roles in many diseases like cancer, heart diseases, and birth defects. Epidemiological studies unveil that certain minerals like iron, calcium, and iodine along with vitamins such as folate, vitamin E, B_6, and A are a significant part of the diet. Vitamin B_{12} along with folic acid protects from heart diseases. An important component is vitamin D, which

has a significant role in bone diseases and prevents osteoporosis and certain cancers, also. Vitamin E supplement in a 100 IU daily dose for two or more than 2 years prevents health-related risks, whereas vitamin C supplementation for nearly 10 years reduces the risk of cataracts. Vitamin C deficiency can lead to scurvy or unhealthy teeth and gums. Similarly, vitamin A deficiency can cause night blindness. Thus, vitamins play an important role in prevention of diseases and maintain nutrition for proper functioning of the body (Houston, 2005).

Polyphenols: Polyphenols are plant-based phytocompounds that are produced as secondary metabolites in plants to circumvent photosynthetic stress and oxidative stress. Derived from various food sources like vegetables, fruits, legumes, cereals, whole grains, coffee, tea, cocoa, and wine, there exist nearly 8000 polyphenols including the flavones, flavonols, flavanones, flavan-3-ols, and anthocyanins. Most of the polyphebolic compounds are synthesized by phenylpropanoid pathway. Polyphenols can be classified on the basis of their phenolic links and major elements that bond these phenolic rings. Phenolic acids constitute a major portion of a polyphenol rich diet including hydroxybenzoic acid derivatives (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid) and hydroxycinnamic acid derivatives (chlorogenic acid, caffeic acid, coumaric acid, sinapic acid, ferulic acid). Usually kiwi, berry fruits, apple, cherry, pear, coffee, and chicory are sources of high phenolic acids. The foremost class of polyphenols present in human diet is flavonoids. These can be subclassed into six types of flavonoids including flavones, isoflavones, flavanols, flavanones, and anthocyanins. The natural sources of flavonoids are berries, cherry, red cabbage, black grape, strawberry, and red wine. In vitro cell studies revealing the effect of dietary polyphenols on cellular processes are of current interest because substantial evidence in vitro has suggested that they can affect numerous cellular processes like apoptosis, gene expression, intercellular signaling, and platelet aggregation advocating their anticarcinogenic and antiatherogenic associations (Duthie et al., 2003). Apart from these, polyphenols exhibited enormous therapeutic effects, for example, antiinflammatory, antioxidant, antimicrobial, antidiabetes, and cardioprotective activities, which prevent neurodegenerative diseases (Scalbert et al., 2005). The pharmacological effects of polyphenols are restricted due to their pharmacokinetic profile as low bioavailability of polyphenols also influences the biological activity of polyphenols. The pharmacokinetic profile of polyphenols depends on various factors like chemical properties, preparation processes, gastrointestinal digestion, intestinal absorption, and metabolism via enzymes along with conjugation

and reconjugation in the intestines (Yang et al., 2001). Polyphenols are mainly excreted from the body via urine and bile. After undergoing absorption, distribution, metabolism, and excretion processes, polyphenols accumulate at the target site to produce desired pharmacological effects. Extensive research has exposed an interesting finding related to polyphenols that flavonoids have shown tempering in the expression of an important rate-limiting enzyme γ-glutamylcysteine synthetase participates in glutathione synthesis. As glutathione plays an important role in regulation of transcription factors and signaling transducing enzymes conversely, polyphenol significantly modifies cellular properties, such as xenobiotics detoxification and protein glutathionylation (Moskaug et al., 2005). Some other important cell-signaling pathways modulated by polyphenols are nuclear factor kappa-B (NF-κB), phosphoinositide 3 (PI3), kinase/protein kinase B (Akt), mitogen-activated protein kinases (MAPK), extracellular signal-regulated protein kinase (ERK), nuclear factor erythroid 2 related factor 2 (Nrf2), and activator protein-1 DNA binding (AP-1) (Han et al., 2007).

4 Role of Nutraceuticals as "Dietary Antioxidants"

"Dietary Antioxidants" covers a broad area of nutraceuticals, with wide range of products claiming direct and indirect antioxidant effect. The Panel on Dietary Antioxidants and Related Compounds of the Food and Nutrition Board proposed an official definition of "dietary antioxidants" that it can be defined as a dietary substance that suppress reactive oxygen species (ROS) and reactive nitrogen species (RNS) effect on the cells to maintain the free radicals' equilibrium for normal functioning of human body. Paradoxically, the ROS and RNS produce oxidative damage in the normal cells to create an oxidative stress. Prolonged oxidative stress can result in permanent damage to the cell and aggravate disease condition in the body such as neurological disorders, cardiovascular disease, endocrinological disorders, and fatal cancers (Jain and Ramawat, 2013; Cornelli, 2009).

4.1 Prooxidant Activity of Antioxidants

The basic mechanism behind antioxidants' action is that they accept the unpaired electron to intervene in the process of free radical propagation. In a way, antioxidants delay the

autoxidation process by means of several mechanisms: (1) inhibition of free radicals that initiate peroxidation, (2) chelation mechanism to interfere in generation of free radicals, (3) inhibition of peroxides formation by quenching O_2, (4) infringing on the chain process of autoxidation, and (5) decreasing localized O_2 level in cells (Cornelli, 2009; Brewer, 2011). Influenced by the mechanism of action of the antioxidants, the chemical nature of antioxidants also varies. Thus, chemical characteristics and lipophilicity decides the effective antioxidation (Dubey, 2014). Antioxidant efficacy is also directly related to oxidation–reduction potential, activation energy (inversely proportional to antioxidation efficiency and directly proportional to bond dissociation energy) and rate constants (Brewer, 2011). The most relevant effect is shown by antioxidants with aromatic or phenolic rings in their chemical structures, as these antioxidants act as reducing agents by donating their H atom to the free radicals and form an intermediate radical. These intermediate radicals are resonance-stabilized electron delocalization in the aromatic ring and consequently, forming quinone structures (Lü et al., 2010). In addition, chemical structure of flavonoids does not provide any suitable attack sites for molecular oxygen attack. Dietary antioxidants inhibit peroxides' formation by quenching free radical oxygen, whereas antioxidant property and mechanism of carotenoids varies with oxygen tension and concentration. At low oxygen tension β-carotene acts through chain-breaking mechanism and at high oxygen tension β-carotene exhibited prooxidant nature by autoxidation (Fiedor and Burda, 2014).

For antioxidant combinations (food intake), there exists a complicated relationship between diet and chronic diseases that can relate to an antioxidant-rich diet. It is well observed in studies that a low weekly dose of antioxidants can reduce the oxidative stress in healthy volunteers. A higher dosage of antioxidants or an increase in the intake of antioxidants with food did not substantially modify the oxidative markers, despite a significant increase in α-tocopherol, carotenoids, and vitamin C in serum. Projecting toward the higher doses or long-term consumption of antioxidants like vitamins C and E alone or in combination does not make any significant contribution in the antioxidant capacity. However, an improved profile was obtained in volunteer groups with the combination of two vitamins in respect to resistance to lipoprotein oxidation. Antioxidant vitamins may prevent hypertension. Supplementation with combination of vitamin and slow release vitamin C slows down atherosclerotic progression in hypercholesterolemic (Fiedor and Burda, 2014).

5 Nutraceuticals for Self-Care and Longevity

Detailed surveys from the past decade unveiled the mind-set of consumers regarding health promotion products. Consumers are more aware about health issues and self-care. The transformation of the "kitchen cabinet into medicine cabinet" had engendered present market ideas for promotion of health foods and nutraceuticals. Consumption of healthy food circumvents disease and infection; therefore "self-care" is the promotional trend by multinational food brands with huge consumer acceptance. Demographic reports in recent years also highlight the changes in the patterns of existing human populations. The increase in the population of people who are age 65 years and above is distinct evidence of improved longevity. Overall, an increase in the average lifespan of the human population adds a burden of medical issues to healthcare systems due to the progression of chronic diseases attributed to the aging process, such as Alzheimer's disease, heart disease, osteoporosis, cancer, and age-related macular degeneration. The estimated healthcare cost for treatment of chronic diseases has already been raised to $659 billion per annum in the United States. Prevention is always more effective than seeking a cure, thus preventative stratagems for healthcare could reduce annual healthcare costs by $60 billion using a nutrition-based paradigm. Consequently, nutrition has a direct effect on prolonging life expectancy, which is very well envisaged in researches on dietary supplementation with antioxidants and functional foods. An illustration of the effect of an antioxidant-rich diet is observed in the Mediterranean lifestyle. Western civilization along the Mediterranean Sea has generated a diet of seasonal foods such as vegetables, fruits, beans, seeds, nuts, healthy fats, whole grains, and red wine. The resultant effect is a reduction of chronic diseases like cancer, cardiovascular disorders, metabolic syndromes, and neurodegenerative diseases. The process of aging was defined through various theories; among them a prevalent concept is the free radical theory of aging (FRTA). FRTA conjectures that free radicals are related to the cellular damage responsible for aging. In the normal state of the body, free radicals and antioxidants are in a state of equilibrium. Free radicals are produced in human body as a component of eukaryotic cell redox processes, which increases oxidative stress in the body, leading to an imbalance between reactive oxygen species (ROS) and antioxidant level, a major cause for cell damage and may lead to DNA damage increasing cell ageing process and resulting in various diseases (Thring et al., 2011; Nussler and Billiar, 1993; Sun et al., 2012; Box et al., 2012; Minotti and Aust, 1989; Torel et al., 1986). This oxidative damage caused by

ROS are counteracted by antioxidant therapy. Antioxidants are reported to interfere with the oxidation process by different mechanisms including chelation of free radicals and by acting as oxygen scavengers. By virtue of such attributes antioxidant supplementation received huge attention and market acceptance. Antioxidant therapy requires scientific evidence for better elucidation of the mechanism of action for the cell aging process, governing markers for redox process for regulating oxidative damage and progress of antioxidants, therapeutic indexing of antioxidant supplements, and exploiting the prooxidant role of antioxidants (Berger, 2005). Polyphenols have been shown to possess an important antioxidant activity toward these radicals, which is principally based on the redox properties of the phenolic hydroxy groups and the structural relationships between different components of their chemical structure. Thus, there has been increasing interest in finding naturally derived antioxidants to prevent oxidative damage with lower cytotoxicity. Among them, several are introduced as antiaging food stuffs on the basis of their antioxidant potential and antiinflammatory properties like low-fat protein-rich beans, berries, dark chocolate, some sea foods, fish, garlic, aloe vera, nuts, whole grains, and avocados. As new supplementary food, an Indonesian arboreal dioecious plant, *Gnetum gnemon*, with dimeric stilbenoid compounds as active constituents has been explored for it's antiaging effect. The key constituents gnetin C, gnemonoside A, gnemonoside D with active RSV are present in the seeds of *G. gnemon*. Recent studies also reveal that ethanolic extract of *G. gnemon* seeds inhibited the endothelial senescence. Another study carried out by Fleenor et al. recommended gnetin in antiaging therapy for management of arterial aging in humans.

Age-related malnutrition is very common in elderly people as they require some critical nutrients like vitamins B_{12} and D. Nearly 10–30% of the elderly population suffers from deficiency of functional vitamin B_{12} due to type B atrophic gastritis. The state of malnutrition manifests a high risk of neurodegenerative diseases related to vitamin B_{12} deficiency and associated hyperhomocysteinemia. An increasing concern in geriatrics is low bone density owing to vitamin D status deficiency. Studies of the consequences of dietary supplements on increasing lifespan are still in progress (Fusco et al., 2007; Sadowska-Bartosz and Bartosz, 2014).

6 Nutraceuticals in Chemotherapy

Contending with fatal cancer with dietary compounds is a recent functional strategy for both cancer prevention and therapy. Dietary interventions are exploited for cancer prevention

to reduce the cancer risk, whereas, in therapies they are actively involved in treatment of cancer. Thus, there exist two strategies using the same nutraceutical phytocompounds for cancer: chemoprevention and chemotherapy. Inhibition and reversal of instigation of highly aggressive cancer by exploiting phytocompounds or synthetic compounds or biologic chemicals is defined as chemoprevention. The chemoprevention strategy has shown some successful clinical trials in populations with a high risk of cancer. Chemoprevention thus provides an efficient approach for intervening with cancer risk and also adds new agents as potential inhibitors of cancer progression along with understanding new mechanistic pathways for the inhibition of tumorigenesis. Thus, nutraceuticals have evolved into effective chemopreventive agents as well as therapeutic agents. These phytochemicals have various biological targets in cells as well as participating in several processes like inflammatory processes, aging process, oncogenic modulations marked with angiogenesis, metastases, cell cycle control, and apoptotic effect. These chemotherapeutically important anticancer phytocompounds can potentially relieve the toxic effects of the therapy (Singh et al., 2014).

Safety being the first issue for the development of potential chemotherapeutic agents, some pharmacological and pharmacokinetics factors of drug became preferable such as: low or no toxicity, effective at low doses, oral administration, economical, and availability. Synthetic drugs and the semisynthetic analogues possess high toxicity and moreover there are frequent chances of development of drug resistance for therapy. Owing to these extensive limitations, a new perception highlights the nutraceuticals in order to develop a safe therapy with minimum side effects (Schloss et al., 2013; Saldanha and Tollefsbol, 2012).

Epidemiological studies revealed that neutraceuticals included in our diet and consumed as fruits, vegetables, and spices can effectively reduce the chances of various cancers in humans. The polyphenolic compounds derived from natural sources possess a high antioxidant capacity, which is believed to prevent or retard the progress of factors responsible for cancer. The antioxidants interfere with the formation of free radicals in the cells. Therefore, antioxidants in dietary form are preferred as they include natural polyphenols and vitamins, which are known to assist in disease prevention in the human system. In recent years, dietary agents derived from natural compounds lead the market as nutraceuticals and have become the most acceptable form of natural products. Thus, a general public switch to chemopreventive mode using neutraceuticals for effective prevention or

management of diseases like cancer. The common popular and most promising plant-derived natural compounds are vincristine, vinblastine, irinotecan, etoposide, paclitaxel, and curcumin. These phytocompounds exert anticancer properties based on varying mechanisms like interfering with microtubules, inhibition of topoisomerases enzymes, alkylation of DNA, and intruding in signal transduction process. To date, various dietary agents and plant-based products have been investigated for anticancer effects to identify effective chemopreventive and therapeutic agents. Some triterpenes such as lupeol, betulinic acid, ginsenosides, and oleanolic acid are new potential anticancer agents exhibiting chemopreventive activity against multiple cancer types like colon, bladder, prostate, and breast cancers. Nature bestows us with anticancer phytomedicines that are not yet completely explored. Researchers are working on this key challenge of exploiting these phytocompounds to extract the best possible outcomes and, also, investigating possible synergism by combining two or more phytocompounds to access maximum therapeutic effect at comparatively lower doses to reduce the related toxicity of cancer chemotherapy (Sarkar, 2011).

7 Using Natural Compounds Combinations in Nutraceuticals

Natural compounds in combination are a novel dimension in modern nutraceutical research, with resources allocated to identification of new phytocompounds along with detailed study of their modes of action and related bioactivities. Newer advancements in the field of nutraceuticals bridged the drug delivery with natural compounds. This link brought forth an affordable and economical technology for development of therapeutic agents. The concept of using phytocompounds in combination results in a dramatic synergism even at low concentrations with the accomplishment of multipurpose objectives such as dose reduction of active compounds and related drug toxicity in a very economical manner. This synergistic effect is due to an increase in drug bioavailability depending on the mechanism of action of the phytocompound added (Ji et al., 2009). In the modern scenario allopathic drugs and antibiotics are a preferred choice for every disease and unnecessary consumption of antibiotics has raised the chances of rejection of drugs in therapy. The continuous use of antibiotics generates resistance to the drug and thereby the situation worsens at the multidrug resistance stage (Worthington and Melander, 2013; Coutinho et al., 2009). The

multidrug resistance occurs due to overconsumption of antibiotics and may be caused by:

- Low oral bioavailability of the drug
- Limited cell uptake of drug at target site
- Predominantly, drug efflux by transporter proteins

Thus, the therapeutic level of drug falls below what is required and as a result we need to increase the dose of drug, and therefore the related toxicity is also amplified and causes multidrug resistance (Worthington and Melander, 2013).

Combination therapy is the most appropriate solution for this problem. The synergistic mechanism of two therapeutic molecules reduces the dose of drug. Still, in the case of a synthetic drug, even after reducing the dose, associated toxicity and side effects persists. Thus, synergism through such nonantimicrobial/nontoxic compounds was an urgent need for the success of this concept. Synergism through codelivery of two or more phytocompounds enhances the therapeutic output with multitargeted action that lowers the dose of the drug, ultimately reducing the side effects associated with therapy (Rai and Kon, 2013). Previous studies detailed that phytocompounds when used alone required a higher dose to administer for desired effect therapy, thus causing an excessive load on the metabolic system with expected unsafe consequences. Preliminary research on phytocompounds strongly suggests that a combination approach of natural compounds can produce synergistic effects in vitro and in vivo. Among these phytocompounds there are many natural compounds that augment the bioavailability of the drug, when coadministered even at very low doses. Thus, these are called bioenhancers, as these chemical entities promote and augment the bioavailability of the drugs on mixing with them with or without any synergistic effect (Atal and Bedi, 2010). Nutraceuticals as bioenhancers were first reported in the 1920s by Dr Bose, through his work on the polyherbal formulation of *vasaka* (*Adhatoda vasica*) with enhanced antiasthmatic effect on concomitant administration of long pepper. In 1979, the Regional Research laboratory, Jammu, India (nowadays, known as Indian Institute of Integrative Medicine), coined the term "bioenhancer" for such phytocompounds for those effects in increased bioefficacy in combination. They provide a scientific background to this concept and validate piperine as a potential bioenhancer (Dudhatra et al., 2012; Atal and Bedi, 2010).

8 Nutraceuticals as Bioenhancers

The concept of bioenhancers had its roots in the Ayurveda system of medicine. "Trikatu" is a common ayurvedic preparation used in almost every ayurvedic formulations. The formula of

Trikatu is a combination of black pepper (*Piper nigrum* Linn.), long pepper (*Piper longum* Linn.), and ginger (*Z. officinale* Rosc.) (1:1:1). Trikatu is a Sanskrit word referring to three acids. The principle component of Trikatu is piperine, which is now a well-established bioenhancer (Atal and Bedi, 2010). Bioenhancers are very important in chemotherapy due to their widely accepted behavior. These molecules have tremendous potential to combat resistance against active drugs and antibiotics. The ultimate objective of bioenhancers is to prolong the effective lifespan of drug inside the body and considerably delaying the resistance process to prevent the multidrug resistance stage. These bioenhancers as drugs offered some novel features like (1) at low concentration, in combination with active drug augment the bioefficacy of the drug, (2) safe due to their nontoxic nature, (3) easily formulated, and (4) participate in modulation of drug–transport and ultimately enhance the cell uptake of drug, and in response absorption of drug increases, resulting in enhanced bioefficacy. Such a concept can be developed as a prudent solution for concerned molecules with huge limitations.

The term *bioenhancer* is defined as "Bioenhancers are referred to such chemical entities, which in combination with active drugs results in enhancing the bioavailability of the drugs. This effect of bioenhancers may or may not be related to any expected synergistic effect with the drug or nutrient" (Atal and Bedi, 2010). In the struggle of utilizing the latent aspects of drugs or nutrients limited due to bioavailability issues, interest toward novel approaches in the use of natural bioenhancers gains in popularity. Enhancing bioavailability results in increased plasma concentrations and in response the bioefficacy. Bioenhancer codelivery with drug or nutrients results in making any therapy more reasonable and affordable. Poor bioavailability of drugs restricts them, as a larger part of dose is unavailable for delivery or at the target site, therefore a subtherapeutic state is maintained throughout because of low plasma concentration. On increasing the dose further there will be chances of serious side effects. Thus, another important output of the use of bioenhancers is that along with enhancing the dose of a drug it will also reduce the dosing frequency of that drug (Randhawa et al., 2011; Atal et al., 1985).

Bioavailability can be limited due to factors like:

- Low aqueous solubility of drug results in poor dissolution profile
- Low permeability through intestinal membrane
- Susceptibility of intestinal degradation, presystemic intestinal or hepatic metabolism
- Loss of some essential nutraceuticals with the progress of therapy, thus the bioenhancers also improves nutritional balance by effecting the bioavailability/bioefficacy of nutraceuticals like metals and vitamins

Factors promoting the bioavailability of drug are:

1. Absorption of the drugs from GIT
2. Rate of biotransformation of drugs in the liver or intestines
3. Immunomodulation to decrease the requirement of the drug
4. Drug transport mechanism, such as, P-gp efflux mechanisms for anticancer and antimicrobial drugs
5. Modifying the signaling process between host and pathogen ensuring increased accessibility of the drugs to the pathogens
6. Affinity of target to receptor and potential binding
7. Mode of action of drug

8.1 Mechanism of Action of Bioenhancers

Bioenhancers dramatically act via diverse mechanism to exert bioavailability enhancing effect on any drug molecule. The detailed mechanism of action of bioenhancers is yet to be explored. The mechanism of action of natural bioenhancers varies with different phytocompounds (Ali et al., 2012). These were reported to influence the gastrointestinal fate of a drug: bioenhancers inhibited gastric emptying (GE) in rats and gastrointestinal transit (GT) in mice in a dose- and time-dependent manner. Here GE inhibitory activity is totally unrelated to gastric acid and pepsin secretion. Commencing into the details of thermogenic function and bioenergetic mechanisms, the bioenhancers interact with thermoreceptors and trigger the release of catecholamines, and also show agonist activity at beta 1, 2, 3. The thermogenic receptor also facilitates antiobesity and antidiabetic effects and elevates the synthesis enzyme thyroid peroxidase, resulting in increased plasma concentrations of triiodothyronine (T_3) and thyroxine (T_4) (Reanmongkol et al., 1988; Kawada et al., 1988). Bioenhancers also modulates the activity of γ-glutamyltranspeptidase, cell uptake of amino acids. Piperine, an important bioenhancer, induces the activity of γ-glutamyltranspeptidase at concentration 25–100 μM and thereby changes the permeability of rat intestinal epithelial cells in in vitro experiments (Johri et al., 1992). Some of the reported mechanisms are listed in Table 3.2 and also elaborated as follows:

1. Bioenhancers augment gastrointestinal absorption of drugs on oral administration by elevating the blood supply.
2. Bioenhancers actively modulate the drug transport mechanism through inhibition of P-glycoproteins (P-gp) represented in Fig. 3.3. P-gp efflux pump prevents the drug from moving to its target site (Breedveld et al., 2006).
3. Bioenhancers circumvent the drug metabolism, avoiding the first pass effect as they inhibit drug metabolizing enzymes like CYP 3A4,CYP1A1, CYP1B2, CYP2E1, housed in several organs

Table 3.2 Mechanism of Action of Bioenhancers

Inhibitors of P-gp Efflux Pumps	Suppressor of CYP-450 Enzyme and Its Isoenzyme	Regulators of GIT Function to Facilitate Better Absorption	Bioenergetic Properties
• *Carum carvi* (caraway) • Quercetin • Genistein • Sinomenine • Cyminum • Naringin • Cuminum	• Naringin • Quercetin • Gallic acid and its esters	• *Aloe vera* • Niaziridin • *Z. officinale* • Glycyrrhizin	• Piperine

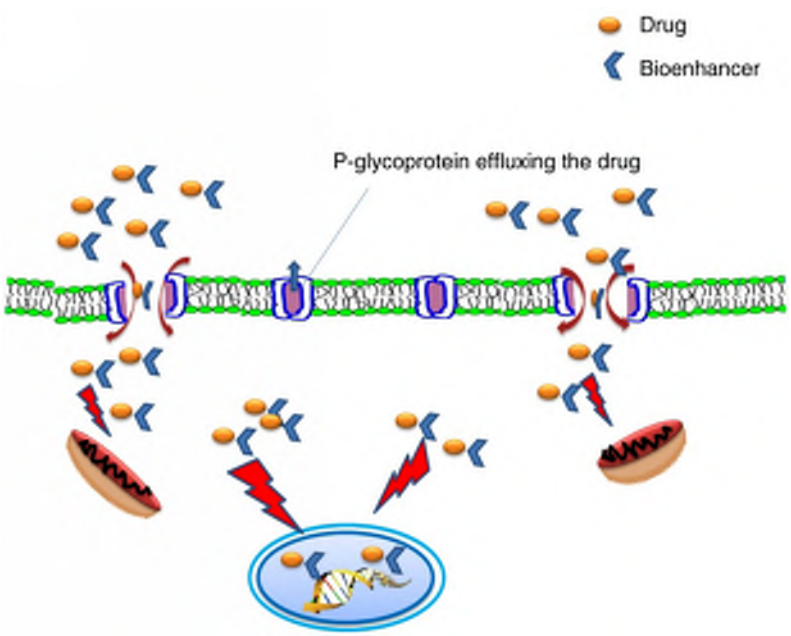

Figure 3.3. Representation of mechanism of action of natural bioenhancers by modulation of cell membrane permeability and inhibiting drug efflux mechanism by P-glycoprotein.

and localized specifically in liver, gut, lungs, and also reduce the drug elimination process resulting in longer retention of the drug in the body.

Conversely, supplementing bioenhancers with therapeutic agents had its traditional backgrounds, where polyherbal Ayurvedic

formulations served the purpose of bioavailability enhancing. However, novel tactics were devised for exploiting herbal bioenhancers for escalation of bioavailability of drugs to design a safe therapy with low dose and frequency regimen (Atal et al., 1985; D'Arcy et al., 2012).

Major categories of drugs including respiratory, heart-related, CNS, GIT, antibiotics, and antitumor effectively offer bioavailability enhancement. Several classes of drugs exhibit bioavailability potentiation with natural bioenhancers like antituberculosis drugs including rifampicin, ethambutol, pyrazinamide, and some antibiotics like tetracyclines and sulfadiazine. Other important drugs for CNS, antioxidants, and anticancer drugs are curcumin, vasicine, phenobarbitone, carbamazepine, phenytoin, nimesulide, indomethacin, coenzyme Q_{10}, ciprofloxacin, dapsone, amino acids, glucose, and β-carotene. Nowadays, bioenhancers were formulated in polymeric systems for nano- and microdelivery accomplishing dual functioning: by consuming bioenhancers for potentiation along with polymeric delivery system for ensuring active uptake of drug in order to upsurge the oral delivery of drug. Thus, the codelivery of bioenhancers is urgently needed for successful delivery of drugs with low bioavailability. Investigations are in progress for identification of novel and potent bioenhancers and innovative methods are also in progress to develop new techniques for formulating codelivery system for bioenhancers with anticancer drugs. Drug development of low bioavailable drugs is a challenging issue merging the codelivery of bioenhancers along produce more hurdles such as sustained action, enhanced surface area, rescue of bioenhancers from degradation, and target specificity. Also, scaleup of nanoformulations imposes some practical issues regarding regulatory control of herbal bioenhancers, physicochemical, and pharmacokinetic behavior.

8.2 Details of Some Important Natural Bioenhancers

The concept of bioenhancers was well documented in Ayurveda as "Yogavahi," which means there is a coadministration effect in enhancing the bioavailability, efficacy, and moreover tissue distribution of a drug. "Trikatu" is the commonest example of a bioenhancer comprising *P. longum* (long pepper), *P. nigrum* (black pepper), and *Z. officinale* (ginger) (Atal and Bedi, 2010).

8.2.1 Piperine

Piperine (1-piperoyl piperidine) is an amide alkaloid extracted from *P. longum* (long pepper) and *P. nigrum* (black pepper) found in plants of Piperaceae family. Piperine is an herbal product

generally recognized as safe (GRAS), and the content of piperine in *P. longum* is approximately 5–9%. Piperine is extensively used as a food spice and as a flavoring agent in Asia. *Piper* species were already in use as a folk medicine for the management of severe diseases, like convulsions. Piperine is bestowed with enormous biological outputs including antiinflammatory activity (Tasleem et al., 2014; Srinivasan, 2007), antifungal activity, antipyretic activity, antioxidant activity, analgesic activity, antidiarrhoeal activity, antimetastatic activity, antithyroid activity, antimutagenic activity (El Hamss et al., 2003; Srinivasan, 2007), antitumor activity, antidepressant activity, antiplatelet activity, hepatoprotective activity, antihypertensive activity, antiasthmatic activity, anticonvulsant (D'Hooge et al., 1996; Srinivasan, 2007), and fertility enhancement (Srinivasan, 2007). Piperine also exhibited severe toxic profile with noticeable effects on hepatocytes and hippocampal neurons in vitro. It also shows immune-toxicity and reproductive toxicity in Swiss albino mice (Ahern et al., 2006). Piperine is a strong antioxidant and inhibits lipid peroxidation and balances the glutathione transport thereby maintaining thiol redox (Flora, 2009; Brewer, 2011). In a recent experiment on rats fed a high-fat diet, piperine effectively reduced the thiobarbituric acid reactive substances (TBARS) level, and it also balanced the enzymes superoxide dismutase (SOD), glutathione-S-transferase (GST), glutathione peroxidase (GPX), glutathione (GSH), and catalase (CAT) in levels nearly equal to control rats (Panda and Kar, 2003). Piperine also reported to have chemopreventive effect through inhibition of lipid peroxidation and raising the antioxidant capacity (Samykutty et al., 2013). Studies revealed the anticancer effect of piperine on benzo(*a*)pyrene-induced lung carcinogenesis in albino mice model by suppressing the cell proliferation along with reducing DNA damage (Aggarwal and Kunnumakkara, 2009; Selvendiran et al., 2006). A combination of gallic acid and piperine on coadministration exerted enhanced therapeutic effect and reduced the beryllium-induced hepatorenal dysfunction along with oxidative stress (Zhao et al., 2007). In earlier reports piperine was found to increase the bioavailability of rifampicin by about 60% and hence reduce the dose from 450 to 200 mg (Randhawa et al., 2011). In another study piperine had enhanced the bioavailability of nevirapine when combined with it (Kasibhatta and Naidu, 2007). Piperine was also found to increase the bioavailability of curcumin. Piperine increased the bioavailability of curcumin by 20 times on coadministration in a lower concentration of 20 mg with human dose of curcumin (Kasibhatta and Naidu, 2007). Piperine as a dietary supplement is safe up to a broad dose range. The effective dose of piperine in order to enhance the gastrointestinal nutrient absorption is 0.0004–0.15 mg/kg per day

and 5 mg/person per day is the recommended daily dose for a healthy adult on oral administration. The effective dose as bioenhancer of piperine is 15 mg/person per day and no more than 20 mg/day approximately in divided doses or 10% (w/w) of the active drug (Dudhatra et al., 2012). Recent scientific studies on drug efflux by transporter proteins during cancer chemotherapy provides a strong background for piperine as a potent inhibitor of human P-glycoprotein and investigations also revealed its effect on metabolizing enzymes CYP3A4 (CYP: Cytochrome P450) (Atal and Bedi, 2010). This directly correlated that dietary piperine modulates the plasma concentrations of P-glycoprotein and CYP3A4 substrates in humans, especially when these drugs are the orally administered. Some other important metabolizing enzymes effectively inhibited or induced by piperine include CYP3A4, CYP2E1, CYP1A1, CYP1B1, and CYP1B2 (Bhardwaj et al., 2002). Thus, eventually drugs metabolized by these enzymes will inevitably be influenced by bioenhancers.

8.2.2 Ginger (*Z. officinale*)

Ginger (*Z. officinale*) is also an important herb with gingerol as its major component. Gingerol modulates GI tract function and facilitates absorption through the GI tract (Evans, 2002). The effective dose of gingerol as bioenhancer is 10–30 mg/kg body weight (Wildman et al., 2006). The gingerol was reported to increase the bioavailability of rifampicin by 65%, ethionamide by 56%, and some other anticancer drugs like 5-fluorouracil by 110%. Gingerol is a promising chemopreventive agent with a quite safe therapeutic profile and can be used as a better alternative for nontoxic chemotherapy. Extract of *Z. officinale* exhibited numerous biological effects like antiulcer activity, antithrombotic activity, antimicrobial activity, antifungal activity, antiinflammatory activity, antidiabetic activity, antiemetic activity, anthelmintic activity, analgesic and antipyretic activity, antioxidant, and anticancer activity (Evans, 2002; Dudhatra et al., 2012). Gingerol is a strong GI modulator and regulate intestinal functioning to effect the intestinal absorption (Bode and Dong, 2011). Earlier studies on bioenhancer effect of *Z. officinale* detailed that it offers a bioenhancer effect nearly in the range of 30–75% and in combination with piperine the range of 30–85%. Effective dose of *Z. officinale* is 5–15 mg/kg body weight, preferably 30 mg/kg body weight (Dudhatra et al., 2012).

8.2.3 Quercetin

Quercetin is an abundantly available flavonoid, usually present in various fruits, vegetables, leaves, and grains. Quercetin is

extensively used as a strong antioxidant and also exhibits enormous pharmacological effects: antiinflammatory, antiatherosclerotic, antiviral, anticancer, and antioxidant effects (Kumar and Pandey, 2013). Quercetin is a P-glycoprotein inhibitor with the same effect on CYP3A4 (Choi et al., 2011). The effect of quercetin as a bioenhancer was studied on bioavailability of paclitaxel in an in vivo experiment on oral administration of paclitaxel in rats pretreated with quercetin. The pharmacokinetic profile of paclitaxel (40 mg/kg) concomitantly administered with quercetin (2, 10, 20 mg/kg) represented an exponential increase in bioavailability of paclitaxel (Choi et al., 2004). In another experiment of coadministration of quercetin (5.0 and 15 mg/kg) with verapamil (10 mg/kg) to rabbits reflects significant variation in pharmacokinetic profile of verapamil. In comparison to control rats, quercetin (15 mg/kg) coadministration affects in augmentation of C_{max} and AUC of verapamil by twofold in rabbits (Choi and Han, 2004). Quercetin also amplified the effect of diltiazem; the bioavailability of diltiazem (15 mg/kg) was increased in the rabbits pretreated with quercetin (2, 10, 20 mg/kg) in correlation with the control (Choi and Li, 2005).

On investigation of pharmacokinetic parameters of tamoxifen (10 mg/kg) concurrently with quercetin (2.5, 7.5, and 15 mg/kg) orally, a significant ($P < 0.05$) increase in the K_a, C_{max}, and AUC of tamoxifen was observed. The tamoxifen with quercetin displayed elevated absolute bioavailability from 18.0 to 24.1%, much higher than the control group, 15.0% ($P < 0.05$). Also, the relative *bioavailability* of tamoxifen was found to be 1.20–1.61 times increased in control group when coadministered with quercetin. Though coadministration of quercetin with tamoxifen had no significant effect on the terminal $t_{1/2}$ and $t_{max,}$ so, there may be intestinal absorption modulation by quercetin, which plays a substantial role to rescue tamoxifen from first-pass metabolism and contributed to bioavailability of tamoxifen (Shin et al., 2006).

Epigallocatechin gallate (EGCG) is a popular polyphenol with anticancer effect found in green tea. The pharmacokinetic study revealed that plasma C_{max} in rats supplemented with green tea extract along with quercetin raised from up to 94.44 ± 1.59 ng/mL, than green tea extract (55.29 ± 1.70) alone. The other pharmacokinetic parameters were: the $AUC_{0–24\,h}$ of green tea extract was 510.16 ± 9.88 ng h/mL and green tea with quercetin was 794.08 ± 15.27 ng h/mL ($P \leq 0.05$), corresponding $t_{1/2}$ elimination were 2.04 ± 0.2 h and 2.28 ± 0.049 h, respectively (Kale et al., 2010). The similar experiments conducted on doxorubicin and etoposide emphasized the bioenhancer effect of quercetin due to increased bioavailability of EGCG in rats (Li and Choi, 2009).

8.2.4 Naringin

Naringin is a flavonoid glycoside that is commonly present in grapefruit, apples, onions, tea, and so forth. Naringin had been reported to have large pharmacological outcomes like antioxidant activity, antiallergic effect, antiulcer, and anticancer potential, and it also reduces blood lipid level (Dixon and Steele, 1999). Naringin is a P-gp modulator and also reported to inhibit CYP3A4. Effect of naringin was investigated on pharmacokinetic parameters of diltiazem after oral administration of diltiazem (15 mg/kg) with naringin (5 and 15 mg/kg). On comparing with control administered with diltiazem alone, the C_{max} and AUC of diltiazem revealed a nearly twofold increase in rats administered with naringin. Though diltiazem displayed no explicit variation in the t_{max} and terminal $t_{1/2}$, still, absolute bioavailability and thereby the relative bioavailability were higher ($P < 0.05$) in the treatment group with coadministration with diltiazem and naringin than the control group, thus highlighting the effect of naringin on inhibiting the metabolism of diltiazem (Choi and Han, 2005).

In addition, the effect on the pharmacokinetics parameters of intravenous paclitaxel by orally administered naringin was determined in rats. In rats, pretreated orally with naringin (3.3 and 10 mg/kg), 30 min prior intravenous administration of paclitaxel (3 mg/kg), the AUC was enhanced up to 40.8 and 49.1%, respectively, whereas, Cl_B was decreased by 29.0 and 33.0% decrease, respectively, compared to controls. There must be significant inhibition of paclitaxel metabolism through CYP3A1/2 by naringin. It also inhibited hepatic P-glycoprotein contributing in substantial increase in AUC of intravenous paclitaxel (Lim and Choi, 2006).

8.2.5 Glycyrrhizin

The biological source of glycyrrhizin is roots and stolon of liquorice (*Glycyrrhiza glabra*). It is a glycoside utilized for various ailments including bronchitis, asthma, peptic ulcers, gastritis, rheumatism, allergies, inflammation, and sore throat. It is a strong immunomodulator and maintains the liver functioning for detoxification of drugs. Moreover, it is also a laxative, diuretic, and exerts a stimulation effect on the adrenal gland. Glycyrrhizin is a natural sweetener with nearly 50 times more sweetness than sugar. Glycyrrhizin is primary a remedy for peptic ulcer and some other stomach diseases. In recent studies, glycyrrhizin displayed enormous therapeutic activities like antiinflammatory, antiviral, hepatoprotective, and anticancer activity (Dudhatra et al., 2012). The concentration range for bioenhancing effect varies with the activity of the drug such as glycyrrhizin, which is utilized in the

range of 0.05–50% for antibacterial drugs, 0.25–20% of antifungal agents, and nearly 10- to 10,000-fold to the weight of anticancer compound. In vitro and in vivo investigations of glycyrrhizin revealed that it enhances the anticancer effect of "Taxol." Glycyrrhizin in combination with paclitaxel exhibits a multifunctional behavior by suppressing the cell proliferation much higher, nearly fivefold, than paclitaxel in breast cancer cells MCF-7. Furthermore, glycyrrhizin at concentration of (1 μg/mL) with antibiotics displayed a bioenhancing effect. It exponentially increased the bioactivity of antibiotics in common use like tetracycline, ampicillin, rifampicin, and nalidixic acids against gram-positive bacterias (*Bacillus subtilis* and *Mycobacterium smegmatis*) and gram negative bacteria (*Escherichia coli*). It also exerts its effect on clotrimazole, an antifungal drug, which showed increased activity against *Candida albicans* (Khanuja et al., 2005).

9 Role of Nanotechnology for Anticancer Nutraceuticals

To assess the latent physiognomies of nutraceuticals, researchers introduced nanotechnology as a promising technique. Being potent and safe agents for chemoprevention and chemotherapy, the phytochemicals are still far from being recognized as a "panacea for all ills." The efficacy of phytocompounds suffers limitations in terms of bioavailability, even with well-known anticancer phytocompounds like curcumin, and EGCG oral bioavailability is a major issue. Despite enormous results regarding management of chronic diseases, such phytocompounds are still struggling to overcome their limitations and to generate a wide market acceptance due to their high dose and cost. Correspondingly, similar circumstances are faced by nutraceuticals used for chemoprevention with very limited success. These consequences are attributed by many factors like low aqueous solubility of phytocompound and minimal systemic bioavailability. Nanotechnology has its extensions in multifunctional fields like medicine, biology, chemistry, and engineering. Nanotechnology also productively invades cancer fields with nanoscale drug-delivery systems for diagnosis and treatment of cancer. Cancer nanotechnology is a new armament against cancer with old targets. Novel nanodelivery systems eradicate the demerits of drugs such as solubility and bioavailability for active or passive targeting of tumors. Nanodrugs not only target the tumor but also can be developed into surface engineered nanoformulations to add unique features in the nanoformulation along with high specificity. Recent reports on the

development of cancer nanotechnology revealed the interest of the scientific community in biodegradable nanoparticles loaded with natural products/compounds including curcumin, resveratrol, green tea extract (GTE), pomegranate extract, and epigallocatechin-3-gallate (EGCG). Being effective chemopreventive agents, the natural products loaded in biodegradable nanoparticles broadcasted an improved way of chemoprevention termed as *nanochemoprevention*. In actuality, though nanochemoprevention is a promising strategy but clinical trial experiments of the product still aren't recognized due to: (1) genetically different credentials of every patient with probable risk, (2) food habits of patient may vary, and (3) drug delivery issues with natural products including bioavailability. To overcome these limitations, different approaches are functionalized to sustain the release of chemopreventive agents meant to improve bioavailability and for reducing the dose of drug. Thus, the maximum outputs of natural products were assessed with minimized toxic effects (Garti and McClements, 2012; Singh et al., 2014; Siddiqui et al., 2009). The advances in nanotechnology have now released tremendous potential of the phytocompounds from bioavailability to specific targeting of tumors. Through nanotechnology, nanovectors are designed for drug delivery in cancer. Application of nanotechnology is considered to have great potential due to the ability to engineer devices with unique therapeutic potentials that because of their tiny size can penetrate tumors extremely with a high level of specificity. "Nanochemoprevention" is engendering a worldwide recognition among eminent food researchers, biologist, and pharma research groups as a high throughput technology for cancer prevention (Siddiqui et al., 2010).

Drug delivery of nutraceuticals: Progress in drug delivery enabled design of novel delivery systems for complicated drug molecules. Among these, controlled-release microencapsulation process is extensively used for delivery of bioactive agents of functional foods (Kuang et al., 2010). Consequently, viable bacteria are a dominant choice in drug delivery. Probiotics loaded with lactobacillus strains aimed to benefit the disorder related to spastic colon or irritable bowel disease. Such probiotics had gained a continuous interest for local delivery of antiinflammatory drugs and also investigations for bioefficacy in murine models of colitis (Clarke et al., 2012; Carroll et al., 2007). Polymeric formulations of probiotics are developed to encourage colon specific delivery and many other formulations were by microencapsulation and spray coating techniques were also attempted for an absolute exceptional product. Formulation of raw drugs into delivery system is a convoluted technique as it involved manifolds of excipients of generally regarded as safe (GRAS)

category that functioned to maintain the function along with viability of the cells. Stability of food material is of the utmost priority in formulations along with its specific delivery (Champagne and Fustier, 2007). Recently developed technologies including probiotic delivery in chitosan-coated alginate gels are promising demonstrations of microencapsulation. In consequence of this approach, natural products rescue probiotics from gastric acid degradation and prevent contact with bile exposure to be developed as stable functional foods. The inclusion of nanotechnology in food industry has already revolutionized the nutraceutical market. Likewise, bioactive lipids are encapsulated in nanoemulsion system for oral delivery with enhanced absorption and bioavailability (Chandramouli et al., 2004). Attempts were also made to incorporate vitamins and important minerals in polymeric matrices to improve their performance. One such example is a vitamin B_{12}-loaded emisphere (New Jersey, USA) Eligen® carrier formulation for effective oral delivery of vitamin B_{12} in B_{12}-deficient patients (Castelli et al., 2011; Brayden and Baird, 2013). Toxicological evaluation of the carrier system substantiated the safety profile of the carrier. Moreover, spray-dried emulsions of omega-3 fatty acids can be best alternated to combat taste- and smell-related problems. The check points for food-based delivery systems are fabrication and manufacturing process, physicochemical properties, flavor and aroma, delivery site for formulation, stability parameter for long-term and accelerated stability studies with account of forced degradation and packaging requirements (Brayden and Baird, 2013). For the increase of nutraceuticals in drug-delivery systems, food science researchers and drug-delivery scientists need to work out detailed data on food and polymer interactions, especially regarding functional foods and dietary supplements. Formulation of combinations of phytomolecules or extracts requires impeccable encapsulation of multiple components in a single core or in multiple layers. To date, health and nutrition is a prime issue for every nation and in some countries foods related to health and nutrition fetch the highest fundings and research interests and are an utmost priority. Pharmacological studies have already laid coherent and mechanistic bases for therapeutic assertions associated with nutraceuticals. Thus, the formulation of the nutraceuticals can be realized and translated into products with delivery aspects.

10 Regulations for Nutraceuticals

The field of nutraceuticals and its potential health benefits is quite new to the health industry. Health-promoting foods were generally not questioned when functional ability and safety of

foods were discussed. In recent years, consumers became more health conscious and more concerned about nutritional values and food safety issues. This shift in food choices owing to an increase in health education and food habits made the relationship of health and diet much more clear to consumers. Health foods are now well recognized by many consumers, who show preferences for powerful antioxidants, digestive fibers, and body-building proteins over junk food with high fat, salt, sugar, and cholesterol content. As an effect, manufacturers are emphasizing the promotion of functional foods, cashing in on extra benefits and popularity. This constructs a scenario of health-centered foods—nutraceuticals with chemopreventive and chemotherapeutic potential—being chosen for more than just being plant-derived medicine. Many nutraceuticals were already in household use and some others are sold on strong health claims of bioactives without any regulatory oversight or scientific evidence. The results interpreted from significant intervention studies throughout the world strengthen the status of health claims with clear evidence regarding dietary supplementation though it provide nourishment but may or may not show positive effects in prevention of diseases. Good food with proper nutrition is essential for the maintenance of health and prevention of disease, but the level of effect of nutraceuticals in packaged dosage form such as tablet, capsules, pills, powder, or any other form needs to be explored with scientific proofs using experimental models.

Regulatory requirement for nutraceuticals is a prime requirement for investigation of phytochemicals under claim and for quality control of functional foods and other nutraceuticals. Nowadays, different developed and developing countries are deciding upon certain regulations for food and nutraceuticals to improve the quality of product meant for public health. Strong legislative framework can solve the purpose of effective food control system. Usually, "food law" governs legislation requirements for manufacturing, handling of food, and safety in food trade chains including the regulation for food quality control and safety. In food law, quality requirements ensure food safety and check any possible adulteration. Most of countries follow an explicit set of regulations for supplements and nutraceuticals (eg, United States, European Union, Association of South East Asian Nations, India) grounded in a food-based regulatory paradigm (Heasman, 2007). In Japan, the functional foods are categorized in FOSHU and non-FOSHU. FOSHU are foods with specified health use that gain official clearance from the Ministry of Health, Labor, and Welfare on label claims and there are more than 200 functional foods listed by FOSHU, including the common FOSHU foods that are enumerated in Table 3.3. The non-FOSHU foods include green tea

Table 3.3 List of Some Important FOSHU Foods

Carbohydrates	Proteins	Minerals	Miscellaneous
Poly dextrose	Casein phosphopeptide	Phosphorus	Rice globulin
Indigestible dextrin	Casein dodeca peptide	Calcium	Eucommia leaf glycoside
Galacto oligosaccharides	Soy protein	Heme iron	Lactobacillus
Lactulose			
Lactosucrose			
Isomalto oligosaccharides			
Maltitol			
Palatinose			
Soybean oligosaccharides			
Xylo oligosaccharides			
Wheat bran			

extract, prebiotics, fish oil, glucosamine, coenzyme Q10, and others with no specific health claims (Fulgoni, 2007; Heasman, 2007; Joseph, 2007; McNamara, 2007). In the USA, the Food and Drug Administration (FDA) is the regulatory body for governing nutraceuticals and drug products. It is noticeably mentioned in the Dietary Supplement Health and Education Act from 1994 (DSHEA) that safety of nutraceuticals is entirely the manufacturers' responsibility before its marketing. Thus, the FDA can take action against the manufacturer if the product is found unsafe or the product label information is found to be unfounded or not trustworthy (Fulgoni, 2007; Heller, 2007; McNamara, 2007).

Food legislation was administrated by the European Food and Safety Authority (EFSA) in the European Union. EFSA explained the legislator requirement for "food supplements and other nutraceuticals" with health benefits. All new food products from Europe were passed through strict European development and quality requirements. Consequently, European nutraceutical are among the highest quality products (Ottaway, 2007). Legislation for nutraceuticals in Canada and Australia is more similar to drug than to food products (Fitzpatrick, 2007; Allen et al., 2007). In India, the Food Safety and Standards Authority of India (FSSAI) monitors science-based standards for articles of food. Food Safety and Standards Act (FSSA) passed in 2006 by the Government of India mandated for manufacture, storage, distribution, sale, and import

of nutraceuticals to certify safety of food for human consumption (Joseph, 2007; McNamara, 2007; Tee, 2007). In Latin America, the legislation requirement varies as per the registration approaches in Colombia, Brazil, and Argentina, and based on notification in Mexico and Chile. Whereas, in countries like Brazil, China, and Taiwan, clinical studies on animal and human are mandatory for product registration. In some countries, regulations for nutraceuticals are more related to food category (Heasman, 2007; Joseph, 2007; Lajolo, 2007; Mansour, 2007; McNamara, 2007).

11 Future of Nutraceuticals

According to reports in 2007, the comprehensive analysis of the nutraceuticals market in Asia-Pacific, European, and Latin American countries provides an outline of the future of the global nutraceuticals market and is anticipated to reach nearly $74.7 billion at an AAGR of 9.9%. The present global markets of nutraceuticals have grown to US$117 billion (Street, 2015; Chauhan et al., 2013). The effective strategies for utilizing latent aspects of nutraceuticals from dietary components are required to extract maximum output from nutraceutical agents. Strong regulatory requirements are urgently needed to develop phytocompounds and dietary stuffs like functional foods and supplements into paramount health promoters and chemopreventives. Above all, scientific research on bioenhancers had already revealed the fact that bioenhancers significantly increase the bioavailability and bioefficacy of the therapeutic agent on coadministration or pretreatment along with reducing the effective dose of the therapeutic agent, and thereby reducing the treatment period. Besides, multidrug resistance and drug related toxicity or any adverse effects are other important subjects of concern, where nutraceuticals can significantly contribute benefits with concomitant therapy. Altogether, nutraceuticals are worthwhile in terms of cost, dose, bioefficacy, and safety and can be developed into future substitutes for medicines with more acceptances. Novel approaches like delivery systems and nutrigenomics are crucial to magnify the in vivo performance of nutraceuticals. Nutraceuticals-based drug delivery systems ultimately maximize their effects as therapeutic agents and as bioenhancers.

12 Conclusions

Extensive advancement in area of food research avails new dimensions in food technology. Currently, nutraceuticals are directed more toward “functional foods” and “dietary supplements.”

Efforts have been made for functional approaches of nutraceuticals through identification of possible bioactive compounds and mechanisms, to promote foods with health benefits. Thus, nutraceuticals reduce healthcare costs and improve the quality of human life. Advancing from food supplements to active chemotherapeutic agents is the emerging trend in drug delivery based on nutraceuticals using bioactives and bioenhancers in combination therapies. This breakthrough could eventually make it feasible to develop food into medicine for prevention of chronic diseases related to heart, bones, and cancer. With these entire positive sides regulatory requirement regarding the health claims for promotion of nutraceuticals is a major concern. Surely, proper strategies for quality control and regulations of nutraceuticals are required to develop nutraceuticals as the best substitute for conventional therapies for chronic diseases.

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4

POTENTIAL OF NANOTECHNOLOGY IN NUTRACEUTICALS DELIVERY FOR THE PREVENTION AND TREATMENT OF CANCER

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1 Introduction

Cancer is one of the leading causes of death worldwide, and it is a unique genome disease, where cells undergo several gene mutations including, for instance, abnormal cell growth, which stimulates angiogenesis and apoptosis resistance, leading to the formation of a mass of cells, known as a malignant tumor. Based on the tumor site, cancer is classified into several types such as brain cancer, breast cancer, lung cancer, liver cancer, colon cancer, and so forth. Cancer development occurs via three stages: the first includes gene mutations that result in alteration of various gene functions but without changing the DNA sequence. The second stage involves promotion of and reversible change in the genome expression by promoter-receptor interactions. The final stage is the cancer progression, which is characterized by propagation of genetic errors leading to increasing the cell proliferation and formation of malignant tumor (Hanahan and Weinberg, 2000). Hanahan and Weinberg reported that cancer has six common hallmarks including (1) self-efficiency, (2) antiapoptosis, (3) rapid angiogenesis, (4) resistance to growth/inhibitory signals, (5) uncontrollable proliferation, and (6) invasion and metastasis (Hanahan and Weinberg, 2000, 2011).

Clinical evidences reveal that cancer development is strongly influenced by environmental factors such as dietary patterns, food

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type, body mass, and physical activity. This is based on the fact that cancer metabolic phenotype is a result of the interaction between the genome and environmental factors. Some studies show that diet and related lifestyle factors are ranked among the factors most highly correlated with high cancer incidences. In fact, it was estimated that around 35% of cancer mortalities are related to the diet and around 70% are dependent on cancer site (Wiseman, 2008). The studies also revealed that the morbidity and mortality of cancer varies geographically due to the variation of environmental and cultural factors. Indeed, it was reported that food, nutrients, and related lifestyle factors play important roles behind the geographic variation of cancer incidences. For instance, in developed countries, both colorectal and the hormone-related cancers are more common and are strongly related to various environmental and cultural factors such as diet and lifestyle, while in developing countries, infectious diseases are the main cause behind the development of various cancers. Several studies also demonstrated that fruit and vegetable intake is strongly decreasing cancer risk as well as aiding in cancer therapy (Kris-Etherton et al., 2002; Knekt et al., 1997).

The conventional approaches of cancer therapy include surgery, chemotherapy, and radiotherapy, either singly or in combination. The commercially available chemotherapeutic agents provide temporary relief from symptoms, prolong survival, and occasionally cure. An ideal chemotherapeutic agent should selectively kill cancer cells without harming neighboring normal cells. However, this ideal treatment is unachievable because the vast majority of cancer therapeutics possesses poor efficiency and causes severe systemic toxicity. Nevertheless, the advances of technology allow the synthesis and modification of different drugs, but most of these synthetic drugs showed a relatively small therapeutic enhancement compared to the prototype. Accordingly, there is an urgent need to find new prototypes that can be used as a template in the design of an effective cancer therapy. Recently, ongoing research focusing on the anticancer effects of various nutraceuticals in the form of crude extracts or as isolated compounds has offered an appealing array of novel natural compounds. The evidence emerging from such research shows that various nutraceuticals have pivotal roles in the prevention of cancer as well as showing a significant potential in cancer therapy in vitro and in vivo (Surh, 2003; Bingham et al., 2003; Nobili et al., 2009).

1.1 Nutraceuticals

Nutraceutical is a term derived from the words “nutrition” and “pharmaceutical,” and could be defined as “any substance that

is a food or a part of a food and provides medical or health benefits, including the prevention and treatment of diseases" (DeFelice, 2002). Based on this definition, nutraceuticals categories could encompass dietary supplements such as functional foods, medicinal foods, and phytochemicals (Go et al., 2012). *Phytochemical* is a term that refers to a variety of plant-derived compounds with therapeutic activities such as anticarcinogenic, antimutagenic, antiinflammatory, and antioxidant properties (McGuire, 2011). Nutraceuticals have a strong historical background and significant biomedical applications due to their therapeutic properties. These therapeutic properties were associated with the presence of many different compounds in fruits, vegetables, and plants such as carotenoids, flavonoids, organosulfur compounds, isothiocyanates, indoles, monoterpenes, phenolic acids, and chlorophyll. To date, various studies showed that nutraceuticals possess numerous advantages in cancer prevention and therapy along with aiding in improving overall health. Chemoprevention is a term that was first introduced by Dr. Michaelsporn, which refers to intake of a natural remedy to decrease the incidence and mortality of cancer. This is based on the fact that decreasing the incidence of a disease relying on prevention rather than a cure. Chemoprevention is an emerging research area of oncology that focuses on preventing of cancer using naturally occurring agents. It was shown that chemoprevention plays a pivotal role in inhibiting invasion and metastasis in tumors (Mateen et al., 2013). Accordingly, several nutraceuticals are being used alone or as adjuvant therapy together with conventional therapy for inducing synergic anticancer effects. Studies also showed that nutraceuticals possess a strong anticancer effect against various cancers without inducing toxicity, and are also capable of prolonging one or more stages of carcinogenic process as well as regulating various molecular pathways involved in carcinogenesis process. Various nutraceuticals are known to suppress carcinogenesis via multiple mechanisms (Fig. 4.1) such as controlling DNA damaging factors, DNA transcription, blocking transcription nuclear factor kappa B (NF–kB) activation, and so forth. Example of nutraceuticals possessing potent anticancer activities are curcumin (turmeric), green tea (catechins), silymarin (artichoke), propolis [caffeic acid, phenethyl ester (CAPE) and chrysin], vitamin D3, capsaicin (red chilli), and genistein (Hasler and Brown, 2009; McGuire, 2011). Understanding the anticancer mechanism of these nutraceuticals will provide useful information for their possible applications in chemoprevention and as adjuvant therapeutics. In the following section, the anticancer activity of some nutraceuticals with emphasis on their role in prevention and treatment of cancer is comprehensively discussed.

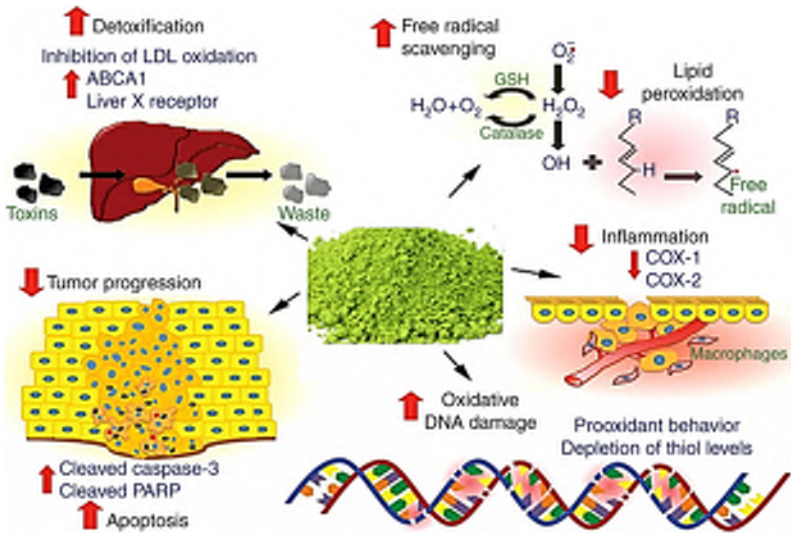

Figure 4.1. Nutraceuticals possess anticancer activity via targeting multiple cellular events such as inhibiting tumor growth, activation of detoxifying enzymes, inducing of DNA damage, and/ or causing apoptosis, as well as possessing antioxidant and antiinflammatory activities. Adapted from Gundala and Aneja (2014).

2 Nutraceuticals for Cancer Prevention and Treatment

2.1 Curcumin

Curcumin is a polyphenol (1,7-bis(4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione) derived from turmeric, a rhizome from *Curcuma longa* Linn. herb (Fig. 4.2). Historically, curcumin was used as an effective therapy over centuries for various diseases such as asthma, bronchial hyperactivity, allergy, anorexia, coryza, cough, sinusitis, and hepatic diseases, in Ayurvedic, Chinese, and Hindu regions. Extensive research on curcumin showed that it

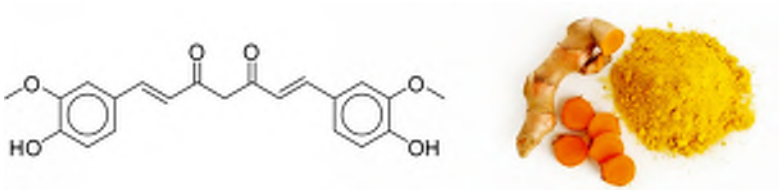

Figure 4.2. Chemical structure of Curcumin and its natural source, turmeric.

has potent therapeutic properties such as infectious, antioxidant, antiinflammatory, hepatoprotective, cardioprotective, thrombosuppressive, antiarthritic, chemopreventive, and anticarcinogenic activities (Chen et al., 2006a,b; Chan et al., 2005; Divya and Pillai, 2006).

Studies revealed that curcumin can be used as a chemopreventive due to its ability to reverse, inhibit or prevent the development of cancer through modulating multiple cellular molecular targets involved in carcinogenesis. In fact, it was found that curcumin can enhance tumor necrosis factor related apoptosis–inducing ligand (TRAIL)-induced apoptosis. TRAIL is a member of the tumor necrosis factor (TNF) superfamily that is capable of selectively inducing apoptosis in cancer cells without harming normal cells. TRAIL causes programmed cell death in various cancer cells by interacting with death-domain containing the receptor TRAIL-R1 (death receptor 4 – DR4) and/or TRAIL-R2 (death receptor 5 – DR5). Therefore, by expressing immune cells such as lymphocytes T and macrophages cells, TRAIL plays a significant role in immune surveillance and defense mechanism against cancer cells and thus enabling the immune system to cope effectively with malignancy (Shankar et al., 2007). Other studies demonstrated that curcumin has antiangiogenic effect by inhibiting the synthesis of vascular endothelium growth factor (VEGF) (Lin et al., 2007). In a recent study, curcumin has also shown to control various cellular transcription factors such as NF-kappa B, angiopoitein-1, inducible nitric oxide, and blocks the activity of Jun N-terminal kinase. The studies also demonstrated that curcumin has an antioxidant activity, which scavenge the reactive oxygen species (ROS) and electrophiles preventing DNA damage and inhibit the metabolic activation of cytochrome P450 mediated carcinogenesis, thus significantly reducing the risk of carcinogenesis (Shanmugam et al., 2015).

Recently, curcumin was considered a promising candidate either used alone or in combination with other conventional cancer therapies for cancer prevention and treatment. Recent studies showed that the anticancer activity of curcumin is associated with its ability to decrease the activity of β-catenin/TCF transcription leading to reduction of the amount of nuclear β-catenin and TCF-4 proteins in several cancer cell lines such as gastric, colon, and intestinal cancer cells. Sarkar et al. (2010) revealed that curcumin reduces the nuclear expression of disheveled and β-catenin proteins as well as inhibit transactivation of β-catenin/TCF/LEF complex resulting in altering the expression levels of GSK-3β and E-cadherin. Also, Wang et al. (2006) pointed out that curcumin is capable to downregulate Notch-1 mRNA level, causing

inactivation of NF-κB DNA–binding activity in pancreatic cancer cells and leading to cancer cells death. Subramaniam et al. (2010) also reported that curcumin and its analogue DiFiD suppress the Notch signaling pathways by inhibiting expression of Notch-1 receptor and its ligand Jagged and γ-secretase complex in pancreatic cancer cells. Although 5-flurouracil (5-FU) is still the drug of choice for colon cancer, it has shown a limited success in colon cancer therapy, and Yu et al. (2009) have shown that curcumin boosts the effect of 5-FU via mediating growth inhibition of cancer cells and hence supporting the use of curcumin as adjuvant therapy for colon cancer.

In addition, Aggarwal et al. (2009) reported the potent antiinflammatory effect of curcumin that is considered a promising therapeutic property in the treatment of cancer due to its significant involvement in the carcinogenesis process through mediating cell proliferation, survival, invasion, metastasis, and angiogenesis. This study also found out that the antiinflammatory property of curcumin was ascribed to the suppression of NF-κB signaling pathway. Several studies conducted on normal and cancer cells showed the notable importance of NF-κB, which modulates the expression of about 450 genes involved in all main signaling pathways (Singh and Aggarwal, 1995; Aggarwal et al., 2009), including tumor cell proliferation (cyclins), angiogenesis growth factors (epidermal growth factor (EGF), tumor necrosis factor (TNFa), invasion potential (matrix metalloproteinases [MMP], adhesion molecules), and many antiapoptotic genes (Bcl-2 and X linked inhibitor of apoptosis (XIAP)) (Karin and Greten, 2005; Lin et al., 2007; Notarbartolo et al., 2005). Moreover, it was revealed that curcumin affects other molecular targets implicated in inflammation and tumor promotion such as inflammatory cytokines (TNFa, interleukines IL-1, IL-6, and IL-8), inflammatory transcription factors (STATs), and inflammatory enzymes (Cyclooxygenase (COX)-2, 5-lipoxygenase (LOX)) (Shao-Ling et al., 2009; Moon et al., 2006). Due to the extraordinary anticancer activity of curcumin in suppressing cancer cells via multiple mechanisms, its clinical use could provide various opportunities for both cancer prevention and treatment (Teiten et al., 2010).

2.2 Propolis

Propolis is a resinous material collected by honeybees from the buds and exudates of conifer trees and plants. Propolis is a Greek word, where *pro* means "in defense" and *polis* means "city." Historically, propolis has been used in folk medicine in the treatment of various diseases due to its diverse pharmaceutical properties

such as immunomodulatory, antiinflammatory, antioxidant, antibacterial, antiviral, antifungal, and anticancer properties. There are different types of propolis based on geography, including Europe, New Zealand, and Brazil (Sforcin, 2007). Propolis contains different chemical compounds such as flavonoids, fatty, aliphatic and aromatic acids, steroids, aminoacids, and vitamins, such as B1, B2, E, C, polyphenolic, alcohols, terpenes, sugars, and esters (Gardana et al., 2007; Watanabe et al., 2011). Epidemiological and preclinical studies claimed that propolis has a chemopreventive activity, thus leading to an increased propolis intake among people for decreasing the risk of cancer.

The anticancer effect of propolis and its isolated components are frequently mentioned in the literature. In fact, it was found that ethanol extract of propolis (EEP), one of the richest sources of phenolic acids and flavonoids, exerts anticancer effect on human breast cancer (MCF-7) cell line through inducing apoptosis. The apoptosis induction of EEP was a dose-dependent manner where EEP 0.125 and 0.063 mg/mL showed more potent anticancer effect than EEP 0.25 and 0.5 mg/mL (Vatansever et al., 2010). Although studies support the anticancer effect of propolis and its components, the exact mechanism behind such effect is still unclear. Several studies revealed that the key mechanisms of cancer manipulation are (1) inhibition of matrix metalloproteinases, (2) antiangiogenesis, (3) prevention of metastasis, (4) causing cell-cycle arrest, and (5) induction of apoptosis. It also reported that propolis possesses in vitro and in vivo anticancer effects based on its immunomodulatory action (Sforcin, 2007). The role of the immune system has become increasingly significant in understanding the mechanisms implicated in prevention of cancer. Propolis has been found to stimulate nonspecific immunity, activated humoral immunity and enhanced cell-mediated immunity. Such enhancement of host immunity would be beneficial for cancer chemoprevention. Moreover, propolis has shown no systemic toxicity or side effects upon in vivo administration to both rats and humans (Szliszka et al., 2009).

Recent studies focused on the chemical composition of propolis in correlation to its biological properties. It was found that the main components that confer anticancer potentials of propolis are caffeic acid phenethyl ester (CAPE), chrysin (Fig. 4.3), artepillin C, and cardanol. Among different compounds, CAPE (Fig. 4.3) was found to possess a potent anticancer effect on various cancers such as human leukemia (HL-60, CI41, U937), human ovarian carcinoma, (SK-OV-3), human lung carcinoma (NCI-H358), human hepatocellular carcinoma (HepG2), human cervical cancer (ME180), human pancreatic cancer (PANC-1, BxPC-3) cells, and

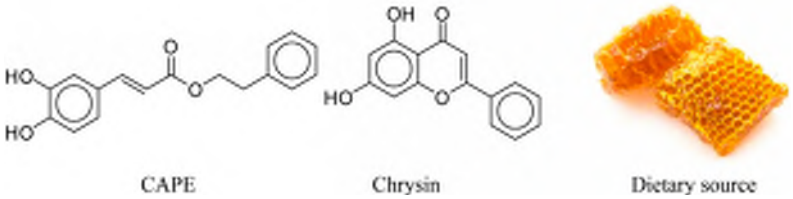

Figure 4.3. Chemical structure of anticancer components: CAPE and chrysin and their dietary sources.

colon cancer (HCT116). Jing et al. have investigated the effect of propolis containing CAPE on breast cancer cells MCF-7 (hormone receptor positive, HR+) and MDA-231 [a model of triple-negative BC (TNBC) tumor growth, both in vitro and in vivo]. This study showed that propolis containing CAPE is capable to induce apoptosis, modulate NF-κB signals, cause cell cycle arrest, and inhibit angiogensis on cancer cells without much effect on normal mammary cells (Wu et al., 2011). Another in vitro study showed that CAPE has a strong potential in activating TRAIL, which induced the death of HeLa cancer cells (Szliszka et al., 2009). Other studies demonstrated that the anticancer activity of CAPE was attributed to its ability to inhibit NF-κB along with inducing apoptosis. The induction of apoptosis is a result of activation of caspase-3, downregulation of Bcl-2, and upregulation of Bax in human leukemic HL-60 cells (Chen et al., 2001).

Another anticancer component of propolis is chrysin (5,7-dihydroxyflavone), which is a natural flavonoid present in some plant extracts, propolis, and honey (Fig. 4.3). It was also reported that chrysin influences the apoptotic process in various cancer cell lines such as liver, colon, and leukemia (Williams et al., 1997). In a study conducted on human histiocytic lymphoma (U937) cells, chrysin induces apoptosis through the inactivation of PI3K/Akt signal pathway as well as downregulation of NF-kB and IAP activation, and in turn activates caspase-3 leading to cancer cells deaths (Woo et al., 2004). In other studies, the pretreatment of human colorectal cancer (HCT116) cell line, human liver cancer (HepG2) cell line, and human nasopharyngeal carcinoma (CNE-1) cells with chrysin and 1 ng/mL TNFα influences the intrinsic apoptosis pathway through promoting TNF-α-induced apoptosis via a caspase cascade—activation of caspase-8 and caspase-3 (Li et al., 2010). Overall, propolis extract and its extremely active isolated compounds, such as CAPE and chrysin, induce apoptosis in various cancers, which suggests their significant potential in the development of new anticancer agents (Patel, 2015). These findings can open up a new hope for propolis to be used as a

complementary medicine, which could be implemented in the prevention and treatment of various cancers.

2.3 Green Tea (Catechins)

Green tea is among the most studied nutraceuticals for health benefits. Polyphenols such as catechins, are the main constituents of green tea, which is correlated to its preventive effects of cancer. Catechins contain a benzopyran skeleton with a phenyl group substituted at the 2-position and a hydroxyl group at the 3-position. Variation of the stereochemistry of 2,3 substituents and number of hydroxyl groups of catechin results in the variation of catechin structure. The most famous catechins present in tea leaves are (—)-epigallocatechin-3-gallate [(—)-EGCG], (—)-epigallocatechin [(—)-EGC], (—)-epicatechin-3-gallate [(—)-ECG], (—)-epicatechin [(—)-EC], (—)-catechin-3-gallate [(—)-CG], and (—)-gallocatechin-3-gallate [(—)-GCG] (Mukhtar and Ahmad, 1999). Several studies revealed that green tea has a great potential for cancer prevention and treatment due to the presence of (—)-EGCG (Fig. 4.4). (—)-EGCG is the one with most powerful antioxidant activity among catechins and it is about 100 times more effective than vitamins C and E. It was also found that green tea polyphenols, (—)-EGCG affect various biological pathways implicated in cancer development (Chen et al., 2011).

Many studies showed that (—)-EGCG is a promising chemopreventive of different cancers such as skin, breast, prostate, lung, and liver cancer. (—)-EGCG was reported to have an antiproliferative effect via inducing G_2-M arrest in lung cancer cells in vitro and in vivo. (—)-EGCG has also showed a potent antiproliferative effect on human hepatocellular carcinoma cells as well

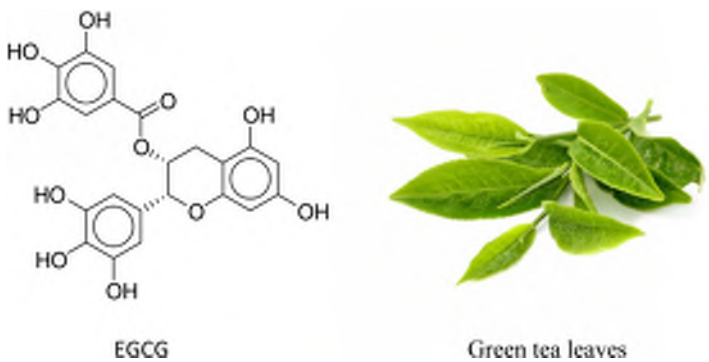

Figure 4.4. Green tea and structure of the most active anticancer polyphenols, EGCG.

as significant migration inhibition on bladder carcinoma cells (Fujimoto et al., 2002; Suganuma et al., 2006). Extensive studies demonstrated that (—)-EGCG exerts multiple inhibitory effects via targeting different cellular molecules. It was found that (—)-EGCG inhibits tyrosine phosphorylation of focal adhesion kinase (FAK) leading to the reduction of melanoma and lung metastases. Studies on breast cancer (MCF-7) cells showed that (—)-EGCG treatment decreases the expression of FAK, membrane type-1-MMP, NF-κB, and VEGF. Real time RT-PCR showed that (—)-EGCG reduces in the expression of integrin receptors α5, β1, αv, and β3 in MCF-7 cells. (—)-EGCG also demonstrated a strong anticancer effect on pancreatic cancer. In vitro also studies demonstrated that EGCG has a dose dependent antiproliferative effect; reduces of caspase-3 expression, resulting in inducing apoptosis of pancreatic cancer (MIA PaCa-2) cell line (Chen et al., 2011; Khan and Mukhtar, 2010).

2.4 Silymarin

Since many years ago, silymarin has been used for the treatment of liver diseases such as hepatitis and cirrhosis as well as protecting liver from toxic substances. Silymarin possesses therapeutic properties such as antioxidant, antilipid, peroxidative, antifibrotic, antiinflammtory, and immunomodulatory. Silymarin is extracted from seeds of milk thistle plant *Silybummarianum* (L.) Gaertn (Asterceae). The milk thistle plant contains about 65–80% silymarin flavonolignans (silymarin complex) with small amounts of flavonoids and about 20–35% fatty acids as well as other polyphenolic compounds (Kroll et al., 2007). Oncology studies demonstrated that silymarin has an antiangiogenic effect against various cancers. Studies also showed that silymarin inhibit the release of vascular endothelial growth factor (VEGF) in prostate (DU145), breast (MCF-7), and (MDA-MB-468) cancer cell lines. Another study showed that the antigenic effect of silymarin was attributed to its ability to inhibit capillary tube formation, inducing cell cycle arrest and apoptosis along with reducing both cancer invasion and migration. The molecular events associated with the antiangiogenic effect include (1) upregulation of Kip1/p27, Cip1/p21 and p53, (2) mitochondrial apoptosis, (3) caspase activation, (4) downregulation of surviving, and (5) inhibition of Akt and NF-kB signaling, and (6) matrix metalloproteinase (MMP)-2 secretion (Jiang et al., 2000; Agarwal et al., 2006; Deep and Agarwal, 2007).

Extensive in vitro and in vivo studies have been conducted on the potential chemopreventive of silymarin against various cancers. Studies showed that silymarin possesses an anticancer

activity due to the presence of active components such as silybin and other flavonolignans including isosilybin, silychristin, silydianin, and flavonoid taxifolin. Silibinin is a 1,4-dioxane ring and possesses the most potent antihepatotoxic activity in silymarin (Ahmed et al., 2003). Moreover, silibinin has shown to possess a powerful anticancer activity against various cancers. In vitro studies demonstrated that silibinin inhibits constitutively active Stat3 leading to apoptosis in prostate carcinoma (DU145) cells. Several studies showed that the use of silibinin along with chemotherapeutic agents such as doxorubicin, cisplatin, and carboplatin showed synergism on human (DU145) cell line, resulting in inducing growth inhibition and apoptotic death (Deep and Agarwal, 2007). Other studies also reported similar synergistic effects of silibinin with doxorubicin and cisplatin on breast and ovarian cancer cell lines (Scambia et al., 1996). Davis-Searles et al. examined other active components of silymarin such as silybin A, silybin B, isosilybin A, and isosilybin B on three different human prostate carcinoma (LNCaP), (DU145) and (PC3) cell lines. This study revealed that all these active components induce potent antiproliferative effects on the three cancer cell lines (Davis-Searles et al., 2005). Another recent study reported that isosilybin B and isosilybin A inhibit cells growth along with causing a strong G1 arrest and apoptosis in human prostate carcinoma (LNCaP) and (22Rv1) cell lines (Deep et al., 2007). These findings suggest that the anticancer and antiangiogenic activities of silymarin are advantageous for future studies in cancer prevention and therapy.

2.5 Capsaicin

Capsaicin, (8-methyl N-Vanillyl-6 nonenamide) is a naturally occurring phytochemical extracted from hot chili peppers of the genus capsicum (family Solanaceae). Nelson first described the structure of capsaicin in 1919 and it has been used for many years in food additives in South Asian and Latin American countries. Capsaicin has also been used in relieving inflammation and pain associated with some diseases and cancer. Extensive studies on the use of capsaicin in cancer prevention and treatment has been widely conducted to investigate its anticancer potential on various cancers (Hayman and Kam, 2008; Elkholi et al., 2014). It was reported that capsaicin possesses anticancer effects on multiple human cancer cell lines such as leukemia, multiple myeloma, skin cancer, gastric cancer, pancreatic cancer, liver cancer, colon cancer, nonsmall cell lung cancer, breast cancer, and prostate cancer.

The anticancer activity of capsaicin is associated with its ability to generate ROS via activating protease and nucleases, altering

various gene expression and mitochondrial membrane and inhibiting mitrochondrial complex activities (Tsou et al., 2006; Bhutani et al., 2007; Zhang et al., 2008; Lin et al., 2013). In vitro studies conducted on liver cancer (HepG2) cell line showed that capsaicin increases the intracellular calcium and ROS leading to cells apoptosis. Kim et al. reported that capsaicin-induced apoptosis in colon cancer cells via generation of ROS, disruption of mitochondrial membrane and via caspase-3 dependent pathway. Jin et al. (2014) also revealed that capsaicin treatment to xenografts in nude mice results in reducing the growth of colon tumor. Other in vitro and in vivo studies also showed that capsaicin causes cell cycle arrest, regulation of transcription factor expression and inhibit the growth signal transduction pathways (Pramanik and Srivastava, 2013). Mori et al. (2006) reported that capsaicin induces apoptosis on three prostate cancer cell lines (LNCaP), (PC-3), and (DU-145) through increasing the expression of p53, p21, and Bax and preventing the activation of NF-kB. Thoennissen et al. revealed that capsaicin induces a potent anticancer effect on breast cancer. Capsaicin has been shown to inhibit the growth of different of ER-positive (MCF-7, T47D, BT-474) and ER-negative (SKBR-3, MDA-MB231) breast cancer cell lines through causing G0/G1 cell-cycle arrest and apoptosis (Thoennissen et al., 2010). In addition, Moon et al. (2012) revealed that capsaicin sensitizes cancer cells to apoptosis through TNF-related apoptosis-inducing ligand (TRAIL) via activating two distinct receptors, death receptor 4 (DR4), and DR5. Taken together, these studies suggest that understanding the anticancer effect of capsaicin will provide a theoretical basis for the therapeutic use of capsaicin in the preventive and treatment of various cancers in the near future (Pramanik and Srivastava, 2013).

2.6 Genistein

Over the past few decades, epidemiological studies provided convincing evidence that isoflavones in soy-rich foods participate in reducing the incidence of breast and prostate cancers in Asian countries such as China and Japan. Genistein (4,5,7-trihydroxyisoflavone) is the predominant isoflavone in soybean-enriched foods, which are highly consumed in China and Japan (Fig. 4.5). Studies have previously demonstrated that women in Shangahi have high isoflavones plasma concentrations, which are inversely associated with the lowering incidence of nonproliferative benign fibrocystic conditions and breast cancer. In parallel, high isoflavones levels have been found in serum, urine, and prostatic fluid of Asian men, which is also contributing in reducing the incidence of prostate cancer. Upon intake of genistein, it is conjugated with

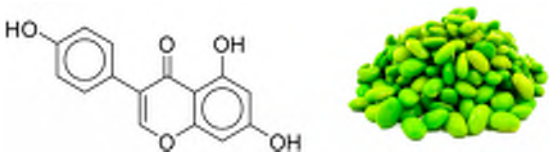

Figure 4.5. Chemical structure of genistein and its source, soyfoods.

glycoside and metabolized by intestine enzymes to dihydrogenistein and 6′-hydroxy-O-desmethylangolensin. Since genistein is relatively hydrophobic in nature, its cellular uptake occurs without cleavage and does not have to be biologically active to exert its inhibitory effects on cancer cell growth (Lampe et al., 2007). Adlercreutz et al. (1993) reported that people who consumed soyfoods exhibit 1–5 μM plasma level of genistein after metabolism and excretion. Phase I pharmacokinetic and pharmacodynamic study showed that following the administration of unconjugated soy isoflavones (containing 43% and 90% genistein, respectively) to cancer patients, the high plasma concentration of genistein was associated with antimetastatic effect (Takimoto et al., 2003). Based on these initial findings, various studies have been conducted and documented the decreased risk of breast and prostate cancers correlated with soy-foods and isoflavones intake (Banerjee et al., 2008).

The structure of genistein is closely similar to the estrogen structure. The fundamental structural feature of isoflavones is the flavones nucleus, which is composed of two benzene rings linked through a heterocyclic pyrane ring. Due to its structural similarity with 17β-estradiol, genistein competes with 17β-estradiol in ER binding assays. Kuiper et al. demonstrated that the binding affinity of genistein to ER-α was 4%, and for ER-β was 87%, as compared to estradiol. Accordingly, genistein interaction with estrogen receptor will block the binding of estrogen and in turn affect estrogen metabolism, thus exerting a pivotal role in the prevention of hormone-related cancers. Indeed, it was reported that genistein inhibited the proliferation of estrogen and androgen receptor positive and negative breast and prostate cancer cells in vitro, as well as in vitro inhibited the estrogen-stimulated growth of breast cancer cells line (Kuiper et al., 1998). Genistein is also a famous protein-tyrosine kinase (PTK) inhibitor, which inhibits the proliferation of cancer cells through inhibiting PTK-mediated signaling mechanisms (Akiyama et al., 1987). Sakla et al. recently showed that genistein suppresses HER-2 protein tyrosine phosphorylation in breast cancer cells and delays onset of cancer in

vivo. This study also supports the potent anticancer role of genistein in breast cancer therapy (Sakla et al., 2007).

Other in vitro and in vivo studies also revealed that genistein inhibits cancer cell growth through modulating genes involved in controlling cell cycle and apoptosis such as inactivation of NF-kB and Akt signaling pathways, which both are known to maintain a homeostatic balance between cell survival and apoptosis. In addition, genistein was shown to inhibit estrogen- and androgen-mediated signaling pathways implicated in carcinogenesis processes. It was also reported that genistein exerts antiproliferative activity through inhibiting other cellular enzymes and proteins such as topoisomerase I and II, 5α-reductase, and protein histidine kinase. Furthermore, it was found out that genistein possesses antioxidant properties that protect cells against ROS by scavenging free radicals, inhibiting the expression of stress response related genes and thus reducing carcinogenesis. Indeed, isoflavones offer an excellent opportunity for prevention of most common cancers globally. Moreover, isoflavones have been shown to sensitize cancer cells toward radiotherapy and chemotherapy and thus offering great avenues for devising novel therapeutic options (Banerjee et al., 2008).

Recently, a novel combination therapy comprising chemopreventive agents and chemotherapeutic agents has gained a great deal of attention in oncology research. Studies showed that genistein could boost the anticancer effect of chemotherapeutic agents against various cancers both in vitro and in vivo in preclinical studies. Sarkar et al. reported that genistein potentiated cancer cell apoptosis mediated by cisplatin, erlotinib, docetaxel, doxorubicin, gemcitabine, and CHOP (cyclophos-phamidine, doxorubicin, vincristine, prednisone against different types of cancers such as prostate, breast, pancreas, and lung cancer (Li and Sarkar, 2002; Li et al., 2005; Mohammad et al., 2003). Similar results were reported in vivo by other researchers showing that genistein boosts the anticancer effects of chemotherapeutic agents such as 5-fluorouracil (5-FU), adriamycin, cytosine arabinoside, and tamoxifen. Hillman et al. also demonstrated that combination of genistein with radiation enhanced the inhibitory effects on tumor growth and progression of renal and prostate tumor in orthotopic models (Jin et al., 2007; Hillman et al., 2007). Genistein also increases radio sensitivity of human esophageal and cervical cancer cells (Yashar et al., 2005). Collectively, both in vitro and in vivo studies clearly demonstrated that genistein is a promising agent for cancer chemoprevention and further suggest that it could be used as an adjuvant therapy along with other conventional cancer therapy owing to its effects on reversing radio-resistance and chemo-resistance.

Figure 4.6. Chemical structure of vitamin D_3 and its dietary sources.

2.7 Vitamin D_3 (Calcitriol)

Calcitriol, 1,25-dihydroxyvitamin D_3, is the hormonally active form of vitamin D_3, which has multiple actions throughout the body (Fig. 4.6). Studies showed that vitamin D_3 plays an important role in controlling and regulating various cellular pathways implicated in carcinogenesis. Vitamin D_3 has been demonstrated to decrease the incidence of human cancers such as breast, prostate, and colon cancer. In vitro and in vivo studies on vitamin D_3 conducted on different cancer cell lines showed that vitamin D possesses a potent anticancer effect via suppressing cell proliferation, stimulation of apoptosis, and inhibition of inflammation, angiogenesis, invasion, and metastasis (Krishnan et al., 2010). Li et al. showed that vitamin D_3 induces differentiation of colon cancer cells through inducing E-cadherin expression and suppressing β-catenin signaling. It was also found out that ligand-activated vitamin D_3 receptor competed with TCF-4 for β-catenin binding and thus decreased the levels of c-Myc, peroxisome proliferator-activated receptor, TCF-1, and CD 44 (Li et al., 2011a). Although the exact role of vitamin D_3 is not fully elucidated, the accumulating results from preclinical studies strongly suggest that vitamin D_3 supplement decreases cancer incidence and improves cancer prognosis (Feldman et al., 2014).

3 Clinical Problems of Nutraceuticals

Although nutraceuticals are considered valuable sources of bioactive compounds, and are among the single most successful discovery of modern medicine due to their potential in cancer prevention and therapy, their clinical advancements are hampered by their poor aqueous solubility, low stability, and poor pharmacokinetics (eg, short half-lives), resulting in low bioavailability in both plasma and tissues. Because of the clinical importance of

nutraceuticals, extensive research has been conducted to utilize nanotechnology for enhancing both the inherent and therapeutic properties of nutraceuticals. Nanotechnology permits encapsulation of various nutraceuticals using safe and biodegradable materials such as proteins, polysaccharides, gelatin, starch, and synthetic polymers, which results in enhancing the pharmacokinetics, biodistribution, and bioavailability of nutraceuticals as well as providing a targeted drug delivery and controlled release behavior. Besides, the advent of nanotechnology makes several nanoparticles-based therapeutic products commercially available. According to the European Technological Observatory (ETO) survey, more than 150 pharmaceutical companies were developing nanoscale therapeutics (Nair et al., 2010; Huang et al., 2010). An overview on the potential of nanotechnology in improving the inherent and therapeutic properties of nutraceuticals and their advantages, as "nanochemoprevention" and "nanochemotherapy" will be discussed in the following section. Several formulation strategies and examples of nanoparticles-based nutraceuticals' platforms as anticancer therapeutics will also be mentioned.

4 Formulations to Enhance Nutraceuticals' Characteristics

The unique characteristics and great versatility of nanoformulations have supported the idea that nanotechnology would soon be able to overcome the problems limiting the use of natural anticancer agents. Nanotechnology could significantly improve the characteristics of nutraceuticals, and consequently allow their clinical use for protection and treatment of different tumors. Nanoformulation of nutraceuticals provides many advantages over standard ingredients or conventional formulations. These include, for instance, increased stability during storage and inside the body, optimized aqueous/lipid solubility, which improves the overall bioavailability, sustained release of the active ingredients, improved therapeutic efficiency, and selective targeting toward tumors (Saraf et al., 2010). Many different nanoformulations are available, which differ in preparation techniques, particle size, structure, morphology, pharmacodynamics, and therapeutic properties. In this section, we will explore some of the main formulations used for delivery of anticancer nutraceuticals.

4.1 Liposomes

Liposomes are bilayer structures consisting of lipids with hydrophobic and hydrophilic parts of the same molecule, which

rearrange spontaneously to keep the hydrophobic parts away from contact with the aqueous environment, forming distinct water-soluble and lipid compartments. Liposomes consist of single or multiple concentric layers; each one of them consists of a double layer of lipids, which encapsulate at their core part of the surrounding microenvironment. Liposomes are able to deliver hydrophilic, hydrophobic, or amphiphilic drugs. In addition, liposomes offer several pharmaceutical advantages including biocompatibility, biodegradability, simple preparation methods, as well as simple targeting toward reticulo endothelial system as an inherent property of most lipids used in the preparation of liposomes.

4.2 Nanoemulsions

Nanoemulsions are colloidal systems consisting of a heterogonous system of lipid phase dispersed as droplets in aqueous phase or the reverse. This system is stabilized by the use of surfactants and can be used to incorporate water-soluble or lipid-soluble drugs. The droplet size of nanoemulsions usually lies in the range of 20–200 nm. Nanoemulsions are normally directed in vivo toward the lymph. The lipid nanodroplets formed after absorption of the emulsion are subject to phagocytosis by macrophages followed by accumulation in the liver, kidney, and spleen and thus they are beneficial when targeting these sites. (Solans et al., 2005)

4.3 Nanoparticles

Nanoparticles are polymeric colloidal systems that usually have a particle size in the range of 10–500 nm, and they may be formulated as nanospheres with the pharmaceutically active substances dispersed in their matrix or as nanocapsules with the active ingredients loaded into their core. These are the most versatile delivery systems with the possibility to attach different targeting moieties to their surface or introduce desired structural modifications.

4.4 Phytosomes

Phytosomes are nanopreparations that are used for the delivery of hydrophilic constituents that suffer from limited bioavailability and absorption either due to large molecular size or very poor lipid solubility that retards their diffusion through lipid membranes. They consist of phospholipids that can form a complex with the loaded substances through chemical bonds. Phytosomes are miscible in water and lipids and thus have good oral bioavailability.

5 Nanoformulation of Anticancer Nutraceuticals

In this section we discuss some specific examples for the nanoformulations that have been used to enhance the different characteristics of anticancer nutraceuticals including the preparation methods, used material, and the prominent characteristics. These examples will be mainly classified according to the main target of preparation.

Researchers always focused on using biopolymers, particularly those of food origin to formulate nutraceuticals. This class of polymers is encountered by our bodies in dietary intake and as a result they are generally recognized as safe materials (Donaldis, 2004). In addition, they are available in relatively large amounts and at low cost, and many of them can be extracted by ecofriendly methods (Hu et al., 2013). Moreover, some of the biopolymers are not only biodegradable and biocompatible but also biofunctional, providing additional beneficial effects in the prophylaxis or the treatment of cancer (Wimardhani et al., 2014). Researchers also focus on the development of simple reproducible ecofriendly synthetic pathways that avoid the use of organic solvents or catalysis.

5.1 Oral Delivery Systems of Nutraceuticals

The oral route is the most commonly used route for the administration of drugs due to its convenience, which improves patient compliance. However, this route can't be used for many nutraceuticals, especially those of protein nature as they are liable to degradation by the strong acidic environment of the stomach and digestive enzymes secreted at various sites of the gastrointestinal tract. As a result, much research effort has recently been dedicated to developing new approaches for the oral delivery of anticancer nutraceuticals for prophylaxis or treatment. For example, in 2008, Sahu et al. prepared curcumin-casein micelle for this purpose. Casein is a milk protein that is known to be biocompatible and biodegradable, which makes it a promising nanocarrier for oral delivery of nutraceuticals. Curcumin-casein micelles were prepared by complexation between curcumin and casein. The resulting micelles showed narrow size distribution below 200 nm and improved curcumin solubility (Sahu et al., 2008). In another study, the oral bioavailability of genistein was improved by loading into pluronic F127 nanoparticles. Pluronic F127 is a triblock copolymer consisting of relatively hydrophilic poly(ethylene oxide) and hydrophobic poly(propylene oxide) blocks providing optimum vehicles for oral delivery of drugs (Kwon et al., 2007). Capsaicin was also loaded in a more recent study into mixed polymeric micelles,

which provided a more than twofold increase in the oral bioavailability and prolonged duration of action. The mixed micelles were prepared using thin-film dispersion method using polyvinyl pyrrolidine, sodium cholate, and phospholipids (Zhu et al., 2014). Capsaicin was also loaded in alginate and chitosan-stabilized double- and triple-layer nanoemulsion to enhance its stability and oral bioavailability. The nanoemulsion provided optimum solubility, which dramatically increased the bioavailability of hydrophobic capsaicin around 130-fold (Choi et al., 2011, 2013). In another trial to improve the solubility and oral bioavailability of capsaicin, Peng et al. have prepared capsaicin-loaded nanoparticles of methoxy poly(ethylene glycol)-poly(ε-caprolactone) through emulsification solvent diffusion method. The nanoparticles provided significantly higher bioavailability and stability. Moreover, such formulation provided prolonged release of capsaicin for 60 h following administration while reducing inflammation of the gastric mucosa (Peng et al., 2014). Silymarin also was a subject of extensive research efforts owing to its importance as a hepatic support medication. Many studies investigated stable formulation of silymarin and its main constituent, silybin for the oral delivery as well as other routes of administration. Similar to capsaicin, silymarin suffers from poor water solubility, limiting its oral bioavailability. In a very recent study, Shangguan et al. aimed to overcome this problem through the preparation of binary-lipids nanostructured carriers incorporating silymarin. They used glycerol distearrate as a solid lipid and oleic acid as a liquid lipid with the use of Tween 80 and lecithin as emulsifiers. Nanostructures were prepared using high-pressure homogenization. This formulation showed very limited drug release in vitro, while after oral administration, the formulation was subject to lipolysis and the loaded drug was released within 1 h with an about 2.5-fold increase in bioavailability compared to the commercially available formulation Legalon® (Shangguan et al., 2014). Another approach for oral delivery of silybin involved the preparation of a supersaturated self-emulsifying drug-delivery system. This delivery system was prepared using simple dissolution of the drug with different constituents including oil and surfactant at 37°C with vigorous stirring forming droplets with a size of around 50 nm. The precipitation of such a formulation is retarded using hydroxypropyl methyl cellulose. The delivery system effectively provided better bioavailability compared to conventional preparations (Wei et al., 2012). Another study employing silymarin was done by Parveen et al., where they prepared nanoemulsion silymarin formulation and tested it against carbon-tetrachloride-induced hepatic damage. The nanoformulation was found to be more effective than

conventional silymarin, which was attributed to better absorption and better bioavailability (Parveen et al., 2011). Different drug delivery system was proposed by Cao and his coworkers by loading of silybin into porous silica nanoparticles prepared by reverse microemulsion and ultrasonic corrosion methods. The porous silica provided sustained release of silybin for 3 days with higher bioavailability than the commercially available formulations (Cao et al., 2012). Similarly, curcumin was also conjugated to silica in another study and the conjugate was shown to be more cytotoxic to HeLa cells than primary cell lines. This conjugate released 50% of the loaded curcumin after 7 h (Gangwar et al., 2013).

5.2 Other Delivery Routes of Nutraceuticals

In addition to the oral delivery of nutraceuticals, some other routes of administration have also gained attention over the last decade. For instance, the transdermal route of nutraceuticals delivery has been investigated as an efficient route for the treatment of skin cancer. Mangalathillam and his coworkers have prepared curcumin-loaded chitin nanogel using nanoprecipitation along with repeated cycles of centrifugation and sonication at working power of 130 W and frequency of 20 KHz. The prepared chitin nanogel improved the transdermal penetration of curcumin fourfold and was found to have specific toxicity on human melanoma cells (A375) rather than human dermal fibroblast cells. Silymarin has also been investigated for delivery through other nonoral routes such as the parenteral or buccal route. Jia and his team have prepared nanostructured lipid carriers for parenteral delivery of silybin using emulsion evaporation method. The prepared nanostructures showed higher absorption and higher accumulation in the reticulo endothelial system of the hepatic cells with prolonged duration of action (Jia et al., 2010). The buccal route was studied by El-Samaligy et al., who prepared silymarin-loaded liposomes from commercially available soybean lecithin using thin-film hydration method. The liposomes provided adequate mucoadhesion of the formulation to the buccal region while the presence of Tween 20 allowed the liposomes to squeeze through the buccal mucosal cells (Takeuchi et al., 1996; El-Samaligy et al., 2006).

6 Advantages of the Nanoformulation of Anticancer Nutraceuticals

6.1 Enhanced Solubility

Many nutraceuticals with highly promising therapeutic activity are not used clinically due to their poor aqueous solubility, which

limits their absorption and distribution. For example, propolis has been shown in many studies to have promising anticancer activity toward various types of cancer; however, its use is limited by the fact that it is highly hydrophobic. Kim et al. attempted to overcome this problem through the encapsulation of propolis into nanoparticles consisting of random copolymers of N-vinyl-2-pyrollidine, N-isopropylacrylamide and poy(ethyleneglycol) monoacrylate. The prepared nanoparticles were easily dispersed in aqueous media while retaining their anticancer activity when measured against pancreatic cancer. The study also demonstrated the safety of the used nanocarrier system (Kim et al., 2008). Enhancing the solubility of nutraceuticals is a primary target aiming to improve absorption and bioavailability as mentioned previously.

6.2 Sustained Duration of Action

Cancer treatment and prophylaxis generally require long-term use of anticancer agents and protective antioxidants. Sustained release formulations provide more convenience to the patient due to the need for less frequent dosing, which improves the patient compliance. Moreover, maintaining steady drug concentration for prolonged period improves its therapeutic efficiency.

Many studies are concerned with the development of prolonged duration of action of nutraceuticals with prophylactic or therapeutic anticancer activity. In this direction, Tsai and his coworkers have encapsulated curcumin in PLGA nanoparticles. The nanoparticles were prepared by high-pressure emulsification solvent evaporation technique. These nanoparticles provided a burst release for the first 12 h followed by the release of around 85% in 6 days. The nanoparticles also provided higher drug absorption indicated by larger area under the curve (AUC). Distribution study of the nanoparticles indicated that the nanoparticles are mainly directed toward the spleen followed by the lung. The study also showed that the curcumin PLGA-nanoparticles could pass through the blood-brain barrier and reach the brain (Tsai et al., 2011). In addition and due to the highly promising activity of curcumin, it was the subject of many other studies. For instance, a recent study investigated the utilization of supercritical fluids for the preparation of curcumin-loaded PLGA nanoparticles. PLGA solution was sprayed into supercritical CO_2 fluid in which curcumin is dispersed using ultrasonic agitation. It was found that increasing the used ultrasonic power results in a smaller particle size, which was found to be around 40 nm at 350 W power with about 35% loading efficiency. The prepared nanoparticles significantly sustained the release of the loaded curcumin. In vitro assessment of the developed formulation showed that around 30% of the loaded

curcumin was released after 10 h (Zabihi et al., 2014). Chin et al. described an interesting simple self-assembly approach for loading of curcumin into mesoporous silica. The particles formed by initial formation of cetyltrimethylammonium bromide micelles, which aggregate to form micellar rods that were used as a template for the formation of silica particles using polymerization or hydrolysis of tetramethylorthosilicate in the presence of acid. The loading capacity of curcumin into the developed mesoporous silica was calculated to be around 0.12 mol/g. The release of curcumin was monitored using UV-spectrometric analysis at 420 nm, which showed prolonged release profile lasting for around 90 h. This approach offers several advantages for sustaining the release of nutraceuticals as it was achieved at room temperature avoiding thermal degradation of active ingredients and provides thermal stability, and adequate prolonged action for loaded curcumin or other nutraceuticals (Clifford et al., 2008). In our study that has been reported in 2011 (El-Sherbiny and Smyth, 2011), swellable microparticles incorporating nanoparticles were developed for sustained pulmonary delivery of curcumin. Curcumin was loaded into PLGA nanoparticles, which were then mixed with PEG-chitosan copolymer solution followed by homogenization at 10,000 rpm. The homogenized suspension was then spray dried using a mini-spray dryer yielding dry hydrogel powder. The size of the resulting nanoparticles was around 243 nm while the PEG-chitosan microparticles loaded with PLGA curcumin nanoparticles had a size range of 3–3.5 μm. The size of this nano-in-micro system increased due to swelling after hydration to a final size range of 30–38 μm. These developed swellable particles have a small size when dry that allows them to bypass the pulmonary defense mechanisms including the ciliary action and the mucosal secretions while, after swelling by hydration, the particles are too large to be engulfed by the macrophage cells or to be expelled during exhalation. Moreover, the use of PEG renders the prepared particles stealth characters to evade the detection by the immune system. This system showed a significant sustained release profile of the loaded curcumin with an initial burst of about 20% followed by a slow release of a total of around 35% in 24 h (El-Sherbiny and Smyth, 2011). In another recent study, V. Saxena and M.D. Hussein have prepared mixed micelles using thin-film hydration for effective sustained release delivery of curcumin toward multiple drug resistant ovarian tumor cells. Multiple drug resistance is caused by P-glycoprotein transporters, which transport xenobiotics including anticancer drugs out of tumor cells. Mixed micelles were formed from a combination of Poloxamers and D-α-Tocopheryl polyethylene glycol 1000 succinate, which are both inhibitors of

P-glycoprotein. The mixed micelles increased the cytotoxic activity of formulated curcumin threefold compared to that of free curcumin and sustained its release for more than 9 days (Saxena and Hussain, 2013).

Other nutraceuticals of anticancer activity have also been the focus of research for the development of sustained release formulations. For example, in 2011, Li et al. have prepared a hydrophobic derivative of alginate by conjugation with oleoyl chloride without the use of any organic solvents. The oleoyl alginate was then used to encapsulate vitamin D3, which resulted in releasing around 55% after 7.5 h (Li et al., 2011b).

6.3 Stability under Storage Conditions

Nanoformulation of nutraceuticals may also be used to improve their stability during the storage. For example, Vitamin D_3 was loaded into zein nanoparticles which were then coated with carboxymethyl chitosan. Zein is a natural protein produced from maize with unique characteristics, and it consists of a small hydrophilic part and a larger hydrophobic part providing optimum alcohol and water solubility. This protein is capable of forming self-assembled nanoparticles that has been studied for the delivery of various active ingredients (Parris et al., 2005; Zhong et al., 2009). Antioxidant ingredients usually suffer from poor stability. Green tea catechins are examples of such substances whose efficiency is limited by low stability and oxidation during storage. They have been the subject of extensive research to enhance their stability and prolong their half-life. Catechins were formulated into polylactic acid-polyethylene glycol nanoparticles, poly(lactic-*co*-glycolic acid) nanoparticles, and chitosan-tripolyphosphate nanoparticles to enhance their stability. Researches also studied the use of reducing agents such as ascorbic acid or tris(2-carboxyethyl)phosphine agents to improve the stability of catechins by being preferentially oxidized (Chen et al., 1998; Siddiqui et al., 2009; Dang et al., 2015). Catechins were also loaded into micelles and liposomes as other methods of formulation for the same purpose (Shpigelman et al., 2010; Fang et al., 2006). For instance, Lee et al. have loaded catechins into liposomes of L-α-phosphatidylcholine and cholesterol stabilized by calcium pectinate. Maximum loading of the prepared liposomes was achieved by coating with hydroxypropylmethyl cellulose, which also provided optimum drug release for around 4 h (Lee et al., 2008). Micellar formulation of catechins was investigated in another interesting study where a group of researchers used a modified oligomer of epigallocatechingallate (EGCG) to form the inner core of micelles with an outer

shell of polyethylene glycol (PEG). The prepared micelles were used for the delivery of proteins, drugs, and flavonoids. The PEG shell of the micelles provided stability against enzymatic degradation, longer duration of action, and controlled distribution, while the inner EGCG could be used for targeting cancer with upregulated receptors. In addition, EGCG provides antioxidant activity, thus conferring more stability for the loaded drugs (Shpigelman et al., 2010). Shpigelmann et al. also improved the stability of EGCG by the coassembly into β-lactoglobulin nanoparticles by moderate thermal treatment. This preparation provided a more than 30-fold improvement in the stability of the sensitive antioxidants (Shpigelman et al., 2010).

6.4 Cancer Targeting

Selective targeting of anticancer agents would provide better safety and higher therapeutic efficiency. In other words, it would overcome the toxicity that limits the therapeutic use of many natural anticancer substances. For this reason, the selective targeting of cancer cells with nanocarriers loaded with nutraceuticals has been the focus of many recent studies. In general, there are several approaches to achieve targeting of cancer cells (Fig. 4.7) while avoiding harming the healthy cells. Specifically, this targeting can be achieved either actively or passively.

Passive targeting is achieved depending on the particle size of the delivery system, which is referred to as the enhanced permeability and retention (EPR) effect, which was first described in 1986. EPR effect depends on structural differences between solid tumors and healthy tissues. Tumors are generally characterized by rapid growth that leads to hypoxia at the core, which results in the

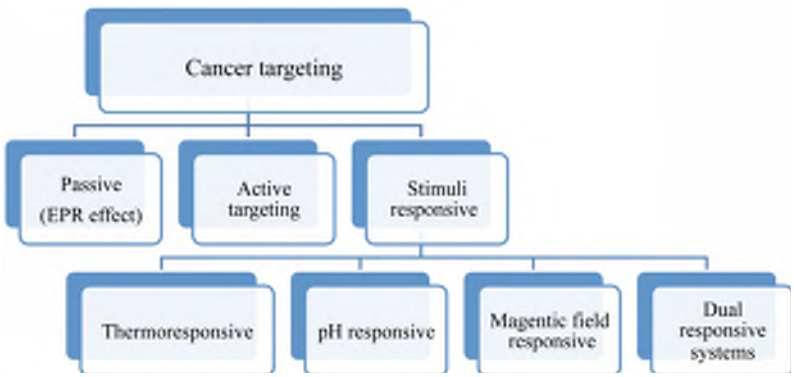

Figure 4.7. Classification of cancer targeting systems.

release of angiogenic factors that stimulate rapid growth of blood vessels to support the growing tumor with nutrients and oxygen. Rapid angiogenesis results in the formation of defective blood vessels with large fenestrations that are leaky toward macromolecules and nanoparticles. It was found that the pores of tumor vasculature have sizes ranging between 380 and 780 nm while normal blood vessels are impermeable to particles larger than 400 nm (Gerlowski and Jain, 1986). Tumors are also characterized by poor lymphatic drainage along with slow venous flow. All these factors result in the accumulation of macromolecules and nanoparticles in tumors rather than healthy tissues (Matsumura and Maeda, 1986). Passive targeting could be of particular importance for the establishment of nutraceuticals as curcumin with suboptimal pharmacokinetic and physicochemical properties as therapeutic agents of clinical use. However, the direct investigation of EPR effect for the delivery of nutraceuticals has unfortunately gained little attention to date.

Active targeting on the other hand is achieved by the use of targeting moieties with special affinity to cancer tissues. This is mainly achieved using a ligand, cytokines, or other nutrients forwhich cancer cells have greater affinity than normal healthy cells. Cancer cells are unregulated rapidly proliferating cells and consequently have greater affinity for folic acid, which is a cofactor involved in the synthesis of DNA and cell division. Folate derivatives have been widely used for selective targeting of different nutraceuticals for cancer treatment. For instance, Thu and his coworkers developed a folate-targeted nano system for selective delivery of curcumin. O-carboxymethyl chitosan was conjugated through its amino group to folate using click chemistry. The modified polymer was then used to encapsulate curcumin using nanoprecipitation technique, where O-carboxymethyl chitosan was dissolved in distilled water followed by the addition of ethanol solution of curcumin under agitation by stirring or sonication till a clear solution is obtained. The nanoparticles were then separated from large aggregates using differential centrifugation. The produced nanoparticles had the size range between 50 and 100 nm with significant targeting and enhanced intracellular uptake at HT29 and HeLa cancer cell lines (Thu et al., 2015). Another recent study provided a very interesting approach for targeted delivery of curcumin. This study utilized dequalinium (Fig. 4.8), which is an antibacterial agent that has been used for more than 50 years. The amphiphilic nature of dequalinium allows it to form vesicles in aqueous medium called DQAsomes.

Preparation of curcumin-loaded DQAsomes was done using thin-film hydration technique. Curcumin formed hydrogen bonds with dequalinium in the formed DQAsomes, which had particle

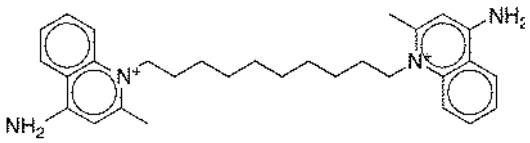

Figure 4.8. Structure of dequalinium.

sizes between 170 and 200 nm. Dequalinium has an intrinsic affinity with mitochondria in addition to its cationic structure, which enhances the intracellular uptake of DQAsomes. Mitochondrial targeting has been confirmed using fluorescence confocal microscopy. This formulation was proposed for treatment of acute lung injury that is normally a consequence of chemotherapy or surgery in treatment of lung cancer. Different targeted sustained release formulations have also been developed for curcumin. Wei and his coworkers at the University of Nebraska prepared targeted nanogel conjugates for intracellular delivery of curcumin. Curcumin was conjugated to hyaluronic acid with the aid of click chemistry through cholesterol linker using EDC and HOBt catalysis. Then, the conjugate was formulated as nanogel using sonication in aqueous medium for 30 min (Wei et al., 2014). The developed conjugate nanogel particles had hydrodynamic diameter of 20 nm, which allows optimum permeability through the mucosa of the gastrointestinal tract and avoids uptake by macrophage cells providing prolonged systemic circulation for adequate tumor targeting. Hyaluronic acid in turn has an intrinsic affinity for CD44-expressing drug-resistant cancer cells. Attachment of the nanogel to the surface of CD44 cells stimulates clathrin-dependent endocytosis that results in internalization of the nanogel and suppression of tumor cells. Interestingly, it was found that the prepared nanogel not only provided prolonged duration of action of curcumin but also demonstrated higher cytotoxic activity than the free drug. The nanogel showed significant enhancement in the stability of the loaded drug with only 10% degradation after 24 h incubation in aqueous medium while the free curcumin was completely degraded within 60 min. The nanogel was also able to resist gastrointestinal degradation and deliver more than 95% of the loaded drug to the systemic circulation. In vivo treatment of human pancreatic tumors in mice model using the developed nanogel resulted in 13-fold tumor suppression suggesting it as an effective treatment as a prophylaxis against tumor relapse (Wei et al., 2014).

A more recent approach for active targeting of nutraceuticals involves the use of stimuli responsive drug delivery systems, which may respond to internal or external stimuli as temperature,

pH, the presence of specific enzymes or substrate, or external magnetic field. Magnetic responsive systems are the most commonly studied among other types of stimuli responsive systems for the delivery of anticancer nutraceuticals. O. Vittorio et al. prepared magnetic catechin-dextran conjugated for targeted treatment of pancreatic tumors. Catechin-Dextran conjugates were prepared using free radical grafting by activation of dextran using H_2O_2 followed by conjugation with the antioxidant catechin. The prepared conjugate was then used to encapsulate the commercially available magnetic nanoparticles Endorem®. The advantage of the use of endorem is its FDA approval confirming its biocompatibility and its efficiency as stimuli responsive particles. The prepared catechin-endorem particles were completely attracted toward external magnetic field resulting in increased local concentration and increased intracellular uptake resulting in apoptosis of around 98% of the tumor cells in vitro (Vittorio et al., 2014). Another trial for targeted delivery of nutraceuticals using magnetic nanoparticles was done by Chin and his coworkers. Curcumin was coloaded with magnetic nanoparticles into a mesoporous silica reservoir, which was previously described as a method for sustained release of curcumin (Clifford et al., 2008; Chin et al., 2009).

Other stimuli-responsive formulations include those that are sensitive to temperature or external irradiation. In 2011, Wu and his coworkers reported the development of a complex drug delivery system that can be used for photothermal therapy along with inducible sustained release of curcumin. The delivery system was based on Ag/Au bimetallic nanoparticles that were then coated with a hydrophobic layer of polystyrene gel followed by a hydrophilic layer of PEG derived gel. The Ag/Au core has strong fluorescence that can be used for imaging and diagnosis and strong absorption of near IR absorption spectrum. The inner polystyrene layer has affinity toward hydrophobic curcumin, allowing high loading efficiency, while the outermost PEG-derived layer provides thermal sensitivity that would induce the release of the loaded curcumin in response to increase in temperature or external stimulation by near infrared radiation (Wu et al., 2011) (Fig. 4.9).

Rejinold and his coworkers also prepared another thermoresponsive system for the delivery of curcumin using ionic crosslinking of chitosan-g-poly(N-isopropyl acrylamide). Lower Critical Solution Temperature (LCST) for the developed copolymeric system was determined to be 38°C. This system released less than 10% of the loaded curcumin after 24 h of incubation below the LCST while around 90% was released when tested above the LCST, proving its efficiency as a thermoresponsive carrier (Rejinold et al., 2011). Thermoresponsive delivery systems have also been manipulated for other

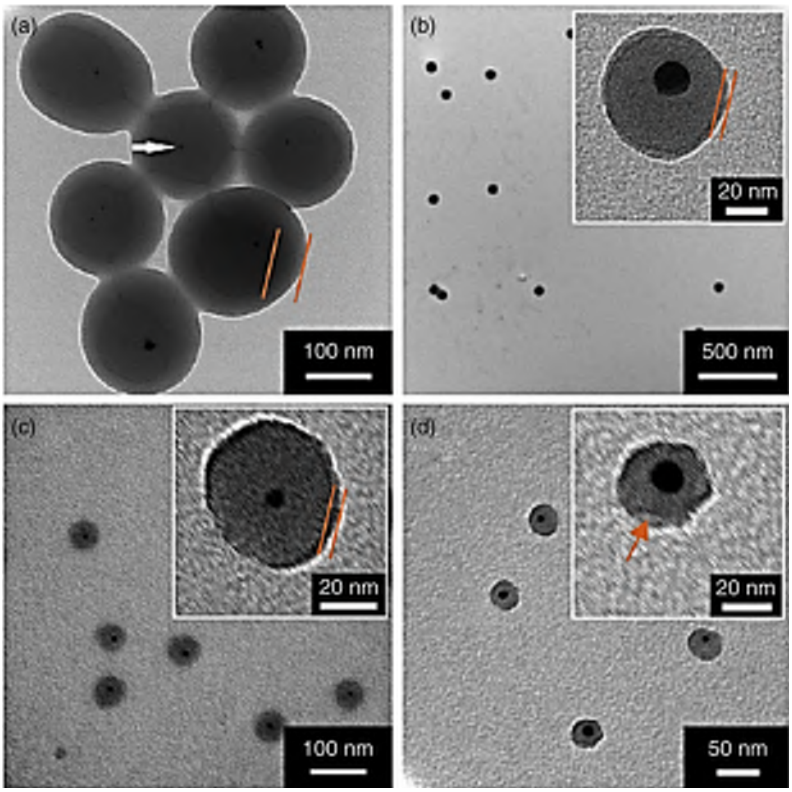

Figure 4.9. SEM images of thermoresponsive Ag/Au bimetallic nanoparticles.
Adapted from Wu et al. (2011).

anticancer nutraceuticals. Thermosensitive antioxidant-hydrogels were synthesized by grafting of catechin as antioxidant and N-isopropylacrylamide as a thermoresponsive species on inulin hydrogel network. The prepared hydrogel showed a phase transition around 31.3–33.1°C, which suggests its use in nonmedical purposes in the food industry (Spizzirri et al., 2011). Another stimuli-responsive carrier system involving catechin was developed by Spizzirri et al., who developed pH-responsive nanogel by coupling reaction between catechin and polyacrylic acid. The hydrogel was able to delay the release of the loaded drug in acidic conditions of the stomach (pH 1) while releasing its content at slightly alkaline conditions (pH 7.4), while catechin was used not as a drug itself but for its antioxidant properties to protect other drugs liable to oxidation, thus suggesting the utility of such a system for the delivery of unstable drugs through the gastrointestinal tract (Spizzirri et al., 2013).

Recently, a group of researchers in China have prepared dual responsive drug delivery system for efficient intracellular delivery

of curcumin. RAFT copolymerization was used to synthesize copolymers that are functionalized with the acid-sensitive cyclic benzylideneacetal groups and attached through disulfide linkage to PEG shell. The nanoparticles were then formed using nanoprecipitation method in the presence of curcumin providing high loading capacity up to 19% and excellent entrapment efficiency reaching 96%. These nanoparticles are rapidly disassembled in response to mild acidic and reducing conditions, which leads to cleavage of the cyclic benzylideneacetal groups and disulfide linkages, respectively. The mild acidic and reducing conditions resemble the microenvironment of the lysosome and the cytoplasm, ensuring efficient intracellular delivery. The developed carrier system was able to entrap the loaded curcumin, releasing less than 15% of its load after 20 h in normal physiological conditions while around 80% of the loaded curcumin was released within 5 h in the presence of mild acidic conditions (pH 5) and reducing environment (10 mM DTT). This carrier was shown to be more efficient in the growth inhibition of EC-109 and HepG-2 cell lines compared to nonresponsive curcumin loaded control and to free curcumin (Zhao et al., 2013).

7 Conclusions

Several nutraceuticals have demonstrated potent and promising activity for the treatment and prophylaxis against cancer. As a consequence, several nutraceuticals as curcumin, propolis, silymarin, and genistein are very promising candidates for the development of clinically useful therapeutics, and some of them are already available in the market. However, several drawbacks limit the clinical use of nutraceuticals such as low solubility, low stability, poor bioavailability, and toxicity. Nanoformulations with their great diversity and unique characteristics pave the way to overcome these drawbacks by conjugation or incorporation of such nutraceuticals into well-designed nanocarrier systems to provide enhanced solubility, improved bioavailability, sustained duration, and selective targeting of cancer cells. According to several recently reported studies, it became obvious that the nanoformulation approach of various anticancer nutraceuticals would be a major step in facing the challenge of cancer treatment.

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5

ADULTERATION AND SAFETY ISSUES IN NUTRACEUTICALS AND DIETARY SUPPLEMENTS: INNOCENT OR RISKY?

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1 Introduction

In recent years, many food active principles have been transformed into pharmaceutical forms such as tablets, capsules, pills, powders, granules, syrups, and so forth, since statistics clearly show that there is great consumer demand throughout the world for health-promoting products, which improve quality of life and boost the health status of the body. Hence, the term "*nutraceutical,*" derived from combination of "nutrition" and "pharmaceutical" in hybridized from, was first coined in 1989 by Stephen De Felice, the director of the Foundation for Innovation in Medicine in New York (Brower, 1998). The nutraceutical market was estimated at US$30 billion and growing by 5% per annum in the beginning of 2000s (Andlauer and Fürst, 2002). A common debate regarding nutraceuticals and dietary supplements worldwide is whether they should be considered to be food or medicine. Actually, nutraceuticals must contain a single component of essential micro- or macronutrients that are the active components of functional foods. In this regard, a large number of nutraceuticals containing phytochemicals from foods including carotenoids (eg, lycopene from tomato), sulfur compounds (from garlic), glycosinolates, phytosterols, curcumin, isoflavonoids, proanthocyanins, essential

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fatty acids (EFAs), vitamins, proteins, peptides (eg, carnosine) and amino acids (eg, arginine), polysaccharides, antioxidants for example, coenzyme Q10, minerals (eg, selenium), and so forth, are now presented in the market (Ferrari, 2004; Nicoletti, 2012).

Nevertheless, since it is difficult to differentiate between nutraceuticals, herbal medicines, functional foods, nutrients or food additives due to their drug-like health-claiming effects, some various other terms are also used to refer these products such as "dietary supplements and medicinal foods." According to Health Canada (Shahidi, 2009; Bishop et al., 2015), a nutraceutical is defined as "a product that is isolated from a food, and believed to have a benefit to health or prevention of chronic diseases." Another definition for a nutraceutical is "any nontoxic food extract supplement that has scientifically proven health benefits for both disease treatment and prevention" (Dillard and German, 2000), while the term "functional foods" has been described as foods that "should have a relevant effect on well-being and health or result in a reduction in disease risk" (Roberfroid, 1999). According to Zeisel (1999), nutraceuticals were described in detail to be "the diet supplements that deliver an isolated form or a concentrated form of a presumed bioactive agent from a food, presented in a nonfood matrix, and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods," which seems to be the most appropriate definition for these products. On the other hand, the American Dietetic Association earlier proposed a little different description for nutraceuticals as "any substance considered a food or part of this and offers health or medical benefits, including prevention and treatment of diseases—vitamins, minerals (selenium), plants (garlic, ginger, *Ginkgo biloba*), and animal (carnosine, carnitine, chitosan) extracts" (Bloch and Thomson, 1995). As aforementioned, all these definitions points out to fact that there is not certainly a consensus on definition of nutraceuticals or dietary supplements. Consistently, we need to emphasize again that it seems to be hard to decide indeed whether any nutrient is classified into food or drug and, in fact, nutraceuticals blur a line between drug and food in terms of efficiency and safety. For instance; tryptophan, an amino acid derivative, is essential for metabolism at low doses, whereas it, in the form of 5-hydroxy-L-trytophan, acts as a drug to treat insomnia through increasing brain serotonin synthesis at higher doses. However, since tryptophan administration led to occurrence of Eosinophilia-Myalgia-Syndrome (EMS), it was legally withdrawn from the market (Belongia et al., 1992). Similarly, cholestin, a cholesterol-lowering supplement made from red yeast rice, is actually a supplement identical to lovastatin. As can be seen from these examples, the difference between drugs and nutraceuticals

is sometimes difficult to define, depending only on regulatory issues. According to regulatory issues like health claims and dosage, the mentioned substance, that is, cholestin, could be either a nutraceutical or a drug (Sener, 2009). Consequently, it is logical to define that nutraceuticals are the pharmaceutical forms containing food phytochemicals as active principles (Espin et al., 2007).

There are drastically rising health risk problems with herbal formulations marketed as nutraceuticals, dietary supplements, or herbal medicines/natural medicines throughout the world due to adulteration of synthetic drugs, contamination of pesticides, microbes, toxins, and so forth, as well as substitution (faked species) issues. Besides, interactions with other herbal products or pharmaceutical drugs due to adulteration expose another risk to public health (Jordan et al., 2010). Although advanced analysis techniques are being developed to detect these illegal issues with herbal medicinal products (HMP), application of Good Agricultural and Collection Practices (GACP) and Good Manufacturing Practices (GMP) might help to diminish the threat of external items into herbal formulations (Zhang et al., 2012). It is obviously the duty of the governmental health agencies to provide safety and to regulate the druglike quality of HMPs as well as herbal raw materials and to avoid intended adulteration due to lack of regulations (Byard, 2010; Sanzini et al., 2011). In this chapter, we have aimed to articulate an introduction on the topic of nutraceuticals with a brief overview on their definitions and regulations along with a major emphasis on adulteration, substitution, and undeclared labeling issues commonly seen with these products.

2 Regulatory Issues with Nutraceuticals and Dietary Supplements

In the United States, nutraceuticals are recognized under dietary supplements, which are regulated by the Dietary Supplement and Health Education Act (DSHEA) established in 1994 by the U.S. Congress, concomitant to the Food, Drug and Cosmetic Act (FDA). Particularly for new ingredients, manufacturers are responsible to give appropriate label information and to conduct safety studies on these products as required by the FDA, and if no indication about this requirement is mentioned, they are required to put the following statement on their labels; “This statement has not been evaluated by the Food and Drug Administration” or “This product is not intended to diagnose, treat, cure, or prevent any disease” (Dillard and German, 2000; Halsted, 2003). Dietary supplements in the United States can be marketed without the FDA being satisfied

that they are safe. This makes it easy for a manufacturer to market a product without investing the time and money required for its safety. On the other hand, nutraceuticals and dietary supplements are considered among "natural health products" regulated and licensed by the Natural and Nonprescription Health Product Directorate (NNHPD) since 2004 in Canada (Smith et al., 2014).

Nonetheless, no precise regulation existed or was listed by any health authorities in Europe to control nutraceuticals until 1997 when Green Paper on Food Law opened a new stimulus to the foundation of European Food Law, which was later on followed by the White Paper on Food Safety in 2000. As explained in excellent review by Coppens et al. (2006) on European regulations on nutraceuticals, dietary supplements, and functional foods, the General Food Law Regulation in 2002 was released in Europe and defined "foodstuff" (and functional foods) through Regulation (EC) No. 178/2002 that led to establishment of European Food Safety Authority (EFSA) and medicinal products are defined by Directive 2004/27/EC amending Directive 2001/83/EC. Thus, food supplements are defined as "foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities" in Directive 2002/46/EC. Despite this definition, it is eventually worth recalling that European legislation does not accept functional foods or nutraceuticals as specific food categories at the present time (Coppens et al., 2006).

3 Intentional Adulteration, Counterfeiting, and Undeclared Labeling in Nutraceuticals, Dietary Supplements, and Other Herbal Formulations

What is adulteration? According to Dhanya and Sasikumar (2010), in order to demonstrate adulteration in food as well as dietary supplements, three different detection strategies can be eventually used as demonstrating (1) the presence of an undeclared substance, (2) that a component is deviated from its normal level (content), and (3) that a profile is unlikely to occur.

Adulteration can occur on purpose fully and/or inadvertently (Villani et al., 2015). Plants, which are the most popular source of

nutraceuticals/dietary supplements, during plantation, manufacturing process, and storage may be easily contaminated by pesticides, microbial agents, fertilizers, heavy metals, and so forth. All of them may cause the food-borne disease or even serious illness—from gastric complaints to liver injury to life-threatening conditions (Pan et al., 2015). Therefore, the quality control of raw and finished products of nutraceuticals is required by determining specifications outlined in certain monographs. In addition, problems with the stability of active compound(s) and microbiological control have also been reported. Numbers of projects have been conducted to evaluate commonly used drugs derived from traditional Chinese medicine (TCM), which has become more and more popular in Western countries and contamination with heavy metals (eg, cadmium, chromium, lead) and pesticides—in some cases at toxic levels—was detected in many cases (Harris et al., 2011). It should also be emphasized that not only adulteration with synthetic drugs, pesticides, or substitute species, but also contamination with dust, pollens, insects, rodents, parasites, microbes, fungi, molds, toxins, and heavy metals is a serious issue with herbal formulations all over the world (Posadzki et al., 2013).

Other serious risks may result from the intentional adulteration supplements or herbal remedies with synthetic compounds, which are undeclared and hence not allowed in order to develop an immediate pharmacological action or to intensify a claimed biological effect (Vaclavik et al., 2014). The motivation behind purposeful adulteration in commercial products is eventually for economic profit (Villani et al., 2015). Natural products have traditionally played a pivotal role in drug development, especially in the therapy of malignant diseases, metabolic syndrome disorders, and immunosuppression (Butler, 2005). Global demand for plant extracts for use in nutraceuticals and other herbal remedies has been continuously on the rise. For instance, the annual turnover of the most important commercially relevant phytopharmaceuticals is valued at over $25 billion in the United States alone (De Luca et al., 2012). The sources of many plants is very often limited, and preparation of extracts enriched with active molecules may be time- and cost-consuming, thus for economic reasons, it can lead to “motivated or intentional adulteration.”

3.1 Adulteration in Herbal Preparations for Sexual Enhancement

The most frequently occurring adulteration of active pharmaceuticals has been observed with herbal products, which are massively used worldwide, as they are considered as effective and

with good safety profile. A common adulteration with phosphodiesterase type-5 (PDE-5) inhibitor analogues, that is, sildenafil citrate (Viagra®, Pfizer) (Fig. 5.1), vardenafil hydrochloride (Levitra®, Bayer), and tadalafil (Cialis®, Elli Lilly) as approved drugs for the treatment of erectile dysfunction (ED) have been detected in dietary supplements containing declared, well-recognized natural constituents such as *Panax ginseng* L., *Astragalus membranaceus* (Fisch.) Bunge, *Schizandra chinensis* (Turcz.) Baill., *Ginkgo biloba* L., and many others. Few of the mentioned inhibitors are well known as prescription-only medicines, but are contraindicated in men taking nitrates (can cause an unsafe drop in blood pressure), and thus need to be administered carefully (Ulloa et al., 2015).

The first adulteration of a PDE-5 inhibitor in an herbal dietary supplement was reported after identification of homosildenafil detected in a food beverage (Shin et al., 2003), which was followed by discovery of two more sildenafil analogues (acetildenafil and hydroxyhomosildenafil) in some other herbal products (Blok-Tip et al., 2004). Then, a new sildenafil analog was explored in an herbal product as 41 mg per capsule in the free base form, which was determined as piperidenafil, where the *N*-ethylpiperazine ring had been replaced by a piperidine ring, using LC-MS and GC-MS by Reepmeyer and Woodruff (2006). The same researchers (Reepmeyer et al., 2007) also found another new synthetic analogue of sildenafil, named methisosildenafil, via LC-MS, GC-MS, and derivatization techniques, where *N*-methylpiperazine moiety was replaced with 2,6-dimethylpiperazine in the new analog. Later, Lam et al. (2008) identified adulteration of another new analogue of vardenafil along with sildenafil, tadalafil, and vardenafil in an herbal product sold in Hong Kong, while the presence of

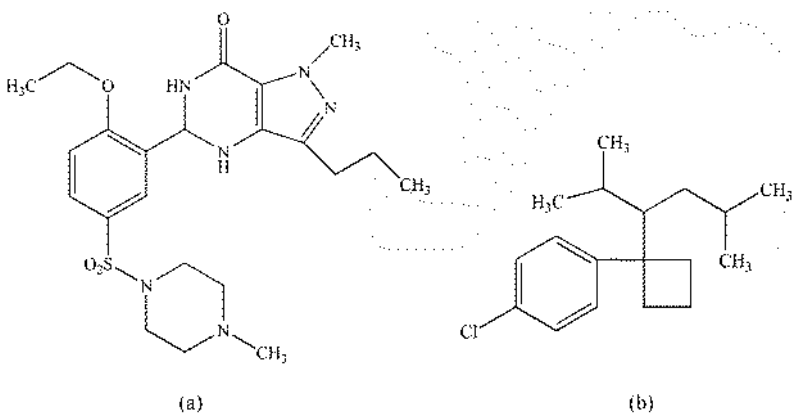

Figure 5.1. Chemical structures of sildenafil (a) and sibutramine (b)

benzamidenafil, found as a new class of PDE-5 inhibitors, was reported to found in another herbal product brand after it was sent to Health Sciences Authority (HSA) of Singapore for analysis (Zou et al., 2008). Thiosildenafil, a thioketone analogue of sildenafil, was also one of those reported as an adulterant in herbal aphrodisiac supplements (Reepmeyer and d'Avignon, 2009). Venhuis et al. (2011) explored another analog named nitroso-prodenafil, a prodrug of aildenafil, in a dietary supplement using advanced analysis techniques such as liquid chromatography-diode array detector-mass spectrum (LC-DAD-MS), MS-MS, high resolution mass spectrum (HR-MS), infrared spectroscopy (IR) and nuclear magnetic resonance spectroscopy (NMR), which was concluded to be carcinogenic, as nitroso derivatives are very toxic. When examining nine dietary supplement brands of herbal origin used for sexual performance enhancement, Balayssac et al. (2012) discovered the presence of adulteration with some sildenafil analogs in four of these brands. In fact, one of them was explored as propoxyphenyl-thiohydroxyhomosildenafil, a new analog, while three of the adulterants were identified to be tetrahydropalmatine (no sexual performance enhancer activity), phentolamine (not approved for oral use), and osthole, an herbal coumarin derivative curing sexual dysfunction. Tandem mass spectrometric analyses of three herbal-originated sexual enhancer products marketed in Kuwait indicated adulteration of these tablet products with sildenafil, tadalafil, and vardenafil (Abdel-Hamid, 2006).

In a large-scale study carried out in the United States that screened ninety-one herbal products claimed to be natural/herbal for potential adulteration with PDE-5 inhibitors, seventy-four of the products contained PDE-5 inhibitor analogs, although none of them indicated the presence of any synthetic inhibitor on their labels (Campbell et al., 2013). In a similar study (Reeuwijk et al., 2013), sildenafil and analogous PDE-5 inhibitors were detected in eighteen of twenty-three herbal dietary supplements sold in the Dutch market.

In a very recent study performed in Canada (Gilard et al., 2015), one hundred and fifty samples of dietary supplements apparently sold as herbal or natural sexual enhancers were subjected to analysis using ^{1}H-NMR and mass spectrometry techniques for any possible adulteration with PDE-5 inhibitors in order to identify and quantify. The results showed that 61% of the dietary supplements contained sildenafil, tadalafil, vardenafil as well as their structural analogs. Moreover, 64% of them contained only one PDE-5 inhibitor, while 36% of them contained at least two synthetic analogs or even more. It should also be mentioned that the dose of synthetic PDE-5 inhibitors found as adulterants in these products was much

higher than their maximum allowed doses, which certainly creates a risk to human health. In fact, only 2.5% of these supplements had the herbal sexual enhancer compounds, for example, osthol and icariin. Shi et al. (2014) searched for the presence of sildenafil, vardenafil, tadalafil, homosildenafil, hydroxyhomosildenafil, noracetyldenafil, acetyldenafil, aminotadalafil, pseudovardenafil, norneosildenafil, and thiosildenafil in eighty-eight dietary supplements or nutraceuticals in mostly capsule form marketed in China using UHPLC-Q-Orbitrap HR-MS technique and found out that eight of them contained sildenafil and one product had noracetildenafil, whose quantities varied between 20.5 and 115.7 mg/g. On the other hand, flibanserin, another synthetic adulterant, was detected in two samples of herbal sexual enhancer for women by the Polish National Medicines Institute, while tadalafil was also found in one of the samples (Poplawska et al., 2014).

To date, more than fifty unapproved structurally modified analogues of PDE-5 inhibitors—that is, piperidenafil, piperidinovardenafil, pseudovardenafil, norneosildenafil, piperidino sildenafil, carbodenafil, aminotadalafil, cyclopentynafil, dimethylacetildenafil, isopiperazinonafil, and hydroxypropylnortadalafil—have been reported as adulterants and the number is continuously growing (Patel et al., 2014). They may cause serious health risk to consumers as their efficacy and toxic effects have not been properly assessed. It is also worth noting that all mentioned molecules added in herbal preparations for adulteration are difficult to detect by routine screening tests and analysis methods as new structurally modified analogues are being developed progressively (Patel et al., 2014; Ulloa et al., 2015). Very recently, Ulloa et al. (2015) identified a new and unapproved analogous molecule of PDE-5 inhibitors named as aminotadalafil [(6R,12aR)-2-amino-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydropyrazino [1',2':1,6] pyrido [3,4-b] indole-1,4-dione] isolated through classical column chromatography from a suspected herbal product marketed in Argentina, which represented the first report in Latin America in terms of finding illegal PDE-5 inhibitors.

Although not of much interest to our topic herein, counterfeiting of PDE-5 inhibitor-containing products is another important issue threatening public health as in the case of that six Viagra® samples in Poland and forty-four Viagra® samples in the Netherlands were found to be counterfeits (Vredenbregt et al., 2006; Maurin et al., 2007; Singh et al., 2009). On the other hand, it should be remembered that not only adulteration of unapproved drugs into medicinal herbal products, but also microbial contamination and heavy metal presence may pose a great public health risk. For instance; Pullirsch et al. (2014) reported

that 23% of the tested illegal samples in Canada were identified with microbial contamination whose levels were above the U.S. Pharmacopeia (USP) and EP limits. Relevantly, the same situation was seen detected in an anabolic steroid-containing preparation and an herbal product sold in Austria.

3.2 Adulteration in Herbal Slimming Preparations

Slimming pills are always trendy for consumers as obesity and gaining weight are major problems worldwide. So-called herbal preparations for slimming purposes are among top-selling products and filling sibutramine hydrochloride monohydrate (an anti-obesity drug molecule that inhibits serotonergic and noradrenergic reuptake) (Fig. 5.1) into these products seems to become troublesome in many countries. For instance; twenty herbal preparations called "Meizitanc" used for slimming purpose were confiscated by police in Poland and sibutramine, an average of 10 mg per table, was detected as the major substance using all conventional analysis techniques, that is, TLC, GC-MS, HPLC-UV/DAD as well as infrared spectroscopy (IR), in 19 of these so-called herbal products (Wiergowski et al., 2007). Wang et al. (2008) detected the presence of phenolphthalein, *N*-mono-desmethyl sibutramine, and sibutramine in 11 dietary supplements out of 22 samples in China. In a similar analysis performed on 15 samples sold in China, 4 were demonstrated to contain sibutramine and *N*-di-desmethylsibutramine (Huang et al., 2008). However, consumers should be aware that adulteration or using synthetic fillers in these products, which are presumed to be safe, may cause some serious effects. For instance; the Chinese herbal medicine called meizitanc marketed in Turkey led to miscarriage in two pregnant women after taking adulterated portions of this medicine (Cayan et al., 2009). Relevantly, two women in Hong Kong were recorded to show mania-like psychosis after taking herbal slimming products due to neuropsychiatric side effects of sibutramine, an adulterant contained in these pills (Chong, 2010). In fact, long before sibutramine, fenfluramine, a drug withdrawn from the market in 1997, was detected in some Chinese traditional medicines used for slimming purposes, and which caused primary pulmonary hypertension and valvular heart disease (Corns and Metcalfe, 2002).

By capillary electrophoresis (CE) method, Cianchino et al. (2015) determined presence of ephedrine, norephedrine, caffeine, and furosemide as possible adulterants in the products (anorexigens, diuretics, stimulants, laxative agents) used in weigh control programs due to their potential toxic effects, while, among 20 weight-loss products sold in France, 16 of them were found

to contain either sibutramine, combination of sibutramine plus phenolphthalein or synephrine, although not declared any of them on the label (Vaysse et al., 2010). Although sibutramine was withdrawn from drug market in 2010 and called an "unsafe drug" due to its serious cardiovascular effects, *N*-desmethylsibutramine was still detected in one sample among 50 slimming pill samples purchased through their international marketing websites (Roh et al., 2011). On the other hand, the same situation with these products was also reported from Lebanon, where most of the 34 brands of dietary supplements were found to be adulterated with sibutramine, caffeine, and phenolphthalein (Sadaka et al., 2011), while 23 out of 25 herbal slimming preparations of Far Eastern origin secured in Poland by police were declared to contain either sibutramine or *N*-desmethylsibutramine hydrochloride, sixteen of which even had an higher amount of these compounds than their maximum daily allowed dose (15 mg) (Stypułkowska et al., 2011). Then, a new analogue of sibutramine identified as 11-desisobutyl-11-benzylsibutramine was detected in another natural slimming preparation (Mans et al., 2013). It is also worth mentioning that acetazolamide, benzthiazide, bumetanide, chlorothiazide, chlorthalidone, clopamide, cyclothiazide, ethacrynic acid, furosemide, hydrochlorothiazide, hydroflumethiazide, metolazone, probenecid, and lorcaserin are also synthetic adulterants more or less encountered in herbal slimmers (Bogusz et al., 2006; Hachem et al., 2014). Besides, addition of thyroid hormone and phencyclidine was demonstrated in weight-reducing herbal preparations of Chinese origin (Khazan et al., 2013), while 9 samples of herbal slimming pills of different country origins sold in Turkey contained either sibutramine, caffeine, or temazepam analyzed at the Laboratory of Forensic Medicine and the Scientific and Technological Research Laboratory (Ozdemir et al., 2013). Very recently, in a similar case report in Turkey with a young female patient (aged 17) having palpitations, dizziness, anxiety, and insomnia, she reported taking pills of an herbal slimming preparation named "La Jiao Shou Shen—pepper pill," which was found to contain sibutramine (Pamukcu Gunaydin et al., 2015). Actually, a remarkable number of the case reports with sibutramine-associated psychosis and other adverse effects have been demonstrated. In a retrospective study carried out between 2004 and 2009 in Hong Kong by Chen et al. (2010), all of the cases diagnosed with psychosis at the hospital were proven to be caused by herbal slimming pills adulterated with sibutramine or its analogs. Moreover, 13% of these cases attempted suicide as an adverse drug reaction of sibutramine, while some other adulterants such as phenolphthalein in 9 samples, fenfluramine, mazindol,

and animal thyroid tissue in 2 samples, and hydrochlorothiazide and spironolactone in 1 sample were detected. In a case report from the Netherlands (Bertholee et al., 2013), a 43-year-old woman who showed serious symptoms of psychosis was admitted to the hospital and found to have sibutramine-contaminated herbal products along with Brazilian slimming coffee, which caused a severe adverse reaction in the case of this woman. Moreover, 3 cases of panic attacks caused by illegally sibutramine-containing herbal weight-loss products have been recently reported from Turkey by Eraslan et al. (2015). Hence, these findings underline how serious a situation of adulterated or counterfeited medicinal herbal product use may go.

In a similar study carried out in Switzerland in 2010 (Mathon et al., 2014), possible incidents of adulteration by sibutramine or its analogs were searched in 39 herbal slimming preparations marketed through Internet sales in which no sibutramine was declared on their labels. The findings demonstrated that sibutramine was detected in 17 of the preparations (44%), with an amount varying between 3 and 35 mg per capsule. After withdrawal of sibutramine from the market in 2010, the same research group performed analogue work again on 13 herbal slimming preparations purchased from the Internet in 2012 using HPTLC-UV densitometry method and 69% of them was found to have adulteration with sibutramine per se or its derivatives. This time, the amounts were between trace to 10 mg, which was reduced relatively in comparison to the findings of the previous study of these authors in 2010. In another study carried out by De Carvalho et al. (2012), the presence of amfepramone, sibutramine, fenproporex, fluoxetine, paroxetine, sertraline, and bupropion was investigated in a total of a 106 herbal weight-loss products sold in the Brazilian market, and only 4 products were found to contain fenproporex or sibutramine detected by capillary electrophoresis (CE).

Considering microbial contamination and heavy metal presence in medicinal herbal products, Zin et al. (2014) performed a screening on 10 brands of herbal slimming products sold in Pakistan using inductively coupled plasma-mass spectrometry (ICP-MS) and only one out of 10 products was found to contain the highest total daily intake of heavy metals, whereas the rest had acceptable amounts of these elements (As, Cd, Pb, Co, Cr, Cu, and Zn). In the same study, *Bacillus cereus* and *Pseudomonas aeruginosa* were detected in two separate samples, which led to the conclusion that all these products must be used carefully.

Undoubtedly, these cases might be enlarged. Adulteration, particularly with sibutramine as well as its analogs in weight-loss supplements of herbal origin, seems to constitute a dramatic

health problem, which also causes serious side effects and, hence, should be controlled more strictly by regulatory authorities.

3.3 Adulteration in Nutraceuticals and Dietary Supplements Used for Other Therapeutic Purposes

As mentioned previously, adulteration of herbal preparations by undeclared synthetic drugs is quite common. For instance, approximately 24% out of 2,609 herbal samples analyzed in Taiwan were found to contain synthetic adulterants that act as real drugs (Huang et al., 1997). Among them, the most widely used adulterant drugs are nonsteroid antiinflammatory drugs (NSAID), steroids, and analgesics.

Consequently, another instance of the inclusion of synthetic drugs in herbal products that should be mentioned is the adulteration of diabetes treatments. Based on the active ingredients, dietary supplements are believed to regulate blood sugar levels and to improve the life quality of patients without causing the serious side effects (eg, hypoglycemia, liver injury, gastric complains) characteristic of well-known and popular drugs. Unfortunately, gradually more drugs are adulterated among herbal/botanical supplements and glibenclamide as well as metformin have been the most commonly detected contaminants (Chen et al., 2009; Li et al., 2010; Zhu et al., 2014). In addition to these drugs, the presence of chlorpropamide, gliclazide, glimepiride, glipizide, pioglitazone, tolazamide, and tolbutamide has been also detected in herbal antidiabetics, while codeine, indomethacin, ketoprofen, morphine, oxyphenbutazone, paracetamol, phenylbutazone, diclofenac, dipyrone, ibuprofen, mefenamic acid, salicylamide, and salicylic acid were the analgesic compounds mostly often found as adulterants in dietary supplements (Bogusz et al., 2006). Adulteration of anabolic steroids and hormones, for example, androstendione, betamethasone valerate, betamethasone, clenbuterol, dexamethasone, flumethasone, hydrocortisone, prednisolone, prednisone, testosterone propionate, testosterone isocaproate, testosterone phenylpropionate, and testosterone decanoate has been also commonly reported from the analyses of herbal preparations (Bogusz et al., 2006).

Wang et al. (2009b) applied an HPLC method coupling with electrospray ionization MS for simultaneous determination of four known, synthetic drugs, for example, phenformin, rosiglitazone, glibenclamide, and glimepiride, which are considered as the illegal additives in dietary supplements and traditional Chinese medicines for diabetes mellitus. Synthetic antidiabetic drugs have an obviously fast effect, but also a series of common side

effects including hypoglycemic, lactic acid intoxication, and gastrointestinal upset, while herbal drugs are believed to regulate the level of sugar without causing any danger, even when overdose occurs (Ching et al., 2012). However, in order to demonstrate high and instant efficacy, these substances are still added as unwanted, unlabeled adulterants in herbal preparations. Similarly, 9 synthetic antidiabetic drugs were found in 14 samples from 30 collected Chinese medicines and dietary supplements. Glibenclamide followed by metformin, rosiglitazone, glimepiride, phenformin, gliclazide, chlorpropamide, nateglinide, and mitiglinide were the most common ones (Li et al., 2010). The adulteration rises up sometimes to highly toxic levels as in the following example that metformin in a concentration about 93% was detected in Indian pills contain herbal ingredients and declared as an antidiabetic formulation (Kumar et al., 2011).

Kim et al. (2014) analyzed two hundred and fourteen so-called herbal preparations marketed in south Korea for presence of any NSAIDs, steroids, and analgesics by LC-MS-MS and reported that 53 of these products contained acetaminophen, diclofenac, ibuprofen, indometasine, naproxene, and pyroxicam, while ibuprofen was the most used adulterant in this study (1.06 and 233.40 mg/g). Besides, presence of NSAIDs was confirmed in 73.6% of the samples. In a similar work (Silva et al., 2013), ranitidine and a mixture of orphenadrine citrate, piroxicam, and dexamethasone were detected in a Brazilian herbal pain killer remedy using HPLC-UV-SPE-NMR as well as 1D and 2D NMR techniques. In India, dexamethasone and diclofenac were determined using LC-MS-TOF (time of flight) method in a total of 10 Ayurvedic herbal formulations, while piroxicam was detected in one product. The authors concluded that all adulterated products were marketed by healthcare practitioners, whereas nonadultered ones were sold in pharmacies (Savaliya et al., 2009).

A lot of adulteration issues have been also reported with other popular herbal medicines such as *Ginkgo biloba* L. Eight samples of the dietary supplements containing the leaf extract of *G. biloba*, used for cerebrovascular diseases, tinnitus, and Alzheimer's type of dementia, were demonstrated to be adulterated with free flavonol aglycones (quercetin and kaempferol) as well as genistein, the isoflavone derivative that is not actually found in *G. biloba* (Wohlmuth et al., 2014). In fact, genistein has been suggested lately as an analytical marker for the discovery of adulteration in *G. biloba* extracts with the fruits and/or flowers of Japanese sophora (*Styphnolobium japonicum* L. Schott., syn. *Sophora japonicum* L.), that is rich in genistein since it is a common way to contaminate *G. biloba* extracts (Avula et al., 2015). In a similar work

by Demirezer et al. (2014), various dietary supplement brands of *G. biloba* marketed in Turkey were analyzed by HPLC and rutin was demonstrated to be the main adulterant flavonoid to be found in the total flavonoid quantity that was labeled.

Considering isoflavones, five samples of soy-based dietary supplements were earlier analyzed by our research group in order to check whether the claims on the label concerning amounts of ingredients were true, and three of them failed to contain the claimed amount of the isoflavones, that is, genistein and daidzein (Orhan et al., 2007). For instance, one of them claimed that it contained 60 mg of isoflavone based on genistein per tablet, while we detected only 1.538 mg of genistein per tablet after our HPLC analysis.

In another interesting case, abietic acid, although natural, was stated as an adulterant in an herbal preparation used for the treatment of psoriasis, as it is not an approved drug molecule up to date (He et al., 2012). In a large-scale study by Park et al. (2015), 212 herbal products marketed for the treatment of joint pain and bone aches in Korea were investigated for any corticosteroid-types of adulterants and ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS), and only 3 of them (1.4%) were found to contain dexamethasone, which may become a serious adulterant in later preparations of this kind of herbal preparations.

3.4 Use of Novel and Advanced Analysis Techniques for Morphologically Substitute Species: Contamination and Adulteration in Medicinal Plant Species

Use of morphological substitutes (fakes) of some medicinal plant species such as notoginseng adulteration in *Ginseng* is another serious health problem (Wang et al., 2009a; Niu et al., 2011). As well known, the true ginseng is the roots of *Panax ginseng* C. A. Meyer (Araliaceae), which is known as "Asian or Korean ginseng," while the roots of *Panax quinquefolius* L. (American ginseng) and *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (Siberian ginseng) are also known and substituted to true ginseng, whose pharmacological profiles are more or less similar. Nevertheless, their phytochemical contents are somewhat different, although ginsenosides (dammarane-type of saponins) such as Rb1, Rb2, Rc, Rd, Rg2, and so forth are common in two *Panax* species. In this regard, only the marker compound has been established as Rf, which is found only in *P. ginseng* (Korean ginseng) and not present

at all in *P. quinquefolius* (American ginseng). Therefore, it is rather important to use the true medicinal species with the highest pharmacological and phytochemical quality in preparation of herbal medicines/nutraceuticals/dietary supplements. On the other hand, the roots of *Mandragora officinarum* L. (Solanaceae) might be adulterated again with *Panax ginseng*, which it resembles closely. However, *M. officinarum*, also known as a toxic plant species, has completely different pharmacological effects and phytochemistry, as it contains tropane alkaloids and anolide derivatives such as mandrogorolides A and B. Similarly, the roots of *Pfaffia panaculata* (Mart.) Kuntze (Amaranthaceae), named as "Brazilian ginseng or suma root," look like *P. ginseng* roots in appearance, but have a totally different phytochemical content (ecdysteroids) from that of *P. ginseng*. In another case, Yu et al. (2014) efficaciously applied HPLC coupled with hierarchical cluster analysis and principal component analysis performed on 43 American ginseng samples by determining their ginseng-specific saponosides to determine the region of cultivation, whether Asian or American ginseng, two of which were elucidated as Asian ginseng.

It is also worth to mention that *Anthemis nobilis* L. and *Matricaria chamomilla* L. (Asteraceae), the medicinal chamomile species registered in European Pharmacopeia, can be easily confused by its substitute species from the family Asteraceae including *Tanacetum parthenium* (L.) Sch. Bip., *Tanacetum cinerariifolium* (Trevir.) Schultz Bip., *Tripleurospermum callosum* (Boiss. et Heldr.) E. Hossain, *Bellis perennis* L., and *Leucanthemum vulgare* Lam., which have very similar appearances to those of *Anthemis nobilis* or *Matricaria chamomilla* in nature. In the case of use of these latter four species, either some unexpected side effects or toxic outcomes may occur or expected pharmacological effects may not be observed. According to a very recent study by Guzelmeric et al. (2015), a new high performance thin-layer chromatographic (HPTLC) method was developed in order to detect adulteration in chamomile species. This may also happen unintentionally since the flowers of many Asteraceae species look much alike and contain very similar botanical characteristics, which consequently cause miscollection of them from nature. For this purpose, apigenin 7-O-glucoside was selected as a marker compound for the officinal chamomile species, which led to identification of the following plant species, that is, *Anthemis* spp., *Bellis* spp., *Chrysanthemum* sp., and *Tanacetum* sp., used wrongly as chamomile. HPTLC in combination with HPLC and densitometry was successfully applied for phytochemical fingerprinting of *Lawsonia inermis* L., known as "henna," as well as *Cassia obovata* Collad., and *Indigofera tinctoria* L. (Gallo et al., 2008). The results of the study

indicated that not all herbal products sold as "henna" in the market contained *L. inermis*, but instead it was substituted by *C. obovata* and *I. tinctoria*. Hence, these techniques have been stated to be feasible, rapid, and comprehensive for detection of adulteraton or wrong identification of botanical species.

Another good example in this direction is bilberry fruits (*Vaccinium myrtillus* L., Ericaceae) recognized as one of the richest sources of anthocyanins. The major sources of bilberry are Nordic European countries and the United States. Fresh berries should be properly harvested, freeze-dried, adequately extracted, and subjected to ion exchange chromatography in order to obtain high-quality anthocyanin-rich bilberry extracts. Additionally, a relatively small region of growth for bilberries makes the product nowadays one of the most expensive herbal extracts in the phytopharmaceutical industry. Unfortunately, different species of wild berries, extracts of black rice, black soybean hull, and red sorrel flower are commonly used as adulterants (Govindaraghavan, 2014). As another example, turmeric (*Curcuma longa* L., Zingiberaceae) is now gaining importance globally as a mighty cure of a vast range of serious diseases, but is one of the spices probably most subjected to adulteration since it is frequently sold in ground form. Artificial colors such as metanil yellow or orange II are popular additives, used to increase the product color intensity. Turmeric powder is frequently adulterated with rhizomes of cheaply available related species containing the pigment curcumin from the *Curcuma* genus, which are toxic in nature (Dhanya and Sasikumar, 2010).

Very rich in numerous bioactive polyphenols are grape products, which were consequently associated with the prevention of numerous diseases including cardiovascular and neurodegenerative diseases. Villani et al. (2015) tested a series of grape-seed-containing drugs and found out that some of the tested products contained no detectable quantities of grape seed extract, and, in contrast, were composed primarily of peanut skin extract. The next few drugs were adulterated with significant amounts of peanut skin. It is definitely worth mentioning that peanut skin extract is not only a cheap and widely available high-volume byproduct, but also one of the most common allergens, hence adulteration with a common allergen represents a considerable risk to public safety.

Obviously, classical botanical methods based on morphological and anatomical features of the plants are now too primitive for detection of synthetic adulterants as well as quality control and, hence, much more advanced techniques must be developed. In this regard, hyphenated techniques like liquid chromatography tandem mass spectrometry (LC-MS-MS), gas chromatography-tandem mass spectrometry (GC-MS-MS), and other conventional

tools are still very useful (Haneef et al., 2013), while metabolomics and DNA-based authentication techniques have also emerged as more modern approaches (Coutinho Moraes et al., 2015). For example, flow injection tandem mass spectrometry method (FI-MS-MS) as a hyphenated technique was applied for detection of several PDE-5 inhibitors (tadalafil, sildenafil, and vardenafil) in 13 dietary supplements, which led to identification of 1 sample with adulteration of tadalafil and sildenafil (Song et al., 2012). On the other hand, electrospray tandem mass spectrometry (ESI-MS-MS) technique was successfully employed in detection of adulteration of PDE-5 inhibitors in herbal preparations sold as sexual enhancers (Abdel-Hamid, 2006).

Metabolomics is generally defined as both qualitative and quantitative analysis of all metabolites in an organism, at given condition. The metabolomics approach is a tool for macroscopic view of a plant metabolome and is of great interest in system biology studies in all kind of biological processes (Kim et al., 2011). Metabolomics along with the other "omics" approaches form a holistic functional approach, in which the synergism of this powerful combination opens novel avenues for accelerated lead finding and drug discovery, quality control assessment, and mode of action of healing herbs (Yuliana et al., 2012). Furthermore, the innovative biochemometric relationships between metabolomics and bioactivity data are established to evidence possible synergy effects and to find extracts with optimal chemical and biological profiles in view of the development of phytopharmaceutical preparations with pharmaceutical significance and prominent therapeutic value (Inui et al., 2012). For instance, metabolomics approach has been recently applied to *Sambucus* L. (Adoxaceae) plants (Zahmanov et al., 2015). *Sambucus* plants have been traditionally used in folk medicine of Western Europe, the Balkan Peninsula, and Middle East, hence several commercial products based on the plant species are currently available on the market. Sambucol®, for instance, is now widely available on the market and is promoted as a supplement to support the immune system. The *Sambucus* species, however, also accumulates cyanogenic glycosides (ie, sambunigrin; Fig. 5.3), whose presence is undesirable. NMR-based metabolomics approach along with principal component analysis revealed by Zahmanov et al. (2015) that sambunigrin was not detectable in any *Sambucus* samples as well as that NMR-metabolomics platform could be eventually used for quality control assessment of herbal supplements.

The tuber extract of *Harpagophytum procumbens* (Burch.) DC. ex Meisn. (devil's claw, Pedaliaceae) has been used for treatment of degenerative rheumatoid arthritis, osteoarthritis, tendonitis,

kidney inflammation, and heart disease (Georgiev et al., 2013). At present, devil's claw is currently listed in the European Pharmacopoeia for the treatment of arthritic ailments and rheumatism; commercial devil's claw products should contain at least 1.2% harpagoside (Fig. 5.2) (Georgiev et al., 2013). *H. zeyheri* Decne, a closely related species, is also commercially harvested, though *H. procumbens* is preferred because it has been reported to contain higher levels of biologically active constituents including harpagoside (and concomitant iridoid glycosides; Mncwangi et al., 2014). By combining metabolomics approach (both MS- and NMR-based) with chemometric analysis, Mncwangi et al. (2014) found out that *H. procumbens* and *H. zeyheri* are not chemically equivalent, and in particular harpagoside is not always present in *H. zeyheri*, which suggests that the pharmacological properties may differ; hence, both species should not be interchangeably used. It is clear that standardization is necessary to ensure safety, to optimize efficacy, and to guarantee reproducible pharmacological and clinical outcomes as well as to advise on individual dosage regimens that enhance the sustainability of traditional medicinal plants (see Fig. 5.3).

As an application of metabolomics, real-time MS was applied to detect possible adulteration or substitution in commercial samples of *Berberis aristata* D.C. (Berberidaceae), known as "daruharidra," with *Berberis asiatica* Roxb. ex. D.C., *Mahonia borealis* Takeda, and *Coscinium fenestratum* (Goetgh.) Colebr. (yellow

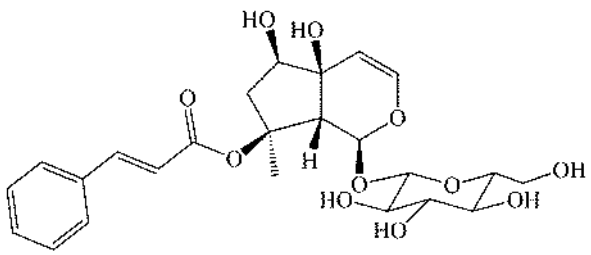

Figure 5.2. Chemical structure of harpagoside.

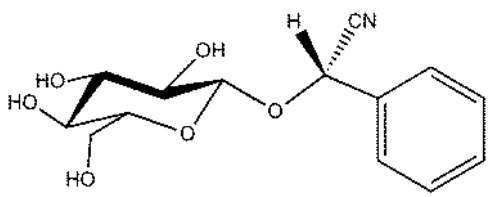

Figure 5.3. Chemical structure of sambunigrin.

vine), the common substitutes to *B. asiatica*, through identifying the specific alkaloids, for example, berberrubine, berberine, jatrorrhizine, ketoberberine, palmatine, dihydropalmatine or 7,8-dihydro-8-hydroxyberberine, berbamine, and pakistanamine (Bajpai et al., 2015). The real-time MS was also used for detection of synthetic antidiabetics in herbal preparations, which led to identification of metformin in two products without informing on their labels (Zhou et al., 2011).

Black cohosh, referred to the rhizomes and roots of *Cimicifuga racemosa* L. (syn. *Actaea racemosa* L.) from Ranunculaceae, has adulteration problems investigated by Masada-Atsumi et al. (2014). According to their results, the metabolic profiling of 25 black cohosh products analyzed by LC-MS-MS as an hyphenated analysis method pointed out that *C. dahurica* (Turczaninow ex Fischer & C. A. Meyer) Maximowicz and possibly *C. foetida* L. are the substituted species for adulteration of *C. racemosa*. One of those classical but advanced methods, TLC-image analysis method was successfully applied for fast detection and quantification of sibutramine hydrochloride in possibly adulterated herbal slimming products (Phattanawasin et al., 2012). In a recent study by Guo et al. (2014), a new comprehensive and sensitive technique, orbitrap high-resolution mass spectrometry (UHPLC-Q-Orbitrap HR-MS), was developed to detect adulterants in herbal antidiabetics.

Another common adulteration issue is counterfeiting of *Illicium verum* Hook. F. (Chinese star anise) with *I. anisatum* L. (Japanese star anise) or some other *Illicium* species. For detection of adulteration, anisatin, a rare neurotoxic sesquiterpene dilactone present in Japanese star anise, is a merker compound and for this purpose, a new real time (DART) ambient ionization coupled with orbitrap HR-MS was developed, which allowed measurement of adulteration down to 1% (w/w) (Shen et al., 2012).

On the other hand, DNA barcoding-authentication technique has been successfully used to distinguish *Ganoderma lucidum* (Curtis) P.Karst. (Polyporales), "the reishi or lingzhi mushroom" used for cancer treatment, from other *Ganoderma* species (Sadava et al., 2009), whereas it was earlier used to differentiate two *Ginseng* species (Mihalov et al., 2000) and identify true medicinal species of *Dendrobium* Sw (Orchidaceae) (Lau et al., 2001). Lately, DNA barcoding was also employed by Xin et al. (2015) as a supervisory method in 82 commercial *Rhodiola* products sold in China to find out how many of them contained *Rhodiola crenulata* (Hook. F. et Thoms.) H. Ohba from the family Crassulaceae recorded as the medicinal species in Chinese Pharmacopeia. The analysis exerted that 38.9% were authenticated as *R. serrata* H. Ohba, while

R. rosea L. (golden root, rose root, king's crown), the medicinal species recognized in the US Pharmacopeia was identified in 10% of the samples. In a similar work by Seethapathy et al. (2015), adulteration rates were detected as 50% in *Senna auriculata*, 37% in *Senna tora*, and 8% in *Senna alexandrina*, which are sold as laxatives in the Indian herbal product market using effectively common DNA barcode regions including ITS, matK, rbcL, and psbA-trnH. Relevantly, Newmaster et al. (2013) screened the authenticity of 44 herbal preparations produced by 12 companies in the North American market by DNA-barcoding method and found out that most of them had very poor quality since 59% of the products possessed DNA barcodes from other plant species, which were not given on the labels as ingredients. The herbal products containing the *Sida* L. species (Malvaceae) are some of the most adulterated preparations sold in India and therefore DNA-barcoding was again used to differentiate unrelated species used as adulterants or distinguish if it is *S. cordyfolia* or *S. acuta*. and also to elucidate the substances in those preparations (Kumar et al., 2015). As DNA-based techniques have been employed as common authentication methods for herbal drugs, another DNA-based technique, called PCR-RFLP, is also used for identification of correct species as in the case of *Boerhavia diffusa* L. (Biswas et al., 2013).

As aforementioned in some cases of adulteration and also reviewed in detail by Moreira et al. (2014), CE has been also successfully applied nowadays for screening and determination of synthetic adulterants such as antipyretics (acetaminophen, bucetin, etc.), analgesics, sedatives, anticonvulsants, stimulants like caffeine, hypoglycemics, antidepressants, cannabinoids in herbal health products, while adulteration with tadalafil or sildenafil, mixed adulteration (both of tadalafil and sildenafil), and adulteration with analogues of these drugs in herbal products sold in the United States was determined using another new technique called atmospheric solids analysis probe (ASAP) developed by Twohig et al. (2010). Attenuated total reflectance-infrared (ATR-IR) spectroscopy has also been one of the novel techniques to analyze adulteration of sibutramine in medicinal herbal products or dietary supplements, which led to a minimum level of false positive results (Deconinck et al., 2014).

3.5 Adulteration in Essential Oils

Many plants possessing essential oils are popularly used as spices and are of high value export oriented commodities, thus both synthetic and natural constituents are used as common

adulterants. Thus, adulteration is also considered as a serious problem with essential oils. This is a result of the increasing demand for pure essential oils worldwide. Essential oils have a high commercial value as they have been widely used not only as phytomedicines, but also in the flavor and fragrance industry including perfumery and cosmetics as well as food technology. As the prices of pure natural essential oils sometimes are usually very high, a logical consequence from an economical point of view is mixing them with cheaper essential oil with the same olfactory note. One of the most known examples is the adulteration of essential oils from citrus by sweet orange essential oil, which is the cheapest citrus oil, or mixing the expensive pure oil from *Lavandula angustifolia* Mill. (Lamiaceae) with almost six times cheaper essential oil of other species of genus lavender (Do et al., 2015; Koenig and Hochmuth, 2004). Very expensive essential oil produced from the *Melissa officinalis* herb (balm oil), containing (–)-citronellal as the main constituent, is very often adulterated with cheaper citronella oil (*Cymbopogon winterianus*), which contains enantiomeric mixtures of citronellal (Koenig and Hochmuth, 2004).

Sometimes, inexpensive synthetic volatiles from other natural sources are added in order to meet industrial requirements. Synthetic α-irone and β-irone is added to iris (*Iris* sp., Iridaceae) oil to a degree that may double the commercial value of the product, and linalyl acetate or linalool is added to improve the olfactory quality of bergamot or lavender oil (Do et al., 2015, Koenig and Hochmuth, 2004). Similarly, cheap vegetable oils are added to increase weight, while lemongrass oil is diluted with coconut oil, and sandalwood oil with polyethylene glycol (Do et al., 2015). Adulterations can also involve substitution of part of the original plant by other plants, or the addition of nonvolatile products. All these not only decrease the quality, but also can lead to safety issues. Cinnamon bark essential oil can be adulterated by leaf cinnamon essential oil with the same olfactory notes to reduce the amount of cinnamaldehyde—a well-known allergen, but also to increase the volume (Do et al., 2015). Neroli oil made from the flowers of *Citrus aurantium* L. spp. *amara* L. var. *pumilia* (Rutaceae) is often mixed with cheaper petit-grain oil made from leaves (Do et al., 2015).

4 Conclusions

Herbal preparations and natural compounds used either as phytotherapeutics or nutraceuticals and dietary supplements are in great demand by consumers all over the world. Although

evidence-based phytonutrients are pharmacologically effective, nutraceuticals and dietary supplements of plant origin appear to be a popular aspect of disease-preventive food components in pure form as single components as in nutraceuticals and in the form of extracts or enriched fractions in dietary supplements. These products are generally effective in helping to maintain healthy status of our bodies and, hence, leading to disease prevention. However, the risks and benefits of nutraceuticals/dietary supplements/herbal medicinal products is not as well known as for conventional drugs, and the fact that there is an absence of reported adverse effects and effects of drug interactions does not mean that the mentioned products are devoid of these properties. On the other hand, all we should be aware of is the fact that the pharmaceutical quality of herbal preparations seems to be inadequate since their regulatory and licensing mechanisms are weak in many countries due to the great amount of adulteration or substitution (Fig. 5.4). Our literature survey indicated that many products claiming to be "herbal or natural" have been actually identified with compelling evidence of adulteration of "synthetic adulterants" or "undeclared synthetic drugs"—in other words, contamination has become a dangerous phenomenon in various parts of the world. Therefore, uncontrolled use of herbal preparations along with pure compounds obviously poses a public health risk and all countries are recommended to implement more strict regulations and licensing procedures; to develop regulatory standards for all kinds of analysis and to create adequate pre- and postmarketing inspections to protect their community.

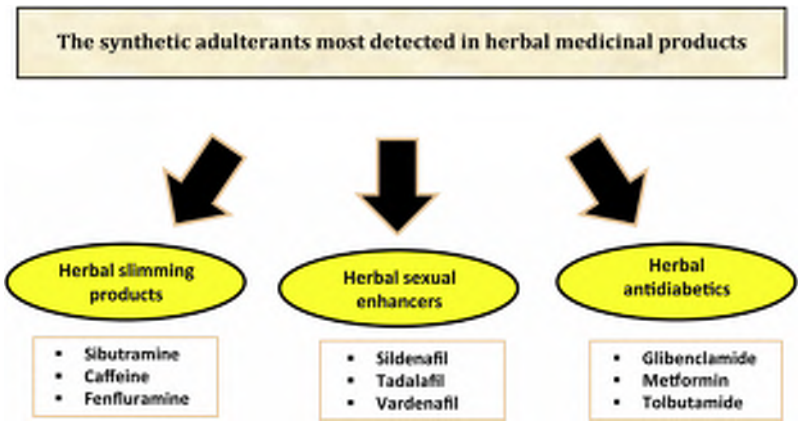

Figure 5.4. Some of the synthetic adulterants detected in herbal formulations.

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6

REGULATORY PERSPECTIVES ON NANOTECHNOLOGY IN NUTRACEUTICALS

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1 Introduction

Nanomaterials (NMs) have unique functional properties due to the larger surface to mass ratio compared to bulk materials. Because of their peculiar properties, NMs are widely used in many application fields offering new and innovative products for daily life. Food and agricultural sectors are taking advantage of nanotechnology, and an increasing number of NM-containing products can already be found on the market (Bouwmeester et al., 2014). The new technological developments have already opened up a multibillion-dollar industry in recent years, and the global market is expected to reach US$1 trillion by 2015, employing around 2 million workers (Roco and Bainbridge, 2001). In terms of market value and number of food nanotechnology applications, the European market is currently small in comparison to the U.S. and Japanese markets. Frans Kampers, Coordinator of Innovative Technologies at the Wageningen University and Research Center, said that encapsulation and delivery of nutrients appears to be a dead end in Europe because of European Union regulations (eg, Novel Foods regulation), and the difficulty of having health claims approved (Newsome, 2014).

The use of nanotechnology in food and food-related applications are directed to: (1) improve sensory perceptions such as flavor/color enhancement, and texture modification, (2) increase absorption and targeted delivery of nutrients and bioactive compounds, (3) stabilize active ingredients such as nutraceuticals in food matrices, (4) extend shelf life with smart packaging, (5) improve food safety by using nanobased sensors and tracers, (6) kill

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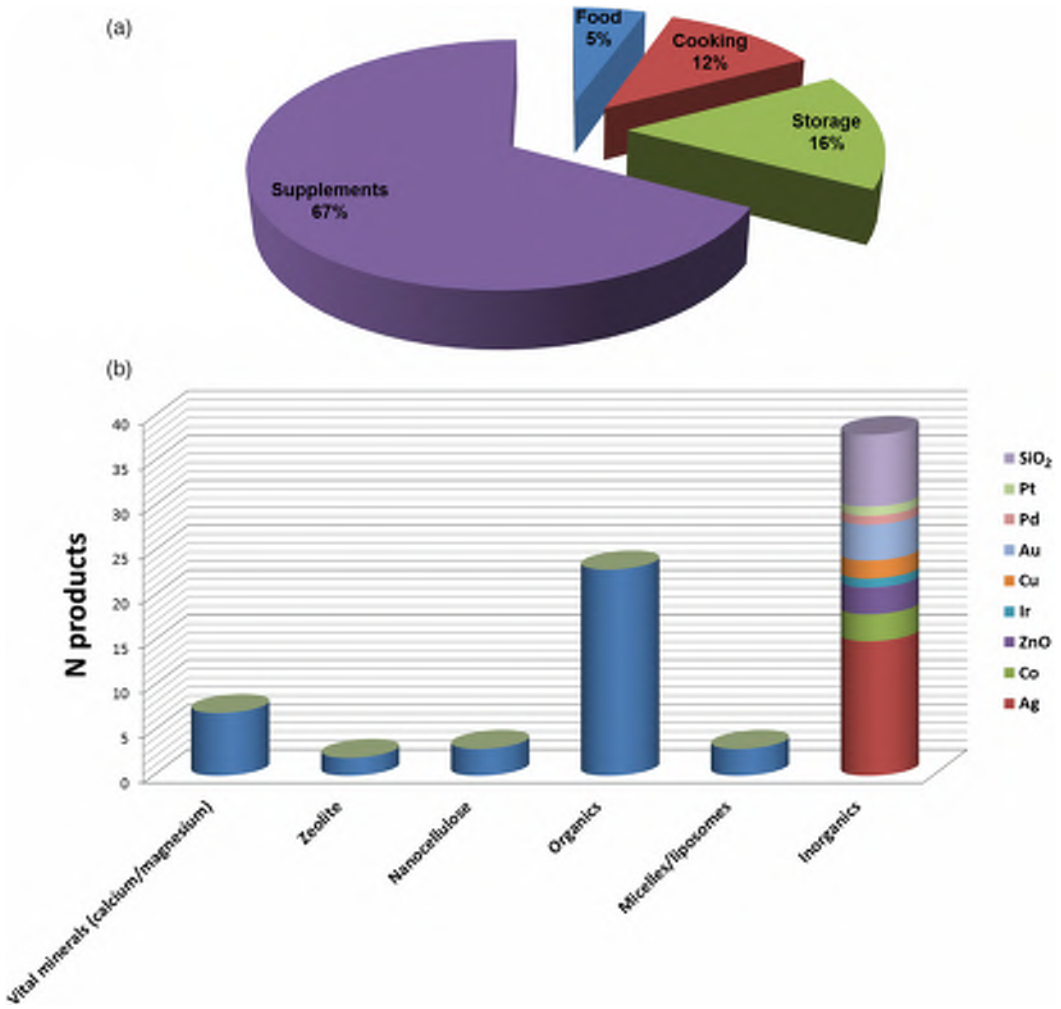

Figure 6.1. Nanotechnology in marketed food products, as reported by the Woodrow Wilson Center: Project on Emerging Nanotechnologies (2013). (a) Statistical graph on nanotechnology-based products in the food sector introduced in the market. (b) Chemical composition and abundance of NMs used in food supplements.

pathogenic bacteria in food. The Woodrow Wilson Center Inventory reports that nanotechnology-based products for the food sector include food, cooking, storage, and supplements (Fig. 6.1a) (Woodrow Wilson Center, 2013).

The extensive use of nanotechnology in food supplements depends on the increasing consumer demand for healthy food products. The growing usage of nutraceuticals led companies and institutes worldwide to apply tools and knowledge in nanotechnology for addressing issues relevant for food and nutrition. In particular,

nanotechnology is applied to nutraceuticals for improving bioaccessibility and bioavailability of active ingredients. An example is represented by the nanotechnology-based delivery systems developed to improve curcumin bioavailability. Curcumin possesses diverse pharmacological effects including antiinflammatory, anticancer, antioxidant, antiproliferative, and antiangiogenic activities but it exhibits poor bioavailability in free form. Curcumin complexed with soy-based nanoparticles showed water solubility 98,000-fold higher than free curcumin, and an improvement of pharmacological parameters as stability, bioaccessibility, and bioavailability (Chen et al., 2015).

The term nutraceuticals was coined in 1989 by Stephen L. DeFelice, and it combines "nutrition," and "pharmaceutical" words (Andlauer and Fürst, 2002; Kalra, 2003). Nutraceuticals refers to the food or food ingredients that have well-defined beneficial physiological effects. In contrast to functional foods that must remain foods (Contor, 2001; Gulati and Ottaway, 2006), nutraceutical products do not easily fall into the legal categories of food or drug and often reside in a grey area between the two. As previously described, many nutraceuticals have poor oral bioavailability, which significantly lowers their efficacy as disease-preventing agents. Nanotechnology represents an innovative way to effectively increase oral bioavailability of nutraceuticals, eg, preserving them from digestion. During the passage through the gastrointestinal tract (GIT), nutraceutical formulations are indeed exposed to dramatically different environments with possible changes in their physical state, location, and chemistry. These changes may cause reduced bioaccessibility and bioavailability of active ingredients, decreasing their efficacy. While bioaccessibility is defined as the amount of an ingested nutrient available for absorption in the gut after digestion, oral bioavailability of a nutraceutical is defined as the fraction of the ingested nutraceutical that actually reaches the site of action in its active form (Parada and Aguilera, 2007). Nanotechnology favors both bioaccessibility and bioavailability, preserving active ingredients from physico-chemical transformations occurring in the presence of adverse gastrointestinal conditions. The use of nanotechnology is also important for a controlled release of active ingredients at a specific site of action, preventing their degradation and promoting their bioconversion and bioefficacy.

According to the currently available information, NMs used in food supplements include both inorganic and organic substances depending on their chemical composition (Fig. 6.1b) (Woodrow Wilson Center, 2013). Inorganic NMs include metals and/or metalloids, and they are the active ingredients themselves. The most common inorganic NMs used in nutraceuticals include silica, silver,

copper, platinum, palladium, gold, zinc oxide, cobalt, and iridium, vital minerals such as calcium and magnesium, and zeolite. Examples of inorganic NMs used in nutraceutical formulations with their beneficial effects are: (1) nanoselenium, which is marketed as an additive to a green tea product with a number of (declared) health benefits resulting from enhanced uptake of selenium; (2) nanosilver, used in a number of consumer products including food and health food, for example, for stimulating the immune system.

Organic NMs can be categorized as lipid-based or nonlipid-based materials, depending on the presence of lipids in the composition of these NMs (Yao et al., 2015). Nonlipid-based organic NMs are mainly composed of biopolymeric nanogels and nanoparticles, organogel based nanoemulsions, and protein-based micelles (Zimet and Livney, 2009; Matalanis et al., 2011; Paramera et al., 2011; Haham et al., 2012; Yu and Huang, 2012; Xu et al., 2013; Schiborr et al., 2014; Zhou et al., 2014). Lipid-based NMs are liposomes, micelles, nanoemulsions, and solid-lipid nanoparticles (Borel et al., 1998; Herrera and Barbas, 2001; Weiss et al., 2008; Thanatuksorn et al., 2009; Huang et al., 2010; Gong et al., 2012; Qian et al., 2012; Mignet et al., 2013; Xiao et al., 2013; Yang and McClements, 2013; Chen et al., 2014; Cho et al., 2014; Yao et al., 2014; Zou et al., 2014). A wide range of materials, such as chitosan, polymers, cyclodextrins, and dendrimers have been used as carriers to improve bioavailability. An example is represented by poly(lactic-*co*-glycolic acid) (PLGA) material, which is approved by U.S. Food and Drug Administration (FDA) for use in therapeutic devices for its biocompatibility and biodegradability (Astete and Sabliov, 2006). PLGA can be used as an efficient carrier of functional foods and for drug delivery, as recently shown for PLGA nanoparticles loaded with curcumin (Xie et al., 2011).

Organic NMs can be either the active ingredients themselves, as inorganic NMs, or act as delivery systems of active molecules. Self-assembling omega-3 fatty acids belong to the former class, while liposomes and micelles loaded with molecules such as vitamins and CoQ10 are delivery systems.

The growing use of nanotechnology in consumer products raises questions about their impact on human health. The greater chemical reactivity of NMs, responsible for the interesting physicochemical properties, leads to an increased interaction with biological systems. Once in the body, NMs may easily cross biological barriers and reach those parts usually protected from entry of (larger) particulate materials. While organic NMs should not raise any severe health concern since they are composed of biocompatible and biodegradable macromolecules, a number of knowledge gaps on safety of inorganic NMs still remain. Inorganic NMs can be

composed of essential and/or nonessential elements, as is the case with nanoselenium and nanosilver. Selenium is an essential trace element, while silver is a nonphysiological element able to specifically interact and alter the homeostasis of essential elements such as copper, selenium, and sulfur (Benetti et al., 2014). Inorganic NMs, also termed *hard*, are nonbiodegradable and potentially biopersistent. The exposure to insoluble, indigestible, and potentially biopersistent NMs might lead to their accumulation in target organs, high local concentrations and long-term side effects. It is well documented that internalized NMs may cause a wide range of biological effects such as inflammatory response, oxidative stress, DNA damage, and apoptosis (Song et al., 2009; Stebounova et al., 2011; Huang et al., 2013; Kodali et al., 2013; Sahu et al., 2013; Stoccoro et al., 2013; Steuer et al., 2014; Ucciferri et al., 2014).

The use of nanotechnology in food and food-related goods might have an impact on consumers' perceptions and opinions. Consumers are considerably uncertain, anxious, and increasingly critical about the safety of food. This is mainly due to several food scandals and scares that have occurred all over the world, such as mad-cow disease, dioxin crisis, avian flu, and the (H1N1) new influenza, and the presence of genetically modified products in the food chain (Bánáti, 2011). Currently, available information about nanotechnology is not sufficient for consumers to make their decisions. Generally, the perception of nanotechnology is positive, but at the same time consumers are worried about the use of nanotechnology in the field of food production (European Commission, 2005). To address some of these worries, the introduction of new principles such as the risk analysis framework, and the separation of risk assessment and risk management provided a more efficient, science-based system in Europe. The EU, indeed, has one of the highest food safety standards in the world—largely thanks to the solid set of EU legislation in place, which ensures that food is safe for consumers. In light of these considerations, there is an urgent need for regulatory systems capable of managing risks associated with nanofoods and the use of nanotechnology in food industry. In this chapter, we present and discuss the regulatory framework related to nanotechnology in functional food and nutraceuticals.

2 Regulatory Aspects on the Use of Nanomaterials in Nutraceuticals

For the scope of this paragraph, the term *nutraceuticals* includes all food that is intended to provide a nutritional and beneficial physiological effect. Two main categories can be

identified: (1) dietary supplements composed of nutrients or active ingredients delivered in specific form such as capsules or extracts; (2) functional food containing new or more ingredients added in conventional form and resulting in enhanced function and improvement of health.

2.1 Regulatory Framework

There is no international harmonized regulatory framework when it comes to food safety. It is possible to identify three main aspects that are critical for food safety and the use of NMs in food. The first aspect is the regulation of "placing on the market," which can be allowed after a premarket authorization, a notification, or direct commercialization, with different safety information requirements and publicly available to the consumers. The choice is often linked to the "traditional" and already authorized products versus the "new" nature of the food or ingredient. In all cases, a postmarket surveillance by the national authorities is in place. The second aspect concerns the assessment of product efficacy. This is absolutely relevant for nutraceuticals since the "claim" of beneficial effects for a given formulation (eg, iron "fights against anemia/tiredness"; EFSA Scientific Committee, 2010) can be evaluated by providing information at different levels, from in vitro tests to human studies. As it is difficult to scientifically prove a claim for normal substances, it is even more difficult to prove benefits with NMs due to lack of general knowledge about their interactions with the human body. Another important issue for the use of NMs in food is public information, normally provided through labeling. Labeling is not always mandatory, and the identification of substances to label as "nano" in the ingredient list depends on the regulatory definition of nanomaterial.

2.1.1 International Regulatory Framework

All countries have a regulatory framework to ensure the safety of food for human consumption. Regulation may vary in different countries, but a similar general approach in terms of information requirements and approval procedure (including separation of risk assessment and risk management) exists. Magnuson et al. (2013) published a review on food regulatory framework and the status of NMs regulation of the main industrialized countries, such as EU, USA, Canada, and Australia (Table 6.1). Table 6.1 highlights (1) the existence of NMs definition, (2) the Novel Foods regulation, (3) the food/supplements authorization to commercialization process, and (4) the nanolabeling requirements. The authorization procedure is described as notification and registration. Notification is a

Table 6.1 Extract of Relevant Information on Food Regulatory Framework and the Status of NMs Regulation as Published by Magnuson et al.

Country	Nanomaterials	Novel Foods	Food Authorization Process	Nano Labeling
Argentina	Not explicitly considered	Definition: no Specific regulation: no	Registration	No
Australia/New Zealand	NMs are defined and safety evaluation is foreseen	Definition: yes Specific regulation: yes	Notification, listed	No
Brazil	Not explicitly considered	Definition: yes Specific regulation: yes	Registration	No
Canada	NMs are defined, use not yet regulated, safety assessment foreseen (general guidance)	Definition: yes Specific regulation: yes	Registration	No
China	NMs are defined, use not yet regulated	Definition: yes Specific regulation: yes	Registration	No
USA	NMs are defined, use regulated as for other substances used in food	Definition: no Specific regulation: no	Registration, notification	No
European Union	NMs are defined, and specifically regulated	Definition: yes Specific regulation: yes	Registration, notification	Yes

Source: Magnuson et al. (2013).

simpler procedure, where only some limited information is provided to the national authority, normally excluding safety data, and the review process is fast(er). Registration includes safety information, and implies a premarket review process.

2.1.1.1 USA Approach

In the United States, the Food and Drug Administration (FDA) regulates dietary supplements and new dietary ingredients (which can be translated as nutraceuticals) in a different way with respect to "traditional" food ingredients (FDA, 2015a). For "traditional" food ingredients Companies and Importers are not obliged to have FDA approval before placing a dietary supplement product on the market. The only requirement is a notification of the intention to market a product, and the assurance of safety is the responsibility of the producer. The information used to evaluate the product's

safety is not available to the public or FDA, therefore it is not possible for consumers to know the considerations by which a specific dietary supplement is considered safe for human consumption. Producers can use guidelines about good manufacturing practices, information requirements for safety assessments, and use basic labeling rules to comply with good safety assessment approaches.

New dietary ingredients and products (NDI) are subjected to a premarket review process, where a dossier with safety data is evaluated by FDA within 75 days before the foreseen marketing date (FDA, 2014a). FDA can ask the producer for more safety data if deemed necessary to assure the NDI safe use. Guidelines are available about notification procedure and information requirements. According to FDA, modified traditional food and food ingredients, also with nanotechnology, require to be evaluated as NDI. Therefore, all food/ingredients in nanoform need to be considered NDI. NMs applied in new dietary supplements are evaluated with the existing rules, though individual premarket review (ie, registration) procedures may require additional data on safety or effectiveness, as applicable (FDA, 2015b). To avoid misunderstanding in considering food or food ingredients as NMs subjected to NDI process approval, FDA encourages producers to consult with the agency on safety matters before marketing a product (FDA, 2015b).

Concerning labeling rules and information about the product (eg, accompanying material, leaflets, etc.), all ingredients must be reported. Besides the need to report information on product safety, there is no obligation to report an ingredient as NM. Concerning the physiological effects of supplement and related claims on the label, it is forbidden to sell a dietary supplement as a way to prevent, cure, or treat a disease or a condition. The label can report only three types of claims: (1) health claims, (2) nutritional claims, and (3) structure/function claims. Claims can be used only after FDA approval, which occurs on the basis of scientific evidences (FDA, 2014b).

2.1.1.2 Canadian Approach

In Canada, nutraceuticals are grouped in the category of Natural Health Products (NHP). The approach for NHP authorization changed recently, with the aim to rationalize the legislation of food included in the dietary supplements category. The placing on the market of NHP is subjected to premarket authorization, or licensing, which is provided after safety and efficacy (claim) assessment by Health Canada (National Authority) on the basis of a dossier submitted by the firm interested in marketing the product. A guidance document is available to support dossier preparation

with relevant information for NHP safety and efficacy, as well as for product labeling.

The approval process is based on three risk categories depending on NHP ingredients and health claims (potential risk for consumers in terms of curing/preventing simple versus serious health conditions). Class 1 includes the most well-known nutraceuticals (lower uncertainty), such as vitamins, minerals, and certain botanicals. The review process of this class is shorter, and the amount of information is low. For this class there are the so-called "monographs" that represent a sort of positive list of substances. The second class (Class 2) includes products moderately characterized, as already authorized products for which the producer wants to add a new claim. Class 3 includes products with little information already available, and declared as acting on serious health conditions. For each class, an incrementally greater amount of information is requested in order to demonstrate safety and effectiveness. Additional information ranges from data available in monographs to human studies with clinical evidences (Harrison and Nestmann, 2014; Health Canada, 2015).

Currently, there are no specific regulations for authorization and labeling of nanotechnology-based health products. Health Canada is addressing NMs in nutraceuticals by using the existing regulatory framework. As for FDA in the United States, Health Canada suggests to address nanotechnology-specific issues from the beginning, encouraging a presubmission meeting with the responsible department, providing information on: (1) intended use of NM, including any end product in which it will be used; (2) manufacturing methods; (3) characterization and physico-chemical properties of NM, such as identity, composition, and purity; (4) toxicological, eco-toxicological, metabolism, and environmental fate data that may be both generic and specific to the nanomaterial if applicable; (5) risk assessment and risk management strategies, if considered or implemented (Health Canada, 2011a).

2.1.1.3 Australian Approach

In Australia, nutraceuticals are considered "complementary medicines" (CMs). CMs are a particular category of medicines regulated by the same law regulating proper medicinal formulations. CMs are used for therapeutic purposes, and include vitamins, minerals, herbal materials, essential oils, sugars, and amino acids. The approval process foresees a two-tiered premarket authorization. Products with lower risk, for example, preapproved ingredients, and making limited claims can be directly included in the approved products list (Listed), while products with higher risk must be tested for safety, efficacy, and quality, before being

approved for marketing (Registered). A Code of 2007 reports the guidelines for labeling and advertising CMs (Australian National Audit, 2011).

In Australia, nanotechnology is not explicitly addressed in regulation. The Therapeutic Goods Administration (TGA) considers current regulations sufficient to address risks of nanotechnology in prescription medicines (Australian Government Department of Health, 2014). This concept can be extended to CMs, as they are regulated in the same framework. However, TGA is reviewing the existing regulations for adapting them to new developments, as well as keep developing high-level scientific expertise.

2.1.2 European Regulatory Framework

In the European geographical and political area, food policy and food legislation have been substantially developed in the past decade. Several principles linked to traceability and risk analysis were implemented, with a clear separation between risk assessment, done on a scientific basis, and risk management, done on a socioeconomic (essentially political) basis (Bánáti, 2011). This new approach led to the Regulation 178/2002, that is, the General Food Law (The European Parliament and the Council of the European Union, 2002a). Its main aim was to provide the basis for assuring high protection level of human health and consumers' interest in relation to food, by laying down the principles for food and feed safety at European and national level, and establishing the European Food Safety Authority (EFSA). The Regulation is applicable to the whole food supply chain, from production to distribution, while it cannot be applied to medicines and cosmetics.

It should be noted that nanofoods clearly fall within the regulatory scope of General Food Law, though the adequacy of conventional procedures for establishing safety of such foods remains unclear. According to Art. 7 of Reg. 178/2002, the presence of potential harmful effects and high scientific uncertainties require the application of Precautionary Principle to food risk management. This principle seems applicable to nanotechnology-based food, when available exposure and hazard information are not sufficient to draw a conclusion about food safety. Moreover, Art. 8 of Reg. 178/2002 affirms that consumers shall be informed about the food they are consuming to avoid fraudulent or deceptive practices, food adulterations, and any other misleading practices for consumers. These principles are key elements for food labeling and consumers' right to know the nature of ingredients. With the aim to assure that the food placed on the EU market is safe for human consumption, and in light of this regulation, existing laws were modified and new laws approved. This process is currently still ongoing.

Nutraceuticals and functional foods in the EU are covered by a long list of European and National laws: legislation on food supplements (Directive 2002/46/EC) (The European Parliament and the Council of the European Union, 2002b), legislation on fortified foods (Regulation 1925/2006) (The European Parliament and the Council of the European Union, 2006a), legislation on food for special purposes (Directives 2006/141/EC, 2006/125/EC, 96/8/EC, 1999/21/EC, substituted by the Regulation 609/2013 from Jul. 2016) (The European Parliament and the Council of the European Union, 2013), health and nutritional claims (Regulation 1924/2006) (The European Parliament and the Council of the European Union, 2006b), novel food (Regulation 258/97, recently revised (the European Parliament and the Council of the European Union, 2015); for a short discussion: Nutrafoods 2016 15(1), in press) (The European Parliament and the Council of the European Union, 1997), and food labeling (Regulation 1169/2011) (The European Parliament and the Council of the European Union, 2011). Legislation is covered by both directives and regulations. While regulations are directly implementable in all Member States, directives require National adaptation that leads to different interpretations of law requirements.

Directive 2002/46 addresses the placing on the market of food supplements, defined as:

> *foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities.*

As "nutrients," are considered only vitamins and minerals, and the directive establishes a positive list of admitted nutrients (Directive 2002/46, Annex I), and purity criteria (Directive 2002/46, Annex II). Directives have to be translated into the national legislation of each Member State.

Regulation 1925/2006 addresses the addition of vitamins and minerals to conventional food or food for special purposes, defining the need to assess if substances added to food increases consumers' risks due to ingested doses greatly exceeding the expected amount through a varied and balanced diet. A procedure was established to add such substance in a negative list (prohibited substance) or a regulated list (substance use allowed only under given conditions).

Food for special purposes—regulated be several legislations—includes all food that must fulfill particular nutritional

requirements of persons with metabolic disorders and delicate physiological conditions. In addition, the law allowed dietetic food to be commercialized without a premarket evaluation, hampering the safety assessment of products and the scientific basis of claims (Coppens and Pettman, 2014). To overcome these gaps, Regulation 609/2013 will enter into force in Jul. 2016, controlling: (1) infant formula and follow-on formula, (2) processed cereal-based food and baby food, (3) food for special medical purposes, and (4) total diet replacement for weight control. The Regulation establishes a procedure to create and update a positive list (Union List) of ingredients. Other requirements (eg, product notification procedure, composition requirements, labeling requirements) had to be decided by delegated acts of the European Commission within Jul. 2015. The important aspect for NMs is that the verification of suitability and the ability to satisfy nutritional requirements of food composition, for persons whom use is intended, shall be demonstrated on the basis of adequate test methods. Also, in the recital (23) it is clearly stated that substances produced with a different method, or with a different size, even if already authorized by this regulation, should be considered different and re-evaluated first according to the Novel Food regulation, and second according to the Regulation 609/2013.

Regulation 1924/2006 lays down the requirements and procedures to approve and use nutritional and health claims. A nutritional claim is defined as "any claim which states, suggests or implies that a food has particular beneficial nutritional properties," while a health claim is "any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health." Although there is no direct mentioning at NMs, this Regulation is relevant since NMs may increase the efficacy of nutrients leading producers to ask for a claim linked to specific nutritional or health effects of substances in nanoform.

Health claims must be authorized by EFSA prior product commercialization, on the basis of high quality scientific evidences such as human studies. The economic burden of this kind of studies for Companies, especially small and medium-sized enterprises, is hampering the approval of such claims. This is also true for NMs, which would need standardized methods not yet always available to study the relationships between the nutrient and the health effects (eg, ADME-administration, distribution, metabolism and excretion, long-term toxicity, etc.).

Novel Food Regulation (Regulation 258/97) aims at regulating the placing on the market of novel food and novel food ingredients. Novel food is defined as a food not significantly used for

human consumption before the entry into force of this Regulation (May 15, 1997).

Novel food is defined as:

1. Foods and food ingredients with a new or intentionally modified primary molecular structure;
2. Foods and food ingredients consisting of or isolated from microorganisms, fungi, or algae;
3. Foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices and having a history of safe food use;
4. Foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism, or level of undesirable substances.

Novel food commercialization approval is addressed directly by Member States national authorities for categories 1 and 4, while categories 2 and 3 are evaluated and authorized directly by the European Commission at EU level. If food in categories 2 and 3 are considered substantially equivalent to exiting foods in terms of composition, nutritional value, metabolism, intended use and the level of undesirable substances, a notification to the Commission is sufficient for placing on the market the new product. The equivalence status is determined by the Member States.

Regulation 258/97 does not refer explicitly to NMs, though categories 1 and 4 can be regarded as potentially including NMs. Moreover, labeling rules establish the need to indicate modified characteristics or properties, together with the method by which modifications were obtained. This indication can be used to inform consumers on the use of nanotechnology or NMs to produce novel foods or ingredients. However, these considerations on NM in Novel Food Regulation are linked to the spirit of the law, giving space to different interpretations.

In order to better address the NM issue, a revision of the Novel Food Regulation was concluded in December 2015. European Parliament defined NM in the revised Novel Food Regulation (which concerns the food risk assessment) by using the definition included in labeling regulation (Regulation 1169/2011, see later on). This approach is going contrary to the Commission general intention, which is to harmonize all definitions with the Commission Recommendation (The European Commission, 2011). Another aspect of the regulation revision is that NMs should always be considered novel food, and to be evaluated according to the Novel Food Regulation prior to be evaluated according to the relevant

specific legislation (eg, special food regulation). The European Parliament is also urging EFSA to develop appropriate toxicological methods and risk assessment approaches to support the safety assessment of NMs in food. NMs should be included in the Union list of approved substances only after development of ad hoc methods by EFSA.

Novel Food Regulation revision includes among novel foods soft particles, such as micelles, which would otherwise be excluded from the safety assessment due to the interpretation of the term *particle*, as used in Commission Recommendation nanomaterial definition.

Regulation 1169/2011 defines the rules for food labeling, including nutraceuticals. This Regulation establishes the general principles, requirements, and responsibilities governing food information, and in particular food labeling. The regulation is also providing a definition of NMs (see later on). Every ingredient falling within the definition shall be labeled with the word "nano" in brackets after the name of the ingredient. In the list of nutraceutical ingredients, nanoforms have to be clearly indicated in agreement with the Regulation. As seen in the last two Regulations, one of the main issues in the safety assessment of NMs is the regulatory definition. An overview of the nanomaterial definitions around the world and specifically in Europe is reported in Section 2.2.

2.2 Nanomaterial Definition

The need to have a regulatory definition for NMs arose in Europe in 2009, when the European Parliament published a report about their regulatory aspects (The European Parliament, 2009). The European Parliament highlighted a significant lack of information about NM safety, leading to disagreement already at the level of definition. Therefore, the European Parliament appointed the European Commission to introduce a scientific-based definition of NMs useful both for EU horizontal and sectorial legislation, and to promote the development of an international harmonized NM definition.

The importance to discuss NM definition resides in regulatory and practical reasons. For legislators new technologies pose new challenges in terms of health and environmental risks, especially if applied to consumer products. Therefore, new legislations or the adaption of existing ones may be necessary, and the regulatory authority has to define what is specifically regulated (Lövestam et al., 2010). It is also important to assure similar

procedures in different regulations for the same NM. While variation in NM definition for sectorial legislation due to the different applications (eg, relevance of exposure scenarios) is appropriate, it is important to avoid situations in which substances are considered either NM or not NM depending on the application field. Differently from food, in cosmetic products, indeed, reference to "nanotechnology" means the use of biopersistent nanoparticles as ingredients.

The development of a scientific-based and applicable NM definition presents some difficulties. NMs are complex objects with different measurable properties, many of them relevant for safety concerns. However, some measurands are not easily assessed, especially in complex matrices such as food. NMs are produced and used in products to exert innovative specific functions linked to their unique properties. To date, no defined size limit at which specific properties appear has been identified. While some evidences show that in certain conditions 30 nm in diameter is the size limit below which nanospecific regulatory attention should be posed (Auffan et al., 2009), the National Cancer Institute currently recommends 10 nm as the lower limit since it is the threshold for first-pass elimination by the kidneys (Nair et al., 2010). In real conditions NMs are often a mixture of particles with different sizes, and an applicable NM definition should consider the substance size distribution.

NM definition is useful for Companies to better select the best ingredients for specific functions, thus balancing costs (eg, to prepare a dossier and meet nanosafety data requirements), optimizing product efficacy (less functional product per unit), and identifying market share (eg, specific consumer target). For consumers, NM definition is useful to allow an informed choice. Uncertainties reduction in the regulatory framework is a strong prerequisite for a faster transfer of the new technology benefits to consumers, and the development of nanotechnology-based market.

2.2.1 International and National definitions

The definition of NMs, as first step toward the identification and characterization of nanosafety issues, has been addressed by several countries and international organizations. This definition was based on the size of material and/or its novel properties. Some examples of international and national definitions are reported in Tables 6.2 and 6.3. A complete list of definitions is in the JRC report "Considerations on a Definition of Nanomaterial for Regulatory Purposes" (Lövestam et al., 2010). All these definitions are horizontal, meaning they are generic and applicable to

Table 6.2 International Horizontal Definitions of Nanomaterials

Item	Definition	Source
Nanomaterial	A material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale. Nanoscale is a size range from approximately 1–100 nm	ISO/TS 80004-1:2010
Nano objects	Material with one, two, or three external dimensions in the nanoscale	CEN ISO/TS 27687
Nanomaterial	A chemical that is either a nanoobject or is nanostructured. Size range typically between 1 and 100 nm	OECD (in EUR 24403 EN)
Nanomaterial	Any form of a material composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less	SCENIHR, 2007

all sectors/products, and aim to harmonize research efforts, and as standardization tool. However, the use of generic terms, such as *approximately*, as in the ISO definition of the term *nanoscale* (ISO/TS, 2010), makes them unsuitable for regulatory purposes.

2.2.2 Regulatory Definitions in EU

The European Union is the only geographical area with regulatory definitions of NMs. There are two definition levels: (1) horizontal, covering all sectors and potential applications; and (2) vertical, or sectorial, concerning specific NMs applications and based on horizontal definition. In this paragraph, both definition levels with their characteristics and limitations are described.

2.2.2.1 Horizontal Definitions

There are horizontal definitions at both European Union level (Commission Recommendation) (The European Commission, 2011) and national level as established in national registries.

2.2.2.1.1 EU Level Recommendation At European Union level there was a growing awareness of the need of regulatory definition of NMs for answering the regulatory questions on their safety in different application fields. In Oct. 2011, the European Commission issued a Recommendation on NM definition, as "a reference for determining whether a material should be considered as

Table 6.3 National Horizontal Definitions of Nanomaterials

Country	Item	Definition	References
Australia	Industrial nanomaterial	Any industrial materials intentionally produced, manufactured or engineered to have unique properties or specific composition at the nanoscale, that is a size range typically between 1 and 100 nm, and is either a nanoobject (ie, confined in one, two, or three dimensions at the nanoscale) or is nanostructured (ie, having an internal or surface structure at the nanoscale)	NICNAS Handbook (Australian Government Department of Health, 2014)
Canada	Nanomaterial	Any manufactured substance or product and any component material, ingredient, device, or structure that: (1) it is at or within the nanoscale in at least one external dimension, or has internal or surface structure at the nanoscale; (2) it is smaller or larger than the nanoscale in all dimensions and exhibits one or more nanoscale properties/phenomena	Health Canada Policy Statement (Health Canada, 2011b)
USA	Nanomaterial	Nanomaterials can exhibit unique optical, mechanical, magnetic, conductive, and sorptive properties different than the same chemical substances in a larger size	US-EPA (accessed 07/2015) (EPA, 2012)
USA	Nanomaterials	Both materials that have at least one dimension in the size range of approximately 1–100 nm and certain materials that otherwise exhibit related dimension-dependent properties or phenomena	FDA (2012)
Denmark	Nanomaterial	Nanomaterials: a natural, incidental, or manufactured material that contains particles in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm (nanometers). The definition follows Commission Recommendation 2011/696/EU on the definition of nanomaterial	Danish EPA (The Danish Environmental Protection Agency, 2014)

a "nanomaterial" for legislative and policy purposes in the Union." The Commission Recommendation is applicable to all application fields by stakeholders operating within the European Union market, though it is not legally binding. The definition recites:

> *Nanomaterial' means a natural, incidental, or manufactured material containing particles in an unbound or an agglomerate state, where 50% or more of the particles size distribution is in the size range 1 nm–100 nm. In specific cases and where warranted by concerns for the environment, health, or safety the number size distribution threshold of 50% may be replaced by a threshold between 1% and 50%.*

The definition identifies a NM on the basis of fraction in number of particles having an external size range between 1 and 100 nm. The size was chosen as main parameter since it is easily measurable. However, specific surface area was selected as a complementary criterion in defining NMs. This parameter is very useful for some NMs including fullerenes, graphene flakes, and single-wall carbon nanotubes, which are considered NMs even though they have one or more dimensions below 1 nm in size. The definition was revised by JRC to adapt it to scientific advancement. The first part of revision collected comments and instances related to the application of the current definition (Rauscher et al., 2014), while the second part evaluated the information, highlighting the main issues and potential solutions (Roebben et al., 2014).

Among the identified main issues, it is worthy to mention: (1) the only inclusions of particulate materials with the exclusion of nanostructured materials such as nanoemulsions (which could be relevant for food industry and nutraceuticals); (2) the impact of using particles' number instead of mass as a metric (potential definition as NM of a substance in which nanoparticles represent only a small fraction in mass); (3) the selection of a clear-cut threshold, which leads to borderline cases; and (4) the flexibility in the threshold definition (in the range 1–50%), depending on sectors and exposure scenarios. Finally, some substances that are considered NMs are not included with the current definition (eg, nano clays, nanostructured materials), and vice versa (eg, pigments). The JRC report also describes potential solutions to improve the definition.

The third JRC report, published in Jun. 2015, is focused on technical and scientific recommendations for the clarification of the definition and its implementation (Rauscher et al., 2015). According to the JRC, the broad scope of the definition including natural NMs should be maintained. The definition revision by JRC keeps the focus on particles, without extending the definition to

nanostructured materials where the particle size fraction as main criterion is not valid anymore. The main suggestion is to maintain particle size as the only criterion for NM definition, making the volume-specific surface area a criterion for screening only. Regarding the size distribution, it is suggested to fix the 50% threshold as a limit, removing threshold flexibility. In this revision, JRC highlighted the importance to clarify the term *particle*. Currently, the definition defines particle as having a well-defined physical boundary. A clarification is essential to define the meaning of "physical boundary," because in the case of phase boundary (ie, an interface where one or more physico-chemical properties change abruptly), then it can include also micelles and liquid droplets usually used in food as nanoformulations. Another misunderstood term to be clarified and/or modified is *contained*, which could suggest that a NM-containing product is a NM itself. By replacing this term with *mainly consisting*, doubts should be eliminated. A relevant issue concerns the exclusion criteria of a substance as NM, thus avoiding including unintended substances in the NM category. Substances with wide particle size distribution—from <1 nm to >1 mm—make the determination of complete size distribution difficult. JRC suggested using an additional criterion such as minimum mass of particles in the nano fraction to discriminate between NMs and nonnanoforms, and to extend the "derogation list" to include explicitly considered NMs as for carbon nanotubes.

Although Commission Recommendation is not legally binding, it is used as a starting point for sectorial European legislation and national registries.

2.2.2.1.2 National Registries Some Member States considered the existing EU framework development not sufficient to guarantee information transparency on commercially available NM-containing products, and established national registries for monitoring NMs commercialized at national level.

The first national registry was established in France, and entered into force from Jan. 1, 2013 (Ministry of Ecology, 2012; The Minister of Social Affairs and Health et al., 2012). The definition of NM is based on the 2011 Commission Recommendation, but it is limited to intentionally produced NMs. It also includes NMs in mixtures, in unbounded state or in materials that are foreseen to intentionally release NMs under normal or reasonable conditions. The French registry includes the intentional use of NMs in nutraceuticals.

Last year, other two Member States established national registers: Denmark and Belgium. In both cases NM definition is based on the Commission Recommendation, and both substances and

NM-containing products are considered. These registries exclude NMs used in food sector, and therefore they are not reported here.

2.2.2.2 Other Regulatory Nanomaterial Definitions

The European Commission is including NMs in several new regulations for different sectorial legislations, such as biocides and cosmetics. Concerning food and nutraceuticals, NMs are defined as reported in the Regulation 1169/2011 (see Section 2.1.2 or the Regulation content description). This definition recites:

> *"engineered nanomaterial" means any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale. Properties that are characteristic of the nanoscale include: (i) those related to the large specific surface area of the materials considered; and/or (ii) specific physico-chemical properties that are different from those of the nonnanoform of the same material.*

The main aspects of the definition compared to the Commission Recommendation are the limitation to engineered NMs (ie, intentionally produced), the extension to all materials and not only particles, the absence of particle number threshold, and the application of specific physico-chemical properties together with size for distinguishing NMs from nonnanoforms. Since this definition is very broad, making its application difficult, the Commission is intending to change it through a delegated act. A first delegated act was rejected by the European Parliament due to provision excluding food additives from the "nano" labeling if already authorized (ie, included in the Union lists) and thus on the market since some time (The European Commission, 2013). A second delegated act, currently in draft status, eliminated that provision and changed the definition as follows:

> *engineered nanomaterial' means any intentionally manufactured material, containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm to 100 nm.*
>
> *By way of derogation, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm shall be considered as engineered nanomaterials.*

For the purposes of the definition set out in the first paragraph:

1. *"particle" means a minute piece of matter with defined physical boundaries;*
2. *"agglomerate" means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;*
3. *"aggregate" means a particle comprising of strongly bound or fused particles;*
4. *"intentionally manufactured" means that the material is manufactured to perform/fulfil a specific function or purpose;*

The important aspects of this new proposed definition are: (1) the intentionally manufactured NM are meant to fulfill any specific function or purpose in food, thus not only limited to a new or nanospecific function or purpose; (2) a clear fraction threshold in terms of particle size distribution, with 1 nm as lower limit; (3) the absence of nanostructures, or materials with specific nanoproperties since "particle" is defined as having defined physical boundaries. This definition of particle could exclude micelles and other soft particles depending on the revision process by the European Commission and European Parliament.

2.3 Nanofood Safety Assessment

All the European Union legislations, from the General Food Law to the Novel Food Regulation, are based on assuring the safety of marketed food for consumers. Up to now, there are no specific indications to evaluate in a specific way the safety of nanoforms of approved foods and food ingredients. However, there is the need to prove the safety of NMs-containing nutraceuticals on the market, and producers must present relative dossiers to national authorities on demand. Besides the hard law tools represented by European Directives and Regulations, there is a whole "soft" way of regulating nanotechnology safety that is increasingly used in the EU. One example is represented by the Delegated Acts of the Commission (see the Regulation 1169/2011, amendment of the nanomaterial definition). Another soft approach to nanomaterials safety regulation is exerted by EFSA, through activities foreseen by its institutional role. The scientific opinions, the risk assessment guidelines, and the technical support to Companies and notifiers, carried out as an independent authority and under the law, are influencing the EU approach toward nanosafety in food. An EFSA guideline (eg, how to measure and report a specific parameter) is not an enforced law, but it should be considered as such because it is the current and approved thinking of the assessing authority. This aspect is valid both for the Member States and for the food/feed value-chains actors (Salvi, 2015).

Concerning the nanomaterial safety in food, to produce appropriate data following a sound risk assessment approach EFSA published in 2011 a guideline to perform risk assessment of NMs used in food and feed chain (EFSA Scientific Committee, 2011). This guideline has to be considered in addition to the existing guidelines for traditional food. This recommendation is based on the risk assessment paradigm, including material characterization, exposure and hazard assessment, and risk characterization. The risks of NMs are due to chemical identity, physico-chemical properties, interaction with tissues, and potential exposure levels.

Physico-chemical characterization of a substance has to be done along the five stages ranging from food production to consumption, such as manufactured (pristine state), delivered for use in food/feed products, in the food/feed matrix, in toxicity testing, and in biological fluids and tissues. Since nature and characteristics of NMs can change in different environmental conditions, a full characterization is necessary to properly assess the risks. The guideline identifies a set of physico-chemical parameters, by using the ones established by the Organization for Economic Cooperation and Development (OECD)–Working Party on Manufactured Nanomaterials (WPMN) (OECD, 2010a). While some of them are always required (eg, chemical composition, morphology, size, solubility, reactivity), others are essential only for specific NMs (eg, viscosity for liquid dispersions, surface chemistry for coated particles). Specifically, particle size is a primary parameter because it allows the identification of a substance as a NM, according to the Commission Recommendation.

Once a substance has been identified as NM in at least one of the previously described steps, exposure assessment is required. EFSA guideline developed this tiered approach to simplify the data burden. However, it is often complicated to obtain the required information due to lack of validated methods, the inherent complexity of real matrices, and the high reactivity (ie, high interaction) of NMs. All aspects concerning characterization, exposure, and hazard assessment methods for NMs are better described in the next parts.

3 Characterization of Nanomaterials for Regulatory Purposes

Identification and characterization of NMs in functional foods and nutraceuticals are key issues to assess their efficacy and consumers' safety, as well as the compliance with the existing regulations. The debate on the selection of the most relevant

physico-chemical parameters to assess exposure and hazard of NMs, and the analytical methods to measure them is still in progress. Several indications and nonbinding recommendations are available as scientific opinions of relevant international organizations, such as EFSA (EFSA Scientific Committee, 2011), the FDA (FDA, 2012), the OECD (OECD, 2010b), and the Scientific Committees of the European Commission (eg, the Scientific Committee on Emerging and Newly Identified Health Risks–SCENIHR) (SCENIHR, 2010).

In the framework of NM safety assessment, there is a good agreement on the physico-chemical properties to be considered and quantitatively evaluated. They are particle size and size distribution, chemical composition including impurities and surface chemistry (ie, coating, capping agents, surface functionalization), structural information such as crystal structure and crystallinity, shape (eg, aspect ratio), surface area and charge, agglomeration status and its distribution, and persistence/reactivity in terms of solubility, UV- and thermal-stability (Card and Magnuson, 2009; Bouwmeester et al., 2011). It is well known that many of these characteristics are strongly influenced by the interactions with exposure media and the environment, therefore primary and secondary characterization—referring to the nanoobjects in the pristine form and in the relevant surrounding environment, respectively—is warmly recommended (EFSA Scientific Committee, 2011; OECD, 2012). This issue is particularly relevant for NMs used in food-related applications as nutraceuticals due to the complexity of their life cycle, encompassing NM manufacturing, preparation of the commercial formulation, incorporation in the food matrix, conservation, consumption, and digestion by consumers. In the first stages of their life cycle, NMs used in nutraceuticals interact with the formulation and food matrix components with consequences on their solubility, stability, and bioavailability (Yada et al., 2014). Thereafter, during the digestion process NMs are exposed to different biological fluids and large pH variations, which might induce significant transformations such as changing in morphology, size, and chemical composition (Mwilu et al., 2013; Pornwilard et al., 2014). A reliable characterization of NMs along life cycle plays a pivotal role in guiding the safety assessment (Bouwmeester et al., 2011; EFSA Scientific Committee, 2011; Cockburn et al., 2012; Martirosyan and Schneider, 2014). Despite the large investments done in the last years, estimated in more than 100 M€ annually until 2012 if considering funds for research projects supplied by Europe and Member States, methodological uncertainties and scientific gaps in assessing physico-chemical properties, behavior and effects of NMs still remain. Validated approaches and widely accepted risk management guidelines are

still missing, causing great difficulties for risk assessors, risk managers, industries, and consumers (Handford et al., 2014; Szakal et al., 2014a; Dasgupta et al., 2015).

3.1 State of the Art

Characterization of NMs in food, beverages and nutraceuticals represents a challenge due to the presence of complex matrices and possible interferences (Peters et al., 2011; Cockburn et al., 2012). Moreover, analytical methods to characterize NMs often need sample preparation, including extraction or separation from the matrix (Ladner et al., 2012; Zhang et al., 2012; Geiss et al., 2013; Gray et al., 2013; Peters et al., 2014a; Singh et al., 2014; Dan et al., 2015; Lim et al., 2015), with possible generation of artefacts (Tiede et al., 2008; Singh et al., 2014). For these reasons, difficulties in analyzing NMs increase moving from primary to secondary characterization (Szakal et al., 2014a), that is, along the five stages of the life cycle of NMs in the food chain outlined by EFSA (EFSA Scientific Committee, 2011) (see Section 2.3). To overcome these technical limitations, the use of a multiple approach to characterize NMs is required (Powers et al., 2006; Richman and Hutchison, 2009; Dudkiewicz et al., 2012; Singh et al., 2014; Szakal et al., 2014a; Szakal et al., 2014b). Analytical techniques used for measuring the relevant physico-chemical endpoints are mainly imaging and microscopy-related techniques, separation methods, spectroscopy-related techniques, and mass spectrometry (Luykx et al., 2008; Tiede et al., 2008; Dudkiewicz et al., 2012; Lin et al., 2014). All of them present strengths and weaknesses, and they cannot be universally considered useful for all kinds of NMs. Generally, these techniques are more appropriate for inorganic NMs, while a general deficiency in analytical methods to detect, quantify, and characterize properties of organic NMs exists (Szakal et al., 2014b). The need of using complementary instrumentations and techniques for NM characterization implies a heavy financial burden (Szakal et al., 2014a). For example, it has been estimated by a food company that the notification at the French registry (Ministry of Ecology, 2012) required about 1,500 h of work, roughly consisting in 2 work days per substance, whilst a chemical industry declared it spent between €3,000 and €10,000 per substance to collect the information for the mandatory fields in the same database (Risk and Policy Analysts, 2015). Therefore, the development of an analytical toolset capable of detecting, quantifying, and characterizing NMs in food in an inexpensive, rapid, and less labor-intensive or expertise-intensive ways represents a research priority for the successful growth of nanotechnology (Singh et al., 2014).

3.2 Validated Methods

The state of the art of internationally available testing guidelines has been recently updated by the OECD-WPMN (OECD, 2014a), who organized a workshop to assess the applicability of already existing OECD Test Guidelines (TG) on physical–chemical properties of manufactured nanomaterials, as well as to identify needs for updating current or developing new OECD TG and/or OECD guidance documents relevant for safety and regulatory decision-making. The TG developed by OECD and ISO, and available methods to characterize NMs in a regulatory safety assessment framework are summarized in Fig. 6.2.

With the exception of the octanol–water partition coefficient, which is not relevant for NMs since their distribution is not governed by the mechanisms it describes, all discussed physico-chemical end-points have regulatory relevance for classification purposes in agreement with the current regulation, and for evaluating fate and exposure and/or for hazard assessment. Generally the need for new TG which can also refer to existing ISO standards, or for revision of existing TG [such as OECD TG 105 (OECD, 1995)] has been remarked upon for almost all the physico-chemical end-points, as well as the need for defining relevant dispersion and sample preparation protocols.

Since regulation of NMs is not homogeneous at the international level, the same nanomaterial/product can be looked differently around the world with different legal implications and provisions (Newsome, 2014). Nevertheless, some key questions to address for both product development and safety assessment in a sound regulatory perspective should be laid down: (1) presence of NMs in sample, (2) NMs typology, (3) amount of NMs in sample, (4) extent of exposure to NMs, and (5) safety of exposure (Newsome, 2014; Szakal et al., 2014a). Currently, no validated analytical methods for answering these questions are available. A generic approach for validating methods to detect and quantify nanoparticles in food samples has been proposed by Linsinger et al. (2013). The proposed parameters were the same used in the classical analytical chemistry, as: identity (determination of chemical species and size); selectivity (discrimination against matrix components, particles with different chemical species, chemically equivalent particles from different producers or batches, dissolved/bulk species); limit of detection (the smallest detectable mass/number fraction and particle size of NM); limit of quantification (the smallest quantifiable mass/number fraction and particle size of NM); working range/linearity (with respect both mass/number fraction range and particle size range); precision, recovery and trueness, and ruggedness (in terms of mass/number fraction and particle size).

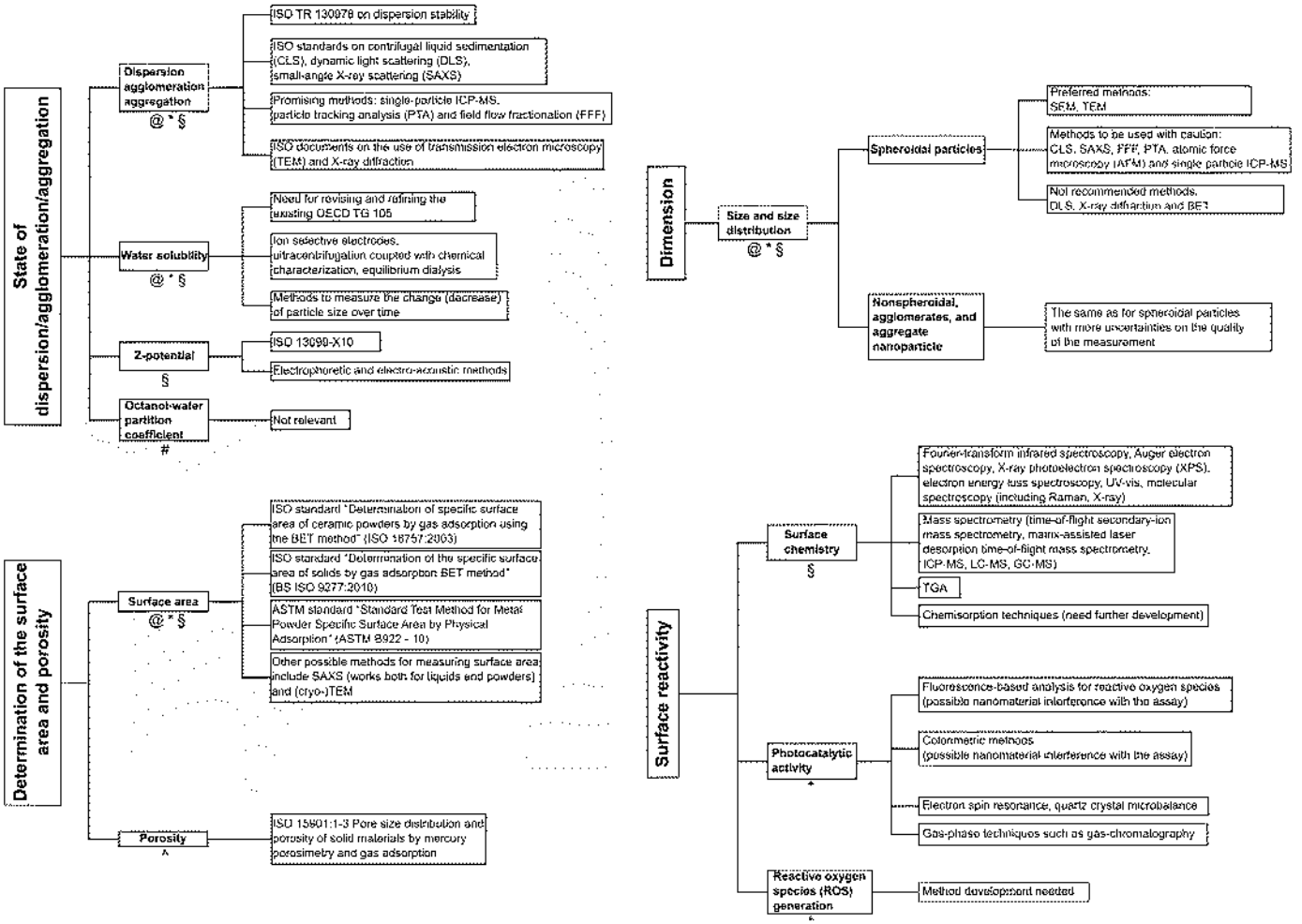

Figure 6.2. Summary of existing TG developed by OECD and ISO, and available methods to characterize NMs in a regulatory safety assessment framework. Symbols under boxes represent the regulatory relevance of the physical–chemical end-point: categorization (@), fate and exposure (§), hazard (*), none (#), for nanostructured materials (^). Adapted from OECD, 2014a.

Improvements in analytical methods have been gained by the results of specifically dedicated research projects, mainly in the EU FP7 Project Nanolyse–Validation of methods for the detection and quantification of engineered nanoparticles in food (www.nanolyse.eu); in NanoDefine–an integrated analytical approach to implement the EC definition of nanomaterial (www.nanodefine.eu); and in the NanoRelease Food Additive (NRFA) project of the International Life Science Institute (ILSI) Center for Risk Science Innovation and Application, (http://www.ilsi.org/ResearchFoundation/RSIA/Pages/FoodAdditiveMainPage.aspx). Relevant results in terms of development and standardization of testing strategies and instruments for risk assessment, characterization, toxicity testing, and exposure measurements of nanomaterials are coming from the project NANoREG, a common European approach to the regulatory testing of Manufactured Nanomaterials (www.nanoreg.eu), aimed also to develop liaisons to global standardization and regulation institutions in countries like the United States, Canada, Australia, Japan, and Russia.

3.3 Determination of Size and Size Distribution of Nanoparticles

Size and size distribution of the particles are the driving properties in the EU regulatory framework of nanotechnologies. The assessment of these end-points allows investigating the question about the presence of NMs along the six exposure scenarios proposed by EFSA (see Section 4 and Fig. 6.3). Particle-sizing methods are based on (i) different physical principles, such as light diffraction and particle transport in various media, or (ii) electron microscopy. These different techniques rely on different theoretical bases for particle size and particle size distribution (eg, number, size, surface area, volume, or intensity-based), and their results are not directly comparable. For the current European regulation (The European Commission, 2011), particle size and particle size distribution have to be expressed in terms of number size distribution, therefore results obtained with many techniques [eg, dynamic light scattering (DLS), flow-field fractionation (FFF) coupled with inductively coupled plasma-mass spectrometry (ICP-MS), laser diffraction (LD), centrifugal liquid sedimentation (CLS)] require a conversion to be useful from a regulatory point of view. This generally can result in potentially large errors due to the assumptions regarding shape and density (Pena et al., 2014). A review has been recently published on the capabilities of currently available measurement methods for determining particle size measurements based on European Commission definition (Linsinger et al., 2012). Authors

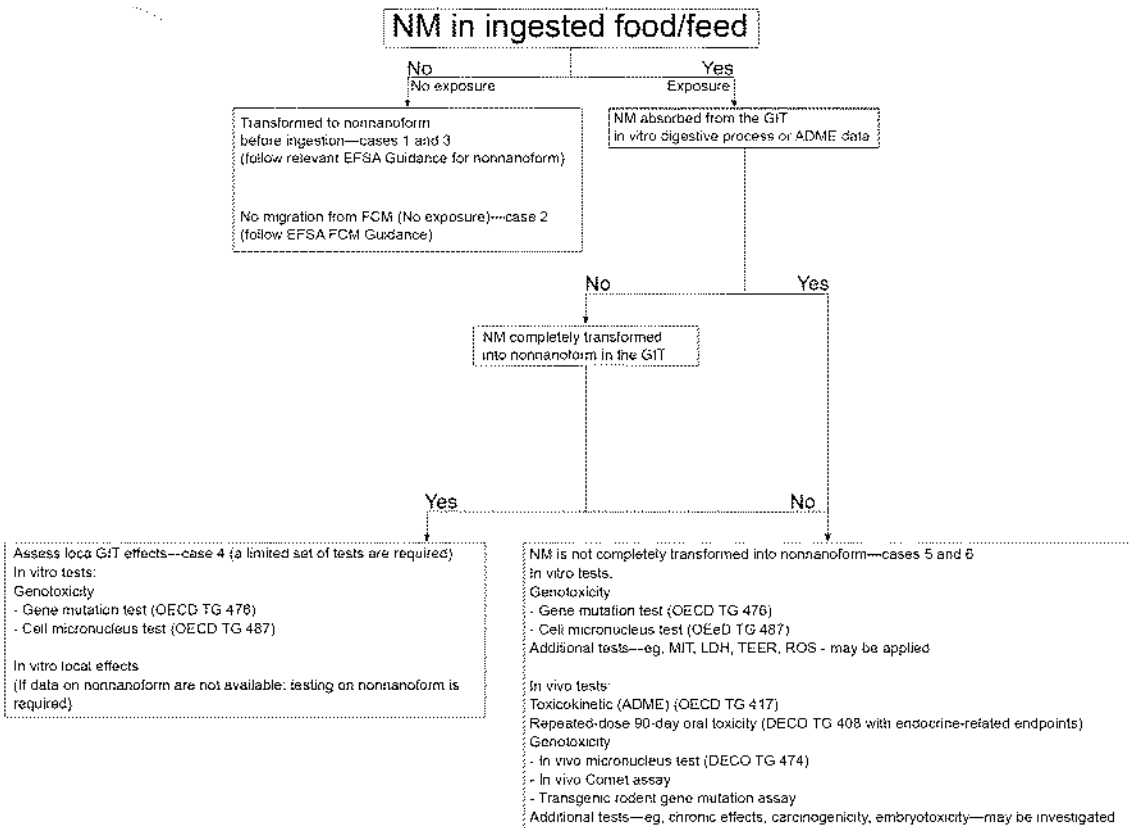

Figure 6.3. Decisional tree for testing toxicity with indicated in vitro and in vivo tests. The six exposure scenarios proposed by EFSA are also reported. Adapted from EFSA.

considered several aspects related to sampling, sample preparation, and quantification, as well as quality assurance and confidence in particle size measurements, and compared the Electron Microscopy (EM), LD, DLS, CLS, and BET methods. They concluded that EM is necessary for the application of the EC recommended definition because it is the only method able to distinguish between primary/constituent particles and aggregates/agglomerates, as well as to determine appropriate size parameters for nonspherical particles. However, Transmission EM (TEM) is an expensive and time-consuming technique and presents significant inherent difficulties in terms of preparing a representative sample of well-dispersed particles, image interpretation, and determination of appropriate size parameters. Therefore, it is not a commonly accessible technique, and some promising techniques are emerging for screening tests, such as FFF and Particle Tracking Analysis (PTA).

Currently many technical limitations still exist in assessing the size and size distribution of nanomaterials. For example, the size detection limit is significantly larger than 1 nm for many techniques: Peters et al. (2014b) found that for titanium dioxide nanoparticles it is 50 nm for single particle-ICP-MS and 20 nm for Asymmetrical Flow FFF-ICP-MS and routine Scanning EM analyses (SEM). The exclusion of such a relevant dimensional range (1–20 nm or more) introduces a certain and perhaps significant bias showing the inability of current state of the art methods to support the European Commission Recommendation for the definition of nanomaterials. Moreover, none of the available methods can be universally applied to all kinds of potential nanomaterials (organic and inorganic) nor reliably distinguish between a single large particle and an aggregate without a sample preparation procedure (Linsinger et al., 2012).

Nonetheless, EM is recognized as the most advanced and robust method able to provide information on particle size distribution as required by the EC definition (OECD, 2014a; Pena et al., 2014), and the only technique to characterize NM shape. Many studies have been done to improve the reliability of TEM and validate its methods. In particular, these studies focused on the quantitative characterization of agglomerates and aggregates (De Temmerman et al., 2012; Verleysen et al., 2014); the semiautomatic size measurement of primary particles in aggregated NMs (De Temmerman et al., 2014b); the assessment of uncertainties in size measurement, shape, and surface topology of near-monodispersed and near-spherical nanoparticles (De Temmerman et al., 2014a); and the determination of the volume-specific surface area (Van Doren et al., 2011). EM is largely applicable to the characterization of NMs in food (Dudkiewicz et al., 2011), including organic NMs after proper sample preparation (Peters et al., 2011). On the basis of EFSA recommendations, it should be considered as a benchmark method for NMs measurement in foods (EFSA Scientific Committee, 2011). Recently, the application of the two main EM methods (scanning and transmission) for measuring NMs in solid and liquid food matrices has been partially validated (Dudkiewicz et al., 2015). They found that both methods were able to measure (stable) NMs in food (chicken paste and tomato soup) with typically an expanded uncertainty of around 21–27%, while much greater expanded uncertainties may be expected for samples containing particles undergoing constant transformation (eg, aggregation and/or dissolution). From the analysis of the contribution of different analytical stages to the NM size measurement uncertainty, authors found that the major contributing step was sampling, while the number of measured particles and small sample intake were only secondary contributors.

3.4 Other Relevant Parameters

Chemical identity of NMs and quantification of their presence are relevant endpoints both for exposure and hazard assessment, though they have not direct implications in the regulatory framework. The most widely applied technique for characterizing NM chemical composition is mass spectrometry (Dudkiewicz et al., 2012). Its coupling with different ionization techniques such as ICP, Matrix Assisted Laser Desorption Ionization (MALDI), Electron Spray Ionization (ESI), and with appropriate separative methods as Size Exclusion Chromatography (SEC), Hydrodynamic Chromatography (HDC), and FFF, enable mass spectrometry to analyze both metal-based (Helfrich and Bettmer, 2011; Mitrano et al., 2012; Loeschner et al., 2013; Fabricius et al., 2014; Peters et al., 2014a), and organic nanoparticles (Helsper et al., 2013). Moreover, signal acquisition with an innovative mode called single-particle allows ICP-MS to assess size and size distribution of nanoparticles in suspension (Mitrano et al., 2012; Loeschner et al., 2013; Peters et al., 2014a).

Some of the methods for chemical characterization such as FFF-ICP-MS and sp-ICP-MS are suitable for assessing solubility of NMs, another regulatory relevant endpoint from the risk assessment point of view. Tantra et al. (2015) have recently reviewed the potential techniques for measuring NM solubility, evaluating their performance against a set of analytical criteria (ie, selectivity, accuracy, repeatability, sensitivity, commercial availability, robustness/ruggedness, and low-time consuming). The compared techniques were separative methods (eg, filtration, centrifugation, dialysis, ultrafiltration, high-performance liquid chromatography, ion exchange technology, capillary zone electrophoresis, and field-flow fractionation), detection techniques of free ions and labile fractions (eg, electrochemical methods, colorimetric and fluorimetric assays), and detection techniques for the measurement of total dissolved species [eg, ICP-MS and ICP-OES (optical emission spectroscopy), atomic absorption spectroscopy (AAS)]. The authors found that no universal method exists, since among the wide variety of techniques available none owns the capability to measure total dissolved species and free ions simultaneously, and they recommended a complementary measurement approach.

4 Hazard Identification and Characterization

The use of nanoscience and nanotechnology in food and feed chain requires a practical approach for assessing potential risks. As previously anticipated, EFSA developed a guidance of the risk

assessment of NMs in food and feed chain, including food additives, enzymes, flavorings, food contact materials, novel foods, feed additives, and pesticides (EFSA Scientific Committee, 2011). The toxicity testing strategy developed by EFSA relies on the six general cases, for which appropriate in vitro and in vivo studies for hazard identification and characterization are foreseen. For clarity, the proposed testing strategy is shown in Fig. 6.3.

The first three cases refer to the absence of exposure to NMs since they are completely degraded/solubilized to nonnanoform before ingestion (cases 1 and 2) or no migration from food contact materials to food occurs (case 3). Complete degradation/solubilization of NMs in nonnanoform materials—that is, ions, molecular or bulk forms—implies the application of EFSA Guidance for nonnanoforms for the specific intended use. On the contrary, cases 4, 5, and 6 regard the oral exposure of consumers to NMs. During digestion, NMs could undergo physico-chemical transformations depending on their chemical composition (Noack et al., 2012; Peters et al., 2012; Walczak et al., 2012; Böhmert et al., 2014; Yi et al., 2014; Walczak et al., 2015). A list of potential effects of digestive process on inorganic and organic NMs is shown in Table 6.4.

EFSA approach requires the evaluation of physico-chemical transformations occurring during digestion, with particular attention to degradation, dissolution, and solubilization. Several in vitro digestive models have been developed to simulate human gastrointestinal tract conditions. The use of in vitro models instead of in vivo ones depends on ethical, technical, and financial reasons. Indeed, they are in compliance with the 3Rs principle

Table 6.4 Potential Effects of Digestive Process on Chemically Different NM

Inorganic NM	Organic NM
Oxidative dissolution	*Enzymatic digestion* (*degradation*)
Surface passivation (*persistence*)	*Structure destabilization* (*degradation*)
Ion release (*solubilization*)	Interaction with small molecules
Surface charge modification	Surface charge modification
Protein adsorption	Protein adsorption
Interaction with small molecules	Active principle release

In the present table are listed some alterations occurring to inorganic and organic NMs during the digestive process. In italic are reported transformations affecting persistence of NMs along digestive tract, thus having an important role in the application of EFSA testing strategy. NM, nanomaterial.

(replacement, reduction, and refinement) on protection and welfare of animals. In vitro models present some advantages such as the standardization of experimental conditions, good reproducibility and repeatability, easy sampling, and the possibility to perform kinetics. On the contrary, they are not able to fulfill in vivo complexity. To date, harmonization between the different in vitro models used throughout Europe is still lacking, and there is the need to validate them with respect to in vivo data. Within the Cost Action INFOGEST (www.cost-infogest.eu), data on in vitro/in vivo (pigs) validation will be available by the end of 2015. In vitro digestive models vary from very simple to sophisticated, taking into account physiological parameters such as temperature, peristalsis, volumetric ratio and transition time, and digestive fluids composition (salts and enzymes). They can simulate different physiological conditions such as adult, kid, fasted, and fed. Flexibility is achieved by modulating chemical and enzymatic composition of artificial gastrointestinal fluids, and changing volumetric ratio and transition time through the different compartments. Chemical composition of simulated gastrointestinal fluids in different physiological conditions is also described in United States Pharmacopeia 33-28NF (2010) and European Pharmacopeia 7.0 (2010). Further, in vitro digestive process is a very useful method to study the behavior of nanocarriers during digestion, with special focus on the release of active ingredients.

Hazard identification and characterization of completely dissolved/degraded NMs during digestive process are based on data for the nonnanoform substance, as long as they are not absorbed before dissolution/degradation (case 4). In this case, a limited number of in vitro and in vivo tests are sufficient. Persistent NMs in food/feed matrices and gastrointestinal fluids require the testing approach proposed by EFSA and, when available (case 5), the comparison with nonnanoform information. This comparison is important to identify major differences between NMs and their nonnanoform, with the aim to establish if more toxicity testing is required. Indeed, further investigations are required in case of increased hazard or in the absence of nonnanoform information (case 6), while no further testing is needed when NMs are less toxic than their nonnanoforms.

To date, no validated in vitro methods are available for hazard assessment of NMs (Park et al., 2009). Conversely from nonnanoform, NMs may interfere with in vitro assays through optical interferences with the readout, adsorption of assay components or culture medium components onto NM surface, and NMs reactivity with assay molecules (Bregoli et al., 2013). Therefore, possible artifacts deriving from potential interferences should be

investigated. In the United States, the Nanotechnology Characterization Laboratory adapted some in vitro test protocols for NMs, such as MTT, LDH, and caspases 3/7 activation. Taking into account these limitations, in vitro testing may provide information on the hazards of NMs, giving indications of their potential toxicity and mode of action. Several ex vivo (eg, tissue explants as Ussing chamber) and in vitro models have been developed to simulate the gastrointestinal barrier. Among these in vitro models, differentiated human colorectal adenocarcinoma (Caco-2) cells cultured on semipermeable support represents the most reliable in vitro barrier model (Kirkpatrick et al., 2007; Kandárová and Letašiová, 2011). Caco-2 system is characterized by polarized cells with microvilli on the apical side and tight junctions between cells. Differentiated Caco-2 cells produces small intestinal hydrolase enzymes such as isomaltase, lactase, aminopeptidase N, and dipeptidylpeptidase IV (Audus et al., 1990; Artursson and Karlsson, 1991), cytochromes and active transport proteins such as *P*-glycoprotein (Chantret et al., 1988; Polli et al., 2001; Thiel-Demby et al., 2004). Caco-2 in vitro system permits researchers to assess barrier integrity and permeability using a number of parameters including cell viability (MTT assay), membrane damage (LDH leakage), *trans*-epithelial electrical resistance, paracellular flux, inflammatory mediators, and radical species generation. This system can be improved by coculturing goblet cells to secrete mucus, and microfold (M) cells specialized in taking up antigens, and transporting them to intraepithelial macrophages and lymphocytes for immunological response. Goblet cells and produced mucus are a very important determinant in NM uptake, while M cells that represent only 1% of intestinal cells are the major gateway through which materials from the nanosize range can be absorbed. Caco-2 system has become the gold standard to correlate drug permeability to oral drug absorption (Hidalgo et al., 1989), and both FDA and EFSA recommended this in vitro model. Caco-2 in vitro model is also recommended by EFSA for the evaluation of NM safety for food applications (Löbenberg and Amidon, 2000; Committee for Proprietary Medicinal Products, 2001; Yu et al., 2002; EFSA Scientific Committee, 2011). In parallel to safety evaluation, this in vitro model is also useful to investigate the efficacy of nanobased formulations in high-throughput screening fashion. Indeed, the application of nanotechnology to nutraceuticals would improve bioavailability and efficacy of active ingredients. Caco-2 system permits the investigation of apical-to-basolateral translocation of active ingredients, identifying absorption and secretion molecular mechanisms such as paracellular, trancellular, and persorption uptake and P-glycoprotein-mediated efflux (Fig. 6.4). The

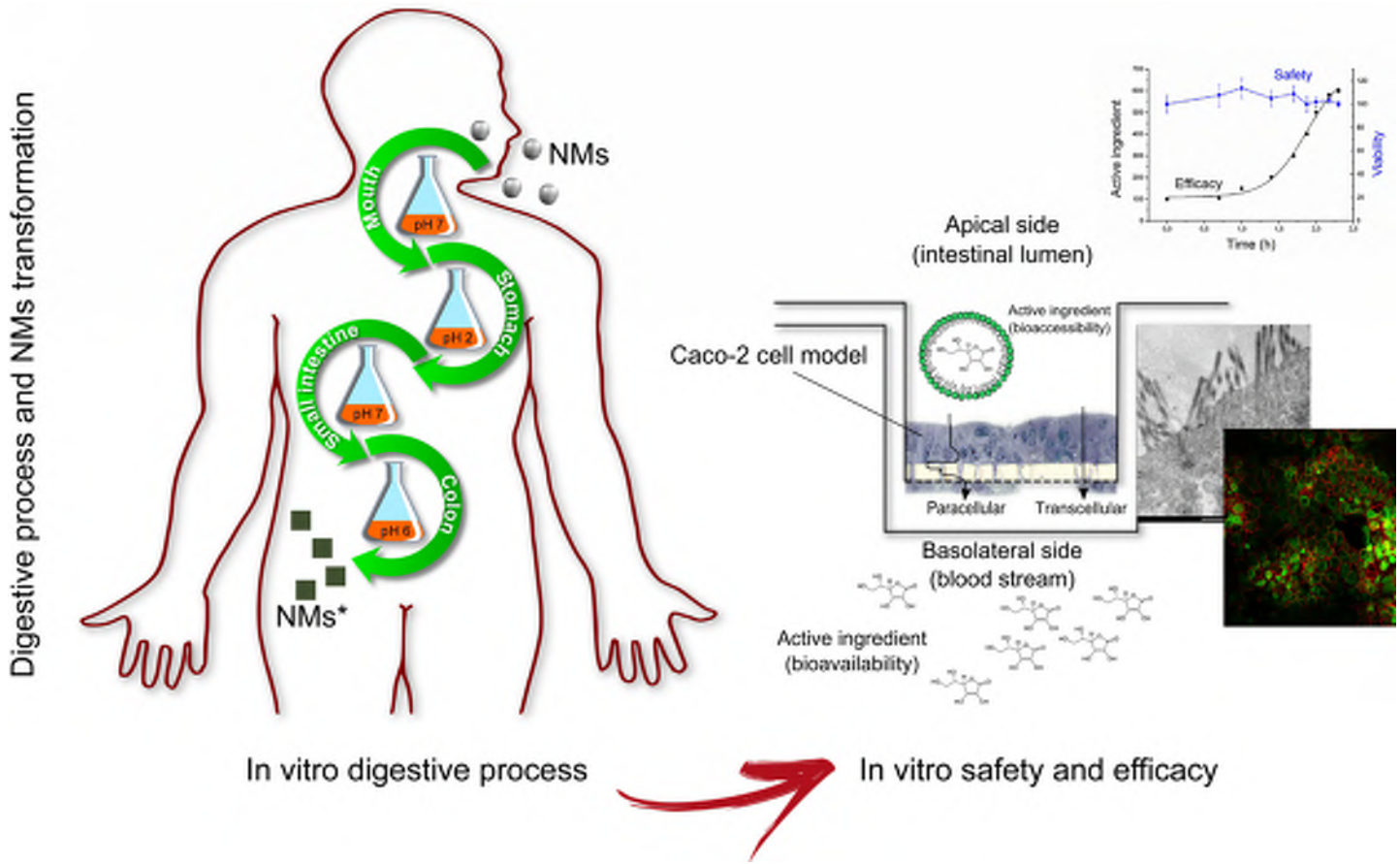

Figure 6.4. Schematic representation of the in vitro digestive process and Caco-2 system for evaluating safety and efficacy of nanotechnology-based nutraceuticals. NMs (*circles*) are ingested and they are exposed to transformations by digestive process (NMs*, *squares*). Caco-2 cells are a good intestinal-like epithelium model, since once differentiated they develop microvilli *(M)* and tight-junctions *(TJ).* They are cultured on semipermeable membrane with apical side representing intestinal lumen and the basolateral side corresponding to bloodstream. In the figure are reported the two main transport mechanisms: transcellular and paracellular. Caco-2 cell model is used for studying safety and efficacy of nutraceuticals, as represented by absorption and viability curves.

understanding of absorption and excretion mechanisms is important for improving formulation efficacy, and as a consequence on nutritional and health claims (The European Parliament and the Council of the European Union, 2006b).

To assess immune response to NMs, the whole blood assay can be adopted. This model provides information on immune responses—immunostimulation or immunosuppression—upon exposure to NMs (Langezaal et al., 2001a; Langezaal et al., 2001b). If in vitro results indicate altered parameters on cell viability, epithelial permeability, release of inflammatory mediators, and immune response compared to appropriate controls, in vivo studies should be considered.

Another important endpoint considered by EFSA in evaluating NM safety is genotoxicity. Two in vitro tests are required to investigate genotoxicity of NM: (1) in vitro mammalian cell gene mutation test, preferably the mouse lymphoma *tk* assay with colony sizing (OECD test guideline 476) (OECD, 1997); (2) in vitro mammalian cell micronucleus test (OECD test guideline 487) (OECD, 2014b). In the presence of reactive oxygen species, soluble or very small NMs, a bacterial reverse mutation test might be still informative. Positive results from in vitro genotoxicity tests as well as the presence of interferences require in vivo genotoxicity evaluation. The appropriate in vivo genotoxicity test should be chosen case-by-case. Any of the following tests may be suitable for investigating NM genotoxicity: (1) mammalian erythrocyte micronucleus test (OECD test guideline 474) (OECD, 2014c); (2) in vivo Comet assay; (3) transgenic rodent gene mutation assay.

In vivo testing is useful to generate information on ADME of ingested NMs, to study adverse effects (eg, genotoxicity) and determine dose-response relationships. Oral toxicity can be studied adding NMs to animal feed, to the drinking water, or by gavage. Each method presents some limitations that should be taken into consideration before starting in vivo experiments. While oral gavage guarantees a well-defined administered dose and permits the definition of dose-response relationship of possible adverse effects, it prevents transformations occurring during the first phases of digestive process. ADME studies are essential for evaluating safety of NMs, since they can have altered toxicokinetics and tissue distribution compared to non-nanoforms. Indications for toxicokinetics studies are described in OECD test guideline 417 (OECD, 2010c). For the main ADME study, a minimum of two NM concentrations should be used. These concentrations should be set with a pilot study in order to avoid highly toxic doses. The biological persistence of NMs in organs and tissues may correlate with long-term adverse effects. Additional studies

to investigate localization of NMs in mononuclear phagocyte system, such as liver and spleen, and in gut associated lymph tissue, as Peyer's patches and mesenteric lymph nodes, are important for understanding potential effects on immune responses. The minimum requirement for determining in vivo effects of NMs is a repeated-dose 90-day oral toxicity study in rodents, as described in OECD test guideline 408 (OECD, 1998), modified accordingly to OECD test guideline 407 (OECD, 2008) to include endocrine-related endpoints. Results from repeated-dose 90-day oral toxicity can be used to obtain benchmark dose lower confident limit (BMDL) or a no-observed-adverse-effect-level (NOAEL). Since these data come from laboratory species, additional tests for target species might be needed. Accumulation of NMs in tissues and organs may require long-term toxicity testing to reveal progressive toxic effects or delayed toxicity. Identification of major chronic effects, carcinogenicity, and dose-response relationships following prolonged and repeated exposure should be made following OECD test guideline 453 (OECD, 2009). Further studies on the impact of NMs on reproduction and development are described in OECD test guidelines 414, 415, and 416 (OECD, 1983, 2001a,b).

Besides hazard identification and characterization of NMs, the increased bioavailability of active ingredients due to nanotechnology-based delivery systems also requires a toxicological evaluation. The assessment of a nanoscale delivery system should include the analysis of the entire system, with and without active ingredient, and the active ingredient in free form in the food.

5 Concluding Remarks

Food industry is taking advantage from nanotechnology to improve taste, flavor, color, texture, and consistency of foodstuffs, as well as their quality and safety, and efficacy (ie, increase absorption and bioavailability of ingredients). In nutraceuticals, nanotechnology is mainly used to ameliorate bioaccessibility, bioavailability, and efficacy of active ingredients. Besides the beneficial effects, the use of nanotechnology in food and the feed chain raise safety concerns. Therefore, a science-based regulation is useful to support companies in developing new and safe products.

Generally, nutraceuticals safety is regulated by the same approaches and tools as general food legislation. While traditional and already authorized ingredients and foods only need a notification to put a product on the market, new products/ingredients (including authorized or traditional ingredients in nanoform) require specific information addressing any potential safety issue. Producers are therefore encouraged to voluntarily state that the

ingredient is in nano form, and to engage the competent authority. Concerning new foods/ingredients, the regulatory approach always includes a premarket authorization pending a full risk assessment (excluding some special cases). In the European Union, new foods/ingredients are regulated by Novel Food Regulation, but NMs are not explicitly included in this category. Upcoming regulations in the European Union will address this aspect, considering any food/ingredient in nano form (even modified versions of authorized ingredients) as novel food, and therefore subjected to a full safety assessment. In the meantime, safety assessment of foods/ingredients in nanoform should be done by following the spirit of the law, using the available guidelines (ie, EFSA guideline), and engaging the National Authorities to assure that all safety concerns are addressed.

A limitation in the safety evaluation of nanotechnology-based products is the lack of a clear definition of NMs. European Union has recommended a legally nonbinding regulatory definition based on the size and size distribution of particles in the material. These end-points are thus relevant to evaluate the law compliance in Europe, although they are not sufficient to perform the risk assessment of NMs used in food and food related applications as nutraceuticals. Conversely, other countries such as the United States and Canada consider NMs (besides the dimension generically lower than 100 nm) as materials exhibiting one or more nanoscale properties/phenomena, and require neither specific labeling nor specific procedure for their authorization.

Characterization of NMs is challenging per se, and even more when they are in complex matrices, such as foods and nutraceuticals, cells, tissues, and biological fluids as digestive fluids. The international scientific community is working for the improvement or the development of reliable testing guidance. Many analytical progresses have been achieved in the few last years due to large efforts spent in specifically dedicated research projects, although no validated characterization methods still exist at present. The main analytical issues to be addressed concern the improvement and validation of: (1) sample preparation procedures, both for solid and liquid matrices; (2) suitable methods for the assessment of size and size distribution over the complete size range considered by the NM definition, and complementary with EM (considered as benchmark technique for EFSA, JRC, and OECD); (3) techniques for characterizing organic NMs; (4) TG and standard operating procedures for the most relevant end-points and NMs. These goals will be completely reached after the proper development of standard reference materials, which will enable the quality assurance/quality control of the applied protocol.

As characterization of NMs is essential to define exposure, hazard identification and characterization represent another important aspect in safety assessment of nanotechnology-based products. EFSA developed testing approaches to identify and characterize hazards arising from NMs used in food applications. EFSA testing strategy is based on a decision tree with appropriate in vitro and in vivo tests at each stage. "Recommended testing protocols" refers to conventional methodologies (OECD TG) needing adaptations to study NMs. Many efforts are required to overcome in vitro models and tests limitations deriving from the peculiar NM properties. While in vitro models should be reproducible, reliable, and predictive, suitable in vitro tests should be free from interferences and artefacts. Consideration also needs to be given to what should be used as negative and positive controls. To date, several international projects, such as NanoValid (Developing Reference Methods for Nanomaterials) and NANoREG (a common European approach to the regulatory testing of manufactured nano materials) are working to develop reference methods for assessing NMs safety.

The accessibility for companies of rapid, inexpensive, and less labor-intensive or expertise-intensive analytical toolset and standard operating procedures will allow for the successful growth of safe nanotechnology in the nutraceutical sector.

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7

ELUCIDATING THE THERAPEUTIC POTENTIAL OF NUTRACEUTICALS

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1 Introduction

Globally, functional foods and nutraceutical products are becoming popular due to increased concerns of consumer for healthy foods. A substantial accentuation is given onto avoiding side effects of medicines by the use of nutraceuticals. Among them, essential oils of different spices, due to their rich bioactive components, find applications in diet-based therapies.

- Functional foods and nutraceuticals
- Extraction of bioactive components
- Encapsulation of nutraceutical compounds
- Bioactive components delivery
- Therapeutic potential of nutraceutical compounds

2 Functional Foods and Nutraceuticals

Currently, the establishment of links between health and food has been diverting consumer focus toward plant-based functional and nutraceutical products instead of synthetic medicines for curing numerous physiological disorders (Chauhan et al., 2013b). Changing lifestyles of people along with poor cultural habits have forced researchers to find diet-based therapies that are cost effective and safe. In this regard, functional foods and nutraceuticals not only fulfill nutritional requirements but also provide medicinal benefits. Nowadays, functional foods and nutraceutical products has become an integral component of diet owing to

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their therapeutic potential against various physiological disorders like cancer, hypercholesterolemia, cardiovascular diseases, and hyperglycemia. Researchers have confirmed that various phytochemical and bioactive component are present in these indigenous herbs and medicinal plants that ensure their medicinal attribute and thus are an important part of modern functional foods and nutraceuticals. Researchers have also determined that a substantial number of phytochemical, bioactive moieties, and antioxidant potential are present in herbal plants.

Through the development in scientific research and user consciousness, the basic idea of food is also shifting from just nourishing individuals to facilitating people in prevention of diseases. There is increasing concern in the consumer, researcher, and commercial health official about diet and health relationship. For this purpose, a trend toward utilization of functional foods and nutraceutical products has been building (Hu and Willett, 2002).

A nutraceutical is a substance that is either a food or part of food that provides health and medicinal benefits along with treating or preventing disease occurrence. Such substances may include dietary supplements, genetically modified designer foods, herbal products, isolated nutrients, and industrially processed foods like soups, beverages, and cereals. Currently, more than 470 functional foods and nutraceutical products are commercially available with proven health claims (Parthasarathy et al., 2008).

Nutraceuticals are food products that provide nutrition together with health and therapeutic interests including prophylaxis and cure of ailments. Phytochemicals are nutraceuticals that provide prevention of diseases such as cardiovascular, diabetes, high blood pressure, and cancer. Antioxidants deliver a helpful role in prevention of prolonged disorders (Hafidh et al., 2009).

Nutraceuticals occasionally cited as functional foods have prompted heated discussion as they blur the traditional separating line between medicine and food. Nutraceutical slightly differ from functional foods. When food is being prepared though scientific intelligence with or without awareness of why or how it is being used, the food is called as functional food. Thus, functional food delivers required amounts of fat, vitamins, carbohydrates, protein, and so forth to the body, needed for good health. When functional foods help in the avoidance and/or management of disorder and/or diseases other than anemia, then it is called nutraceutical. Most of the functional foods help in the cure of anemia in some way or the other, so anemia is considered the dividing line between functional foods and nutraceuticals. Vegetables, citrus

fruit, and fortified dairy products are some common examples of nutraceuticals (Raja et al., 2011).

Nutraceutical and functional foods are gaining much popularity and capturing the global market. The statistics analysis of market shows that the value of nutraceuticals and functional food were estimated above $196 billion and US$29–60 billion in Japan and the United States, respectively. These products are used not only to get health benefits, but also because they have preventive and curative effects for many ailments ranging from cardiovascular disease to cancer (Girgih et al., 2013).

The additions of processed foods, altering living patterns and unbalanced dietary behaviors, are the foremost reasons for physiological ailments and diet-related complexions like diabetes, cancer, CVD and high cholesterol level increasing day by day. Prevention of these disorders is a major public health concern worldwide, especially in developed and undeveloped nations. The involvement of phytochemicals in diet for disease prevention was widespread and documented from ancient times due to their safe and high pharmacological values (Chen et al., 2006).

The therapeutic potential of nutraceutical products has been proven by different studies. Nutraceuticals have been noted to have physiological advantage or deliver defense against several syndromes and/or found to act as cardiovascular mediators, antiobese causes, antidiabetics, anticancer agents, immune booster, chronic inflammatory relief, and relief from disorder degenerative disease. Among these ailments, common ones are obesity, CVD, high serum triglyceride level, declined level of high density lipoproteins (HDL), and hypertension tolerance (Misra et al., 2008).

3 Extraction of Bioactive Components

Plants synthesize bioactive components for their own defense, which are used to impart color, flavor, and acerbity to food products. These bioactive components are important in scavenging free radicals that are accountable for the rise in chronic multicausative maladies (Park et al., 2014).

Medicinal plant extracts, which contain phenolic terpenes, flavonoids, and phenolic compounds, exhibit properties of antimalarial, antiallergic, antioxidative, antineoplastic, antiinflammatory, anticancer, antiulcerogenic, antithrombotic, antimicrobial, and also assist in deterrence of cardiovascular and blood circulatory diseases (Rojas-Garbanzo et al., 2012). Thus, bearing in mind therapeutic effects and economic value of these

bioactive components, a much suited abstraction procedure should be adopted to extract these bioactive compounds from raw material.

Extraction may be defined as "separation of soluble from insoluble materials with the help of specific solvent." Extraction rates are controlled by the diffusion of solute at the interfacial layer of liquid. Extraction include removal of active components of plant from inactive portion with the help of suitable selective solvents in standard abstraction procedures (Sapkale et al., 2010).

For obtaining higher yield of active components from plant sources, different techniques of extraction are going to be studied and applied. There are different methods used for extraction of active components as follows:

- Infusion
- Percolation
- Decoction
- Maceration
- Digestion (Remington et al., 2006)

In Ayurvedic preparations like *arista* and *asava*, fermentation process is used for extracting the bioactive components. The process of extraction includes soaking of powder for a specific time period; in the meantime, it goes through the process of fermentation, resulting in the production of alcohol aiding in extraction and also acting as a preservative. The counter current method has been used for minimum amount of extract without applying higher temperature and at less time. Pulverization of the bioactive components is done by wet condition and water neutralizes the heat generated, avoiding exposure to heat for heat-labile components (Shah and Minal, 2013).

4 Supercritical Fluid Extraction

Powerful chemical solvents considerably decrease the market value and acceptance of compounds by consumers, and are driving researchers to use safe abstraction procedures. The best-suited technique for this purpose is supercritical fluid extraction in which supercritical fluids possess lower surface tension, viscosity, density, higher diffusivity in contrast with orthodox organic solvents used for plants components extraction (Abbas et al., 2008). Supercritical fluid extraction can be a clean, efficient, and fast method for abstraction of bioactive components from different natural products. Selective recovery of different components becomes easy due to simple operating conditions (Herrero et al., 2010; Mendiola et al., 2013).

The assortment of solvents for extracting bioactive components depends upon the nature of the aforementioned compounds. There are a variety of solvent systems available for extraction of these bioactive components, including dichloromethane for lipophilic compounds extraction, polar solvents—for example—methanol for hydrophilic compounds, and removal of chlorophyll is done by using hexane (Brusotti et al., 2011; Cesari et al., 2013).

5 Advantages and Disadvantages of SFE

SFE is environment friendly with sophisticated operating equipment that requires neither higher nor lower temperature and operates at ambient temperature. It also requires low amounts of solvents and shows advantages over other techniques by needing less time (Tonthubthimthong et al., 2001). Bioactive components from raw materials are extracted in nondegradable form and with no contamination by solvents. The extraction efficiency of oils with phenolic and bioactive compounds has been tremendously increased by SFE. Supercritical fluids solvation power can be adjusted by changing pressure and temperature both (Tsao and Deng, 2004).

Bleve suggested that this is a method best suited for abstraction procedures of heat-sensitive compounds and the end product is also solvent free (Bleve et al., 2008). From a laboratory or research point of view, where a small amount of sample is required and extracted by loading a few milligrams of sample with the possibility of higher volatile compounds concentration (Wang et al., 2008), the solvent used in SFE can be recycled and reused to lessen the cost and waste generation.

Posh equipment and high critical pressure are the most prominent disadvantages of SFE. The main snag of SFE is application of high pressure and its related perils that have impeded its further development. Because of the lipophilic and nonpolar nature of SC-CO_2, its performance can be curbed by polarity. To boost its performance, the use of cosolvent for increasing extraction selectivity and operating process at lower pressure (Brunner, 2005). In spite of day by day increment in application of SC-CO_2 for extracting valuable components from different sources, but SFE is still considered as novel technology because every new application requires a specific design, which is costly when compared to other conventional technologies that are more optimized (De Lucas et al., 2003) (Table 7.1).

Table 7.1 Applications of Different Essential Oil

Essential Oils	Applications
Artemisia afra, *Pteronia incana* oils, *Lavandula officinalis* and *Rosmarinus*	Preservatives in aqueous cream formulation
Australian tea tree oil	Recurrent herpes labials
Basil	Treatment for acne
Blend of the spices capsicum, cinnamon, anise, and oregano oil	Feed complement for piglets
Chamomile	Treatment of inflammatory dermatoses
Cinnamon, clove	Yogurt conservation
Cinnamomum zeylanicum (cinnamon), *Mentha piperita* (peppermint), *Ocimum basilicum* (basil), *Origanum vulgare* (origanum), *Teloxys ambrosioides* (the flavoring herb epazote), *Syrygium aromaticum* (clove) and *Thymus vulgaris* (thyme)	Inhibition of fungal development in maize
Cinnamon bark (*Cinnamomum zeylanicum*), lemongrass (*Cymbopogon flexuosus*), savory (*Satureja montana*), Roman chamomile (*Chamaemelum nobile*), rosewood (*Aniba rosaeodora*), spearmint (*Mentha spicata*) and tea tree (*Melaleuca alternifolia*)	Selected bacteria, fungi, and viruses
Cymbopogon citratos	Inhibitor of storage fungi
Eucalyptus citriodora, *Eucalyptus hybrida*, and *Ocimum basilicum*	Nematicidal activity
Laurus nobilis, *Eucalyptus globulus*, and *Salvia officinalis*	Preservatives in O/W skin cream, a hydrogel and a nonalcoholic hydrolyte
Lavender	Bites, stings, boils, burns, stretch marks, rashes, spots, cold sores, sunburns, allergies
Lemongrass	Stored grain protection
Oregano	Fish food conservation
Pyrethrum and neem	Pesticides
Rosemary, sage	Commercial food preservative
Tea tree oil and eucalyptus oil	Herpes simplex virus type 1
Thyme	Nosema disease in honeybees
Vetiver essential oil	Protection of wood against termites

Source: Martín et al. (2010)

6 Encapsulation of Nutraceutical Compounds

To prepare a nutraceutical product containing bioactive compounds means facing a lot of technological challenges. A technology named microencapsulation provides a promising tool to control the release of bioactive components and assist in preparing functional foods and nutraceuticals. Microencapsulation may be defined as "the coating of a substance (solid, liquid or in gaseous form) by wall material into microcapsule that provide stability and help in control release of bioactive components." Such technologies assist the food technologist to produce functional nutraceuticals. Microencapsulation is used to control color, flavor, preservation properties, or texture along with addition of bioactive ingredients providing health benefits. Addition of bioactive components presents challenges like stability of bioactive ingredients along the path of food supply chain. Microencapsulation technique is also helpful in fulfilling requirements of control release of bioactive components during the passage of gastrointestinal tract.

For the preparation of nutraceutical and functional foods, sometimes it is suggested that addition of bioactive compounds may not affect the sensory properties of food, for example, flavor and color.

7 Microencapsulation Technologies Used for Bioactive Food Ingredients

7.1 Microbial Products

Probiotics may be defined as "upon administration of proper quantity of microorganism into food, if health benefits are provided to host, the additives are called probiotics." Addition of probiotics to food for making nutraceuticals are at the forefront especially in dairy products. Preparation of nutraceutical products containing probiotics presents two problems: size and probiotics must be kept alive in nutraceutical products for proper health benefits. There are five different technologies available for encapsulation of probiotics: emulsion, spray-drying, spray-coating, gel particle technologies, and extrusion. Many techniques for microencapsulation are based on spray drying due to liquefied encapsulating or bioactive compounds. In the process of spray chilling, the matrix that contains bioactive components is atomized to form fine droplets as in spray drying. Cold air was injected into vessel for the

solidification of gel particles, it discriminated it from spray drying in which hot air is injected to form fine powder. Bioactive components are entrapped into matrix due to solidification.

The most common used technologies for microencapsulation are gel particle and spray coating (Ubbink et al., 2003; Doleyres and Lacroix, 2005). Solidified core material is kept in motion by the injection of air during spray coating. Core material is coated by liquid coating material by spraying and later solidification process causes formation of coating material layer on core material. The least expensive encapsulation technology for production of gel particles is spray chilling (Gouin, 2004). Unfortunately, very little research has been conducted for utilization of spray chilling and it deserves more attention because of the generation of smaller beads, which might be desired by food industries. Beside the gel particle and spray-coating technologies, spray drying, emulsion, and extrusion have also been used for microencapsulation of probiotics. Of these three, spray drying is more feasible because the other two are not easily applied to probiotics due to larger particle size.

7.2 Nonmicrobial Products

Spray drying is the most commonly used technology for encapsulation of fatty acid and vitamins (oil based) (Augustin et al., 2001; I Ré, 1998). A delivery system can be achieved by many emulsion (W/O/W or O/W/O) that present novel encapsulation and delivery properties. The bioactive components can be coated within inner phase, which may be oil or water as accords with the situation. By the use of many emulsions, a single delivery system may provide multiple bioactive components. Coacervation and cyclodextrins are the technologies that are used rarely for microencapsulation of nonprobiotics components (Hedges et al., 1995).

8 Bioactive Components Delivery

8.1 Microbial Products

Nutraceuticals containing encapsulated probiotics offer several technological benefits. Recent research has shown that alginate encapsulated cultures present more resilience to the gastric environment, maintain integrity and are released in GI tract (Chandramouli et al., 2004; Sriamornsak, 1999; Iyer et al., 2004). So, encapsulation not only improves delivery of probiotics but also improves survival and release at target site. The available nutraceuticals containing encapsulated probiotics are fermented milk, yogurt, and cheese, which are expected to present opportunities

for innovative dairy nutraceuticals (Hayes et al., 2006). Free cells of powder get hydrated when entering the stomach and high viability losses of free cells explain the importance of encapsulation in nutraceuticals. Encapsulation presents a potential for nutraceuticals by reducing the effect of food matrix on viability of culture during food preparation and consumption. From this point of view, encapsulation proves to be a miraculous technology. Results have shown that viability of probiotics is affected greatly by freeze drying and production processes that are being introduced into vegetable juices, biscuits, and frozen cranberry (Reid et al., 2007). Encapsulation may also be used to entrap prebiotics and improve the possibility of encapsulation for delivery of multiple bioactive components. However, research has suggested that the minimum quantity of prebiotics for significant effect on viable culture in gastrointestinal tract is 3 g, which is rarely achieved by encapsulated products. That's why encapsulating prebiotics did not seem highly appreciated. On the other hand, encapsulating technology may be used to coat antioxidants, immune-enhancing polymers, or peptides. Antioxidants with coencapsulation could be a great combination as antioxidants provide protection to the viability of culture media, for example, bifidobacteria, which show sensitivity to oxygen. Coencapsulating material like bacteriocins can also improve antimicrobial activities of probiotics. In short, coencapsulation proves not only to enhance possibility of delivery of multiple bioactive components but also to provide selectivity in different ingredients interactions (Champagne et al., 2008).

8.2 Nonmicrobial Products

Fluidized bed coating and spray chilling for encapsulation of water-soluble vitamins and spray drying for fat soluble vitamins are recommended. Unfortunately, Glass transition properties of encapsulating material were not given proper attention in performing spray drying encapsulation. It is believed that these properties are crucial for providing stability to the products and low-water-mobile matrices are generally recommended. Amorphous glassy matrices that have low water mobility slow down the infusibility of oxygen and may oxidize the bioactive components (Table 7.2).

Encapsulation enhances the delivery of minerals and vitamins by avoiding interaction of these compounds with other food ingredients; for example, iron bioavailability is severely affected by interaction with antinutrients present in food (phytates, tannins, etc.). Moreover, iron also catalyzes rancidity of fats and vitamins (Champagne and Fustier, 2007).

Table 7.2 Beneficial Effects of Probiotic Microencapsulation

Benefits	Products
Improved survival on heating	Biscuits, powder
Improved survival during storage	Yogurt, mayonnaise, milk
Protection against yeast contaminants	Fermented milks
Improved stability during storage in dried form	Nutraceutical
Improved survival on freezing	Ice cream, milk-based medium, cranberry juice
Facilitates the recovery of centrifugation-sensitive cultures	Dried probiotic culture
Improved retention in the finished product	Cheese
Improved acidification rate	Dried sausages
Improved survival on exposure to gastric solutions	Nutraceutical
Protection against bacteriophages	Fermented milks
Facilitates the production of oxygen-sensitive	Dried probiotic culture
Improved survival on exposure to bile solutions	Nutraceutical
Less contamination problems	Dried probiotic culture
Facilitates the recovery of high EPS-producing cultures	Dried probiotic culture
Cultures can be air-dried	Dried probiotic culture

Source: Champagne and Fustier (2007)

9 Therapeutic Potential of Nutraceutical Compounds

Food and medicine originating from the same source have similar benefits for human beings; however, the natural foods also have the ability to overcome the diseases as pharmaceuticals. Currently, the establishment of links between health and food has been diverting consumer focus, toward plant-based functional foods and nutraceuticals products instead of synthetic medicines for curing numerous physiological disorders (Chauhan et al., 2013a). In this regard, functional and nutraceuticals are becoming popular all over the world owing to their health-promoting perspectives. The potential of nutraceuticals and food supplements for mitigating health-related risks like hyperlipidemia, hyperglycemia, cardiovascular diseases, and cancer are widely documented.

Plant-based foods rich in polyphenols are gaining importance due to their effectiveness against health-related disorders.

Functional foods and nutraceuticals not only fulfill nutritional requirements but also provide medicinal benefits. Nowadays, functional foods and nutraceuticals have become an integral component of diet owing to its therapeutic potential against various physiological disorders like cancer, hypercholesterolemia, cardiovascular diseases, and hyperglycemia. Researchers have confirmed that various phytochemicals, bioactive components, and antioxidant potential are present in these indigenous herbs and medicinal plants that ensure their medicinal attributes. Among herbal plants, cardamom has potential against cardiovascular diseases and is thus an important part of modern functional foods and nutraceuticals.

Through the development in scientific research and increase in user consciousness, the basic idea of food is also shifting from merely nourishing the individual to facilitating in hindering diseases. There is an increasing concern in the consumer, researcher, and commercial health official about diet and health relationship. For this purpose, the trend toward functional foods and nutraceuticals shows increasing activity in recent years (Hu and Willett, 2002).

The additions of processed foods altering living patterns and imbalanced dietary behaviors are the foremost reasons for physiological ailments and diet-related complexions like diabetes, cancer, cardiovascular diseases, and high cholesterol levels that are increasing day by day. Prevention of these disorders is a major public health concern worldwide, especially in developed and developing nations. The widespread involvement of phytochemicals in diet for disease prevention is documented from ancient times due to their safe and high pharmacological values (Liu et al., 2007).

Recent research has proven the therapeutic properties of diet to improve human health. Nutraceuticals have been known to have physiological advantages or deliver defenses to counter several syndromes like cardiovascular mediators, antiobese causes, antidiabetics, anticancer agents, immune booster, chronic inflammatory, and disorder degenerative disease. Among these ailments, common ones are obesity, cardiovascular diseases, high level of low density lipoproteins (LDL), high triglyceride level, declined level of high density lipoproteins (HDL), and hypertension tolerance (Misra et al., 2008).

Worldwide, the burden of diseases is rapidly increasing, such as cardiovascular diseases, cancer, diabetes, and obesity. Cardiovascular diseases are the name for the group of disorders of heart and blood vessels, hypertension, coronary heart disease,

cerebrovascular disease, and heart failure. In 2010 cardiovascular diseases alone contributed to a third global death in developing countries. Majority of the CVD are preventable and controllable. Thus, the researchers are taking a keen interest in the identification of natural remedies to handle the physiological disorders.

10 Cardamom

Cardamom refer to herbs *Amomum subulatum* (black) and *Elettaria cardamomum* (green) that belongs to family *Zingiberaceae*. Among spices, cardamom is ranked third after saffron and vanilla. It is native to southwestern parts of the Indian Peninsula and also grown in Sri Lanka, Thailand, Guatemala, Laos, Nepal, Vietnam, Costa Rica, El Salvador, Mexico, and Tanzania. Cardamom seeds are used to provide distinctive flavor and fragrance in various foods. It is used not only as flavor modifier but also as folk medicine and food preservative. The seeds give spicy sweet flavor and the bioactive constituents mainly 1,8-cineole, and α-terpinyl acetate contribute to its pleasant and pungent aroma. The bioactive components present in the essential oil of cardamom, have been reported to exhibit antiinflammatory, hypocholesterolemic, hypoglycemic, and antimutagenic activities. Recent studies have demonstrated that cardamom oil contains various bioactive components such as 1,8-cineole (10.7–28.4%), α-terpinyl acetate (21.3–44.3%) and linalool (6.4–8.6%). The essential oil consists of monoterpenes (97.6%) instituting α-terpinene (2.2%), 1,8-cineole (89.6%), and *cis*-ocimene (3.7%). 1,8-cineole was found in all samples ranging from 29.4% in heated to 89.4% in unprocessed oil sample. Extracted oil is sensitive to heating as temperature about 110°C produces citronellol, linalyl acetate, limonene-1, 2-epoxide, Linalool, thujyl alcohol, *trans*-pinocarveol, and nerol takes place that effect quality. Similarly, generation of linalyl acetate (17.8%) and borneol (12.1%) increases when oil is exposed to sunlight. The chemical analysis of cardamom seed powder on dry weight basis enumerates its constituent's, that is, moisture (9%), protein (12.72%), crude fiber (8.50%), starch (49.05%), volatile oil (8.79%), and ash (6.97%). Cardamom is said to be loaded with a variety of health-promoting nutrients such as iron, calcium, and phosphorous.

10.1 Antibacterial/Fungal Activity

Cardamom extract has shown significant antibacterial activity and is also very useful in the discovery of novel antibiotics. The essential oil of cardamom exhibits durable antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*,

and *Salmonella typhi* microorganisms (Singh et al., 2008; Kaushik et al., 2010). Similarly, food-borne fungi *Aspergillus terreus* activity was inhibited by methanol and ethanol extracts of cardamom so it has strong antifungal activity. Cardamom extract exhibited antimicrobial activity against oral pathogens like *Candida albicans* and *Streptococci mutans*. Hence, cardamom can be used as a caries preventive agent as it stimulates salivary flow (Aneja and Radhika, 2009).

10.2 Large Cardamom Activity as Antidiabetic

Diabetes is an abnormal rise of glucose level of blood. Diabetes is of two types: insulin dependent and insulin independent. Mostly, it has been considered that glucose and fructose malabsorption occur due to additional fructose in the diet and superior raises in TG and cholesterol matched to other sugars. The key position is the capability of fructose to avoid the chief controlling stage of glycolysis, the change of gluctose-6-phosphate to fructose 1,6-bisphosphate, controlled by phosphofructokinase. Therefore, glucose breakdown is damagingly controlled by phosphofructokinase. So, fructose can yield glucose, glycogen, lactate, and pyruvate, provided that both the glycerol and acyl shares of acyl-glycerol particles (Verma et al., 2010).

These specific substrates, and the subsequent extra vitality flux due to free fructose breakdown, will stimulate the overproduction of TG and shrinkage of β cells of islets of Langerhans. *A. subulatum* extracts exposed a noteworthy increase of serum insulin levels, and advanced decrease in hyperglycemia when compared with the diabetic control rats. The histological revisions of the endocrine section of pancreas of diabetic animals show the decline of β cells of islets of Langerhans. *A. subulatum* exposed restoration of β-cells and have substantial possessions on harmful overproduction of glucose. This action might be due to occurrence of phenolic-like protocatechuic acid that renovates the β-cells (Vavaiya et al., 2012).

10.3 Cardio-Adaptogen Activity

A. subulatum has a defensive effect against severe or acute anxiety persuaded myocardial indemnities (Verma et al., 2010). Consistent ingesting of large amounts cardamom may consequently be valuable in treatment for patients with ischemic heart disease, facing steady traumatic situations.

10.4 Cardamom Antioxidant Activity

Cardamom contains phenolic and flavonoid phytochemicals, which are essential for good health in biological systems. The

essential oil and oleoresin showed significant antioxidant activities against all assay, such as peroxide, thiobarbituric acid, p-anisidine, total carbonyl, ferric thiocyanate, and the 2, 2′-diphenyl-1-picrylhydrazyl radical scavenging method due to presence of total phenolic contents. Owing to its antioxidant properties, cardamom can increase levels of glutathione and antioxidant enzymes in the body. The essential oil has better antioxidant activity by comparing with butylated hydroxytoluene (Kapoor et al., 2008).

Antioxidants interfere with free radicals production and ultimately inactivate them, thus protecting the biological systems against deleterious effects of oxidative processes. These substances are mostly natural products like ascorbates, carotenoids, tocopherols, polyphenols that subsidize the prevention and cure of diseases owing to oxidative stress. Recently, curiosity regarding plant-based food spices has grown mostly artificial antioxidants experiences from numerous disadvantages. Additionally, plant extracts have been revealed to retain health-protective assets. In this study, hydro-distilled extracts from cardamom, cumin, and ginger were judged for iron (II) chelation, 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity, total phenol content, antioxidant (iron III) reduction, prevention of linoleic acid peroxidation, and shyness of 2-deoxy-D-ribose degradation of hydroxyl radical-mediated site and nonsite-specific activities (Deeba et al., 2009).

The extract quintessence product of the methanolic extracts was predictable to be 9.8% w/w, which is brown in color and with oily texture. Early screening examination of the powdered methanolic seed extracts revealed the presence of tannins, carbohydrates, flavonoids, alkaloids, cardio active glycosides, terpenes, and saponins. Presence of phytochemicals in large cardamom provided the antibacterial effect against many pathogenic infections, that is, bacterial infections (Tijjani et al., 2012).

The preliminary phytochemical and pharmacological studies on *Amomum subulatum* were done to explore its beneficial effects. The preliminary phytochemical studies, macroscopic and microscopic characters, physicochemical assessment, and pain-killing action of the kernels estimated. These clarifications would be of huge worth in the botanic credentials and directive of the medication in crude system and would help differentiate the drug from its other species. Phytochemical calibration constraints such as moistness content, total ash, alcohol soluble, water soluble, and water decipherable extractives were observed. Initial documentation of phyto elements was done. GC-MS study of the volatile oil attained from the kernels was conceded out and seven compounds were parted. The studies of mass fragmentation exposed the incidence of caryophyllene and 1,8-cineole in the volatile oil.

Ethyl acetate extract and methanolic extract of seeds of plant were inspected for painkilling activity by hot plate method and thrashing method (Shukla et al., 2010).

Ethanol and aqueous extracts assessed for the antioxidant activity by the β-carotene bleaching assay, 1,1-Diphenyl-2-picrylhydrazyle (DPPH) free radical scavenging activity and total phenolic substances procedures. Ethanol extract presented additional antioxidant activity than aqueous extract. Ethanol extracts total phenolic contents and antioxidant activity showed significant result respectively as compare with ascorbic acid and BHA. This study presented that the cardamom ethanol extract consumption could possess useful effects due to its antioxidant activity (Khare et al., 2012).

Cardamom extract comprises of phenolic and flavonoid combinations that showed antioxidant activity. Flavonoid contents, higher phenolic, and important antioxidant activity showed mostly ethyl acetate extracts in the entire sample. Ethyl acetate extract was highly effective in scavenging DPPH compared to standard antioxidant that of due to high phenolic content. Inferior IC value indicates higher DPPH radical scavenging property of the four cardamom varieties. Cardamom has higher reducing power, higher phenolic flavonoid contents, and antioxidant activity. The scavenging result of extracts was of countless standing in biological system. Convincingly, this spice is appreciated cause of a number of natural flavonoids and phenolic contents, which are vital for fitness (Kamatani et al., 2010).

10.5 Cardamom Antihyperlipidemic Activity

The decrease in LDL-cholesterol has been considered to lower the serum cholesterol. It might be due to reduction in hepatic cholesterol biosynthesis, perhaps via lessening the action of 3HMG-CoA reductase. The aforementioned enzyme catalyzed the rapid restraining of cholesterol synthesis. Ultimately, it triggers the amplified expression of functional LDL receptors on cells that dribs serum cholesterol (Adler and Holub, 1997).

Coping with hyperlipidemia and oxidative stress, *A. subulatum* essential oil may have beneficial effects. It may possess oxidative stress by activating antioxidant enzymes that may reduce unfavorable oxidation of LDL cholesterol. Essential oil treatment has increased HDL ratio while triglyceride, total cholesterol, phospholipid, LDL and VLDL in the serum were significantly lowered (Joshi and Joshi, 2007). The cardamom extract have antiatherosclerotic potential as it causes a decrease in total cholesterol, triglyceride and phospholipid insides of aortic tissues (Liao et al., 2000).

The antiatherogenicity of *A. subulatum* extract might be accredited to its straight antioxidative properties. It has the capacity to trigger antioxidant enzymes that can help to inhibit certain diseases such as atherosclerosis, which can be carried on by oxidizing low-density lipoprotein cholesterol. Recent study showed an important upsurge of TBARS (measurement of lipid peroxidation) action in aorta by cardamom extract. The high level of TBARS in the aortic stenosis is a famous key of lipid peroxidation that highlights the incidence of vascular oxidation. Results exposed that administration of *A. subulatum* extract along with cholesterol triggered major decline in the level of TBARS in aorta representing antilipid per oxidative nature of plant extract (Austin, 2000).

Glutathione (GSH) is a tripeptide of amino acids that is vital to uphold physical and functional veracity of the cells. GSH is a main free radical scavenger that improves the defense from oxidative damages as a result of *A. subulatum* extract actions. Likewise, a noteworthy growth in catalase movement after concurrent direction of *A. subulatum* was experiential in aorta of rabbits as matched to hypercholesterolemia rabbits. Triglycerides are an important contributor to cardiac disease. Lipid nourishment raises the absorption of serum triglyceride, which can be due to decrease in lipoprotein lipase (LPL) movement, an enzyme which is important in the uptake of triglyceride rich lipoproteins by additional hepatic flesh. Hydrolysis of triglycerides of VLDL and chylomicrons to produce glycerol and free fatty acids is initiated by lipase. *A. subulatum* extract administration causes decrease in serum triglycerides at the diverse amounts that effect reduction in VLDL synthesis or rise in LPL activity resulting in dropping of triglycerides (Inoue et al., 1999).

10.6 Cardiovascular Diseases

Cardiovascular disparities are the major cause of mortality around the world. Unhealthy lifestyle and poor dietary habits lead to progression of various maladies like hypercholesterolemia, hypertension, coronary heart disease (CHD), and heart failure. Cardiovascular diseases occur due to inflammation of blood vessels, smoking, high levels of sugar and cholesterol in blood. Oxidation of low-density lipoproteins in cholesterol initiates the endothelial dysfunction resulting in the formation of complex plaques. These plaques cause resistance to arterial blood flow thus reducing the flow of oxygen-rich blood to the heart resulting in angina (chest pain). When plaque ruptures, the blood cells (platelets) stick to the site of injury and clump together to form blood clots. These

blood clots can further narrow the coronary arteries and worsen angina, ultimately causing heart attack (Hansson, 2005; Murphy et al., 2013).

A class of diseases that involve hearts and arteries or both is called cardiovascular diseases. It refers to any disease that leads to malfunction of the cardiovascular system, especially peripheral arterial disease, cardiac disease, and vascular diseases of the brain and kidney. Hypertension and atherosclerosis are the common causes among many other diverse causes. With the passage of time, a lot of morphological and physical changes lead to increases in the chances of cardiovascular diseases by affecting cardiovascular systems (Jamal et al., 2005). Almost 60 million U.S. citizens have high blood pressure or hypertension, representing a large contribution to heart diseases (Kamatani et al., 2010).

Avoidance of smoking tobacco, exercise, and healthy eating are recommended to decrease the occurrence of cardiovascular diseases. Therefore, functional foods, nutritional supplements, and nutraceuticals have received extensive consideration from scientists. Many important cardiovascular risk factors are modifiable by lifestyle change, social change, drug treatment, and prevention of Serrano's Cardiac Triad: hypertension, hyperlipidemia, and diabetes.

10.7 Pharmacological Potential/ Ethnobotanical Uses

Conventionally, *A. subulatum* has been used in baked food items as a spice by people on a daily basis and has proved to have a preventive role in the occurrence of gastrointestinal disorders and respiratory problems. Its specific aroma increases palatability and renders foods more digestible that ultimately serves to maintain good health. The seeds are certified for medication in the Ayurvedic pharmacopoeia and are advertised under the name "large cardamom" (Rahmatullah et al., 2009). Large cardamom also has been used for the treatment of throat trouble, irritation of eyelids, peptic illnesses, and lung tuberculosis (Verma et al., 2010).

Cardamom used in situations like dyspepsia, nausea, inflamed spleen, stomach pains, and rectal disease, and mouth contagions. For the heart and liver, the kernels extract is used as a tonic and also cures bowel astringent and has mouthwatering properties. Large cardamom has analgesic effects and its oil has the potential to reduce inflammation problems. The decoction of the seeds is useful as a gargle to cure infections of teeth

and gums while the pericarp is useful in headache and stomatitis (Shukla et al., 2010).

- Antimalaria
  The mixture of cumin (*Cuminium cyminum*) and large cardamom (*Amomum subulatum*) used in treatment of malaria (Thakur et al., 1989). The seeds are also useful as an antidote to scorpion sting and snake bite (Bisht et al., 2011). Ashes of burned leaves are mixed with mustard oil and taken twice daily for cough and sexually transmitted diseases (Rahmatullah et al., 2009).
- Antidote to snake venom
  The usage of large cardamom has been reported as antidote to snake and scorpion venom (Parthasarathy et al., 2008)
- It has been used for treatment and control of mastitis in Pakistan when given orally to dairy animals (Dilshad et al., 2010).
- One teaspoon of fruit/seed powder of *A. subulatum*, if taken with honey twice a day, will be beneficial to patients suffering from ischemic heart diseases (Sarkar, 1986).

On a daily basis, seeds and fruits of black cardamom are used to bake food stuffs and can play a defensive role in the incidence of stomach ailments, breathing difficulties, and by increasing deliciousness and taste of foods makes them more palatable and aid in sustaining health (Rahmatullah et al., 2009).

11 Cinnamon

Cinnamon (*Cinnamomum zeylanicum*), belonging to family *Lauraceae*, has been utilized as a potential therapeutic agent in various cultures for centuries. Historically, cinnamon bark is among the oldest known spices used against gastrointestinal complaints, chronic bronchitis, and inflammation of eyes in Ayurvedic medicine for over 6000 years. Commercially important volatile oils attained from the bark and leaf of *Cinnamomum zeylanicum* offer a number of volatile components that vary considerably in their chemical composition and are broadly classified into sesquiterpenes, monoterpenes, and phenylpropenes. The major constituents of cinnamon bark oil includes cinnamaldehyde (75%), cinnamyl acetate (5%), caryophyllene (3.3%), linalool (2.4%), and eugenol (2.2%). Cinnamaldehyde (3-phenyl-2-propanal) represents the main constituent of the cinnamon bark oil that contributes to about 49.9–62.8% of the total amount. It provides protection against metabolic syndromes such as cardiovascular complications and diabetes.

11.1 Antioxidant Activity

Generally, food deterioration is caused by lipid rancidity. An antioxidant property of cinnamon inhibits lipid rancidity and used in food as a preservative for many centuries (Wu et al., 1994). Cinnamon polyphenols possess a strong antioxidant potential, which is attributed due to the free radicals scavenging activity, modulatory effect on xenobiotic bio activation, and detoxification processes and inhibition of certain prooxidant enzymes, like lipoxygenases and nitric oxide synthase. Moreover, it induces the activity of various glutathione dependent antioxidant enzymes like glutathione reductase, glutathione S-transferase, and glutathione peroxidase.

11.2 Antidiabetic Activity

For the treatment of diabetes, cinnamon is reported to have curing effects in Ayurveda and folk medicines. Moreover, it also helps in the reduction of level of blood glucose in diabetic patients of noninsulin-dependent type. In the recent studies, potential therapeutic effect of cinnamaldehyde has been proven as an antidiabetic agent. It improves the functionality of insulin receptors by virtue of enzyme activation (insulin receptor kinase), which is responsible for insulin binding to the cells. Moreover, it is responsible for hindering the enzyme activity that impedes this process (insulin receptor phosphatase), which ultimately leads to the maximum phosphorylation of insulin receptor, which is associated with improved insulin sensitivity (Parthasarathy et al., 2008).

11.3 Hypolipidemic Potential

Hypolipidemia is a major risk factor for cardinal issues due to increased serum lipid levels resulting in atherosclerosis and cardio vascular diseases. Hypolipidemic effect of cinnamaldehyde is mainly due to its influence on dietary fat absorption and cholesterol transportation. Cinnamaldehyde administration in STZ-induced diabetic male wistar rats lowered the serum total cholesterol and triglycerides levels from 246.7 to 113.5 mg/dL and 38.0 to 17.5 mg/dL, respectively. Increments in HDL levels ranged from 38.5 to 54.3 mg/dL in study group. HDL being an antiatherogenic lipoprotein acts as a protective factor against coronary heart diseases by transporting cholesterol into the liver. The increase in HDL-cholesterol is attributed to the improved activity of lecithin cholesterol acyl transferase (LCAT), resulting in regulation of blood lipids.

11.4 Antipyretic and Analgesic Effects

In vivo study, antipyretic effect of cinnamon had been observed in mice. Cinnamaldehyde or sodium cinnamate had also been produced hypothermic and antipyretic effects in anesthetized guinea pigs and dogs (Xu et al., 2002). Cinnamon had also caused hypotensive effect. Analgesic effect has also produced accredited to cinnamaldehyde (Wang et al., 2006).

11.5 Antibacterial Activity

Cinnamon bark (*C. zeylanicum*) oil showed an inhibitory effect against the gram +ve bacteria: *Enterococcus faecalis*, *Micrococcus luteus*, *Bacillus cereus*, and *Staphylococcus aureus*, and the gram −ve bacteria: *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, and *Alcaligenes faecalis*, and also against the fungi *Rhizopus oligosporus* and *Aspergillus niger* (Chao et al., 2000).

11.6 Antiinflammatory Activity

Antiinflammatory activity has also been shown by cinnamon. Acute inflammation in mice was treated by ethanol extract of cinnamon (Kubo and Kinst-Hori, 1998).

11.7 Antimicrobial Activities

Extracts and oil of cinnamon showed various antimicrobial activities against several fungi and bacteria. Influenza virus replication was inhibited by cinnamon aqueous extract (Mancini et al., 1999).

11.8 Nematicidal Activity

Nematicidal activity (*Bursaphelenchus xylophilus*) of cinnamon was also reported (Park et al., 2005).

12 Turmeric

Turmeric (*Curcuma longa* L.), a perennial rhizomatous herb belonging to family *Zingiberaceae*, originated in India and now widely cultivated in tropical as well as subtropical regions around the globe. Pakistan is ranked second in terms of turmeric production throughout the globe. Among various regions of Pakistan, district Kasur accounts for more than 80% of total turmeric

production. The compositional profiling of turmeric enumerates carbohydrates (69.4%), moisture (13.1%), protein (6.3%), fat (5.1%), and minerals (3.5%). The main active constituents of turmeric are curcuminoids and essential oils. The extracted essential oil from turmeric rhizome is classified into sesquiterpines (53%), zingiberene (25%), α-phellandrene (1%), cineol (1%), sabinene (0.6%), and borneol (0.5%). Commercially, turmeric possesses great significance in food and pharma business as a spice, condiment, culinary additive, medicinal, and cosmetic ingredient.

12.1 Hypocholesterolemic and Hypoglycemic Potential

Numerous researchers have reported hypocholesterolemic potential of curcuminoids by lowering serum cholesterol and triglyceride levels through fecal excretion. Likewise, long-term intake of curcumin subsequently modifies genetic expression, that is, it is involved in cholesterol homeostasis. Diabetes is also linked with dyslipidemia, that is, it is characterized by high triglyceride and LDL levels secondary to insulin resistance. Furthermore, curcumin has peroxisome proliferator-activated receptor gamma (PPAR-γ) ligand-binding activity that enhances transcriptional activity of insulin responsive genes thereby improves insulin sensitivity in type II diabetes mellitus.

12.2 Antiinflammatory Activity

Turmeric constituents (especially curcuminoids) possess antiinflammatory activity and antiinflammatory activity of turmeric extract and its volatile oils have been reported in different experimental models conducted on different animals like pigeons, rabbits, mice, and rats (Arora et al., 1971).

12.3 Antioxidant Effect

Turmeric holds considerable antioxidative potential owing to its polyphenols. Curcuminoids are natural phenolic compounds with potent antioxidant properties, which were reported by (Sharma, 1976).

12.4 Antimutagenic and Anticancerous Property

Inano and his colleagues has studied the anticancer action of curcumin in rat mammary gland tumor that was induced by a standard model of radiation (Inano et al., 2000).

12.5 Antiviral, Antimicrobial, and Antiparasitic Activities

Anti-HIV effect of curcuminoids has been reported in experiments on a limited number of human (Lin et al., 1994). Antimicrobial properties of curcuminoids have also been reported. The antibacterial effects of alcoholic extract of turmeric, curcumin and oil from turmeric have been studied by (Shankar and Murthy, 1979).

13 Ginger

13.1 Antiinflammatory and Anticancer Properties

The pungent phenolic contents of ginger possess antioxidative and antiinflammatory activities. Ginger has also been reported for antitumor promoting activity that was studied with the help of two-stage skin carcinogenesis in mice. Ginger, a natural dietary component has anticarcinogenic properties (Parthasarathy et al., 2008).

13.2 Antiplatelet Effect

Inhibition of accumulation and release of arachidonic acid and platelets of rabbit which were induced by collagen mainly depends on the concentration of ginger. Whereas, level of ginger had no impact on the platelets which were induced by activating factor (U46619) and thrombin (Guh et al., 1995).

13.3 Antioxidant Effect

Some ingredients of ginger were studied by Kikuzaki and Nakatani (1993) for their antioxidant potential. The antioxidative activity of ginger rhizome was observed by its nonvolatile fraction (that is extracted by dichloromethane) on linoleic acid in the buffer solution of ethanol-phosphate (Kikuzaki and Nakatani, 1993). The portion was refined through chromatographic technique (Nakatani, 2003).

13.4 Antiulcer Properties

Antiulcer components (three monoacyl digalactosyl glycerols and 6-gingesulphonic acid) of ginger rhizome was detected in Taiwan. Chinese and Japanese people used ginger rhizome in dried form to treat nausea, headaches, colds, and stomachache (Nakatani, 2003).

13.5 Anticonvulsive and Analgesic Effect

Ginger rhizome has been used in Chinese traditional medicine to prepare cardiotonic, analgesic or anticonvulsive or antipyretic. It has also been evaluated for cardio and analgesic tonic by some researchers (Nakatani, 2003).

13.6 Cardiovascular Effect

Aqueous extract of ginger has been reported for its vasodilator, cardio-suppressant, endothelium-independent and dependent and hypotensive (Ghayur et al., 2005).

14 Cassia

14.1 Antiulcerogenic Activity

Cassia has been reported for its antiulcerogenic effect. Improvements in gastric cytoprotection and its blood flow produced the antiulcerogenic effect. Cassia contains propanoic acid and its O-glucoside has shown antiulcerogenic activity (Tanaka et al., 1989).

14.2 Antifungal Activity

The inhibitory effect of essential oil of *C. tamala* was observed against *Fusarium moniliforne*, that is, the source of postharvest spoilage in cereal crops. The active compound "eugenol" has been found in leaf oil of cassia that exhibited inhibitory effect against *A. parasiticus* at 1000 ppm and against *Aspergillus flavus* at 3000 ppm (Ravindran et al., 2003).

14.3 Hypoglycemic and Hyperlipidemic Effects

In an experimental model, rats were induced to hyperglycemic by Streptozotocin to check the effect of leaves extract of Cassia. Upon administration, lowering in plasma glucose level has been observed and its extracts have also shown antihyperglycemic and antihypocholesterolemic effects in rats (Sharma et al., 1996).

14.4 Pesticidal Activity

The inhibitory effect of *C. cassia* oil has been observed against the adult beetle *Lasioderma serricorne*, that is also known as tobacco beetle (Kim et al., 2003).

15 Clove

15.1 Antimicrobial Activity

Essential oils from clove (eugenol) showed various degrees of inhibition effect against *Lactobacillus acidophilus*, *Aspergillus niger*, *B.cereus*, *Mycoderma* sp., and *S. cerevisiae*, that have been evaluated by the paper disc agar diffusion method (Meena and Sethi, 1994).

Inhibitory effect of clove essential oil has also been evaluated against *Pneumomocci* bacteria, *Staphylococci* and *Streptococci*. The clove oil exhibited antibacterial activity against several genera that include pathogenic, spoilage-causing bacteria (Dorman et al., 2000).

15.2 Antioxidant Activity

Clove essential oil has the highest antioxidant property than any other oil. It is also known as food supplement (Parthasarathy et al., 2008).

15.3 Antiinflammatory Activity

The prime component of volatile oil of clove, eugenol, has been regarded as antiinflammatory substance. Synergistic effect of clove extract has been reported in an animal study (Ghelardini et al., 2001).

15.4 Mosquito-Repellent Activity

Repellent activity of colve oil has been reported against *A. dirus*, *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles albimanus* (Trongtokit et al., 2005).

15.5 Insecticidal Activity

The clove leaf and bud oils showed potent insecticidal activity against the human head louse (*Pediculuscapitis*) (Yang et al., 2004).

15.6 Antiviral Activity

Eugenin that was isolated from buds of clove showed inhibitory activity against virus named *Herpes simplex* at 10 μg/mL concentration (Chaieb et al., 2007).

15.7 Toxicity Studies

Cloves cause local skin irritation, pulmonary oedema, mouth sensitivity and sudden lower airway closure (Fetrow and Avila, 2000).

16 Nutmeg and Mace

16.1 Antimicrobial and Antiamoebic Activity

Nutmeg oil showed strong antibacterial activity against 25 genera of bacteria (Dorman and Deans, 2004). The extracts of nutmeg has been reported for its antibacterial activity against pathogenic and nonpathogenic bacteria (*E. coli*) but pathogenic strain of *E. coli* (0157) showed more sensitivity to the β-pinene than nonpathogenic strains (Takikawa et al., 2002). Antiamoebic effect of nutmeg essential oil at 0.5 μL/mL had been observed against *Entamoeba hystolytica* (De Blasi et al., 1990).

16.2 Antibacterial Activity

Nutmeg seeds extract with chloroform had also produced antibacterial effect, for example, gram-negative and gram-positive bacteria (Narasimhan and Dhake, 2006).

16.3 Anticancer Activity

Interference of nutmeg essential oil with host enzymes that are involved in detoxification of many mutagens and carcinogens produce anticarcinogens in animal studies (Parthasarathy et al., 2008).

17 Coriander

Coriander (*Coriandrum sativum* L.) belonging to the *Apiaceae* family is an odorous herb known as Dhaniyaa in Hindi, Dhanya in Sanskrit, and kotthamalli in Tamil. It derives its name coriander from the Greek "koris," meaning bedbug, as it has a bug-like unpleasant odor when the herb and fruit is green. It is native to Mediterranean and Middle Eastern Region, extensively cultivated in the Middle East and North America and increasingly in Western Europe (Mahendra and Bisht, 2011).

The mature fruits are used all over the world in whole, ground, and essence or oil form. Two primary products that are obtained from the coriander plant are employed for the flavoring purposes; fresh green herb and the spice (mature dry seed

capsule or fruit). Whole plant is edible but fresh leaves and dried seeds are usually used in cooking and the stem has little or no use (Wangensteen et al., 2004).

Egyptians called coriander "the spice of happiness." Its seeds were found in the ancient Egyptian tomb of Ramses II. The Greeks and Romans also used coriander to flavor wine and as a medicine. It is also used as conventional treatment for diabetes, insomnia, indigestion, and for renal disorders in Morocco (Aissaoui et al., 2008).

The major constituents of coriander are fiber, carbohydrates, fatty oil, and protein found in ratio of 23–36%, 20%, 16–28%, and 11–17% respectively. Upon distillation of essential oil, the remaining residues are high in fat and protein that are useful as animal feed. Prime constituents of coriander seeds are the fatty oil and essential oil (Coşkuner and Karababa, 2007).

Proximate analysis shows that the moisture content of coriander seed ranges from 7.1% to 18.94% on dry basis. Other constituents are crude fiber, ash contents, crude protein, and crude fat analyzed up to 29.1, 19.15, 21.3, and 6.0% respectively.

Linalool is the key element of essential oil in coriander seed that varies from 50% to 70%. Total lipid content is 28.4% of seed weight. The essential oil and fatty oils are important constituents of coriander seeds. Linoleic acid and petroselinic acid are the main fatty acids which comprise 65% of the total fatty acids. The chemical composition and the percentage of the components in the coriander oil fruits depend on different maturity stages. Composition of coriander seed oil is entirely different from herb oil and the seed oil contains 60–70% linalool hydrocarbons 20% (Msaada et al., 2007).

Dried seed contains essential oil (0.03–2.6%) with linalool and mono-terpenoid as its main components. The fatty oils ranges between 9.9% and 27.7%. In past, the main use of seed was medicinal and also useful in treatment of worms, digestive disorders and joints pain. Different medicinal characteristics of coriander seed have been reported, including stomachic, antispasmodic, and carminative perspectives. Recent studies have also shown that it has hypoglycemic effect and helpful in the metabolism of carbohydrates. Seeds are rich source of useful phytochemical constituents including linalool, carvone, limonene, borneol, geraniol, camphor, and elemol.

Coriander also has flavonoid contents including apigenin, rhamnetin, keampferol, and quercetin. Moreover, coriander seed contains dynamic phenolic components, which are vital in prevention of free radicals produced in the cellular system. The study of antioxidants found in spices is gaining worth and momentum

because these antioxidants are easily absorbed in human system (Rajeshwari and Andallu, 2011).

Phenolic compounds are consumed in substantial amount in daily life and are vital aromatic secondary metabolites of plants. The compositional analysis revealed that polyphenolic phytochemical constituents are influenced by stage of maturity, cultivar and variety, agricultural practices, geographic region, climatic conditions, harvest, storage environment, and processing procedures and parameters. Due to the biological activities some phytochemicals are known to be nutraceuticals. Recently, beneficial effects of phytochemicals on health has driven a range of research activities including antimutagenic, anticarcinogenic, antioxidant activities, and their potential to decrease the risk of coronary disease. Coriander has important place among savory plants and a potential source of phenolic compounds with pivotal biological actions (Rajeshwari and Andallu, 2011).

17.1 Hypoglycaemic Potential

Different studies have shown that the treatment with *Coriandrum sativum* seeds decreased oxidative stress of diabetic patients due to (positively) interacting action of antioxidant phytochemicals, flavonoids, carotenoids, which are found in the seeds. Results of many studies carried out on seed found that is has antioxidant potential, therefore it can be advocated as adjunct to diet therapy to fight against oxidative stress in patients (Naquvi et al., 2012).

17.2 Aflatoxin Control and Insecticidal Effect

Coriander essential oil has been studied for its inhibitory effects against the mycelial growth and *A. ochraceus* NRRL 3174 production of ochratoxin A (Basilico and Basilico, 1999). Coriander oil potential in the control of *L. acidophilus*, *Saccharomyces cerevisiae*, *A. niger*, *Bacillus cereus*, and *Mycoderma* sp. (Meena and Sethi, 1994). Essential oils of coriander was found effective against beetle pests (Pascual-Villalobos et al., 2003).

18 Cumin

18.1 Antioxidant Activity

Antioxidative activity of cumin essential oil has been evaluated on sunflower oil that was stored at 70°C. Cumin essential oil is found to be a better antioxidant agent than butylated hydroxytoluene (a synthetic antioxidant) (Singh and Upadhyay, 1991).

18.2 Reproductive and Hypoglycaemic Properties

It is emmenagogic and antispasmodic. It is believed to increase lactation and reduce nausea in pregnancy (Weiss, 2002). Cumin decreased the glucose tolerance curve and hyperglycaemic peak (Aslam et al., 2003).

18.3 Antimicrobial Activity

Cumin essential oil has been reported for its inhibitory activity against many microbes like *S. aureus*, *L. monocytogenes*, and *E. coli* and the complete death times on exposure were 180, 90, and 20 min, respectively (Gachkar et al., 2007).

19 Fennel

19.1 Antioxidant Activity

In vitro study of aqueous and ethanol extracts of fennel showed strong antioxidative activity (Oktay et al., 2003). In the linoleic acid system, 99.1 and 77.5% inhibition activity was shown by ethanol and water extracts of fennel that is greater than a natural antioxidants (α-tocopherol). Both water and ethanol extracts of fennel have free radical scavenging, metal-chelating activities effective reducing power, hydrogen peroxide scavenging and superoxide anion radical scavenging, that are directly proportional to sample concentrations. Fennel seed is indicated as a natural antioxidant source (Parthasarathy et al., 2008).

19.2 Antimicrobial Property

A study was conducted to check the combined effect of fennel oil with methylparaben or benzoic acid against *S. enteriditis* and *L. monocytogenes*. *Salmonella enteriditis* showed more sensitivity to the combination of fennel oil with methylparaben and bacterial load was reduced to <10CFU/mL after 1 h exposure. *Listeria monocytogenes* showed more resistance than *S. Enteriditis* to each combination but a significant reduction in bacterial load was observed. Both microorganisms showed synergistic inhibition to one or more combinations (Fyfe et al., 1998).

19.3 Anticancer Property

For the prevention and treatment of cancer, anethols from fennel may be used (Aggarwal and Shishodia, 2006).

19.4 Act as a Food Allergen

Food internationalization and changed dietary habits caused increase in spice usage. Prick test was used to evaluate the children allergy to spices (Rancé and Fardeau, 2002).

20 Fenugreek

20.1 Wound Healing

Fenugreek seed extract with water enhanced healing activity significantly and its seed suspension exhibited more potential than its aqueous extract in enhancing wound healing activity (Taranalli and Kuppast, 1996).

20.2 Immunomodulatory Effect

Aqueous extract of fenugreek had been observed to produce immunomodulatory effect in Swiss albino mice (Bin-Hafeez et al., 2003).

20.3 Antioxidant Activity

Fenugreek seed and its aerial parts exhibited scavenging activities of free radical and also exhibited antioxidant activity (Bajpai et al., 2005). Diet supplementation with fenugreek seed lowered down the oxidation of lipids (Kaviarasan and Anuradha, 2007).

20.4 Hypocholesterolaemic Activity

Fenugreek seeds supplementation of nutraceutical and functional foods exhibited therapeutic potential. Supplemented diet with fenugreek seed exhibited hypertriglyecridaemia and hypercholesterolaemia by significant lowering in serum cholesterol, low density lipoprotein, and triglyceride in human patients (Smith, 2003).

21 Chili

Chili pepper is a member of the *Solanaceae* family. Chilies are said to have originated from the Latin American regions of the New Mexico and Guatemala as a wild crop. Chili pepper (*Capsicum* spp.) is an annual plant, however, cultivated in warmer and tropical regions of the world. Common names include red pepper,

chili pepper, paprika, hot pepper, Lal-mirch, pimento, peperone, and so forth (Rangan and Barceloux, 2009).

As far as chilies production is concerned, India is the leading country in the world with 1,299,940 MT produced annually followed by China, Peru, Bangladesh, and Pakistan. Pakistan ranked fifth in chili production with 150,000 MT produced annually. The crop occupies 20% of total vegetable cultivated area, especially concentrated in Layyia (Punjab) and Hyderabad, Kunri, and Tharparkar areas (Sindh) provinces.

Peppers possess numerous health-promoting compounds; carotenoids, vitamin C, capsaicinoids, glycosides, and aglycones of myricetin, quercetin, luteolin, kaempferol, and apigenin. Furthermore, these are rich in vitamins A, E, and C, potassium, magnesium, and folic acid. Pungency is the main feature of chili peppers that depends on the presence of a group of alkaloids belonging to the family of capsaicinoids.

The active components, for example, capsaicinoids, produce pungent taste. Capsaicinoid, particularly capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) crystalline, is an acrid volatile alkaloid in nature and is considered as the principal pungent and irritating constituent of hot peppers, that is, widely used as food additives along with antimicrobial properties (Peña-Alvarez et al., 2009). Capsaicin-rich cayenne and other hot peppers help to reduce inflammation and potentially reduce arthritis, rheumatism, and headache pain with the natural antiinflammatory properties.

21.1 Anticancerous Activity

Capsaicin potential was reported in inhibiting the growth of cancer cells by causing apoptosis, which depends on the mitochondrial oxidative metabolism regulation. It inhibits growth of various solid cancers via TRPV1 as well as transient receptor potential receptor vanilloid 1 (TRPV1) independent mechanisms. It disrupts mitochondrial membrane potential and can cause rapid reactive oxygen species (ROS) overproduction (Skrzypski et al., 2014).

21.2 Antiobesity Potential

Studies have indicated that the consumption of capsaicin can be effective against fighting obesity, as it increases thermogenesis throughout the body. Thermogenesis is the process in which the body raises its temperature, or energy output. Increasing thermogenesis increases the body's metabolism, which forces fat cells to be used as energy. Many studies confirm the presence of capsaicin increases thermogenesis and lipid metabolism, which is beneficial

for the treatment of obesity. Capsaicin also possesses the potential against growth and aflatoxin production of *Aspergillus flavus* in SMKY liquid medium (Masood et al., 1994).

22 Vanillin

22.1 Antioxidant Property

Antioxidant activity of vanillin has been observed in complex foods that contained polyunsaturated fatty acids (Burri et al., 1989). Vanillin also exhibited antimicrobial properties alongside antioxidant activity. Hence, vanillin may be used as a preservative in different food formulations (Naidu, 2000).

22.2 Antimicrobial Activity

Ajowain oil exhibited antimicrobial activity against *Mycoderma sp.*, *A. niger*, *B. cereus*, *L. acidophilus* and *S. cerevisiae* that was observed by agar diffusion method (Meena and Sethi, 1994).

23 Aniseed

23.1 Antiinflammatory and Antibacterial Activity

Aniseed showed antiinflammatory properties. Aniseed extract showed an antiinflammatory effect in mice treated by 12-O-tetradecanoyl phorbol-13-acetate. Aniseed seed extract was found effective against *Helicobacter pylori* (gram negative) at 100 μg/mL (Mahady et al., 2005). Essential oils of aniseed exhibited significant repellency against the *C. pipiens* (adult females of the mosquito) (Erler et al., 2006).

24 Garcinia

24.1 Antioxidant

Extracts of *Gracinia indica* contains gracinol that exhibited not only antioxidant activity but also antifungal properties and had shown strong potential for use in nutraceutical and functional foods as a biopreservative (Selvi et al., 2003).

24.2 Astringent

Fruit hulls of *G. mangostana* has been used to treat diarrhea and also as an astringent (Chairungsrilerd et al., 1996).

24.3 Anti-HIV Agent

Gracinia mangostana fruit peel extract with ethanol exhibited antiviral activity against HIV-1 protease. The fruit peel of *G. mangostana* contained an active compound named mangostin that was responsible for antiviral activity (Chen et al., 1996).

24.4 Antibacterial Agent

G. mangostana rind contains an active compound polyoxygenated xanthones that exhibited antibacterial activity. *G.bancana* also exhibited antibacterial activity due to the presence of biphenyl derivatives (Rukachaisirikul et al., 2003).

24.5 Antiobesity Factor

Hydroxycitric acid (HCA) is known for its ability to induce weight loss. Some species of *Garcinia* contains HCA in its fruit rinds, that is, *G. atroviridis*, *G. cambogia*, and *G. indica*. Presence of HCA increased demand of these species by health practitioners (Jena et al., 2002).

24.6 Analgesic Effect

G. atroviridis decoction of roots and leaves has been used to treat earache. Bowel complaints and rheumatism have also been treated by the decoction of *G. cambogia* fruit rinds (Jena et al., 2002).

25 Curry Leaf

25.1 Antimicrobial

Curry leaf showed more inhibitory potent to bacteria and less toward fungus (Aqil and Ahmad, 2003).

25.2 Antidiabetic

Breakdown of starch to glucose is slowed down by curry leaves. Consequently, it is recommended as food adjuvants for diabetic patients (Grover et al., 2002).

25.3 Pesticidal Properties

Curry essential oil is constituted of many chemicals, that is, sesquiterpene hydrocarbons and monoterpene hydrocarbons that exhibited pesticidal activity (Ray and Srivastava, 2006).

25.4 Antiinflammatory

Murraya koenigii leaves contain three bioactive carbazole alkaloids—mahanimbine, murrayanol, and mahanine—that exhibited significant analgesic and antiinflammatory activities in mice (Dash et al., 2004).

26 Bay Leaf

26.1 Antifungal Activity

Bay leaf essential oil exhibited antifungal activity against species of genus *Penicillium, Aspergillus*, and *Eurotium* (Guynot et al., 2003).

26.2 Anticonvulsant

L. nobilis essential oil has been used in Iranian medicine as an antipiletic and also produce anticonvulsant activity in many experiments (Sayyah et al., 2002).

26.3 Antimicrobial and Insecticidal Activity

Bay leaf has also been observed to produce antibacterial effect against *Escherichia coli* (Dadalioglu and Evrendilek, 2004). Essential oils from laurel were evaluated for fumigant toxicity against all developmental stages of the confused flour beetle (*Triboliumconfusum*) (Isikber et al., 2006).

27 Conclusions

Nutritional imbalances in the diet are causing a number of physiological dysfunctions which lead to the adoption of diet-based therapies as an intervention against various infirmities. Keeping in view the alarming increase in metabolic disorders, a combination of several ingredients and encapsulated essential oils are used to achieve a specific set of goals through their antioxidant, antiinflammatory, antimicrobial, hypocholesterolemic, hypoglycemic, antimutagenic, and anticarcinogenic roles in the living system to combat the physiological disorders. Food products that are prepared to attain higher quantities of various essential bioactive components and phytochemicals as compared to naturally existing components in the reference foods known as “pharma foods.” Phytonutrients and phytochemicals have been extensively used owing to their versatile health endorsing potential in various

cultures since ancient times. Epidemiological as well as scientific studies have proven the connection among diet consumption and health status. Population or individuals consuming bulk quantity of plant-based diet, containing vegetables, fruits, cereals, whole grains, or high seafood consumption have lesser risks of certain kinds of cardiovascular diseases and cancer.

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8

ADVANCED NANOCARRIERS FOR NUTRACEUTICALS BASED ON STRUCTURED LIPID AND NONLIPID COMPONENTS

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1 Introduction

The term *nanotechnology* refers to engineering of particles at the molecular level. The subject that was originally the forte of building nanoscale machineries and devices has, in the recent past, broached the unfamiliar fields of nutrition and pharmaceuticals. Recently with the emerging interest in an alternative medicinal source, nutraceuticals have gained immense importance among both scientists and consumers. The increasing interest in such products is also due to the latent antioxidant activity of these primarily phytochemicals. Compounds possessing antioxidant activities play an important role in strengthening the body defense system against reactive oxygen species. The reactive oxygen species act as toxins for the body and foment cell damage (Gutteridge and Halliwell, 2000). However, intake of nutraceuticals will help to detox the body and help to maintain the normal physiological functions of the body (Kaur and Kapoor, 2001; Record et al., 2001). The term *nutraceutical* is coined for products such as nutrients, food supplements, herbal isolations, functional foods, and so forth, which show immense efficacy in terms of improvement of human health and well-being (Kalra, 2003). A basic criteria for a food form to be categorized as a nutraceutical is that, in its isolated and purified form, it should provide considerable protection against chronic diseases like diabetes, atherosclerosis, cancer, arthritis, neurodegeneration, and so on (Dubey et al., 2009).

However, the protective effect of nutraceuticals against different degenerative diseases shows limited bioavailability and needs

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to be supplemented at a very high dose to acquire the complete benefit of the products (Landete, 2012). Eventually this propels the emergence of undue side effects of the products at high concentrations. The potential of nutraceutical nanotechnology ensures the reduction of the particle size of the bioactive compounds to one thousand millionth of a meter (nanometer). Thus this technique culminates in improving the availability, delivery, and solubility properties of the substance, thus improving its all-round applications (Cushen et al., 2012).

As early as 1959, Richard Feynman (Nobel Prize winner in Physics, 1965) envisioned the theoretical capability of building things in a bottoms-up approach. Based on this conceptualization, the field of nanotechnology was vigorously attempting to reach unlimited heights. In fact, today it has encompassed several fields like the first generation products of dispersed nanostructures, nanostructured metals, polymers, and so forth; second generation products of targeted drugs and 3D transistors; third generation products like 3D networking and robotics; and finally fourth generation products like molecular devices by design. Nanotechnology refers to the measurement of particles at the scale of 1–100 nm. Today in its advanced form it has significant effects on the proliferation of almost all industries including medicine, electronics, devices, foods, cosmetics, homecare products, agriculture, and, more recently, nutraceuticals. This in turn has a positive effect on all the interests of society.

The present chapter gives an overview of the recent developments in the field of nutraceuticals and their fabrication to develop nanocarriers rich in these bioactive compounds. Investigations on the fundamentals of formulations, methodologies, characterization techniques of the diverse nanotized compounds are also discussed here. Nutraceuticals are not limited to any particular food form. Both lipids and nonlipid sources can serve as the provenance for such nutraceuticals in its original or modified contour. Hence the techniques, raw materials and other nuances for nanoconcoction of nutraceuticals are also elaborated in the present chapter. Furthermore a brief review on the several methods for synthesizing different nanocarriers and the methods for their characterization are also confabulated here.

2 Nutraceuticals: Lipid and Nonlipid Origins

Nutraceuticals are basically certain nutrients which can be isolated from natural products of vegetable or animal source. They are known to have vast health benefits along with providing

nutrition to the body. Thus these compounds help to prevent the occurrence of several diseases. In fact such chemicals from natural sources with potential health benefits such as curcumin, resveratrol, and carotenoids have been well exploited by researchers globally. Their efficacy is primarily due to their intrinsic antioxidant property, which affects the immunity of human system in a positive manner. Thus they have been shown to prevent several disorders like cancers and cardiovascular and neurodegenerative disorders like Alzheimers disease (Sivakumar et al., 2013; Mathew et al., 2012).

Synthetic antioxidants like butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate, and so forth, are extremely effective in inhibiting oxidation in the body. Again certain compounds like ethylenediamine tetra acetic acid form chelation complexes with metals and bind with metals which act as catalysts to induce oxidation. For a long time consumption of such products has been quite common. However in the recent past consumers have shown explicit concern about the safety of ingesting such synthetic products along with food (Brewer and Prestat, 2002; Rojas and Brewer, 2008). As an alternative source today the demand for natural products that provide both nutrition and antioxidative protection is immense. In fact several compounds from natural sources like carotenoids, some vitamins like ascorbic acid and α-tocopherol, many herbs and spices like rosemary, thyme, oregano, pepper, clove, cinnamon, and so forth, plant extracts from tea and grapeseed, phytosterols, oryzanol, phenolic compounds of soybean, flaxseed, sesame, and so forth serve the desired function in providing both nutrition and oxidation preventive activities (Hinneburg et al., 2006; Sen Gupta and Ghosh, 2013). These products can efficiently scavenge free radicals or form chelation complexes and thus prevent oxidation of body cells, thus inhibiting autoxidative cell damage, while at the same time being reduced themselves. Furthermore it is essential to have a sustainable source of these natural products and they should definitely be environmentally friendly, being devoid of any untoward side effects (Berger, 2009).

Food lipids consist of saturated and unsaturated fatty acids. These may be in a free form or as a part of the triacylglycerol, diacylglycerol, monoacylglycerol, or phospholipid. However, the unsaturated fatty acids, especially the long-chain unsaturated fatty acids like the omega-3 [(n-3)] fatty acids—for instance, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—are associated with healthy aging. Furthermore they have also been linked with fetal development, improved cardiovascular function, slowing Alzheimers disease, and improving neuronal, retinal, and immune function. These essential fatty acids are not produced by

the body and needs to be supplemented from outside (Swanson et al., 2012). However, a primary drawback of these lipid-based nutraceuticals is their electron-deficiency character at the carbon–carbon unsaturation (C═C) point. These electron deficient regions make the fatty acids susceptible to attack by free radicals and oxidizing agents, thus deteriorating their quality, which at the same time hampers nutritional efficacy (Nawar, 1996). Phospholipids in tissue membranes are enriched with several types of unsaturated fatty acids (C18:4, C20:4, C20:5, C22:5, and C22:6). Each of them is extremely susceptible to oxidation (Elmore et al., 1999). Generation of lipid radical is thermodynamically unfavorable and needs an initiator. The unsaturated fatty acids often decompose to form hydroperoxides (ROOH), which again help in oxidation propagation. Often a shifting of double bonds may result in the formation of conjugated diene system, which stabilizes the fatty acid. But this process is not feasible for all types of unsaturated fatty acids, which thereby stand the chance of decomposition to form off-flavors and other rancid products.

Food components like carbohydrates and proteins also can easily react with singlet oxygen that is formed in the system by changes in temperature, pressure, or presence of transition metals. To control such damage to foods and lipids, certain nutraceuticals with antioxidant properties are often very helpful and effective. The most effective antioxidants interrupt with the free-radical chain reaction and defuse the radical-propagation step, thus derogating autoxidation of the food products. Nutraceuticals containing aromatic or phenolic rings often exhibit the said antioxidant properties. They donate H^+ to the free radicals generated in the system, thus causing resonance delocalization of the electron within the aromatic ring (Nawar, 1996). Some such nutraceuticals include the nonlipid types like phenolic acids (gallic acid, protochatechuic acid, caffeic acid, rosmarinic acid, etc.), phenolic diterpenes (carnosol and carnosic acid), flavonoids (quercetin and catechin) and volatile oils (eugenol, carvacrol, thymol, menthol, etc.) (Shan et al., 2005; Geldof and Engeseth, 2002). There are other lipid-type nutraceuticals like carotenoids, tocopherols, tocotrienols, and oryzanol, which also provide similar antioxidative protection (Khanduja, 2003; Ozsoy et al., 2009). Furthermore there are several spices and herbs, which are used in foods to enhance flavor. It was observed that these, too, have profound medicinal capacities, capable of providing beneficial physiological effects (Lugasi et al., 1995; Muchuweti et al., 2007).

α-Tocopherol, a lipid-soluble vitamin E precursor, is found in membranes in proximity to phospholipids. Dietary supplementation of α-tocopherol helps to incorporate it in the phospholipid

membrane, which serves there as a protector of the oxidation-prone polyunsaturated fatty acids (Formanek et al., 2001; Swigert et al., 2004; Guo et al., 2006; Boler et al., 2009; Lahucky et al., 2010). Jukic et al. (2006) isolated glycosidically bound volatile compounds from nutmeg which were identified to be eugenol and terpinen-4-ol, the active antioxidants. The aqueous extract of rosemary was found to be enriched with phenolic compounds like flavonoids (Chen et al., 2007). A multitude of compounds like camphene, p-cineole, alpha-terpineol, zingiberene, and pentadecanoic acid are isolated from the volatile oils of ginger, which show extensive oxidation protection capabilities (Tiwari et al., 2006; El-Ghorab et al., 2010). Curcumin, dimethoxy curcumin, bis-dimethoxy curcumin, and 2,5-xylenol, the active ingredients of turmeric are phenolic chain-breaking antioxidant, which have been shown to possess extensive free-radical scavenging activities (Zhang et al., 2009). Phytosterols, oryzanol, ferulic acid ester of sterols were investigated for their health-beneficial activities. It was observed that these compounds provided extensive oxidative stability to the edible oils, even at low concentrations. Rice bran oil, which is rich in these nutraceuticals, is a highly recommended cooking oil (Wang et al., 2002). Several such nutraceuticals are widely popular today in terms of consumer application.

An emerging trend today is the production of nutraceuticals, which are chemically or enzymatically modified food that is generated to be a functional food. It serves as an antisense technique for elimination of health-impairing products. Furthermore the overexpression to increase the concentration of specific functional ingredients is done to enhance its bioactivity. Such modifications also simultaneously introduce a new metabolic pathway or side chain. Conjugated linoleic acids and their isomers, either in their natural or synthetic forms, have been associated with diverse health and physiological effects (Philippaerts et al., 2013). Conjugated linoleic acids (CLA) refer to a group of geometrical and positional isomers derived from the modification of linoleic acid (LA) with conjugated double bonds. CLA has been reported to have diverse health benefits and biological properties. Traditional organic synthesis is highly capital-intensive and results in an isomeric mixture of CLA isomers. Biotechnology presents new alternatives to traditional lipid manufacturing methods.

Furthermore, vegetable oils are in general rich sources of different nutraceuticals. A common nutraceutical from vegetable oil includes free phytosterols. These sterols and their fatty acid esters can also be economically synthesized. Most crude vegetable oils contain 1–5 g/kg of total phytosterols. For instance crude soybean oil contains about 3.0–4.4 g/kg of phytosterols. Apart from that

crude corn oil, wheat germ oil, rapeseed oil, and so forth were also found to contain sufficient amount of phytosterols from which phytosterol esters can be synthesized to yield a new kind of nutraceutical. Furthermore structured lipids with a medium-chain triglyceride backbone and linoleic acid built into the triglyceride molecule have been developed to optimize the triglyceride structure that is best for patients, particularly the critically ill. Structured lipids with built-in essential fatty acid components or other polyunsaturated fatty acids promise greater flexibility in patient care and nitrogen support (Babayan, 1987).

The development of dietary supplements to compensate for nutritional deficiencies in individuals in today's busy lifestyle is urgently necessary. Different nutraceuticals need to be developed for that purpose. Lipids are the most important source materials for different important nutraceuticals, which are bioactive in nature. The major challenge facing the food and nutraceutical industries is the large-scale yet cost-effective isolation of bioactives from vegetable oils. There are immense opportunities for the development of better extraction procedures. Such applications are quite common in the pharmaceutical and chemical industries. However, they still need to be extensively utilized in the nutraceutical industries. The need to find environmentally benign techniques for nutraceutical extraction is widely needed today. Different methods of isolation of the lipid and nonlipid components are in practice. Solvent extraction, microwave extraction, and ultrasonic extractions of different flavonoids is common. Solvent extraction is a traditional method for extracting different lignans from plant sources. However, other less polar components present in most plant tissues may interfere with the subsequent separation of lignan if a polar solvent is used. Hence sequential solvent extractions can also be followed.

Long-chain omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) have become an important subject in both the scientific community and our everyday life. We encounter them in pharmaceutical and/or health applications as well as in food applications. Furthermore the presence of such fatty acids in edible oils also lead to their emollient properties due to which they find immense applications in cosmetic industries, too. Among them the PUFA's have attracted special attention, due to their role in human health and nutrition. Essential fatty acids cannot be synthesized de novo by humans and therefore need to be obtained from the diet. With the growing public awareness of the nutritional benefits of PUFA's, the market for such products is expected to grow in the future. Docosahexaenoic (DHA), eicosapentaenoic (EPA), and gamma linolenic (GLA) acids are the most commonly used PUFA

in nutraceuticals and functional foods. They are being used in a wide array of products ranging from dietary supplements to infant formulas. Naturally these fatty acids are associated with other lipophilic compounds and effective separation and isolation techniques are needed to recover them in concentrated forms. With the growing public awareness of the nutritional benefits of PUFAs, the market for such products is expected to grow in the future. Utilizing the melting point characteristic of fatty acids, low temperature crystallization is carried out. This also depends on the chain length and degree of unsaturation. As the chain length increases, the melting point of fatty acids also increases. However, for longer-chain fatty acids, unsaturation results in a decrease of the melting point. At low temperature, short-chain fatty acids crystallize and PUFAs can be isolated from the rest of the fatty acids. Organic solvents like hexanes, acetone, and so forth facilitate separation of fatty acids during crystallization.

A very novel opportunity in the field of vegetable oils is that they may serve as new hosts for the production of recombinant nutraceuticals by application of new biotechnology and enzymatic reactions. Recombinant nutraceuticals produced in vegetable oils are often products from the primary or secondary metabolism of the plant cells. Metabolic pathway engineering in oil crops will in future enable researchers to produce exotic and rare compounds at increased scales. The type of the functional food or nutraceuticals in the desired oil and its aim needs to be considered. In the case of nutraceuticals active components in functional food crops can involve flavonoids, carotenoids, multiple-unsaturated fatty acids, oligosaccharides, fiber, mineral content, and vitamin levels which will be increased by genetic modifications. Oil crops, transgenically producing nutraceuticals, should meet the general requirements for genetically modified organisms, and, additionally, the specific requirements related to the production of the specific medicinal or health compounds.

Currently, a new phase in genetic modification is beginning. This phase can be considered the engineering of plants, not only for the improvement of their agronomic properties, but also to make new or improved nutraceuticals with better extraction yields. This development would enable us to produce higher-value products, for food and feed, for medical and for industrial objectives, which will have a high economic impact. Entering the new phase of engineering plants, there is however a need for the evaluation of medical or health side along the entire production chain. Biosafety assessments therefore need to be carried out on the produced nutraceuticals before the entire production should be aimed toward the ultimate application on humans.

However, a primary hurdle faced today regarding the wide-scale and cost-effective application of the nutraceuticals is that their low bioavailability and poor solubility leads to their inadequate absorption in the body. This results in their reduced biological activity, which presents a major cause of concern for researchers today. The only feasible solution to this problem is providing the nutraceuticals a nanoentity. Development of a nanoemulsion or nanoencapsulation of nutraceuticals, followed by their smart delivery—sustained release from the nanoformulation—are the emerging challenges within the scope of nanotechnology in the food sector. The principles of nanotechnology are therefore applied to the emerging field of nutraceuticals for the stability of oxidation-prone nutraceuticals, proficient delivery of the nutraceuticals to their targeted site, higher nutritional values, low dose of synthetic preservatives, and of course better organoleptic features with the objective to improve their biological activity.

3 Nanocarriers for Pure and Structured Lipid and Nonlipid-Based Nutraceuticals: Nanoemulsions and Nanocapsules

Commercial exploitation of nano vesicles is in a nascent stage in the food and nutraceutical industries (Tarver, 2006). The increasing interest in nutraceutical nanoscience has driven the extensive development of emulsions at nanoscale. According to modern theory, the primary difference between an ordinary emulsion and a nanoemulsion lies in the size and shape of the dispersed particles or droplets in the continuous phase. Nanoemulsions have a particle dimension between 1 and 100 nm, whereas in the case of conventional emulsions it ranges from 1 to 20 μm (Aboofazeli, 2010). In terms of the structure of the two types of emulsions, a spectacular variation is observed. While in the ordinary emulsions the particles are mostly spherical in shape, in case of the nanoemulsions the droplet structures vary between swollen, rounded forms to bicontinuous structures (Devarajan and Ravichandran, 2011). This often makes the distinct differentiation between oil-in-water emulsion and water-in-oil emulsions in the case of nanoemulsions quite irrelevant. Nanoemulsions can be synthesized by two basic approaches: high-energy approach and low-energy approach (Acosta, 2009; Leong et al., 2009; Tadros et al., 2004). The high-energy approach includes processes like high-pressure

homogenization, sonication, microfluidization techniques, and so forth (Gutiérrez et al., 2008; Leong et al., 2009; Velikov and Pelan, 2008; Wooster et al., 2008). Low-energy approaches include membrane emulsification, spontaneous emulsification, solvent displacement, phase inversion point, and others (Anton et al., 2008; Bouchemal et al., 2004; Chu et al., 2007; Freitas et al., 2005; Tadros et al., 2004; Yin et al., 2009). Shelf life of such emulsions is extremely important, in comparison to the conventional emulsions, in terms of either kinetic or thermodynamic stability. This is essential for the apt encapsulation of functional foods followed by their virtuous preservation over extended periods of time (Huang et al., 2010). The essentially prosaic and potent technique for attaining stability is the choice of a proper emulsifier or surfactant. Voluminous work has been done to elucidate a proper formulation of emulsifier/surfactant either singly or as a mixture (of two or three emulsifiers) for development of a stable nanoemulsion. Surfactants are amphiphilic in nature. Hence they have a hydrophilic water-soluble head group and a hydrophobic oil-soluble tail group. The surfactants adsorp at the oil–water interface and this dynamic mechanism galvanizes the stabilization of emulsions.

The application of nanotechnology to medical and pharmaceutical industries is already a well-exploited field. Recently nanotechnology is drawing immense attention from the scientists of the food and nutraceutical industries. The increasing demand of consumers and scientists today is for healthy and safe nutraceuticals based on nanoparticulate matter. A major role of the nanosized cauldrons is to protect and release functional compounds in a targeted manner when consumed. Such fabricated products conform to nanoemulsions or nanocapsules, which serve as potential encapsulating systems for several functional compounds. Importance of such structured contrivance prevents the degradation of the encapsulated nutraceuticals and improves their bioavailability. Different production methods, materials to be used and analytical techniques for identification and characterization of the nanoproducts substantiate the type and nature of the synthesized nanocarrier. The major challenges faced by the nutraceutical industries include (1) stability, especially against temperature and pH changes, oxidation during storage and processing, and so on; (2) taste and color, in keeping with consumer desirability; (3) complete safety of the consumed product, so that it is not associated with any kind of harmful by-products; and (4) ready bioavailability of the nutraceuticals (Hu and Huang, 2013). Nanofabrication of nutraceuticals are used to address each of these relevant problems.

4 Manufacturing Methodologies of Nanonutraceutical Products

The delivery of nutraceuticals using nanotechnology is in a completely nascent stage (Sahni, 2012). Successful formulation of a new nanosubstance entails extensive research and development in this stream. However, the efficacy and safety of the developed nano products are thoroughly investigated (Wiwanitkit, 2012). In vitro experiments to evaluate the behavioral tendencies of the developed products are easy and convenient. In addition, in vivo studies on the delivery, release, and stability of the new products have also yielded promising results. An important factor to be considered about nanocompounds is their method of synthesis. The nano substance, if not suitably manufactured, usually shows a vigorous tendency to aggregate. Hence, this leads to changes in the physical and biochemical properties of the developed nano products. This would in turn affect the expected mode of action of the nanocarriers. When a substance is converted to its nano level, its physical and chemical properties change, leading to the formation of a new kind of product. The novel properties of nanocarriers serve as the basis for the design of different food, cosmetic, pharmaceutical and, recently, nutraceutical formulations. Hence while designing such formulations certain considerations should be addressed before manufacturing the nanonutraceutical product. First, it should be well-approved that the nutraceutical will retain its nascent properties even at the nanolevel. Second, the quality, stability, and shelf life of the nutraceutical should be of a high echelon. Third, the production process and characterizing methods or instruments should not be very cumbersome and extravagant in nature. Finally, the nutraceutical should be free of any long-term adverse side effects or its nano fabrication should not be the genesis of any unwanted product. Hence, in keeping with all these factors in mind the first important step in the synthesis of nanocarriers is the selection of a proper production technology.

Two basic approaches are followed for nanoformulation of nutraceuticals (Fig. 8.1). They are the *top-down* and *bottom-up* approaches (Cushen et al., 2012). Top-down process of synthesis of nanoproducts involve the breaking down of large nutraceuticals to small units of a few nanometers in dimension. The process adopted may be of a physical or chemical nature. A common example of such a top-down process is the well-known mechanical milling (Zhu et al., 2010) or the homogenization technique of size reduction (Shibata, 2002). Here pressure is used to reduce the size of fat globules of an emulsion when synthesizing a nanoemulsion carrier of nutraceutical. This method is most commonly used in

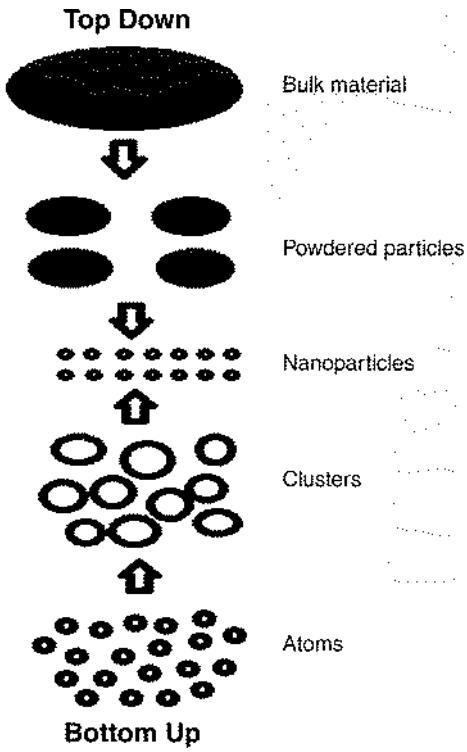

Figure 8.1. A typical pictorial representation of the "Top Down" and "Bottom Up" methods of nanoparticle production.

the dairy industries. Other recent top-down approaches adopted in the industries for mostly packaging purposes include the application of lasers and vaporization followed by cooling (Brody et al., 2008). Chief drawbacks for the top-down process are the critical choice of raw material for synthesis of the nanocarriers, imperfection of the surface structures leading to surface defects, marked crystallographic damage caused by the size-reduction techniques, and finally the lack of development of proper equipment system, both of which limits the application of the top-down process to a great extent (Zhu et al., 2010).

Bottom-up manufacturing technology is the other alternative method of production of nanomaterials. The bottom-up approach refers to the construction of a material from the bottom, starting completely from the base by building atom to atom followed by molecule to molecule and so on to form a complete cluster of a nanomaterial. Methods of bottom-up manufacture include crystallization, solvent extraction/evaporation, self-assembly, microbial synthesis, and so on (Brody et al., 2008). By this approach,

quite complex molecular structures can be designed based on self-organization of biological compounds, which can further be fabricated as per manufacturer's choice. The bottom-up approach also ensures the manufacture of nano structures with fewer defects and more homogeneous chemical compositions.

Nanodispersions and nanocapsules are the most important mediums of nutraceutical delivery. Antioxidants, food preservatives, vitamins, health supplements, antimicrobials, antifungals, and all such functional ingredients can be delivered in the form of these two modes. They not only serve to protect the functional ingredients from degradation or oxidation, but also transfer the nutraceutical to the desired site.

4.1 Namoemulsions or Nanodispersions

Nanoemulsions or nanodispersions are the most common forms of nanocarriers for nutraceuticals. They encapsulate the bioactive ingredients within their droplets, which are minimized to a size of less than 500 nm (McClements and Decker, 2000). Nanoemulsions can be generated using different types of methods, which can further be classified as either high-energy or low-energy methods (Acosta, 2009; Leong et al., 2009; Tadros et al., 2004). High-energy devices use mechanical forces which generate immense disruption within the emulsion system that breaks up the dispersed phase, that is oil, in case of an oil-in-water emulsion and lead to the formation of oil nanodroplets. Instruments that promote such impacts are high-pressure valve homogenizers, microfluidizers, and sonicaters (Gutiérrez et al., 2008; Leong et al., 2009; Sen Gupta and Ghosh, 2014a). The synthesis of nanoemulsions by any of the high-energy technique is primarily governed by the composition of the oil, emulsifier and the nutraceutical to be nanotized. Based on that, the amount of energy to be applied by the chosen technique is deciphered. Hence, nanoemulsions produced through high-energy technique serve as a base for the designing of nanoemulsions according to the manufacturer's and consumer's requirement. Therefore by altering the amount of oil, surfactant/emulsifier, co-surfactant, nutraceutical, and so forth (Anton et al., 2008), the formulation of the predisposed nanocarrier of the specified nutraceuticals is prepared. There are basically three broad groups of mechanical processes for generating nanoemulsions by application of high-energy technique (Anton et al., 2008):

1. *High-pressure homogenization technique*: In high-pressure homogenization technique the oil-water-emulsifier-nutraceutical mixture is subjected to very high pressure which is applied

through a highly restrictive valve. This pressure effectively splits the emulsion in tiny droplets, which is collected in a container attached at the rear end of the valve (Sanguansri and Augustin, 2006; Sen Gupta and Ghosh, 2014b). This method is quite effective in generating an emulsion with uniform droplet-size distribution.

2. *Ultrasound technique*: When oil and water are mixed in the presence of emulsifiers and then subjected to high-frequency ultrasound cavitation, then the dispersed oil droplets in water are minimized in size. Thus small oil droplets are obtained. These droplets are formed by the sudden impact of intense shock waves in the form of ultrasound that causes liquid jets to flow at a high speed and results in the rupture of the emulsion droplets. However, this technology is not very effective in producing nanosized nutraceutical droplets and hence lacks in large-scale industrial applications (Sanguansri and Augustin, 2006; Sen Gupta and Ghosh, 2014a);
3. *High-speed devices*: High-speed dispersion devices are not very efficient in minimizing the size of emulsion droplets, and hence not effective in creating nanocarriers for nutraceuticals. They mostly consist of a rotor or stator which mixes two or more immiscible systems intensely and produces a relatively good dispersion. The energy produced is mostly dissipated and hence cannot participate in size reduction of the emulsion droplets (Anton et al., 2008). Tadros et al. (2004), reported the energy consumption for high-pressure homogenization technique and stated that an efficiency of 0.1% energy consumption was observed, where 99.9% of the energy was dissipated as heat during the homogenization process. As a result an emulsion with droplet diameter of 600 nm was obtained. Hence it is difficult to utilize these calculations for development of such products at a large scale using the high-pressure homogenization technique. Rather it's better to develop these products in situ at the industries and perform their corresponding energy calculations instead of developing the products based on the laboratory scale calculations.

Low-energy approaches are those in which spontaneous nanoemulsions are formed when oil is mixed with water in an oil-water emulsifier system. Here, however, the environmental conditions and the physico-chemical conditions surrounding the mixture are altered. Examples of such systems include phase inversion temperature method and the solvent demixing methods (Anton et al., 2008; Bouchemal et al., 2004; Chu et al., 2007; Freitas et al., 2005; Tadros et al., 2004; Yin et al., 2009). In such approaches, the formation of nanoemulsions is based on phase transitions

techniques, without the incorporation of any kind of energy into the system. Emulsification in such cases is carried out by slowly mixing one immiscible component in the other, and thereby altering the composition, while keeping the temperature unchanged (Usón et al., 2004) or vice versa, that is, temperature is changed while maintaining a constant composition (Morales et al., 2003). Five common methods of low-energy approach that are popular today include:

1. *Spontaneous emulsification*: This process is commonly used when an emulsion between an aqueous and organic phase is prepared. The organic phase consists of a homogeneous mixture of the nutraceutical, proper surfactant and water-miscible solvent whereas the aqueous phase is made of water and a suitable hydrophilic surfactant (Bouchemal et al., 2004). The two phases initially are not in equilibrium with each other. The theory involves the spontaneous mixing of the two phases under a specific set of conditions that may be initiated by stirring, temperature change, developing a concentration gradient, and so forth, to finally develop a homogeneous medium, with nano level specifications. Spontaneous emulsification can be generated by a number of different techniques, namely, diffusion of solutes between the two phases, interfacial turbulence, surface tension gradient, and so on. Apart from that, the compositions of the individual components that compose the nanoemulsion and also the physicochemical characteristics of the different phases influence the emulsification mechanism (Bouchemal et al., 2004).
2. *Solvent displacement*: In this method a water-miscible organic solvent containing the lipophilic nutraceutical is mixed with the aqueous phase containing a suitable emulsifier. The rapid diffusion of the organic solvent deracinates the aqueous phase, in the presence of intense turbulence. This also simultaneously promotes the formation of nanoemulsions. Hence at low-energy, a high yield of nano-sized nutraceuticals is generated. Finally, the organic solvent is evaporated under reduced pressure to let the nano nutraceuticals precipitate. This technique is however limited to only water-miscible solvents (Chu et al., 2007; Yin et al., 2009).
3. *Membrane emulsification*: It is a relatively novel technique of producing nanoemulsions and has a quite low energy requirement. Both single and multiple emulsions systems can be produced by this technique. Here the dispersed phase or the droplets are forced through a microporous membrane directly into a continuous phase. The advantages of membrane emulsification over conventional emulsification processes are

that very fine droplet size and narrow droplet size distributions are attained. Furthermore this method entails the usage of very limited amount of emulsifier, which can be correlated with low requirement of shear stress. Hence shear-sensitive ingredients like starch and proteins can be conveniently used in this process. A chief drawback of this process is its dependence on the dispersed phase flux, which poses a big problem for extending the work up to industrial scale (Sanguansri and Augustin, 2006).

4. *Emulsion inversion point*: In this method the composition of the continuous and dispersed phases are changed. Here a transition occurs in the spontaneous radius of curvature of the surfactant, which can be obtained by changing the volume fraction of the continuous phase gradually. As the concentration of the continuous phase, say water, is increased in comparison to the dispersed phase, say oil containing a particular nutraceutical, the initial O/W emulsion is converted to a W/O emulsion, due to the change in the spontaneous curvature of the surfactant from initially stabilizing an O/W emulsion to a W/O emulsion at the inversion point. This process is common for the short-chain surfactants, which are extensively flexible in nature at the oil–water interface. Also a major iota of this unique phase inversion technique is the nominal interfacial tension which is developed in the system, which allows the phase reversal at a constant temperature. Successful formation of nanosized droplets are achieved, which are also kinetically stable in nature (Anton et al., 2008; Sadtler et al., 2010);
5. *Phase-inversion temperature*: Phase-inversion method refers to a phenomenon where oil-in-water emulsion regresses back to a W/O emulsion or vice versa. In this method explicit behavior of the nonionic emulsifiers which induces them to alter their compatibility to water or oil. The chief functional parameters here are temperature, rate of mixing and concentration of the respective components composing the emulsion (Preziosi et al., 2013). Phase inversion process results in the generation of minute dispersed droplets in a continuous phase. It can be initiated by rapid cooling of an emulsion (Izquierdo et al., 2004) or by dilution with water or oil as the case may be (Anton et al., 2008). The nanoemulsions thus formed are kinetically stable. Hence the degradation or agglomeration of such emulsions does not occur easily. Furthermore an added advantage of this method is that it is a simple process needing low amount of energy and hence it's easy to develop industrial scale products using this technique (Anton et al., 2008).

4.2 Nanocapsules

Nanocapsules are the modern mode of nutraceutical delivery. They have already been utilized in the field of drugs and pharmaceuticals. But they are relatively new born options in the food and nutraceuticals industries. It is basically a technology to pack substances in miniature by the application of nanocomposite, nanoemulsification or nanoestructuration. The final product that is formed is an important medium of controlled release. Apart from that it has several other functionalities such as protection of bioactive compounds (Huang et al., 2010; Sen Gupta and Ghosh, 2012b; Sen Gupta and Ghosh, 2014b). Such nanoencapsulation systems are advantageous due to the fact that they enhance the performances of the nutraceuticals, improve their bioavailability, solubility and stability, as observed both in vitro and in vivo and also prevent unwanted interaction with other materials hence minimizing any de trop side effects.

In fact today the nanoencapsulation technologies have the ability to face the complicated challenges of the nutraceutical industries because of its well-developed targeted delivery and controlled release methodologies. Both lipid and nonlipid based nutraceuticals when encapsulated, bear the capacity to easily disperse in a water-based product, thus improving its ability to be effectively being absorbed by the body. Different types of nanocapsules can be prepared based on the type of nutraceutical to be encapsulated. It is also possible to capacitate the nanocapsules according to the consumer need and they are to be targeted by the bioactive compound. Additionally, nanocarriers display more surface area than the corresponding micro sized capsules and hence have the potential to enhance several functional characteristics. Nanocapsule based nanocarriers can be classified into broad three categories as described below:

1. *Liposomes*: Liposomes find wide-scale applicability both industrially and in the research laboratories due to its immense beneficial properties. It can be produced using natural ingredients like phospholipids, where all types of water-soluble, lipid-soluble and amphiphilic compounds can be encapsulated and released as and when required, that is, based on the molecule targetability (Mozafari et al., 2008; Thompson et al., 2006). Another added advantage of liposomes is their kinetic stability, which ensures a high shelf-life for the encapsulated nutraceutical. Due to this beneficial aspect the liposomes have also been widely used in the food sector. The mechanism of liposome formation is based on the antagonistic interactions between a lipophilic compound namely say, phospholipids, and water molecules. Due to such interactions the polar head groups of

the lipophilic compound is oriented towards the inner and outer water molecules, whereas the hydrophobic hydrocarbon tails are associated with each other and the bilayer structure is formed (Jesorka and Orwar, 2008). Liposomes are unique due to the fact that they are capable of encapsulating compounds of different solubilities and also be tailored for targeted delivery at the desired site of the encapsulated nutraceutical (Mozafari et al., 2008). The common methods to synthesize nanosized liposomes include mechanical processes like extrusion, sonification, high pressure homogenization, microfluidization and nonmechanical processes like reversed-phase evaporation, depletion of mixed detergent-lipid micelles (Schroeder et al., 2009). Followed by the synthesis of the liposomes they need to be stabilized by any of the specified methods like lyophilization, freezing, spray-drying or supercritical fluid technology (Mishima, 2008). It was observed that the stability of liposome nanocapsules produced by the supercritical fluid technology is the highest in comparison with the other methods of stabilization techniques. However, lyophilization is the most commonly used technique for enhancing shelf-life of the liposomes, especially for those containing heat sensitive compounds (Chen et al., 2010). Different sugars like monosaccharides, disaccharides, polysaccharides have been used as cryoprotectants during lyophilization. Although there are innumerable benefits of the liposome delivery system, a major disadvantage of the liposome is its short span of nutraceutical release period. However to overcome this drawback different encapsulants are being developed today to instigate longer release times (Sen Gupta and Ghosh, 2014b).

2. *Solid lipid nanoparticles*: Solid lipid nanoparticles are the emerging encapsulation technique that has attracted attention due to its immense potential as a nutraceutical delivery system. It is already a developed technology in the pharmaceutical industry and is presently being exploited in the food and nutraceutical sectors. Solid lipid nanoparticles consist of a matrix composing the solid lipid shell (Müller et al., 2000a). Compared to nanoemulsions and liposomes, solid lipid nanoparticles are more advantageous in terms of having higher encapsulation efficiency leading to the industrial scale production possibilities. These nanoparticles basically consist of a solid lipid core matrix, which can solubilize lipophilic nutraceuticals. By solid lipid matrix it is indicated that different lipid classes like triglycerides, diglycerides, monoglycerides, fatty acids, sterols, fatty alcohols, and so forth can be used. A suitable emulsifier stabilizes such nanoparticulate matters. Often instead of using

a single emulsifier, a combination of two or more emulsifiers or surfactants has been shown to extend long-term stability to the solid lipid nanoparticles. Furthermore,the avoidance of organic solvents during the synthesis of the nanoparticles is an added advantage for this group of nanoparticles. It was also observed that the produced nanoparticles were capable of providing high flexibility because of which a distinctly controlled release profile of the nutraceuticals could be obtained. This fact can be attributed to the presence of the solid lipid matrix, which was also responsible for the slower degradation rate of the released bioactive materials for prolonged periods of time.

Several methods have been reported for the production of solid lipid nanoparticles. Some of the most common methods for large-scale production are hot homogenization and cold homogenization (Müller et al., 2000a). In the hot homogenization method, the lipid is melted at approximately 5–10°C above its melting point. The bioactive compound or the lipid soluble nutraceutical remains dissolved in the melted lipid. Followed by the dissolution of the nutraceutical in the lipid layer the lipid is emulsified with water containing a suitable emulsifier, to produce a preemulsion. Size-reduction of the preemulsion is then carried out by passing it through a high-pressure homogenizer at a well-mediated temperature. The temperature of the nanoemulsions then is gradually lowered to abet the incorporated lipid molecules, thus resulting in the formation of solid lipid nanoparticles. The recrystallization is generally carried out by lyophilization technique (Müller et al., 2000b). A distinct disadvantage of this process is the loss of any lyophobic bioactive compound, which may be solubilized in water at the high temperature of the process, making the hot homogenization technique not a very efficient one in terms of the incorporation of all the nutraceuticals into the solid lipid matrix. Hence for heat-sensitive nutraceuticals like enzymes or carotenoids, cold homogenization techniques should be applied. In the cold homogenization process, too, like the hot homogenization technique, the bioactive compound or the nutraceuticals is incorporated into the previously melted solid lipid. The lipid melt is then cooled and after solidification is pulverized under pressure. The lipid nanoparticles thus obtained are then dispersed in an aqueous emulsifier solution at room temperature. The medley consisting of the organic and aqueous phases are maintained at a low temperature of 0°C. Due to the solid state of the developed mold, apportionment of the nutraceuticals between the aqueous and lipid phases is markedly deprecated. This stands as the main problem in case of cold homogenization technique (Fathi et al., 2012). In case where the active matter remains in the external shell layer a primary

disadvantage is with the delivery of the bioactive products in correlation with its release, which is due to the exposure of the nutraceutical. However, use of low production temperature and low emulsifier concentration help in decreasing the degradation effect of the nutraceuticals (Müller et al., 2000a).

Another common technique for the synthesis of solid lipid nanoparticles in a small scale includes the emulsification–evaporation method, which is followed by sonication. Here a crude emulsion is initially prepared by mixing an organic solvent, containing the bioactive nutraceutical, along with a suitable emulsifier. The produced preemulsion is then sonicated at a definite temperature. This temperature corresponds to that of a temperature which is higher than the condition at which the lipid melts (Fathi et al., 2012). The ultimate product is originated by enumerating the generated nanoemulsion to cold water, which also contains a hydrophilic surfactant and solid lipid nanoparticles are produced. Finally the solvent is evaporated to get the resultant product (Varshosaz et al., 2010). However, a few disadvantages of the solid lipid nanoparticles include low encapsulation efficiency and probable degradation of the nanoparticles during storage.

3. *Nanostructured lipid carrier*: Radtke and Muller (2001) developed a novel nanocarrier system called nanostructured lipid carrier, which was conceived to make up for the drawbacks of the solid lipid nanoparticles. These nanocarriers can be produced by mixing lipid molecules of different physical characteristics, like solid in nature or liquid lipids that are oils. Thereafter, the nanoparticles are prepared in a similar manner, as is done in the case of solid lipid nanoparticles. The produced lipid base shows a distinct lowering of melting point in comparison to the original solid lipid. In fact by giving the lipid matrix a certain nanostructure, the encapsulation efficiency is considerably enhanced and the size distribution of the nanoparticles is also smaller. Furthermore the problem with the shelf life of the nutraceuticals regarding their degradation was also resolved (Radtke and Muller, 2001). The most important criteria for these nanoparticles is the nature of the nutraceuticals and the type of emulsifier/surfactant chosen for the development of the nanoparticles, which is also responsible for the sustained release of nutraceuticals.

4.3 Release From Nanocapsules

Controlled release of nutraceuticals from nanocarriers, especially nanocapsules is one of the most important criteria that need to be monitored during the synthesis of a nanoparticle. Controlled release (Fig. 8.2) is the basis of targeted delivery of

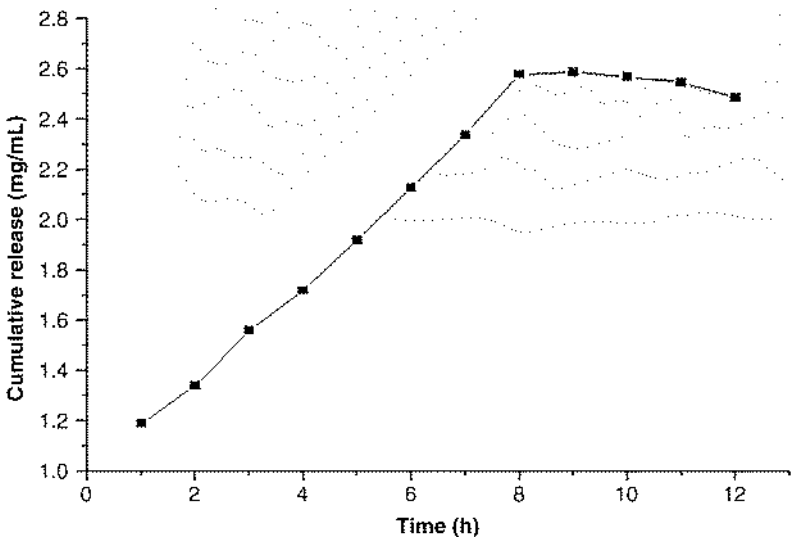

Figure 8.2. A typical graph indicating the cumulative release of nutraceuticals (given by weight of it in milligram per milliliter of the released matrix) from nanocapsules against time represented in hours.

the nutraceuticals at the desired site of action. The release from nanocapsules can be described as delayed release and sustained release. In case of delayed release the dispension of a nutraceuticals is deferred from the nanocarriers by the presence of a suitable encapsulant. Depending on the type of encapsulant, it degrades at a specific time and continues for certain period, which is denoted as the *lag time*. Beyond this time span the release is obstructed. This mechanism is beneficial in the case of developing a compound where the release is supposed to occur only under gastric conditions and not before that. Furthermore the protection of nutraceuticals is also an important factor that is taken care of via this system. In case of the sustained release type, the nanocarriers are so devised that a constant concentration of the nutraceuticals is maintained at its target location. This system is manoeuvred in such a way that clemency of the encapsulated material extends over a long period of time. This fact is particularly useful in perpetuating the release of a certain nutraceutical gradually instead of sudden burst of release of the compound in the blood stream. However there are many factors that control the release of the bioactive matter from the encapsulated material. Some of them are the shape, structure and dimensions of the nanocarrier, compatibility of the nutraceutical with the dispersed phase, continuous phase, encapsulant and finally the environmental impacts like temperature, pressure, pH of the medium, erosion rate, polymorphism of

the lipid based carriers, encapsulation efficiency and so on (Barat et al., 2008; Briones and Sato, 2010). To this date the different encapsulants that have been used with effective results include carbohydrates, protein, or lipid bases (Sen Gupta and Ghosh, 2012a,b, 2014b). The final aim is always to get high encapsulation efficiency of more than 50% and of course a 100% release profile, which can only be possible if an almost perfect nanocarrier is designed, which is devoid of any surface defects. In spite of the choice of a suitable encapsulant, there is always a third factor which controls the behavior of the nanocarriers, be it chemical interferences or media properties, complete control over such delivery system is not possible.

The controlled delivery systems are very important in terms of formulating nanocarrier systems (Sen Gupta and Ghosh, 2015). However it is essential to develop a suitable mathematical modeling for the release process which is essential to understand the mechanism of the nutraceutical release from the nanocarrier. The nutraceutical release is basically governed by one or combination of different mechanisms, like diffusion, erosion and swelling. Both erosion and swelling occur mostly in hydrophilic carriers. Therefore, it is mandatory to understand the basic mathematical model regarding the diffusion mechanism in case lipid based nanocarriers. Fick's law of diffusion helps to understand the progress in the release behavior of nutraceuticals, with time from a nanocarrier system. From Fick's first law of diffusion (Fathi et al., 2012) it can be said that the released components from the nanoparticles commences a diffusion rate which moves from a high concentration region to a low concentration region through a concentration gradient. This concept can be protracted by using Fick's second law of diffusion (Eq. 8.1). This elaborates the changes in the concentration of the released compounds from nanoparticles with the time taken. Fick's equations can thus be represented numerically.

$$J = -D\frac{\delta \Phi}{\delta x} \tag{8.1}$$

where J is the *diffusion flux* (mol/m^2 s); D is the diffusion coefficient (m^2/s); Φ is the concentration per unit volume (mol/m^3); and x is the position in length (m).

Dissolution of capsules to release nutraceuticals which do not disaggregate and release the bioactive component gradually is represented by the zero order models (Hadjiioannou et al., 1993). This is expressed by the (Eq. 8.2).

$$Q_t = Q_0 + K_0 t \tag{8.2}$$

where Q_t is the amount of nutraceuticals dissolved in time t; Q_0 is the initial amount of nutraceutical in the solution at time 0; and K_0 is the zero order release constant expressed in units of concentration/time.

Next the first order model was designed to describe the absorption of some specific nutraceuticals, for which the mechanism of release and absorption is difficult to conceptualize appropriately (Bourne, 2002). The release of the nutraceuticals which follow a first order kinetics can be expressed as follows (Eq. 8.3).

$$\log C = \log C_0 - \frac{K_t}{2.303} \tag{8.3}$$

where C_0 is the initial concentration of nutraceutical; K_t is the first order rate constant at time t.

Again Higuchi (1963) developed a square root of time model to describe the phenomenon of release from spherical units containing nutraceuticals (Eq. 8.4).

$$Q = A\sqrt{D(2C - C_s)C_s\, t} \tag{8.4}$$

where Q is the amount of nutraceutical released in time t per unit area A; D is the diffusivity of the nutraceutical molecules; C is the initial nutraceutical concentration and C_s is the nutraceutical solubility in the matrix media.

Hixson and Crowell (1931) developed a theory for particles with regular area, which is assumed to be proportional to the cube root of its volume. The equation describes the release from a system where there is a change in surface area and diameter of capsules. The equation can be expressed as follows:

$$W_0^{1/3} - W_t^{1/3} = kt \tag{8.5}$$

where W_0 is the initial amount of nutraceutical dosage; W_t is the remaining amount of nutraceutical dosage form at time t; and k is a constant for the surface–volume relation.

Finally the Korsmeyer–Peppas model (Korsmeyer et al., 1983) derived a simple relationship to describe the nutraceutical release from a polymeric system. The relationship can be expressed with the (Eq. 8.6):

$$Mt/M_\infty = K_n^t \tag{8.6}$$

where Mt/M_∞ is a fraction of nutraceutical released at time t; k is the release rate constant; and n is the release exponent. The n value is used to characterize different release rates for cylindrical-shaped matrices.

To find out the mechanism of nutraceutical release, two different concentrations of nutraceuticals at different time periods

were input in the respective equations and were fitted in the Korsmeyer–Peppas model. In this model, the value of n characterizes the release mechanism of the nutraceutical. For the capsules of cylindrical nature, $0.45 \leq n$ corresponds to a Fickian diffusion mechanism, $0.45 < n < 0.89$ to non-Fickian transport, $n = 0.89$ to Case II (relaxational) transport, and $n > 0.89$ to super case II transport (Dash et al., 2010).

5 Characterization Techniques of Nanocarriers for Nutraceuticals

There are several techniques to characterize the nanocarriers of nutraceuticals, which is based on the type of nanocarrier that has been synthesized. These methods can distinguish individual nanocarriers mostly based on their physical properties. The following section describes these methods in brief.

5.1 Dynamic Light-Scattering Technique

Dynamic light scattering (DLS) technique also known as the photon correlation spectroscopy or quasi-elastic light scattering technique is generally used for the rapid determination of the particle-size distribution profile of the nano-sized droplets, mostly in emulsions, colloids, suspensions, or polymer solutions (Fig. 8.3).

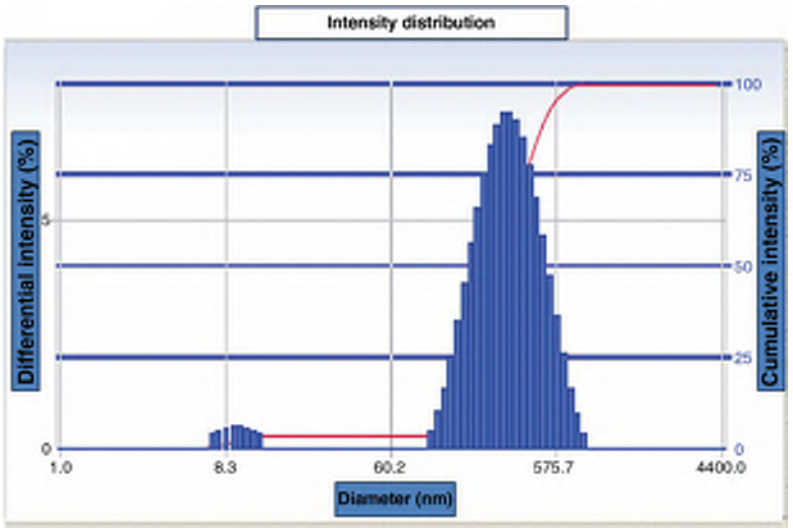

Figure 8.3. A graphical representation of the particle-size distribution as represented by the intensity distribution for a nanoemulsion.

DLS actually measures the Brownian motion of the suspended particles and relates this to the size of the particles. Through DLS illumination the particles utilize the laser for analyzing the intensity fluctuations in the scattered light. DLS provides a quick and efficient evaluation technique of the size of the nanoemulsion droplets along with its stability parameter (Sen Gupta and Ghosh, 2014a).

5.2 Zeta Potential

Zeta potential is used to describe the electrokinetic potential of a colloidal system (Mills et al., 1993). In the case of colloids, zeta potential is a unit to express the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. A value of 30 mV (positive or negative) is taken as the optimum value for determination of the stability of the colloidal system as it indicates the value that separates a low-charged surface from a highly charged one (Preetz et al., 2010). Zeta potential value is related to the stability of the colloidal dispersions, as there is a repulsion between similarly charged molecules in the dispersion. For small droplets, a high zeta potential indicates good stability, indicating no aggregation. When the zeta potential is low, attraction exceeds repulsion and this leads to the breakdown of dispersion leading to flocculation. Nanoemulsions having higher zeta potential values are indicative of an extreme stability while nanoemulsions with low zeta potential values have low stability and hence such emulsions easily break down. Hence zeta potentials from 0 to ±30 mV indicate instability, while zeta potentials higher than ±30 mV indicate stability. Major factors influencing the zeta potential of nano colloids are the presence of different surfactants, type of organic phase, presence of different particles, ionic strength, droplet morphology and size, pH of the solution, and so forth (Simunkova et al., 2009).

5.3 Differential Scanning Calorimetry

Differential scanning calorimetry or DSC is a technique involving the study of heat and mass changes as a function of temperature. Along with the sample a reference standard is also used to correlate the changes observed for the nanoparticle samples. During the analysis the readings corresponding to the sample and the reference standard in terms of their mass and heat changes are recorded at the same temperature. Several such readings are noted at increasing temperatures. The particular temperature at which any kind of drastic thermal or mass changes occur are noted

as this gives a complete idea regarding the nature of the nanoparticle, its melting range, purity, homogeneity, and so on (Venturini et al., 2011). Differential scanning calorimetry is basically used to ascertain the phase transitions involved when a colloidal system is heated over a span of temperature. It helps to ascertain the crystalline nature of the fats or emulsions. This crystalline nature affects the stability of the emulsions, which subsequently depends on the type of emulsifier. Furthermore the DSC data also indicates the nature of the encapsulated nutraceuticals and whether it is in an intact form or has degraded.

5.4 X-Ray Diffraction Technique

X-ray diffraction techniques indicate a group of nondestructive analytical techniques that discloses the crystallographic structure, composition, and physical properties of the emulsion (Fig. 8.4). This is mostly done in the dried form of the emulsions. By X-ray diffraction the scattered intensity of an X-ray beam hitting on a sample is measured along with the diffraction pattern of it, and

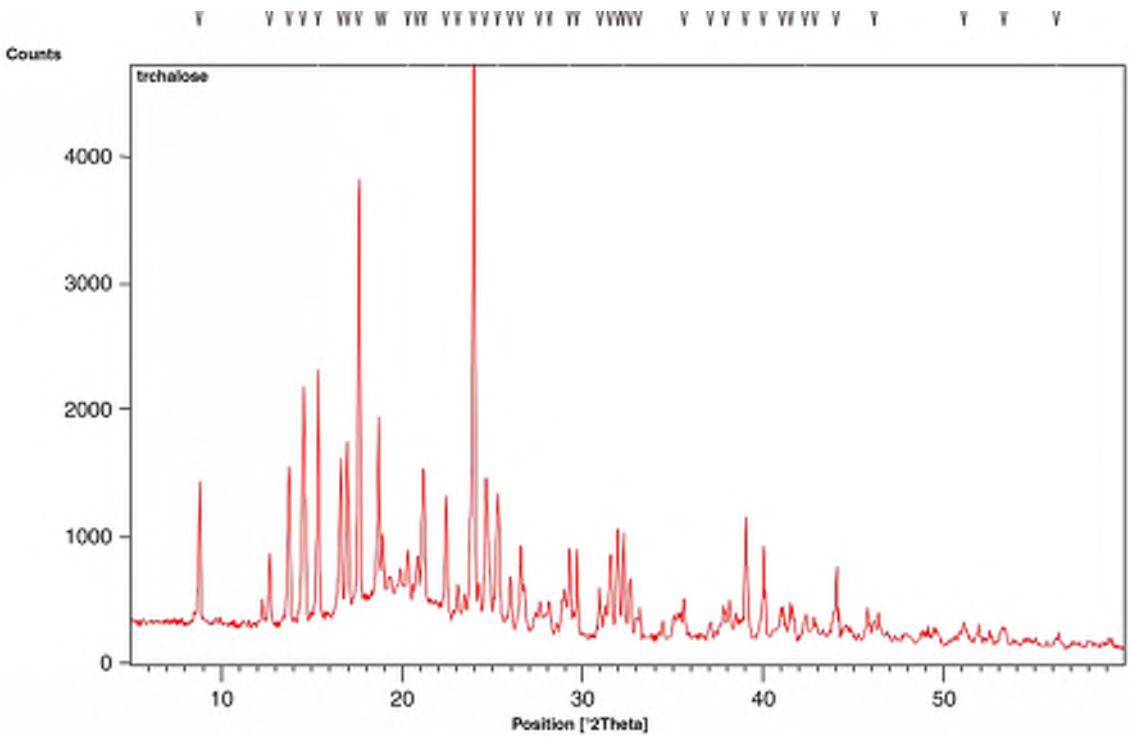

Figure 8.4. X-ray diffraction data for a crystalline compound (trehalose) used as an encapsulant.

based on these data the crystallinity of the compound is determined. Hence by X-ray diffraction technique the crystalline structure helps researchers to understand the structural characteristics of liposomes, solid lipid nanoparticles, or dried nanoemulsions. Also the nature of the nutraceutical, whether in an encapsulated or free state, could also be determined from the diffraction studies by comparing the two diffractograms (Sen Gupta and Ghosh, 2015).

5.5 Imaging Techniques

Microscopy is used as a technique for imaging different solids or colloidal suspensions like nanoemulsions. The type of microscopic technique to be used depends upon the type of product to be investigated. The imaging technique helps to assess the size, shape, and nature of the product being analyzed. Some of the common imaging methods include the following.

5.5.1 Transmission Electron Microscopy

Transmission electron microscopy is the method of analyzing any particle to the range of nanometer (Fig. 8.5). Here a beam of electrons is passed through the sample to be analyzed. An image is formed by the interaction of the electrons with the sample being analyzed, which is magnified and then projected on an imaging

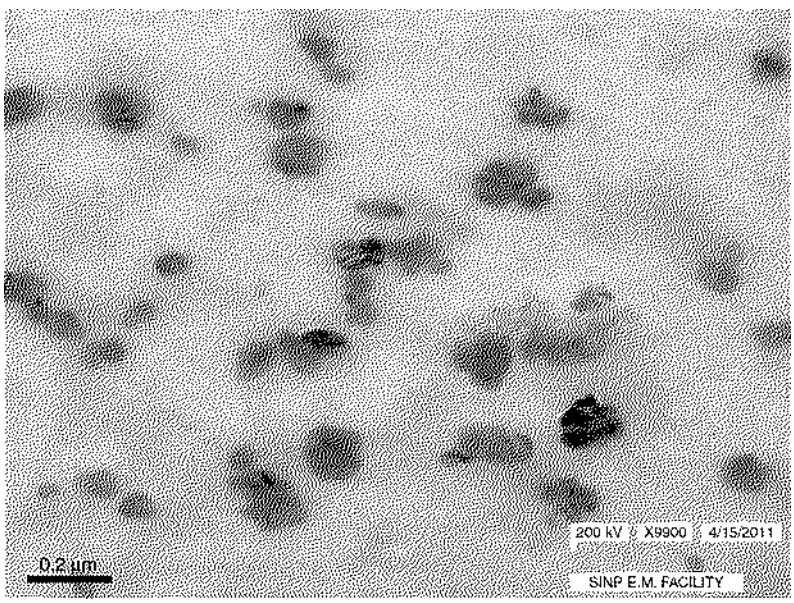

Figure 8.5. Transmission electron microscopic view of a typical nanoemulsion.

device (Sen Gupta and Ghosh, 2014a). A chief disadvantage of this kind of imaging is the extensive sample preparation technique required, which makes it a time-consuming process. However, in the case of nanoemulsions, the TEM studies help to study the exact morphology of the emulsions minutely. Thus it helps to determine the effect of the method of formation of nanoemulsions on the shape and structure of the particle droplets. The principle of the imaging involves the combination of bright field visualization with corresponding magnification, especially for small-sized particles. This property also helps to perform the diffraction and estimation of selected areas of a nanoemulsion and their particle morphology evaluation.

5.5.2 Scanning Electron Microscopy

Scanning electron microscopy is another technique for capturing images of nanosized vesicles at high-resolution (Sen Gupta and Ghosh, 2012b). SEM images have a peculiar three-dimensional appearance and helps in the interpretation of the surface morphology of nano compounds (Fig. 8.6). The resolution power is almost similar to that of TEM. In case of temperature sensitive materials, the imaging is done at a very low temperature by means of liquid nitrogen, the technique being called *cryo-SEM*. However unlike TEM, the SEM imaging provides the basic surface structure of a particular compound, as it does not involve the transmission of electrons technique because of which it is essential for

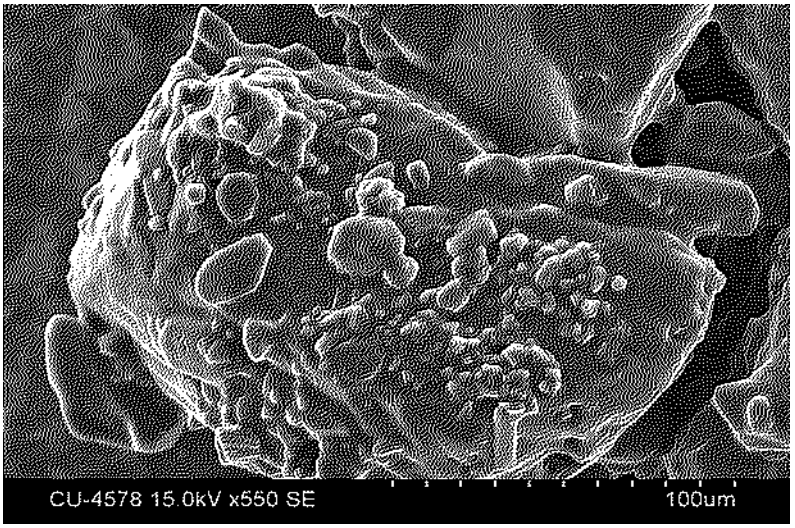

Figure 8.6. Scanning electron microscopic view of a solid lipid unit.

the compound to have a highly conducting surface. This helps in providing a good representation of the surface depth of the compound being analyzed. Hence, a massive number of dimensions of a particular product or an enormous number of particles can be pictured at the same time. These factors make scanning electron microscopy an immensely popular imaging instrument today, though it can be correlated to a costly and high vacuum process.

5.5.3 Atomic Forced Microscopy

Atomic forced microscopy has been recently developed for imaging purposes (Luykx et al., 2008). A very high resolution can be accomplished by the AFM technique, because of which precise and infinitesimal small details like single atoms or molecules that have dimensions of maybe just a few nanometers have been observed by means of AFM. It is based on the rapid scanning of a nanometer-sized sharp probe on a compound which is previously immobilized on a definite surface of mica or glass. A very high-resolution three-dimensional image is obtained. Using AFM liposomes, protein- or carbohydrate-encapsulated nanosuspensions and other nano forms can be visualized (Luykx et al., 2008). Furthermore the structure, morphology, and nature of nanoemulsions can also be studied using AFM. However the difficulty encountered with the use of AFM is that any particle which is too soft or tacky in nature cannot be analyzed.

5.6 Encapulation Efficiency

Nanoencapsulation is the technique of enclosing food ingredients or nutraceuticals by a definite coating agent, to produce tiny capsules displaying several useful properties. The *encapsulation efficiency* indicates the amount of the total nutraceutical or food ingredient actually encapsulated. This term indicates the basic proficiency of the encapsulation procedure or the encapsulant to effectively sheathe the inner core material and generate stable nanocapsules. The efficiency of encapsulation of the nanocapsules can be determined by estimating the amount of the bioactive material encapsulated. The bioactive materials are extracted from the nanocapsules by suitable extraction methods and then evaluated for the actual amount that was collected (Sen Gupta and Ghosh, 2014b). Encapsulation efficiency is given by (Sen Gupta and Ghosh, 2015):

$$\text{Encapsulation efficiency} = [(A - B)/A] \times 100.$$

where, A = actual loading of bioactive product; and B = free bioactive product.

Encapsulation efficiency estimation is an important criterion for estimating the ability of the encapsulant material and encapsulation method for synthesizing the nanocapsules and thereafter modifying either the encapsulant or the methodology according to need.

6 Conclusions

Nanocarriers comprise the most promising medium for improving solubility, bioavailability, and functionality of lipid- and nonlipid-based nutraceuticals. Consumption of nutraceuticals due to their purported health benefits, in addition to the basic nutritional value found in foods, for preventing chronic diseases, improving health, delaying the aging process, increasing life expectancy, and supporting the functions of the body is a growing trend these days. Industrial and consumer demands have encouraged the development of such delivery systems. There are several techniques available to produce and characterize the nanocarriers for the transmittal of bioactive components at the targeted site. Based on the end use and the type of compound, the choice of method for nano formulation is selected. The choice of characterization technique also depends on these factors. The most important criterion for the nanocarriers is the release of the nutraceuticals at the desired site and at the right time. This factor needs to be regulated properly. Nanocarrier formation techniques address the high performance requirements of today. Hence methodologies used include high-pressure homogenization, ultrasonication, liposomes, solid lipid nanoparticles, phase inversion, solvent evaporation, and so forth, for nano concoction of nutraceuticals. The application of nanocarriers to nutraceutical systems, however, still poses a challenge in terms of the production processes, and especially their cost. Furthermore for enhancing the physico-chemical as well as the nutritional qualities of pure lipid-based or nonlipid based nutraceutical products open avenues for structuring the compounds to upgrade their properties. In addition to that, another important factor that must be kept in mind during the preparation of a nutraceutical nanocarrier is the safety aspect. The general acceptance of any product will ultimately depend on the safety of the final product.

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9

ENCAPSULATION OF NUTRACEUTICALS IN NOVEL DELIVERY SYSTEMS

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1 Introduction

The term nutraceuticals resulted from the junction between "nutrition" and "pharmaceutical" and it has been in use since 1989, the time when Dr. Stephen L. DeFelice has used it for the first time (Brower, 1998). According to Dr. DeFelice "a nutraceutical is a food or part of a food, such as a dietary supplement, that has a medical or health benefit including the prevention and treatment of disease" (Kalra, 2003). Nutraceuticals may range from whole foods to single isolated food components, including herbal products, processed foods (eg, cereals, beverages) and genetically engineered or "designer" foods (Kalra, 2003; Wildman and Kelley, 2007). Their use seems to provide more benefits, avoiding several side effects and, as normally they are products used in the human diet, they are also rapidly accepted by the regulatory authorities, as well as by the population (Trottier et al., 2010). Most nutraceuticals are usually isolated from natural nutritional sources, and thus are expected to exhibit relatively less toxicity and less side effects than drugs commonly used to treat similar symptoms (Ting et al., 2014). Nutraceuticals are powerful instruments that help in the maintenance and improvement of human health and act against nutritionally induced acute and chronic diseases, promoting a higher quality of life. In

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some definitions there are differences between diet supplements and nutraceuticals; in summary, a nutraceutical can also be a food supplement but not all food supplements are nutraceuticals, as nutraceuticals, apart from diet supplements, must aid in the prevention and/or disease treatment, and most dietary supplements have no ability to treat or prevent diseases (Ross, 2000; Kalra, 2003).

The development and market implementation of nutraceuticals and related products are growing fast in the food and pharmaceutical industry. This trend is mainly triggered by the current and future consumer concerns, including the preference for natural products in opposition to pharmaceuticals. This can be explained by the several adverse effects, the need for implementation of a healthy and more natural diet contributing for the best health and homeostasis (Nicoletti, 2012).

The focus on nutraceuticals is their acceptance as novel and modern forms of natural substances to benefit human health. The rapid expansion in this area tends to improve the development of several aspects, including legal and market aspects, as the need to develop policies to regulate their application and safety combined with the control in their composition and/or adulteration. The lack of these measures contributes to a negative impact in their market implementation (Silano et al., 2011).

Nutraceuticals are being investigated due to their potential use and effects in human health. Although the research and availability of scientific data is rapidly increasing in this area, the validation of these products remains a major concern. It is crucial to assure the security of the composition, obtained by the useful and adapted analytical approaches. As some nutraceuticals are poorly water-soluble molecules (eg, some polyphenols, omega-3 fatty acids) or may suffer chemical alterations upon exposure to gastric environment (eg, peptides), there is an urgent demand to overcome this issues and one of the solutions has been given by nanoencapsulation. The materials used for the encapsulation of nutraceuticals need to be safe, to guaranty quality, and stability, and to assure the physiological function of the encapsulated compounds. And thus, these materials must be food-grade or nanomaterials "generally recognized as safe" (GRAS) to fulfill the US Food and Drug Administration (FDA) requirements (Huang et al., 2010).

2 Distinction Between Nutrients, Functional Food, and Nutraceuticals

Nutrients are generally defined as substances that are needed by an organism in order for it to survive, grow, develop, and reproduce. The major classes of nutrients are carbohydrates, proteins,

lipids, vitamins, and minerals, although other substances are also needed for the survival of a living organism, such as water, dietary fibers, and oxygen, although these are not usually regarded as nutrients. They are the basis of nutrition and of the daily diet. In the 20th century, one of the major contributions to nutrition was the concept of a balanced diet that was defined as "an appropriate mixture of food items that provides, at least, the minimum requirements of nutrients and a few other food components needed to support growth and maintain body weight, to prevent the development of deficiency diseases and to reduce the risk of diseases associated with deleterious excesses" (James et al., 1989).

At the turn of the 21st century, in a society of abundance, the world faces new challenges, from an increase of life expectancy, exponentially growing costs of health care, and development of new technologies to major changes in lifestyles. Nutrition has to adapt in order to provide maximization of individual physiological functions, well-being, and health and at the same time minimizing the risk of diseases associated with food intake. The concept of functional food has emerged as a concept in the nutrition field and not in the pharmacological field (Roberfroid, 2000). A definition of functional food has been presented as food that is being cooked or prepared using "scientific intelligence" with or without knowledge of how or why it is being used. Thus, functional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates, and so on, needed for its healthy survival (Cencic and Chingwaru, 2010). In order that a food product can be included in the category of functional food it has to obey to several features, as illustrated on Fig. 9.1

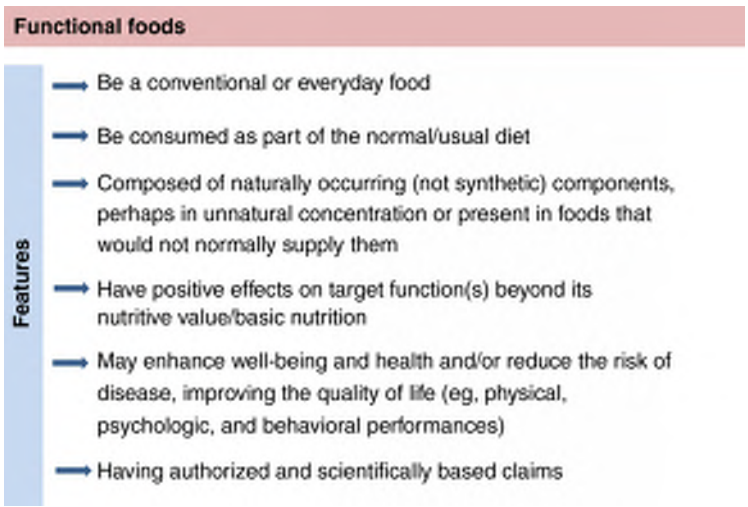

Figure 9.1. Main features that a functional food has to obey.

(Diplock et al., 1999). A functional food must remain food and it must demonstrate its effects in amounts that can normally be expected to be consumed in the diet: it is not a pill or a capsule, but part of the normal food pattern (Diplock et al., 1999; Roberfroid, 2000). A functional food does not need to be "functional" to all members of the population, thus each individual may select functional food according to its biochemistry and physiology. Functional food can be one of the following: (1) a natural food (eg, fruits and vegetables that functions as antioxidants in the body); (2) a food to which a component has been added (eg, fortified margarines with plant sterols that aim to reduce blood levels of cholesterol); (3) a food from which a component has been removed (eg, low-fat dairy products that aim at reducing fat intake); (4) a food where the nature of one or more components has been modified (eg, fermented foods, as yogurt where the type of bacteria and the lactases they possess helps decreasing or modifying the lactose content); (5) a food in which the bioavailability of one or more components has been modified (eg, the presence of oils as food additives resulting in enhanced absorption of liposoluble vitamins); or (6) any combination of these possibilities.

Ultimately, the beneficial health effects of functional foods come from their ingredients that are bioactive compounds exerting their effects on the organisms through a variety of mechanisms, most of them still unknown. Most of the bioactive compounds originate from plants and fruits, although some are derived from animals or microorganisms (Webster-Gandy et al., 2012; Abourashed, 2013).

From the earlier definitions, a group of nutraceuticals includes some functional foods or components of claimed functional foods, although not all claimed functional foods have nutraceuticals as components. This is because the scope of a nutraceutical is different from a functional food, as the *prevention* and *treatment of disease* (ie, medical claims) are relevant to nutraceuticals, only reduction of disease (not the prevention and treatment of disease) is involved with functional foods. And, whereas nutraceuticals include some dietary supplements, as well as other types of foods, functional foods should be in the form of ordinary food (Kwak and Jukes, 2001; Cencic and Chingwaru, 2010). Nutraceuticals also differ from food supplements, as nutraceuticals should not supplement the diet but should act on disease (and/or disorder) treatment and/or prevention (Cencic and Chingwaru, 2010).

Nanotechnology is one of the most innovative scientific research fields. It stands for the production of nanoparticles with special properties, such as the targeting of site-specific, controlled drug release, enhancing content stability over the time and against aggressive environment conditions (eg, pH, temperature, oxygen).

Nanotechnology may bring a revolution in industry in the near future. For this purpose, we describe some nanobased delivery systems that are currently being developed for nutraceutical encapsulation and delivery.

3 Nanotechnology-Based Delivery Systems Applied in the Nutraceuticals Encapsulation

The main advantages of nutraceuticals encapsulation include (1) protection of products from oxidation, (2) taste and odor masking, (3) enhanced delivery, (4) controlled delivery of nanoencapsulated nutraceuticals, (5) possibility to improve nutraceuticals bioavailability and biological action, (6) high precision in food quality, and (7) antibacterial safety and quality (Yao et al., 2015; Ranjan et al., 2014).

The use of nanomaterials to encapsulate nutraceuticals is very extensive, although they share some main characteristics, which are the biotolerability, biodegradability, and the ability to be inert when in contact with biological systems as well with the products to encapsulate (Yao et al., 2015; Ranjan et al., 2014). The majority of the core materials used for encapsulation of nutraceuticals is easily found in the human diet, and thus are easily digested and processed. Many of the materials can be divided according to their nature and chemistry. The choice of the nanomaterial is crucial because it determines the effectiveness of the nanocarriers (Souto et al., 2013). Some examples of nanocarriers are illustrated in Fig. 9.2.

The most important variables that affect bioactive release include (1) nature, shape, and dimensions of the nanocarriers; (2) bioactive diffusivity and solubility; (3) polymorphism form of lipid based carriers; (4) porosity of the nanocarriers; (5) bioactive ratio between the nanocarrier and the aqueous medium; (6) pH of the release medium; (7) encapsulation efficiency; and (8) loading efficiency. The most common materials used for micro- and nanoencapsulation of nutraceuticals, and generally used in drug delivery, are natural polymers, including alginate (Annan et al., 2008; Azevedo et al., 2014; Das et al., 2010; Trabelsi et al., 2013), chitosan (Azevedo et al., 2014; Chen et al., 2013; Cook et al., 2014; Das et al., 2010; Trabelsi et al., 2013), cellulose derivatives (George et al., 2014; Maity et al., 2013), dextrans (Aumelas et al., 2007), and pectins (Dutta and Sahu, 2012). Regarding the lipids, they can be divided in acylglycerols (mono-, di-, and tri-) (Fangueiro et al., 2012, 2014a), fatty acids (Weiss et al., 2012), phospholipids (Abd El Azim et al., 2015; Xia et al., 2015), waxes

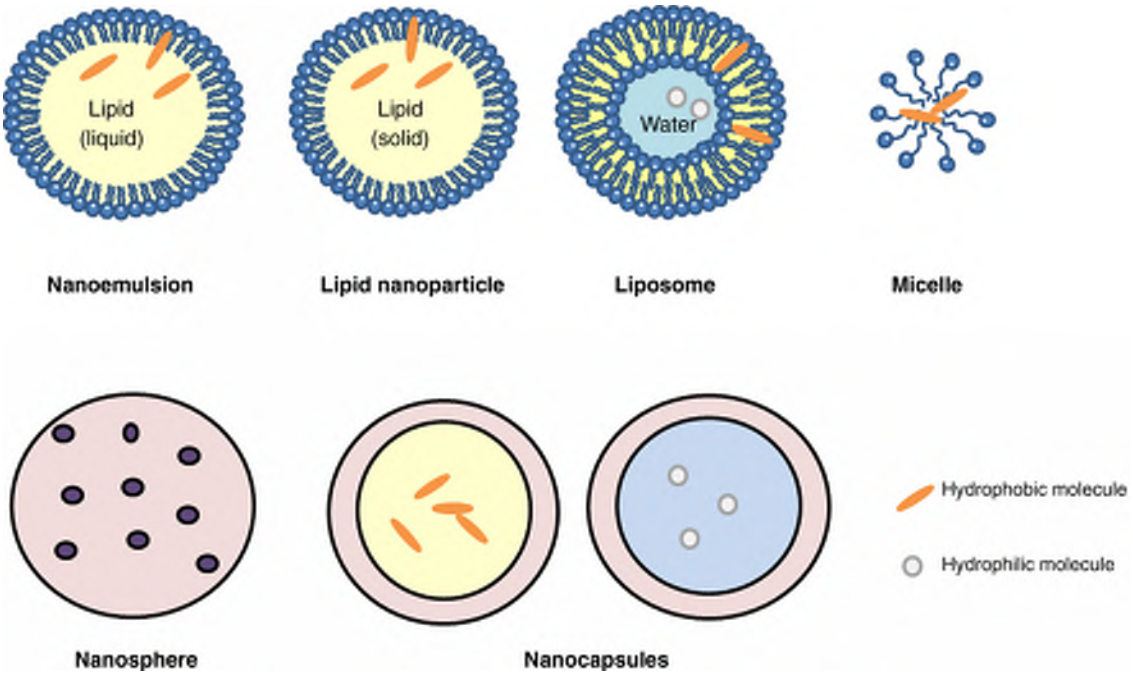

Figure 9.2. Examples of some nanosystems for nutraceuticals encapsulation. Nanoemulsions have a lipid monolayer enclosing a liquid lipid core. Lipid nanoparticles have a lipid monolayer enclosing a solid lipid core. Liposomes with a lipid bilayer enclosing an aqueous core. Nanospheres, solid polymers with drugs embedded in the polymer matrix. Nanocapsules, shell with an inner liquid space loaded with the drug, the inner can be an oil (loading of hydrophobic molecules) or water (loading of hydrophilic molecules).

(Baviskar et al., 2012), and oils (Souto et al., 2013). The synthetic polymers mostly applied are acid lactic derivatives (PLA, PLGA) (Anand et al., 2010; Yallapu et al., 2010; Cook et al., 2014; Stevanovic et al., 2014; Ramalho et al., 2015), methacrylate derivatives (Palao-Suay et al., 2015), and polyethylene glycols (PEGs) (Cheng et al., 2007). The most used emulsifiers or surfactants are nonionic and include lecithins, sorbitans, polysorbates, poloxamers, PVA, Tweens, sugar esters (Souto et al., 2013; Huang et al., 2010), among others. These nanomaterials are being applied in the manufacture of several systems to encapsulate nutraceuticals. Major goals of encapsulating nutraceuticals are dependent on the properties of active compounds, and include the solubility and permeability enhancement, controlled release, and protection from enzymatic and chemical environment (Ting et al., 2014). We discuss the most used nanotechnology-based delivery systems that are being investigated and produced in the food and pharmaceutical industry, highlighting their characteristics, the main production methods, and their proven efficiency for the delivery of nutraceuticals,

along with the main characteristic for the encapsulated nutraceuticals bioactives.

3.1 Nanoemulsions

Nanoemulsions are emulsions with droplet size in the nanometric scale (typically in the range of 20–200 nm). They can be oil-in-water (o/w), water-in-oil (w/o), or even double emulsion (w/o/w), according to the nature of the product to be encapsulated (Fig. 9.2). Nanoemulsions appear to be transparent or translucent to the naked eye and possess stability against sedimentation or creaming due to their nanosized droplets (Solans et al., 2005). Contrary to microemulsions, which are thermodynamically stable systems, nanoemulsions are nonequilibrium systems, and for their formation high-energy input methods are required, because they cannot be formed spontaneously. The emulsification methodology used to produce these systems requires mechanical instruments such as high-shear stirring, high-pressure homogenizers, and ultrasound generators. The most used techniques to produce nanoemulsions include the phase inversion temperature (PIT) method, high-pressure homogenization, ultrasonic emulsification, spontaneous emulsification, microfluidization, inverse w/o method, compressed liquid, or supercritical CO_2 (McClements, 2013). The PIT method seems to produce nanoemulsions with a minimum droplet size and a complete solubilization of the oil in a microemulsion bicontinuous phase (Solans et al., 2005).

Several nutraceuticals have been encapsulated in nanoemulsions and the results seem to be promising, showing advantages, such as (1) enhanced digestion rates, (2) formation of mixed micelles leading to a higher emulsification, (3) higher and controlled release of encapsulated products, (4) rapid diffusion and permeation across the barriers (Ting et al., 2014). The size of the oil droplets is important in the development and efficiency of these systems (Cho et al., 2014). Long-chain triglycerides seem to provide lower droplet sizes than medium chain triglycerides (Komaiko and McClements, 2015). Nanoemulsions encapsulating nutraceuticals, such as coenzyme Q10 (Cho et al., 2014) and curcumin (Zou et al., 2015) showed a higher bioavailability and solubility in smaller droplets for oral administration. These could be explained by the lipid composition of nanoemulsions, which are easily digested when the droplet size is lower because the surface area is higher, providing a higher activity of the lipases. This could lead to higher therapeutic effect and efficiency of the nutraceutical by oral administration. In addition to the droplet size, other formulation parameters, such as oil composition,

could influence its bioavailability (Sun et al., 2015). The in vitro solubility of nutraceuticals in the oil carrier for the development of nanoemulsions seems to be equivalent in terms of capacity of absorption (Calligaris et al., 2015).

The major problems of some nutraceutical products are the stability, because they are very susceptible to oxidation, hydrolysis, epimerization, and other reactions leading to their degradation or transformation in nondesirable products. Nanoemulsions are able to provide protection, for example, curcumin is very unstable at physiological pH 7.4 and encapsulation in nanoemulsions seems to avoid its hydrolysis (Kaur et al., 2015). The factors that influence nanoemulsions stability include the pH, temperature, and salts, being essential for long-term stability (Hategekimana et al., 2015). The temperature is a crucial factor and usually higher temperatures lead to a higher degradation and consequently to a lower stability of nutraceuticals in general (Qian et al., 2012). Additionally, for unstable nutraceuticals, such as vitamins (eg, D, E), the use of a cosurfactant seems to improve the thermal stability. One example used to stabilize nanoemulsions loaded vitamin D was the sodium dodecyl sulfate (SDS) (Guttoff et al., 2015).

3.2 Lipid Nanoparticles

The main lipid nanoparticles (LN) described in the literature are the solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) (Fig. 9.2). These nanoparticles are based on a lipid matrix composed by acylglycerols (mono-, di-, or tri-), waxes, ceramides, fatty acids, or fatty alcohols (Souto et al., 2013). They can be distinguished by the composition of the lipid matrix and polymorphism. SLN are only composed of solid lipids, solid at both room and body temperature, while NLC also integrates liquid lipids (Souto et al., 2013). Both lipid formulations require surfactants, because they are composed of two immiscible phases, lipid and aqueous phases. All lipids and surfactants are classified as GRAS, of recognized biocompatibility and biodegradability, because they are physiological lipids that occur naturally in the organism (Souto et al., 2013).

Lipids are known to promote oral absorption of several products, namely lipophilic compounds, such as lipophilic vitamins (A, D, E, and K) (Kalepu et al., 2013). Despite the physiological nature of LN, these particles present many advantages including their good physical stability, the ability to protect drugs/products in the lipid matrix from environmental factors, such as oxidation, hydrolysis, and possible enzymatic degradation in the gastrointestinal

tract. Also, the controlled release of bioactive compounds seems to present advantages in chronic situations/pathologies (Yao et al., 2014).

The encapsulation of polyphenol nutraceuticals, such as resveratrol (Neves et al., 2013), confirms the advantages listed earlier, because LN are able to protect the antioxidant molecule and provide a controlled release. The LN seem to present high dissolution velocities in relation to high permeability of the bioactive compound through the mucosa, providing additional physical and chemical stability of the encapsulated bioactive (Weiss et al., 2008). Also, LN can accommodate several nutrition agents simultaneously, being beneficial to providing a synergetic effect (Ramon and Danino, 2008).

3.3 Liposomes and Niosomes

Liposomes are vesicular systems within a nanometer size range between 10 nm and 1 μm or greater. Liposomes are colloids with an aqueous core enclosed by phospholipid bilayers (Fig. 9.2). According to the number of lipid bilayers, they can be classified as small unilamellar vesicles (SUVs) or multilamellar vesicles (MLVs). Furthermore, based on the size, liposomes are classified as small unilamellar vesicles (SUVs), giant unilamellar vesicles (GUVs), or large unilamellar vesicles (LUVs) (Souto et al., 2013).

Niosomes are also vesicular nanocarriers composed of nonionic surfactants (majority composed by Tweens, Brijs, and Spans), cholesterol, and derivatives (Moghassemi and Hadjizadeh, 2014). Practically, niosomes have the same advantages of liposomes and the main difference is their composition, thus the raw materials cost is usually lower. The long storage time, stability, and biocompatibility with biological systems are the main benefits of these nanocarriers. Additionally, the controlled release of the encapsulated bioactives, high performance in vivo and in vitro and the nonimmunogenic ability provide potential applications of these systems in food and pharmaceutical products (Choi and Maibach, 2005).

Liposomes and niosomes are versatile carriers with the ability to incorporate both lipophilic and hydrophilic molecules. Hydrophilic bioactives are encapsulated in the aqueous core and the lipophilic bioactives are entrapped along the lipid bilayers of liposomes (Allen and Cullis, 2013). Liposomes and niosomes are being investigated for several years in food industry and they seem to provide a lot of advantages regarding the bioactives stability, solubility, and bioavailability (Xia et al., 2015). A study performed

by Xia et al. (2015) showed that carotenoids encapsulated in liposomes were able to modulate the morphology and size of liposome, and to modulate structure, dynamics, and lipophilicity of liposomal membrane, according to the concentration of the bioactive encapsulated. Also, the authors achieved the liposomes stabilization regarding the particle aggregation and fusion. Niosomes encapsulating antioxidant molecules were able to increase the nutritional quality of food ingredients usually applied in human diets and also to provide a controlled release of these molecules over time (Tavano et al., 2014).

3.4 Polymeric Nanoparticles

Polymeric nanoparticles are colloidal particles in the nanometer range (10–1000 nm) composed of polymers that can be natural or/and synthetic (Rao and Geckeler, 2011). According to their matrix structure, they can be classified as nanopsheres, where the drug is molecularly dispersed in the polymeric matrix and nanocapsules, acting as a drug reservoir consisting of a liquid core (either oil or water) surrounded by a solid material shell (Fig. 9.2). In this case, the drug could be adsorbed onto the surface or encapsulated within the particle matrix (Mora-Huertas et al., 2010). Their main properties are related to their safe use, because they need to present biotolerability and biodegradability, as well as their easily production and scale-up (Arroyo-Maya and McClements, 2015). Additionally, they also provide a controlled release of encapsulated bioactives and have the ability to modify the stability, texture and organoleptic properties of several bioactives (Chung et al., 2013).

A good strategy to enhance bioavailability of encapsulated compounds, using this type of particles, is the use of mucoadhesive polymers, which could improve residence time and absorption in several mucosas. Examples of this type of polymers include chitosan, chitin, alginate, and PEGs (Sosnik et al., 2014). The intimal contact between mucus and mucoadhesive polymer promotes the diffusion through the mucin fibers. The bonding of polymers and mucin can be by covalent bond (eg, disulfide bridging with cysteine residues of mucin) or noncovalent bond (eg, hydrophobic interactions electrostatic attraction, hydrogen bonding, van der Waals bonding) (Khutoryanskiy, 2011).

Several nutraceuticals, such as curcumin (Bisht et al., 2007), green tea polyphenols (Felice et al., 2013), coenzyme Q (Nehilla et al., 2008), have been encapsulated in polymeric nanoparticles providing promising results.

3.5 Micelles

Micelles are self-assembled structures that once reaching a critical micellar concentration (CMC) and temperature, adopt a spherical shape and present nanometer sizes ranging from 5 to 100 nm (Fig. 9.2). Micelles are associations of colloids formed by amphiphilic compounds and show thermodynamic stability in aqueous media (Torchilin, 2007). They can be composed of polymers or even by phospholipids, usually with materials with an amphiphilic nature. Thus, they present a hydrophobic core and a hydrophilic shell. Their main characteristics include (1) the in vivo stability for an optimal long period of time; (2) controlled release of loaded bioactives; (3) biodegradability and biotolerance; (4) improvement of problematic bioactives solubilization; (5) increased water solubility of sparingly soluble bioactives; and (6) improved absorption and permeation through several mucosas (Torchilin, 2004, 2005). All these features lead to the improvement of bioavailability and should be taken into account in bioactive encapsulation.

For instance, casein based micelles are being investigated for the delivery of lipophilic compounds, such as vitamins, and have demonstrated their added value in the potential enrichment of low- or nonfat food products (Semo et al., 2007). Also, the control of cholesterol levels could be achieved by the use of phospholipid micelles, which improves its solubilization and facilitates the transport of lipids though the enterocytes (Kirana et al., 2005).

3.6 Cyclodextrins

Cyclodextrins are colloidal carriers with a cavity promoted by their cyclic structure, composed of cyclic oligosaccharides of α-(1,4) linked glucopyranose subunits (Yameogo et al., 2014). These compounds also can be classified as dietary fibers from the nutraceutical point of view, because they reduce the digestion of carbohydrates and lipids presenting a low glycemic level (Fenyvesi et al., 2016). Regarding their structure, cyclodextrins assume a supramolecular organization that can be matricial, multilamellar, or hexagonal (Kurkov and Loftsson, 2013).

Due to their amphiphilic character, they present a hydrophilic moiety towards the exterior and the interior encloses the lipophilic moiety, which seems to provide advantages for the solubilization of poorly water-soluble bioactives (Oda et al., 2004).

Cyclodextrins are used in food industry due to their classification as prebiotics, improving the intestinal microflora by the selective proliferation of *Bifidobacteria* spp. They are partially digestible and can be fermented by the human microflora

(Fenyvesi et al., 2016). Their main use is related to their ability to control obesity (Jarosz et al., 2013) and diabetes (Merscher-Gomez et al., 2013), which make them attractive nutraceuticals and encapsulating systems. The use of cyclodextrins is not limited to the functions mentioned earlier. Cyclodextrins can also be used to improve food quality during storage time, as they have the ability to remove some specific components or to stabilize and protect components that are present in food and are relevant for a healthy diet (Martina et al., 2013).

4 Micro- and Nanoencapsulation of Nutraceuticals

There are some classifications of nutraceuticals, some according to their availability on the market, others according to the nature of the food, compound, or molecule. Some groups of nutraceuticals are summarized in Fig. 9.3.

The encapsulation of bioactive food ingredients in micro- and nanoparticles is important in food, nutrition, and pharmaceutical investigation and in industry. Nanoscale control of food molecules (and in food incorporated molecules or nanosystems) may result in the modification of some characteristics at the macroscale, such as flavor, texture, taste, processability, and shelf life. At industrial scale, application of nanotechnology in the food industry faces challenges as (1) maintaining food grade, (2) use of inexpensive materials, (3) finding easily scalable methods, together with (4) improved benefits on stability and bioavailability of the encapsulated substances. These technologies are being investigated for bioactives facing problems regarding

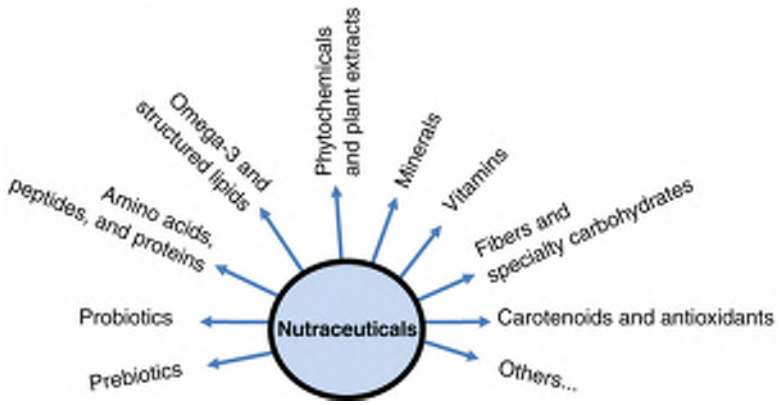

Figure 9.3. Categories of nutraceuticals.

their bioavailability or their stability in vivo and in vitro (Huang et al., 2010). Thus, the potential benefits that these systems provide to bioactives with added value for the human diet can be achieved with the use of nanotechnology. Some of the principal nutraceuticals are presented in the next sections along with the efforts that have been made to improve their bioavailability using nanoparticles.

4.1 Encapsulation of Probiotics, Prebiotics, and Synbiotics

4.1.1 An Overview on Probiotics, Prebiotics, and Synbiotics

Probiotics are defined as foods/substances containing microorganisms that are beneficial to the host organism. Some examples of microorganisms include: (1) lactic acid bacteria (eg, *Bifidobacterium* species, *Lactococcus lactis*, *Enterococcus faecium*), which are the most important probiotics; (2) nonlactic acid bacteria (eg, *Escherichia coli* strain nissle); and (3) yeasts (eg, *Saccharomyces cerevisiae*). The benefits to the host organism are achieved by: (1) modification of the host microbial population, (2) reducing the risk of diseases provoked by pathogens, by aggregation with pathogenic bacteria or by producing bacteriocins (antibacterial substances produced by bacteria to kill or inhibit the growth of other bacteria), (3) modifying the structure and function of the epithelium, (4) production of several substances (eg, organic acids, polysaccharides), favoring the host well-being, (5) modulating immunity, (6) reducing the cholesterol level, (7) reducing lactose intolerance, (8) reducing the risk of some cancers, and others (Burgain et al., 2011; De Vrese and Schrezenmeir, 2008; Patel and DuPont, 2015).

Prebiotics are defined as substances that cannot be digested, i.e., that are resistant to gastric acid environment and to enzymatic digestion, however, they are selectively fermented by the intestinal microflora, promoting the growth and/or activity of beneficial bacteria that colonize the gastrointestinal tract offering benefits upon host well-being and health (De Vrese and Schrezenmeir, 2008; Roberfroid et al., 2010). The compounds that are currently classified as prebiotics include: (1) inulin (a soluble dietary fiber, nondigestible heterogeneous mixture of polymer fructose linked by β(2–1) fructan bonds), (2) oligofructose (OS; inulin subgroup containing 2–10 monosaccharide residues), (3) fructooligosaccharide (FOS; products of inulin degradation or the result of transfructosylation mediated by *Aspergillus niger* or *Aspergillus aculeatus* β-fructosidase action on sucrose), galactooligosaccharides (GOS; short chain polymers of galactose, that act as

soluble fibers and have a bifidogenic effect), and some others that are still under study (Patel and DuPont, 2015; Roberfroid, 2007).

And, *synbiotics* are the combination of a probiotic and a prebiotic that work synergistically as prebiotics, facilitating the survival and the activity of probiotics in vivo, as well as stimulating indigenous anaerobic bacteria (Patel and DuPont, 2015).

4.1.2 Delivery of Probiotics, Prebiotics, and Synbiotic

Probiotics are included in several food products, with emphasis on dairy products as yogurt, cheese, and kefir and in other products (eg, puddings, fruit-based drinks, pharmaceutical products, and in food supplements). The therapeutic efficiency of the added bacteria depends on the dose, the maintenance of their viability (from production, shelf life to the gastrointestinal tract), which is of great relevance in the marketability of food-containing probiotics. Thus, one of the main reasons for the encapsulation of probiotics is to enhance their viability during processing, storage, and delivery (Huq et al., 2013). Micro- and nanoencapsulation can provide major advantages, namely the preservation of probiotics against environmental conditions (eg, pH, fermentation products, temperature, oxygen, moisture), which in part are accomplished by the physical barrier provided by the used nanocarriers leading also to a better improvement of their bioavailability (Riaz and Masud, 2013; Solanki et al., 2013).

Probiotics are usually encapsulated as lyophilized or spray-dried cultures (Huq et al., 2013). Micro- and nanoencapsulation of probiotics depends on the choice of the encapsulating materials because they are influenced by several factors, such as biocompatibility, low cost, availability, and they also have influence on the viability of probiotics during storage and processing, the shape of the particles, and on the final appearance and texture of the product (Riaz and Masud, 2013). Probiotics have been encapsulated using biopolymers such as alginate, chitosan, gelatin, whey protein isolate, and cellulose derivatives. The most used methods include spray drying, extrusion, and emulsion (Huq et al., 2013).

The improvement of probiotics in vitro and in vivo gastrointestinal tract profiles, that is, the survival against the gastrointestinal tract environment, namely the acidic stomach (pH about 2) and the enzymatic environment (eg, pepsin and pancreatin) was achieved with their encapsulation in several matrices, namely polymeric, such as calcium alginate (Khosravi Zanjani et al., 2014), alginate-chitosan (Trabelsi et al., 2013), alginate-gelatin (Annan et al., 2008), alginate-milk (Shi et al., 2013), and lipid matrices (Pedroso et al., 2012). The alginate is the most used polymer, because it has the capacity to resist against stomach degradation,

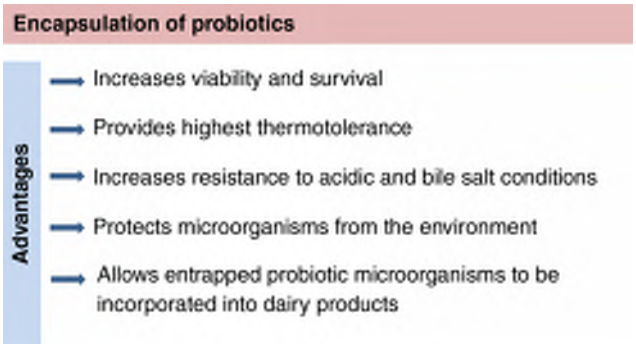

Figure 9.4. Main advantages of probiotic encapsulation.

generally produces microcapsules with smooth surface and also achieves a controlled release (Rayment et al., 2009; Solanki et al., 2013). Additionally, alginate provides a metabolic stabilization to probiotics (De Vrese and Schrezenmeir, 2008). The coatings of the nanocarriers could provide a high mucoadhesion to mucosa tissue in physiological environment of the colon, leading to a prolonged retention time and thus convenient release of probiotics in this area (Chen et al., 2013; Pliszczak et al., 2011).

Overall, the micro- and nanoencapsulation of probiotics seems to be fundamental in the bioavailability, survival, and performance in vivo and in vitro in the gastrointestinal tract, showing several advantages as summarized in Fig. 9.4.

Prebiotics and *synbiotics* include the natural polymers fructooligosaccharides (FOS), galactooligosaccharides (GOS), oligosaccharides (OS), inulin and other molecules. Some of the resulting commercial oligosaccharide products contain a considerable percentage of low molecular weight side resultant sugars, such as glucose, fructose, sucrose, galactose, and lactose, reducing the performance of the end product and being a source of assimilating sugars. As high purity is a demand, methods for purification and encapsulation have been developed in order to reduce the lower molecular weight contaminants and to guarantee the entrapment of the higher molecular weight molecules.

Usually, the prebiotics are mainly used as the matrices, or part of the matrices, to encapsulate the probiotics giving it the name of *synbiotics* (combination of pre- and probiotics in the same system) as these types of fibers protect, transport, and release the probiotics and then serve as "food" to them. Synbiotics are believed to be the best way to deliver probiotics in the gastrointestinal tract, useful to treat or prevent several diseases.

The most widely used matrix for microencapsulation is alginate (a natural anionic polysaccharide composed of D-mannuronic

acid and glucuronic acid residues linked linearly by 1–4 glycosidic linkages), which has been found to increase the survival of probiotics (Annan et al., 2008). And, because of its chemical nature, alginate gel is stable at low pH, and then swell at higher pH (as in the intestinal environment) releasing the cells. Using alginate as coating material in food matrices is also a viable option providing stability and high viability of the encapsulated prebiotics (Sathyabama et al., 2014). FOS can also act as carriers for probiotics, which in combination with whey protein isolate (WPI) or with denatured whey protein isolate (DWPI) produced suitable microcapsules for encapsulating the probiotic bacteria *Lactobacillus plantarum* with reduced particle stickiness and aggregation. These microcapsules showed high encapsulation efficiency, with a better storage stability offering protection to gastric environment, being a promising system for food industry (Rajam and Anandharamakrishnan, 2015).

Dietary fibers include some prebiotics, and other fibers are currently being used to encapsulate food bioactives. Dietary fibers are found in plant derived foods (eg, fruits, vegetables, and whole grains) and have a main role in maintaining a healthy digestive system. They are usually not totally digestible and can be divided into soluble and insoluble fibers; according to water-solubility. The first ones are found in fruits, oats, and beans (eg, pectin, fructo-oligosaccharides, oligosaccharides, inulin, β-glucans) and the second ones are found in whole grains and vegetables (eg, cellulose, hemicellulose, lignin) (De Vrese and Schrezenmeir, 2008; Martino et al., 2013). Note that some dietary fibers are prebiotics. Also, some dietary fibers are being used as matrices for nanotechnology of food products.

4.2 Encapsulation of Oils and Omega-3 Fatty Acids

Oils and fatty acids. Fish, nuts, and seed oils are rich in the omega-3 fatty acids, an essential fatty acid (not synthesized by human but essential in the diet). There are three main omega-3 fatty acids, the eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), present in fish oil, and alpha-linolenic acid (ALA), which is present in vegetable oils and nut. Omega-3 fatty acids have been shown to reduce cardiovascular diseases, to improve visual and mental function (Fulendra et al., 2012) and other conditions such as immunity, as summarized in Fig. 9.5. Conjugated linoleic acid (CLA), a mixture of isomers of linoleic acid (omega-6 fatty acid), play an important role on fat metabolism. A balance between omega-3 and omega-6 is essential to maintain vascular physiology.

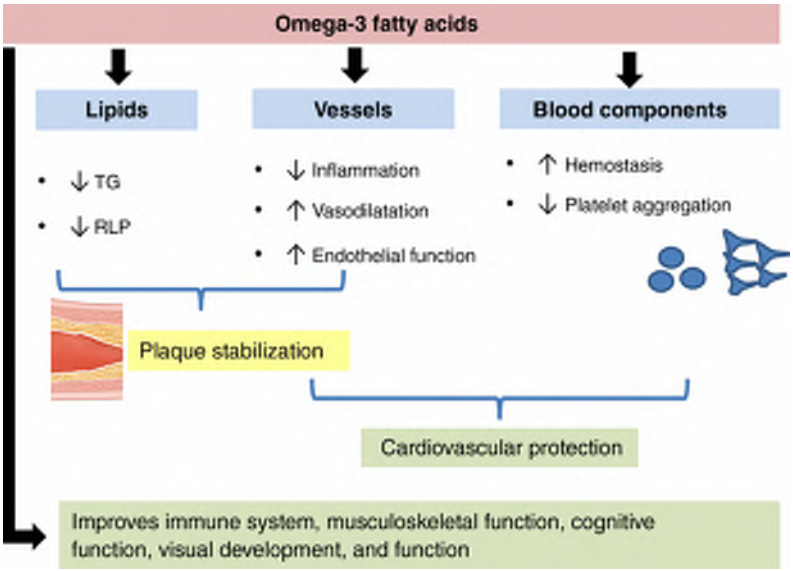

Figure 9.5. Benefits of an equilibrated diet on omega-3 fatty acids. There is improvement of lipid metabolism by reducing circulating triglycerides (TG) and remnant lipoproteins (RLP; independent predictors of cardiovascular disease, namely atherosclerosis). They participate on the synthesis of antiinflammatory mediators that, together with promotion of vasodilation and regulation of hemostasis, improve vascular function which culminates with cardiovascular protection. Omega-3 fatty acids have other beneficial actions, as mentioned, and on maintenance of a healthy skin, equilibrated weight, and others. Modified from Kromhout et al. (2012).

The micro- and nanoencapsulation of this essential oil can provide a range of advantages because its physical and chemical properties limit its use as a nutritional food ingredient. Regarding its highly unsaturated nature, omega-3 fatty acids are very unstable, susceptible to oxidation, which leads to degradation and production of hydroperoxides, leading to organoleptic problems regarding flavor and odor. Due to these characteristics manipulation and storage is not facilitated (Kaushik et al., 2015). Thus, the improvement of their shelf life and ability of masking seems to be two major goals in their encapsulation.

The most common methods for the encapsulation of omega-3 fatty acids include the coacervation (separation of colloidal systems into two liquid phases) and spray-drying and spray-chilling methodologies (Kaushik et al., 2015). The encapsulation of omega-3 fatty acids also requires three essential steps: (1) the selection of appropriate core material (source of omega-3 oils), (2) the selection of material for encapsulation and, (3) the methodology; because these

properties are essential to obtain excellent rates of encapsulation and protection, leading to a high quality and bioavailability of the final product. The main sources of omega-3 oils are the fish, the most used methodology is spray-drying and, for the encapsulating material, many have been used (eg, casein, gelatin, lactose, methylcellulose, gum Arabic, whey protein isolate, lecithin, chitosan, egg white, powder), as reviewed recently (Kaushik et al., 2015).

Success on encapsulating omega-3 oils was obtained using a carbohydrate matrix composed of k-carrageenan/polydextrose, offering a controlled release due the improvement of the diffusion of the oil from the matrix (Paramita et al., 2015). These oils were also encapsulated in lipid matrices, namely SLN, whose material composition and physicochemical properties were studied regarding their crystallization in order to decrease lipid peroxidation (Salminen et al., 2013), more studies are needed in order to use these systems in industrial processes. The use of cyclodextrins also improves the oxidative stability of omega-3 oils compared to nonencapsulated oil (Cao et al., 2011). A synergetic effect between omega-3 fatty acids and probiotic during digestion has been reported (Das, 2002), and their co-microencapsulation seems to provide higher stability.

Food products prepared with microencapsulated omega-3 have been made. Microencapsulation of omega-3 oils, by spray-drying method, using whey protein concentrate, with oil/protein ratio of 0.4 gave good results concerning encapsulation efficiency and protection against lipid peroxidation. Further incorporation of these microcapsules containing omega-3 oils in biscuits was shown, after sensory evaluation and 4 months of packaging to protect the oil along with acceptable physicochemical and organoleptic characteristics (Umesha et al., 2015).

4.3 Encapsulation of Antioxidants

Antioxidants, in general, are molecules/substances (natural or synthetic) that inhibit the oxidation of other molecules. In the category of nutraceuticals, these include vitamins (eg, vitamin A, C, and E), phytochemicals (eg, catechins, carotenoids, anthocyanidins) that neutralize oxidative species, reduce the risk of cardiovascular diseases and cancer, between other actions (Abourashed, 2013; Pandey and Rizvi, 2009). Most of the earlier mentioned nutraceuticals suffer from poor solubility in water, which may lead to poor oral bioavailability and thus reduces their efficacy on disease treatment and/or prevention. Some antioxidants are unstable molecules that can suffer oxidation when exposed to several food components.

4.3.1 Encapsulation of Polyphenols

The class of polyphenols is large and these natural molecules are very interesting from the antioxidant point of view. Unfortunately, the concentrations of polyphenols that appear effective in vitro are not achieved in vivo and their bioavailability is very low. In general, ingested polyphenols revealed problems due to insufficient gastrointestinal residence time, low absorption dissolution within the gut environment, structural instability, due to factors such as temperature, presence of molecular oxygen, light, local pH, digestive enzymes, and the presence of other compounds (eg, fibers, nutrients), contributing to the limitation of their nutraceutical and pharmaceutical activity (Fang and Bhandari, 2010). Another inconvenience of some polyphenols is their unpleasant taste, such as astringency, which needs to be masked. Thus, to circumvent these drawbacks and to deliver these compounds the use of nanocarriers could be a promising approach to provide protection and to maintain the active molecular form until the time of consumption, and delivery in the gastrointestinal tract. Table 9.1 provides a summary of the major polyphenols and their main human health benefits.

Micro- and nanoencapsulation of polyphenols are widely investigated due to the enormous human health benefits attributed to these molecules, in the prevention and treatment of several diseases, such as cancer (Santos et al., 2013; Sun et al., 2014), cardiovascular (Felice et al., 2013; Xu and Si, 2012), diabetes, and associated diseases (Grama et al., 2013; Fangueiro et al., 2014a).

Concerning to *anthocyanins*, these are natural pigments occurring in fruits and plants with several medicinal attributes (see Table 9.1). They have a chemical structure very sensitive to pH (color depends on the pH), oxygen, ionic strength, and temperature, which influence stability and thus their bioavailability. As they can be directly absorbed by the small intestine epithelial cells, methodologies to deliver these phytochemicals directly to small intestine are needed. Good results were obtained by their encapsulation in microspheres (composed of oxidized konjac glucomannan and chitosan oligosaccharides that are dietary fibers), which were able to protect and target delivery of these bioactives into intestines preserving antioxidant activity, and it was also possible to promote a controlled delivery of the anthocyanins (Lu et al., 2015). Concerning to the possible involvement of anthocyanins in modulation of intestinal microbiota, a study with anthocyanins encapsulated in cyclodextrins showed their potential delivery to the colon with increased bioavailability, which was accompanied with a positive effect on intestinal

Table 9.1 Major Polyphenols Indicating Their Names, Sources, and Principal Human Health Benefits That Are Mainly Associated with the Antioxidant Effect

Polyphenols Class	Types (Examples)	Source	Major benefits
Anthocyanins	Cyanidin, delphinidin, peonidin, petunidin, pelargonidin	Fruit, flowers, vegetables	Cardiovascular disease prevention, obesity control, diabetes alleviation, antiinflammatory, antiviral, anticancer properties
Catechins	Catechin, epicatechin, gallocatechin, epigallocatechin, epigallocatechin gallate	Tea	Treatment of metabolic syndrome (eg, obesity, diabetes) Protects against cardiovascular and neurodegenerative diseases Anticancer,
Flavanones	Hesperetin, naringenin, naringin	Citrus	Antiinflammatory, antiproliferative, improve memory
Flavones	Apigenin, luteolin, tangeritin	Fruit, herbs, vegetables	Prevent neurodegenerative, diabetes, cardiovascular diseases Antiinflammatory,
Flavonols	Kaempferol, myricetin, quercetins	Fruit, herbs, vegetables	Regulation of blood pressure and cardiovascular function Antiaging, anticancer,
Isoflavones	Daidzein, genistein, glycitein	Soybeans, peanuts	As phytoestrogens protect against hormone-related disorders ↓ total cholesterol, LDL cholesterol, triglycerides levels
Hydroxybenzoic acids	Gallic acid, *p*-hydroxybenzoic, vanillic acid	Berries, tea, wheat, plants	Antiinflammatory, antimutagenic, colon cancer protection and/or treatment,
Stilbenes	Astringin, piceid, resveratrol, viniferin	Fruit, wine,	Prevent cardiovascular diseases, cancer Treatment of metabolic syndrome (eg, obesity, diabetes)
Tannins (proanthocyanidines)	Castalin pentagalloyl glucose procyanidins	Tea, berries, wines, chocolate	Antiinflammatory, antimicrobial, cardiovascular protection Dermatological benefits (collagen stimulating, skin lightening)

Source: Adapted from Fang and Bhandari (2010).

bacteria population and that several bacteria could metabolize anthocyanins into phenolic acids (eg, ferrulic acid) that are also important bioactives (Flores et al., 2015). In respect to protection of anthocyanins from thermal degradation, gum Arabic nanostructures containing anthocyanins has shown positive effects on their stability under high temperatures, as 80°C (pasteurization temperature) and 126°C (autoclaving temperature) and provided stability to pH degradation effect (pH 5), indicating that this kind of nanostructure might be a good choice for encapsulating bioactives that need further pasteurization or sterilization (Guan and Zhong, 2015).

Catechins, the main polyphenols of green tea with antioxidant, antiinflammatory, chemopreventive properties, have also positive effects on the metabolic syndrome conditions, such as diabetes and obesity (Santos et al., 2013; Thielecke and Boschmann, 2009) among others (see Table 9.1). Catechins are also susceptible to temperature, alkaline pH, presence of free radicals and other agents that may induce their degradation. Catechins encapsulation in lipid emulsions revealed excellent physicochemical properties and these systems were able to provide good stability (Gadkari and Balaraman, 2015). Our research group has also shown good parameters of lipid nanoparticles incorporating catechins, namely epigallocatechin gallate (Fangueiro et al., 2014a). We were able to develop a HPLC method for quantification and stabilization of this bioactive in physiological medium for biopharmaceutical studies (Fangueiro et al., 2014b). Dube et al. (2010) successfully encapsulated catechins in chitosan nanoparticles promoting their stabilization in alkaline solution by the use of reducing agents. Thus, an intelligent strategy to overcome catechins degradation could be the use of antioxidant molecules, to prevent their auto-oxidation, such as ascorbic acid, dithiothreitol or tris(2-carboxyethyl)phosphine (Fangueiro et al., 2014b; Dube et al., 2010). The antioxidant activity of catechins was evaluated after their encapsulation in cyclodextrins and results showed that it is dependent on the inclusion method of the bioactives in the cyclodextrins (Folch-Cano et al., 2010).

Flavonoids comprise a vast group of polyphenolic compounds, characterized by a benzo-γ-pyrone structure, their chemical nature depends on their structural class, degree of hydroxylation, substitutions and conjugations, and degree of polymerization (Fig. 9.6). Flavonoids can be divided in classes, such as flavanones (eg, naringenin, hesperetin), flavones (eg, apigenin, luteolin), flavonols (eg, quercetin, myrecetin), which have differences that are due to the level of oxidation and C ring substitution pattern. Within a class, individual compounds differ in the A and B ring

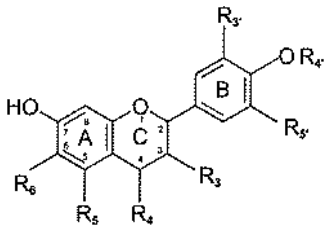

Figure 9.6. Basic structure of flavonoids. The position of benzenoid substituent (B ring) divides the class between flavonoids (2-position) and isoflavonoids (3-position). Flavonols differ from flavanones by the presence of a C2–C3 double bond and a hydroxyl group at the 3-position. Flavonols have a 3-hydroxyl group which is absent in flavones. Flavanones have a saturated heterocyclic C ring.

substitution pattern (Kumar and Pandey, 2013b), see Fig. 9.6. In foods and medicinal plants, flavonoids may occur as aglycones, glycosides (usually linked at 3- or 7-position with D-glucose, galactose, arabinose, L-rhamnose or glucorhamnose), and methylated, while aglycans are easily absorbed by the small intestine the flavonoid glycosides need to be converted into aglycans, which occurs in the small intestine or in the colon, depending on the molecule. The absorption of flavonoids is higher in the small intestine than in the colon (Kumar and Pandey, 2013a), thus most ingested flavonoids are not absorbed because they are eliminated or degraded by the colon bacteria, reducing the probability to exert medicinal properties, thus there is a need to deliver aglycan flavonoids to the small intestine in order to have a nutraceutical effect.

Flavanones have been encapsulated in several nanosystems. *Hesperetin* was encapsulated in solid lipid nanoparticles (SLN, see Fig. 9.2), with an encapsulation efficiency between 39.90 and 63.08%, after investigation of the kinetics of hesperetin release in simulated gastric fluid, a Fickian and dissolution mechanism was observed. Hesperetin encapsulated into SLN also showed to be stable over 30 days, at different temperatures, in aqueous suspension without measurable leakage. It was used to fortify milk and, after sensory analysis, there was no bitter taste (typical from hesperetin) as it is poorly water-soluble (Fathi et al., 2013). Flavanones isolated from *Eysenhardtia platycarpa* were encapsulated in polymeric nanoparticles and in nanoemulsions, which were prepared for topical application. These systems showed sustained release, offering skin permeation and improved significantly flavanone antiinflammatory action, indicating that these systems could be used to encapsulate other flavanones (Domínguez-Villegas et al., 2014) and used for other administration routes. The encapsulation of *naringenin* in liposomes (phosphatidylcholine-based multilamellar vesicles) was shown to be poor (<10%) but it

was highly absorbed onto the surface of these liposomes, increasing stability and antioxidant activity (Kerdudo et al., 2014).

Flavones, namely *apigenin* was encapsulated in distearoylphosphatidylcholine-based liposomes, with an efficiency of encapsulation of about 90%, and was able to exert antibacterial activity due to the intimal relation between these nanocarriers and bacterial membrane, delivery of these systems directly to the site of action could give rise to a new generation of antibacterial chemotherapeutics (Banerjee et al., 2015), either for human use or for food-borne pathogens. *Apigenin* was also encapsulated in modified phospholipid micelles (phospholipids and TGPs [d-alpha tocopheryl polyethylene glycol 1000 succinate]), with 87% of encapsulation efficiency, being stable for more than 90 days after lyophilization. These micelles increased bioavailability, intestinal absorption of apigenin, and showed anticancer effects (Munyendo et al., 2013).

Concerning to *flavonols*, *quercetin* was encapsulated in folate-modified lipid nanocapsules, which were administered to H22 tumor-bearing mice, producing a longer effect of quercetin in vivo showing antitumoral activity (Ding et al., 2014). *Quercetin* was encapsulated in nanostructured lipid carriers (NLC, see Fig. 9.2), with high encapsulation efficiency and the particles also have shown in vitro anticancer activity (Sun et al., 2014), it could be a good system to treat gastrointestinal cancers. *Rutin* was encapsulated in liposomes (phosphatidylcholine-based multilamellar vesicles) with high encapsulation efficiency (>60%) and with high entrapment efficiency, preserving its antimicrobial and antioxidant activity, which is a good promise to further application (Kerdudo et al., 2014). High encapsulation efficiency of *rutin* was also observed in nanoemulsions, showing a prolonged release over time (Macedo et al., 2014).

Nowadays there is a great interest on *isoflavones* (eg, genistein, daidzein), because of their antioxidant activity and estrogen-like activity (phytoestrogens), which may reduce the risk of hormonal-dependent cancers as well as the incidence of hormone-related pathologies (eg, osteoporosis), thus there is a great interest on diet enrichment in isoflavones. Food industry has developed strategies to extract these isoflavone aglycans from soybean with very high yield, but this extract has a low water solubility, which is difficult in its further processing into foods and other health products. Encapsulation of isoflavones in sodium carboxymethylcellulose coating/swelling matrix using spray-drying methodology produced powdered micronized polymeric microparticles that were capable for handling and storage. This methodology improved in vitro dissolution and permeation rates, resulting in higher bioavailability after oral administration

(Sansone et al., 2013), thus encapsulated soy-isoflavones is a promising ingredient to supplement foods. *Genistein* and *daidzein* were successfully encapsulated into a mixture of four types of cyclodextrins by wet-kneading method, showing improved solubility and enhanced membrane permeation (which was investigated in Caco-2 cells that upon confluence form a monolayer that mimic the intestinal epithelium) together with increased bioavailability (Daruházi et al., 2013).

Hydroxybenzoic acids, as *gallic acid*, have been studied for colon cancer prevention (as reviewed, Santos et al., 2013). Gallic acid was encapsulated in matrices of chitosan, β-cyclodextrin, and xanthan gum with high encapsulation efficiency, retaining its antioxidant activity and improving its bioavailability and solubility (Da Rosa et al., 2013). *Syringic acid* and *vanillic acid* were successfully encapsulated in cyclodextrins, enhancing stability and bioavailability, which is a promising result for further food and pharmaceutical applications (Rajendiran and Jude Jenita, 2015).

Resveratrol, a *stilbene*, possesses several biological activities (eg, antioxidant, antimicrobial), however, its efficacy is limited due to low bioavailability, which results from its poor water solubility. Thus, resveratrol encapsulated in methylated-β-cyclodextrins (in a ratio 1:1) improved its water solubility (about 400-fold), thus its bioavailability, maintaining its antioxidant and antibacterial activity (against *Campylobacter*), which is an encouraging result for its application in food industry aiming the control of food-borne pathogen, as well as a nutraceutical (Duarte et al., 2015).

Tannins are present on a variety of foods plant, they can inhibit α-amylase and glucoamylase activity and, thus are modulators of postprandial hyperglycemia decreasing glucose absorption, making them good candidates to treat and prevent type-2 diabetes. However, tannins present several difficulties upon oral administration, because they bind to proteins and have an astringent bitter taste, thus convenient delivery systems are needed to ensure that they reach the small intestine. Tannins were encapsulation in microparticles, made from sorghum kafirin protein by coacervation method, made for oral delivery, showing good encapsulation efficiency, and retaining the antienzymatic activity. This strategy reinforces the use of tannins to treat and prevent diabetes type-2 (Links et al., 2015) and obesity.

4.3.1.1 Curcumin and Derivatives

Turmeric (*Curcuma longa*) is an Indian spice that has been used for centuries as a medicinal plant, as it presents many biological activities (eg, antiinflammatory, anticancer,

antimicrobial, antirheumatic, antioxidant, antidiabetic effects, as reviewed, Santos et al., 2013). Curcumin (turmeric major component), curcuminoids, and curcumin oil encapsulation seems to be an approach to increase their bioavailability due their poor solubility and, thus poorly bioavailability and absorption from gastrointestinal tract after oral administration. Curcumin was encapsulated by emulsion technique in biodegradable nanoparticles, showed a 9-fold improvement in oral bioavailability compared to nonencapsulated bioactive with a permeation enhancer (Shaikh et al., 2009).

The anticancer activity of curcumin was also improved, after its encapsulation in sodium caseinate self-assembled nanoparticles sensitive to pH, against human colorectal and pancreatic cancer cells (Pan et al., 2014). Curcumin encapsulation presented advantages in controlling diabetic retinopathy, because it significantly delayed the progression of diabetic cataract. Curcumin-loaded into poly-(lactide-*co*-glycolic acid), PLGA, nanoparticles administered orally greatly improved its in vivo bioavailability (Grama et al., 2013).

Thus, encapsulation of curcumin not only improved its solubility and permeability with enhanced bioavailability, but also improved the therapeutic effects being markedly investigated as anticancer agent (Santos et al., 2013). The co-activity of nutraceuticals was also investigated, an example of two nutraceuticals, curcumin (lipophilic) and catechins (hydrophilic) were incorporated in a double emulsion (w/o/w), resulting in higher stability in gastrointestinal fluid, as also improved their bioavailability compared to the individual suspended curcumin and catechin solutions (Aditya et al., 2015).

Overall, several strategies are being developed for polyphenols encapsulation to overcome their physical and chemical limitations, and enhance their bioavailability and biological activity.

4.4 Vitamins

Vitamins are an important part of the human diet and a major source of bioactive substances for the maintenance of many biological functions. The consumption of vitamins, in general human diet, is still insufficient, although nowadays we have marketed foods enriched with vitamins, but does this enrichment improve their delivery and bioavailability in the gastrointestinal tract? This is a very important issue, because supplementation with vitamins and minerals requires the maintenance of their integrity and thus bioavailability until the gastrointestinal tract. Thus, to improve their bioavailability and also their stability in food products

some strategies are needed and dependent on the vitamin type. Table 9.2 presents the most important vitamins and their relevant health benefits and food sources (Bender, 2002). Due to the fact that some vitamins are liposoluble (vitamins A, D, E, and K) as well as their derivatives, they present low bioavailability. Also, vitamins present sensitivity to light, pH, temperature, and oxygen and are relatively unstable during processing and storage. These are features or intrinsic characteristics of vitamins that make them attractive molecules for micro- and nanoencapsulation, which is essential to prevent their degradation and also to improve their bioavailability and consequent health benefits. The strategies for encapsulation are in part similar to those presented in polyphenols as they share some characteristics. Some recent advances in their encapsulation are presented.

Vitamin A, in the form of all-*trans*-retinol, suffers degradative reactions that are characteristic of conjugated double bonds resulting in the partial or total loss of its bioactivity. Thus, nanoencapsulation is a solution to preserve its properties. It was encapsulated in solid-state emulsions, made as macroporous silica-lipid hybrid microcapsules, and exhibited improved release, lipolysis kinetics, oral bioavailability, and shelf life, which is an improvement for further nutraceutical applications (Ghouchi-Eskandar et al., 2012). All-*trans*-retinol was also encapsulated into SLN showing enhancement of retinol stability, photostability, and preservation of its antioxidant activity (Jee et al., 2006).

Several *vitamins* from the complex *B* were successfully encapsulated in a variety of nanocarriers, such as liposomes (Abd El Azim et al., 2015), polymeric nanoparticles (Azevedo et al., 2014), with enhanced bioavailability but keeping the nutrition quality. Folic acid or folate is also widely used as nanomaterial for the development of nanocarriers being matrix or coating material or even to conjugate with drugs (Fazilati, 2014) because the folate receptor is overexpressed on the surface of many tumoral cells (Fazilati, 2014; Stella et al., 2000).

Vitamin C has been encapsulated in microspheres (Khalid et al., 2015), emulsions (Khalid et al., 2013), and microcapsules (Comunian et al., 2014; Trindade and Grosso, 2000). Its stabilization is a challenge to the food and pharmaceutical industry. Stabilization of ascorbic acid for more than 10 days was achieved by its microencapsulation in microspheres (fabricated using microchannel emulsification) with good encapsulation efficiency (Khalid et al., 2015). Vitamin C (ascorbic acid) was loaded in microparticle composed of gum Arabic and rice starch, using spray drying method, with high efficiency of encapsulation, thermostability, improved chemical and storage stability (Trindade and Grosso, 2000).

Table 9.2 Examples of the Most Important Vitamins Applied in Human Diet with Description of Their Properties, Sources, and Characteristics

Vitamin	Other Names/ Derivatives	Properties	Main Sources
A	Retinol, retinal Retinoic acid Provitamin A carotenoids Beta-carotene	Maintenance of immune system Good vision Prevention and treatment of skin disorders (acne, wrinkles) Decreases the risk of gastric cancer	Liver, orange, ripe yellow fruits, leafy vegetables, carrots, pumpkin, squash, spinach, fish, soya milk, milk
B	B1—Thiamine B2—Riboflavin B3—Niacin, niacinamide B5—Pantothenic acid B6—Pyridoxine, pyridoxamine, pyridoxal B7—Biotin B9—Folic acid, folinic acid B12—Cyanocobalamin, hydroxycobalamin, methylcobalamin	Carbohydrates metabolism Antioxidant activity Protection of immune system Red blood cells production (B2 and B9) Prevention of hyperlipidemias Production of certain hormones (B5) Antiinflammatory action Diabetes control Normalizes nervous system function Production of hemoglobin (B12)	Pork, chicken, oatmeal, brown rice, vegetables, potatoes, liver, eggs, broccoli, avocados, bananas, leafy green vegetables, pasta, bread, cereal, fish, eggs yolks, and nuts
C	Ascorbic acid	Antioxidant activity Prevention of cardiovascular diseases Collagen, carnitine, and neurotransmitters biosynthesis Anticarcinogenic effects	Fruits (citrus, watermelon, papaya, strawberries, cantaloupe, mango, pineapple, raspberries and cherries), and vegetables, liver
D	D2—Ergocalciferol D3—Cholecalciferol or calcitriol	Protective effects against bones diseases Prevention of muscular weakness Anticarcinogenic effects	Fish, eggs, liver, mushrooms
E	Tocopherols, tocotrienols	Prevention of cardiovascular and neurodegenerative diseases Control of blood pressure Diabetes control and prevention Antioxidant activity	Many fruits and vegetables, nuts, and seeds
K	K1—Phytonadione, phylloquinone, phytonactone K2—Menaquinones K3—Menadione	Protection of immune system; cardiovascular diseases; osteoporosis; biliary obstruction Relief of menstrual symptoms	Leafy green vegetables such as spinach, egg yolks, liver

A, D, E, and K vitamins are lipid soluble (poorly water soluble), the others are water soluble.

Vitamin D encapsulation also revealed some advantages, regarding the instability related to many environmental conditions. Ergocalciferol (vitamin D2) has been successfully encapsulated in shell-core microparticles (composed mainly by alginate guluronic and mannuronic acid) fabricated by ionic gelation to ultrasonic atomization followed by particles drying by microwave (Barba et al., 2015). These shell-core microparticles permitted a high encapsulation efficiency and a sustained release compatible to gastrointestinal pH, being low at pH 1 (about 10%) and high at pH 6.8 (complete release) with minimal degradation of ergocalciferol (Barba et al., 2015). Teng and coworkers (2013) encapsulated vitamin D3 (cholecalciferol or calcitriol) in complex polymeric nanoparticles formed by carboxymethyl chitosan and soy protein isolate, prepared by ionic gelation method, showing high encapsulation efficiency and convenient release (low at gastric pH and high at intestinal pH). This methodology is appropriate to encapsulate nutraceuticals to supplement and fortify foods (Teng et al., 2013). For beverages, the inclusion of nutraceuticals needs to be discrete, without altering its natural appearance, taste, and flavor. Nanoemulsions offer small droplet size, stability against aggregation or precipitation, transparency while increasing bioavailability. Vitamin D was encapsulated in o/w nanoemulsions, increasing its bioavailability, shelf life (more than 1 month at room temperature) without particle aggregation but for high temperatures (>80°C) where particle growth was observed (Guttoff et al., 2015).

As *vitamins E* and *K* are also lypophilic they need the same strategies as *vitamin A* and D, before its their incorporation in functional food and beverages. O/W emulsions, produced by high-pressure homogenization, stabilized with a food-grade natural surfactant (Q-Naturale) isolated from *Quillaja saponaria* Molina tree bark, demonstrated an edible delivery system for incorporation of vitamin E into functional food and beverages (Yang and McClements, 2013). With the aim of increasing intestinal absorption of *vitamin K*, a strategy for its encapsulation in polymeric micelles, composed by a thermosensitive block copolymer, demonstrated in vivo (rat model) improved intestinal absorption with elevated plasma levels of vitamin K (Van Hasselt et al., 2009). Vitamin K1 was encapsulated in SLN (Liu et al., 2010), showing stability more than two in simulated gastric and intestinal fluids and increased storage stability (more than 4 months of storage at 25°C), being a suitable carrier for the oral delivery of vitamin K.

Overall the micro- and nanoencapsulation of vitamins is important to overcome some of the chemical instability of these bioactives improving their stability and providing a higher bioavailability,

which is fundamental due the importance of their nutritional value in human diet. Additionally, the encouragement of these strategies could enhance their intake.

5 Conclusions

The role of nutraceuticals in human lifestyle is an important issue due to the value of the health benefits that these bioactives provide together with the lifestyle of modern societies, leading to exposure to many stress factors, that also increase cellular oxidative stress a trigger for degenerative and chronic diseases. However, due to insufficient intake associated to their chemical properties, such as instability and sensitivity to light, water, temperature, oxygen, and other environmental conditions, affecting their processing and storage, nutraceuticals lose most of their potential therapeutic properties or even do not reach absorption places.

Thus, the micro- and nanoencapsulation of nutraceuticals can provide a variety of advantages regarding their stability, performance in vitro and in vitro, enhanced biological activity and consequent enrichment of their nutritional value and bioavailability. The research on this field still needs further improvement for application of these nutraceuticals in foods and beverages, regarding its bioavailability (thus their nutraceutical effect) and safety of the nanotechnological delivery systems developed.

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10

NOVEL PARADIGM OF DESIGN AND DELIVERY OF NUTRACEUTICALS WITH NANOSCIENCE AND TECHNOLOGY

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1 Introduction

The concept of nutraceuticals is rather very old; it dates back 3000 years ago and it has evolved significantly through the years. Hippocrates, the father of medicine have specified "Let food be thy medicine and medicine be thy food" indicating the significance of food for health and for the prevention of diseases (Bagchi, 2006). It is clear from the statement that physicians paid great attention to the daily diet for health maintenance. It has been quoted in history that during the 16th, 17th, and 18th centuries, crewmen on long voyages across the seas expired because of scurvy. The sea captains (under the direction of the British Admiralty) during 18th century investigated the function of various foods and supplemented seamen's diets with vitamin B and C for maintaining the health of seamen (Barnes and Prasain, 2005). Later in the early 1900s, in United States, the sea salt was fortified with iodine to prevent goiters. In the recent times, there are several compounds (phytochemicals) with functional benefits that have been fortified with food to enhance the nutritional value as well as to prevent diseases. Recently, the scientific support for the nutritional and medicinal properties of nutraceuticals has enabled them to emerge as potentially effective in health-care regimes (Dillard and German, 2000). There are several substantial scientific research data supporting the health benefits of nutraceuticals and the

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results are increasing day by day. In the United Kingdom, Japan, and several countries in Europe, nutraceuticals have already been part of dietary routines and the global nutraceutical market exceeds to be multibillion-dollar business. The United States currently occupies the leading and most rapidly growing functional food and nutraceutical market in the world.

The term "nutraceuticals" was coined by Stephen DeFelice, founder and chairman of the Foundation for Innovation in Medicine in 1989, is the amalgamation of the terminologies "nutrition" and "pharmaceutical" (DeFelice, 1995; Brower, 1998; Gupta et al., 2010). The term "nutraceutical" has a comprehensive meaning. Nutraceutical is defined as any nontoxic food component/extract isolated or purified from foods that has scientifically established potential health benefits for disease treatment as well as disease prevention. Nutraceuticals are the advancing category of natural products that helps to diminish the clear line between food and drugs (Adelaja and Schilling, 1999). Nutraceuticals may include an entire range of products including dietary supplements, isolated nutrients, herbal products (phytochemical), and other processed foods (Andlauer and Furst, 2002). They are often marketed under medicinal forms that are not connected with the foods from which they were either derived or isolated (Zeisel, 1999). Both plant- and animal-based nutraceuticals embraces exciting prospects for the food industries to generate novel food products in the near future.

1.1 Classification of Nutraceuticals

Nutraceuticals can be classified in numerous methods depending on its understanding and application. In a broader way, nutraceuticals can be categorized in two groups: (1) potential nutraceuticals and (2) established nutraceuticals (Pandey et al., 2010). A potential nutraceutical is one that holds a guarantee of a particular health or medical assistance. It can become an established one after obtaining sufficient clinical data to substantiate the promised health benefit. For example, folic acid was categorized as a potential nutraceutical until appropriate clinical research evidence for the prevention of neural tube defects was generated to upgrade it to an established one. It has to be considered seriously that much of the nutraceutical products are still in the "potential" category owing to the lack of substantial research and clinical evidences. Another broad method of classification of nutraceuticals is into three major groups: (1) herbal/natural products (herbs or plant products as concentrates and extracts), (2) dietary supplements (reagents derived from other sources aiding specific functions, such as sports nutrition, weight-loss supplements and meal

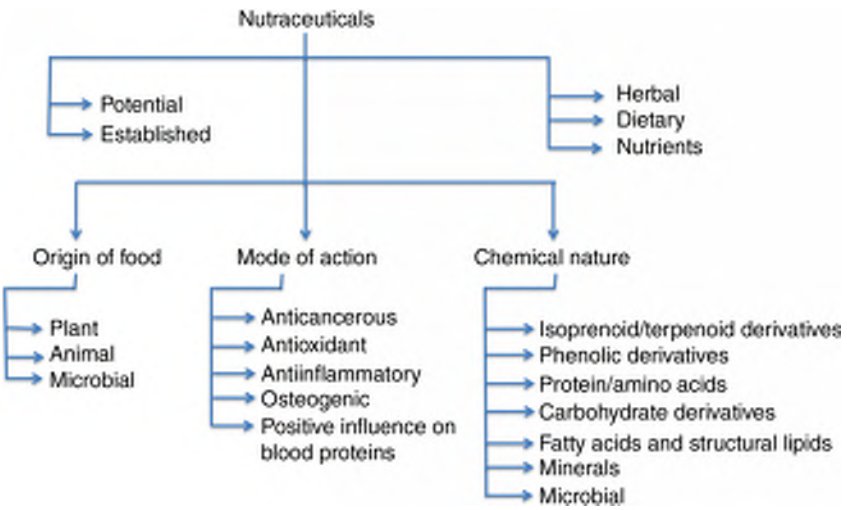

Figure 10.1. Classification of nutraceuticals.

replacements), and (3) nutrients (compounds with established nutritional functions, such as vitamins, minerals, amino acids, and fatty acids) (Rishi, 2006; Hathcock, 2001). The most common strategy of categorizing nutraceuticals is based on food sources, their chemical nature, mode of action, and so on (Fig. 10.1). Based on the origin of food source, nutraceuticals falls under three categories namely, (1) plant-based (lutein, lycopene, gallic acid, etc.), (2) animal-based (conjugated linoleic acids, choline, lecithine, etc.), and (3) microbial (*Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Saccharomyces boulardii*, etc.). Depending on the mode or mechanism of action, nutraceuticals can be grouped under (1) anticancer (curcumin, capsaicin, genestein, etc.); (2) antioxidant activity (ascorbic acid, beta carotene, tocopherols, etc.); (3) antiinflammatory (linolenic acid, curcumin, quercetin, etc.); (4) osteogenic (soy protein, calcium, daidzein, etc); and (5) positive influence on blood profile (beta glucan, resveratrol, tannins, etc.). Another mode of grouping is based on the chemical nature that allows nutraceuticals to be grouped under molecular or elemental groups. It includes (1) isoprenoid/terpenoid derivatives (carotenoids, tocopherols, saponins, etc.); (2) phenolic compounds (coumanins, tannins, flavonols, etc.); (3) protein/amino-based (amino acids, folate, capsaicinoids, etc.); (4) carbohydrate derivatives (oligosaccharides, nonstarch polysaccharides, ascorbic acid, etc.); (5) fatty acids and structural lipids (lecithin, sphingolipids, *n*-3 PUFA, etc.); (6) minerals (Ca, Se, K, etc.); and (7) microbial (prebiotics, probiotics, and synbiotics).

1.2 Concept of Nutraceuticals

Nutraceuticals possess properties for nonspecific biological therapies that are practiced to prevent malignant processes, support wellness, and to control several disease symptoms. The great success as well as the high demand of nutraceuticals can be accredited to their feature of divulging the appropriate therapeutic potential thus reducing the possible side effects, which are linked with the normal drugs used in the treatment of several disorders. There are multitudes of health benefits for nutraceuticals. To mention some, several nutraceutical products not only to lessen the risk associated with heart disease and several types of cancers but also to cure or prevent, hypertension, diabetes, obese conditions, arthritis, osteoporosis, menopausal symptoms, macular degeneration, cataracts, diminished memory and concentration, neurodegenerative diseases, constipation and digestive distresses, antifatigue or for preventing or delaying a number of age-related diseases and higher level of cholesterol (Wildman and Kelly, 2007; Gonzalez-Sarrias et al., 2013; Stauffer, 1999). The concept of nutraceuticals is the method for preventing and treating such diseases and it can be schematically represented as in Fig. 10.2 (Klein et al., 2000; Heyland, 2001; Whitman, 2001; Elizabeth, 2002; Kalra, 2003; Sengupta et al., 2003; Sumi, 2008). Nutraceuticals based on plant-based components such as polyphenols (curcumin, resveratrol, rutin) and carotenoids like beta-carotene existing in vegetables and fruits have fascinated researchers with their potential health and physiological benefits, thus overall improving the immunity.

Among the herbal polyphenols curcumin and its derivatives is one of the well-characterized and studied polyphenols. Curcumin is extracted from plant *Curcuma longa*, (family Zingiberaceae) an herbaceous tuberous plant endemic to South Asia. Chemically curcumin (M.W 368.37) is diferuloymethane ($C_{21}H_{20}O_6$) and it is a mixture of three major curcuminoids—curcumin, demethoxy

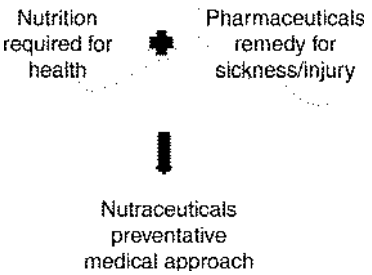

Figure 10.2. Concept of nutraceuticals.

curcumin, and bis-demethoxy curcumin. It has a wide range of biological and pharmacological activity that has been exploited since ancient times. It exhibits antioxidant, antimicrobial, anticholesterol, antiparasitic, antimutagenic antiinflammatory, and anticancer properties (Ringman et al., 2005; Goel et al., 2008; Cartiera et al., 2010). Curcumin can modulate several transcription factors, growth factors, cytokines, kinases, and many enzymes. The anticancerous property of curcumin was ascribed to initiation of apoptosis signaling and blocking several crucial signaling pathways. Curcumin inhibits the expression of Bcl2 and activates Caspase 9 thus leading to apoptosis. Curcumin also blocks several cell proliferation signaling pathways including MAP kinase pathway, AKT pathway, and mTOR pathways (Narayanan et al., 2009; Prajakta et al., 2009; Shaikh et al., 2009; Tang et al., 2010; Banerjee et al., 2010). It also prevents the initiation of angiogenesis (additional blood supply necessary for cancer cell growth) and the regrowth of cancer stem cells that are present at the heart of many tumors. Another most studied phytochemical is thymoquinone, an effective therapeutic agent derived from *Nigella sativa*. It has also several beneficial properties such antiinflammatory, antioxidant, bronchodilator, and anticancer effects (Shah et al., 2010; Woo et al., 2012; Singh et al., 2013). The anticancerous property of thymoquinone can be attributed through several mechanisms such as induction of apoptosis, antiproliferation of cancer cells, cell cycle arrest, generation of reactive oxygen species (ROS), antiangiogenesis, and antimetastasis. Thymoquinone induces apoptosis in tumor cells by suppressing NF-κB, Akt activation, and extracellular signal-regulated kinase signaling pathways (Yi et al., 2008; Sethi et al., 2008; Chehl et al., 2009; Banerjee et al., 2009).

1.3 Conventional Mode of Design and Delivery of Nutraceuticals

Nutraceuticals that are for human consumption are marketed in concentrated forms such as capsules, pills, and powders for oral delivery and tinctures for topical applications or as suppositories containing food bioactive either as a single formulation or as combination formulations (Stephen, 1998). However, the majority of the phytochemicals with potential health benefits exhibits reduced bioavailability. The diminished bioavailability stems from the low solubility, stability as well as the permeability of the bioactive components in the gastrointestinal fluids leading to their partial absorption from the gastrointestinal tract during their first pass metabolism. This often results in their reduced or

almost no biological activity, which is a major concern of the researchers today.

Nutraceuticals are often accompanied with adjuvants or absorption enhancers such as piperine, bile salts, and surfactants for enhanced uptake and for absorption (Shaikh et al., 2009). Liposomes and self-emulsifying drug delivery systems (SEDDS) are most commonly employed for the delivery of lipophilic nutraceuticals for improving its bioavailability. SEDDS have garnered great significance in the recent years owing to their ability to improve the solubility and bioavailability of lipophilic nutraceuticals. SEDDS are isotropic mixtures of oils, surfactants, solvents, and cosolvents/surfactants along with active ingredient that are used for the design of formulations of lipophilic nutraceuticals for enhancing the oral absorption (Charman et al., 1992; Constantinides, 1995; Kumar et al., 2010). SEDDS are designed to form oil-in-water microemulsions with mild agitation provided by the motility of gastrointestinal tract (GIT) followed by solubilization and absorption of active component. It often generates microemulsions of droplet size below 100 nm upon dilution (Pouton, 1985; Shah et al., 1994; Kalepu et al., 2013). This system enhances the absorption of lipophilic nutraceutical from the GIT by stimulating the dissolution process. It in turn assists the formation of solubilized phases by reducing the size of particles (micro or nano) resulting in a solid-state solution within the carrier (Porter and Charman, 2001; Humberstone and Charman, 1997). Thus, it affects the uptake of drug, efflux and disposition by altering enterocyte-based transport (Pramod et al., 2010; Wilson and Mahony, 1997) and augmenting transport of drug to the systemic circulation through intestinal lymphatic system (Charman et al., 1997; O'Driscoll, 2002; Porter et al., 2007). The oil signifies one of the most vital excipients in the formulation of SEDDS. Both long- and medium-chain triglyceride (LCT and MCT) oils with various degrees of saturation have been used for the design of self-emulsifying formulations. The chain length of the triglycerides directs the route of transport. The molecules of long-chain fatty glycerides approach the intestinal lymph than the portal blood (Fig. 10.3). The products of LCT after digestion were resynthesized in the enterocyte, compiled into lipoproteins, and then secreted into the mesenteric lymph. On the other hand MCT were predominantly absorbed directly into the portal blood. The digested lipids of MCT (monoglycerides, fatty acids, and lysophospholipid) with bile salts are subsequently incorporated into a series of colloidal structures, including micelles and unilamellar and multilamellar vesicles and bypass the difficulty of aqueous diffusion layer in GIT. The solubilization and absorptive capability of the small intestine for lipid digestion products and lipophilic nutraceutical is considerably improved owing to the formation of lipid metabolites. Solid dispersions and cyclodextrin

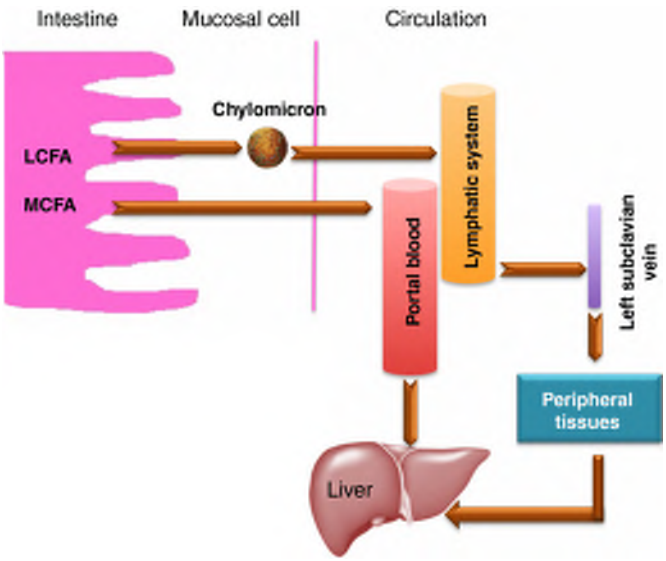

Figure 10.3. Differential LCFA and MCFA transport.

complexation are few of the other promising approaches in expanding the surface area presented for GI fluids and augmenting dissolution rate as well as oral absorption (Yallapu et al., 2010a,b; Abu-Dahab et al., 2013). A representation of formulation strategies to be attempted for lipophilic nutraceuticals as decision tree is presented in Fig. 10.4 (Zaki, 2014).

In the case of curcumin, while it is administrated either intravenously or intraperitoneally, curcumin is metabolized to tetrahydrocurcumin, hexahydrocurcumin, dihydroferulic acid and ferulic acid; however, the orally administered curcumin is excreted mostly through feces in unchanged form (Goel et al., 2008). Another nutrient is coenzyme Q10, also known as ubiquinone or CoQ10, a benzoquinone derivative that is a fat soluble vitamin-like substance produced by the human body, which exhibits antioxidant property (Kendler, 1999). Research signifies that CoQ10 may support health of heart by supporting cellular energy production. However, the bioavailability of the coenzyme Q10 was confronted by its reduced aqueous solubility and slow dissolution rate in GI fluids endowed by its lipophilic character (Balakrishnan et al., 2009). In addition to the reduced aqueous solubility, the permeability owing to its great molecular weight, P-glycoprotein efflux and active transport by several transporters including peptide transporters (PEPT1), organic anion transporters (AE2 and MCTl), and cation/camitine transporters (OCT1, OCTN1, OCTN2, and OCT3) are required (Palamakula, 2004).

In the conventional mode of delivery of nutraceuticals, a portion of the administered nutraceutical dose is absorbed and

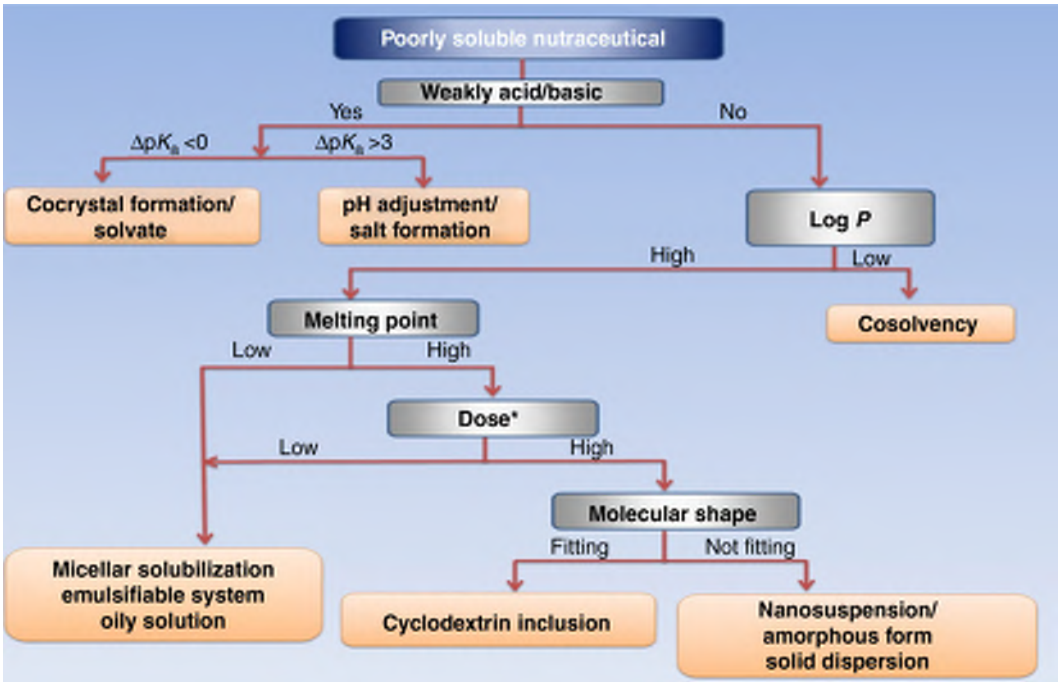

Figure 10.4. Decision tree for the formulation of lipophilic nutraceuticals (Zaki, 2014). The * refers to the dose (high or low) if low micellar solubilization system is to be used and if high formulation is based on molecular shape.

reaches the actual pharmacological site of action where as the remainder portion is either excreted or instigates nonspecific toxicity and adverse side effects owing to undesirable biodistribution. To overcome these issues, principles of nanotechnology have been exploited by researchers for the effective delivery of nutraceuticals with an intention to enhance its biological activity by improving stability and enhancing the solubility and permeability of poorly soluble nutraceuticals (Yallapu et al., 2010c, 2012, 2013). Improvement in efficacy and reduction in the nonspecific toxicity could be achieved by efficient encapsulation and delivery via nanoparticle-based delivery system.

2 Nanoscience and Technology in Nutraceuticals

Nanoscience and technology is the development and application of materials and structures at the nanometer scale. *Nanostructures* refer to materials systems that are in the range of approximately 1–100 nm in at least one dimension. While defining

nanostructures it is essential to distinguish between the numbers of dimensions on the nanoscale. Nanostructures are broadly divided into three classes:

1. Nanoscale in one dimension: thin films layers and surfaces where the thickness of the surface of the material lies is in the range of 0.1–100 nm.
2. Nanoscale in two dimensions: carbon nanotubes, inorganic nanotubes, nanowires, biopolymers where the diameter of the tube is between 0.1 and 100 nm, although its length could be greater.
3. Nanoscale in three dimensions: nanoparticles, fullerenes, dendrimers, quantum dots where the size of the particle ranges between 0.1 and 100 nm in each spatial dimension.

Nanostructures are unique when compared with individual atoms/molecules at a smaller scale and the macroscopic bulk materials. Research in nanotechnology basically centers on the unique properties offered by the nanoscale structures that does not exist in structures of same material composition in bulk materials. Nanostructures exhibit properties different from their macroscopic bulk materials in several properties such as in their mechanical strength, thermal conductivity, electrical conductivity, chemical reactivity, transparency, and magnetic properties. In general in nanoscale materials, the physical, chemical, and biological properties that are significantly different from their macroscopic bulk materials.

Nanoparticle-based materials are already being used in several sectors for example, electronics and engineering, biomedical field (both therapeutic and diagnostic), pharmaceutical, aerospace, defense sector, textile industry, cosmetic, food, and agriculture. Innovations in nanoscience and technology are opening new avenues in the food industry. In general, food and water are naturally made up of nanoscale particles. For example, proteins exist in the nanoscale size range and milk is an emulsion of nanoscale fat droplets. Applications of nanotechnology are increasing in the food industry worldwide and are projected to expand quickly in the near future. Advances in nanoscience have led to the addition of nanoparticles to food to enhance the stability and bioavailability, which could be nanoparticles based on existing ingredients, or entirely novel chemical structures. Although the food industry has started exploiting its applications, nanotechnology offers great potential.

2.1 Concept of Nanodelivery Systems for Nutraceuticals

The concept of nanodelivery system has emerged to widen their application and to develop various modes of administration

so as to decipher the problems related to their absorption. There has been a remarkable progress in the development of nanonutraceuticals and delivery of lipophilic nutraceuticals via nanotechnology, which once were poorly aqueous soluble, that limited their efficiency and bioavailability. The nanoformulations offer targeted delivery of the encapsulated nutraceuticals and also the slow and sustained release from the nanoformulation. Effective solubilization, encapsulation, and delivery based on nanoscience and technology as biocompatible systems are expected to endow with superior absorption in lower doses, reduced frequency of administration, and amended therapeutic index. Nanosystems either with nanocarriers or the absence of nanosized carriers have been developed for the efficient delivery of nanonutraceuticals. Increasing the solubility and dissolution rate can be attained by nanonization in the absence of nanocarrier. The nanonization of products, either in the food industry (eg, nanoencapsulated vitamins) or the drugs for pharmaceutical use, is extremely significant in the economical as well as on the pharmaceutical sector (Junghanns and Müller, 2008). Nanonization is the reduction in particle size to nanodimension that has been developed based on theoretical considerations, such as the Ostwald–Freundlich equation, the Noyes–Whitney equation, the Prandtl equation, and Ostwald ripening (Mosharraf and Nyström, 1995; Müller and Peters, 1998; Müller et al., 2001; Jinno et al., 2006; Kesisoglou et al., 2007; Mauludin et al., 2009; Dolenc et al., 2009; Dong et al., 2009; Sun et al., 2012). On the other hand, nanocarrier-based nanosystems are the most commonly employed nanovectors for the encapsulation and delivery of nutraceuticals; however, biocompatibility and biodegradability-associated concerns are taken to be considered. A number of formulation approaches based on carriers such as nanoemulsions, micelles, nanoparticles, nanocapsules, nanocochleates, and nanocrystals have been utilized for the efficient delivery of the encapsulated nutraceutical. Nanosystems such as nanoemulsion, biopolymer nanoparticles, solid lipid nanoparticles, and nanoliposomes have been studied for their potential role as nanodelivery of nutraceuticals. While designing nanodelivery system in the food-related industry several factors have to be considered so as to make the system highly efficient. Some of the main parameters include the safety, stability, commercial feasibility, compatibility with food matrix, performance and labeling requirements (Fig. 10.5).

An excellent example to demonstrate the advent of nanotechnology for the incredible enhancement in the solubility and the bioavailability of curcumin, a potential nutraceutical with several significant properties including anticancer potential has been

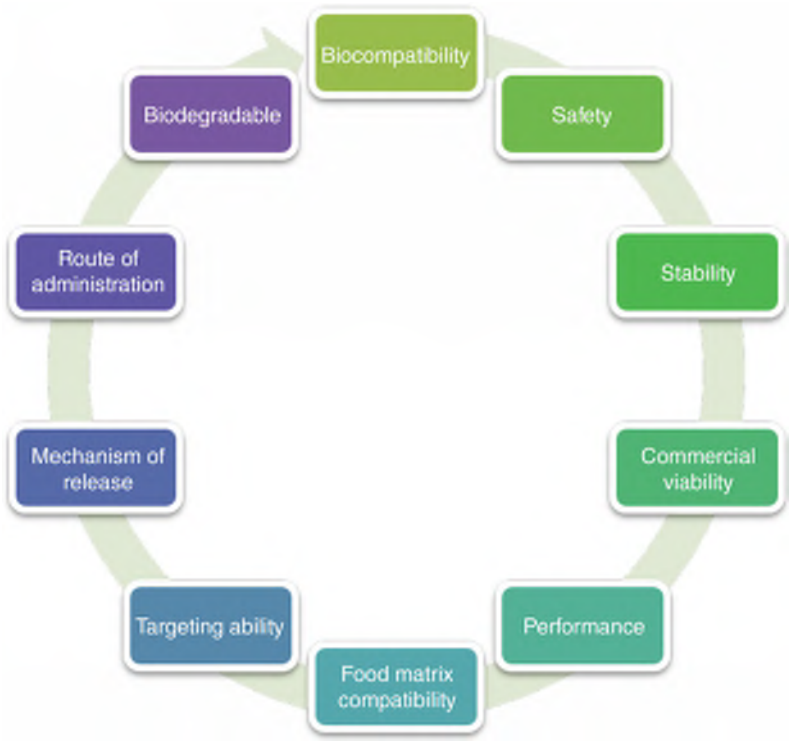

Figure 10.5. Features required for nanoscale delivery systems.

evidenced by various in vitro and in vivo studies, by the development of various nanoformulations like polymeric nanoparticles, liposomes, and micelles. Studies showed that encapsulation of curcumin along with magnetic nanoparticles exhibited excellent reduction in cancer cells in vitro in the synergistic with of magnetic hyperthermia (Sivakumar et al., 2013, 2014, 2015; Balasubramanian et al., 2014). In addition to anticancerous effects of curcumin nanoformulation, effect of curcumin in Alzheimer's disease has been demonstrated by the destruction of amyloid aggregates by utilizing curcumin encapsulated-PLGA nanoparticle (Mathew et al., 2012a,b). Lutein, another potential nutraceutical has also been encapsulated into polymeric nanoparticles, thus enhancing the solubility of the same.

Nanoscale delivery systems are intended to release the lipophilic nutraceuticals within the GIT (mouth, stomach, small intestine, or colon) (Fig. 10.6). The encapsulated nutraceutical in the nanoparticles are fabricated to preserve the potentiality of nutraceutical and by doing that the release mechanism can be controlled. The active component is released under certain situations

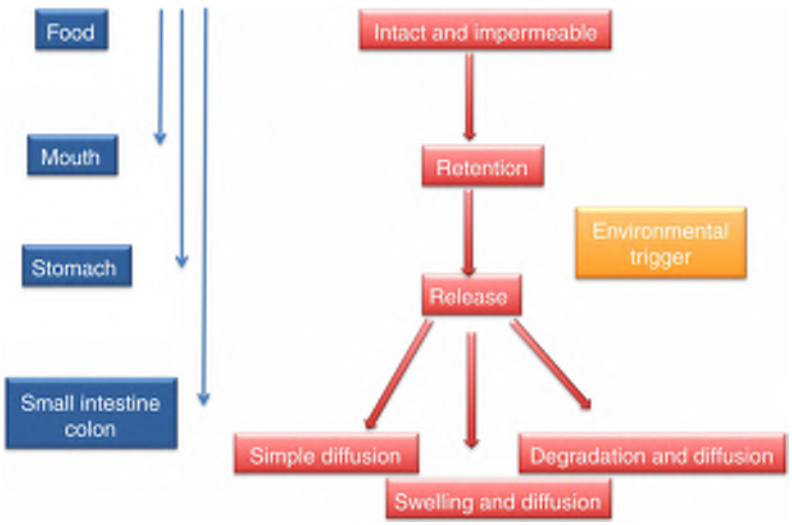

Figure 10.6. Design of nanoscale delivery system to release nutraceuticals under different specific conditions within the food, mouth, stomach, small intestine, or colon.

upon stimulation by pH, temperature, in the presence of enzyme, and so on. For example, if the nanoparticle has to exert its action in the colon, it must be designed in order to overcome all the stress (including enzymatic) in upper GIT. In this context, efficient targeting also plays a major role in enhancing the availability of the nanoformulation. Effective targeting (both active and passive targeting) accelerates the accumulation of drug or nutraceutical components at the precise site of action. Also, it reduces the unwanted distribution thereby lessening the side effects associated with the dug in normal healthy cells (Nair et al., 2010). Several studies recommend that targeting strategies could overcome side effects, drug resistance, and reduce systemic drug administration. Passive targeting is based on the enhanced permeability and retention effect (EPR) of tumor blood vessels and it can be attained by modifying properties of nanoparticles including physicochemical properties, ionic properties, and solubility of nanoparticles in aqueous phase. In tumors, nanoparticles leak from the reticuloendothelial system (RES) and circulate for a prolonged period in the bloodstream, gain more chance for reaching the tumor tissues. The cancer cells develop neovascularization for oxygen and nutrients to the rapidly growing cancer cells (Vaupel et al., 1989). This makes tumor vessels highly disordered with enlarged gap junctions between endothelial cells and improper lymphatic drainage that allows the nanoparticles to reach the target site (Connor et al., 2009).

The addition of a targeting moiety (biomarkers) onto the surface of nanoparticles can upsurge selective cellular binding and internalization via receptor-mediated endocytosis. Ideally active-targeting nanoparticles should possess these properties such as (1) exclusive targeting the cells of interest, (2) receptor mediated endocytosis for rapid uptake of targeted nanoparticles, (3) efficient surface coating, (4) rapid delivery to target site, (5) stability of targeting moieties (ligands), and (6) enhanced retention via compartmentalization. In addition to the active targeting via cellular markers present on the cell surface, subcellular organelle targeting for example, mitochondrial-targeting have been attained. Coenzyme Q10 has been targeted to mitochondria by using a special targeting moiety; membrane-permeable lipophilic triphenylphosphonium cation. The targeting ligand was chemically conjugated to the nanocarrier or the coenzyme Q10 or resveratrol molecule (Cochemé et al., 2007; Biasutto et al., 2008). The conjugation of resveratrol to the lipophilic triphenylphosphonium cation offered transient protection against metabolic conjugation, accumulated into mitochondria and was cytotoxic for fast-growing but not for healthy slower-growing neighboring cells (Biasutto et al., 2008). Mitochondrial targeting of nutraceuticals with antioxidant properties endowed a potential nanotool to arbitrate mitochondrial and cellular redox processes of pathophysiological effects (Porteous et al., 2010). Various targeting moites such as HER2 antibody, aptamer (AS1411), folate, and transferrin have been used to deliver curcumin in cancer cells and demonstrated the enhanced cellular cytotoxicity in cancer cells (Sivakumar et al., 2013, 2014, 2015; Balasubramanian et al., 2014). In the case of Alzheimer's disease, targeted Tet-1 peptide as well as amyloid-binding aptamer attached curcumin–PLGA nanoparticles that destroy amyloid aggregates have been demonstrated (Mathew et al., 2012a,b). A schematic layout of the development of nutraceutical nanoformulations for future clinical application or clinical trials is represented in Fig. 10.7. The nanoformulation developed must be thoroughly characterized before being used. Its structural parameters, chemical composition, encapsulation efficiency of the nutraceuticals, released study and stability must be properly addressed. The mode of release of active ingredient either passive release or triggered release with respect to stimulation such as pH, temperature, mechanical forces, and effect of enzyme must be studied thoroughly. The in vitro studies are significant to study the effect of cell targeting, uptake of nanoparticles with respect to time and targeting moites, migration potential and cytotoxicity imparted by the nanoparticles, thus analyzing the biocompatibility of nanoscale particles encapsulated with

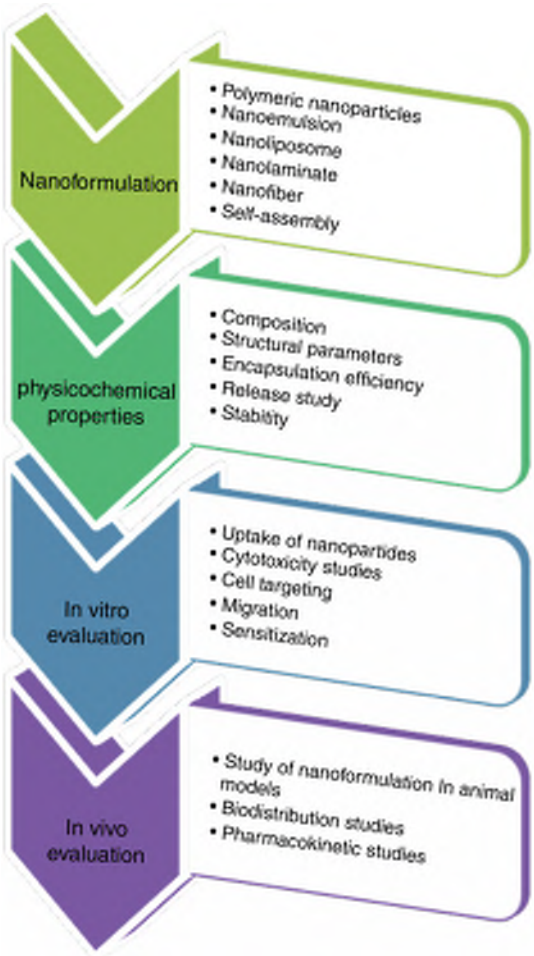

Figure 10.7. Schematic layout of development of nanonutraceuticals for future clinical applications.

nutraceuticals. In vitro studies are also performed to mimic the gastrointestinal environment by simulation studies to analyze the stability of nanocarriers with nutraceuticals. Although, improved bioavailability and therapeutic potential of various nanoformulation of nutraceuticals have been demonstrated in vitro, in order to demonstrate its efficiency in vivo studies are required. In vivo studies are performed to access the biodistribution and the pharmacokinetic studies. The chronic and acute effects of nanoparticles also need to be addressed before being transferred to clinical trials and being marketed. Moreover the toxicity and safety of nanoscale delivery agents to human and to the environment have to be properly addressed.

2.2 Different Methods of Synthesis and Formulation of NPs With Nutraceuticals

Nanosized particles with different shapes, sizes, and properties can be designed or fabricated based on the manufacturing conditions, properties of components used in the reaction, pretreatments to modify the components such as microfluidization, and high-pressure homogenization. The most commonly used nanostructures in food technology are depicted schematically in the Fig. 10.8.

There are two main approaches to synthesize nanoparticles. One is the top-down approach and another is the bottom-up approach. The top-down approach is from top (larger) to bottom (smaller) where small particles are formed through size reduction (by mechanical) processes. The bottom-up approach is from bottom (smaller) to up or top (larger). In this method the nanoparticle is produced by the self-assembly of smaller molecules (chemical processes) (Merisko-Liversidge et al., 1996;

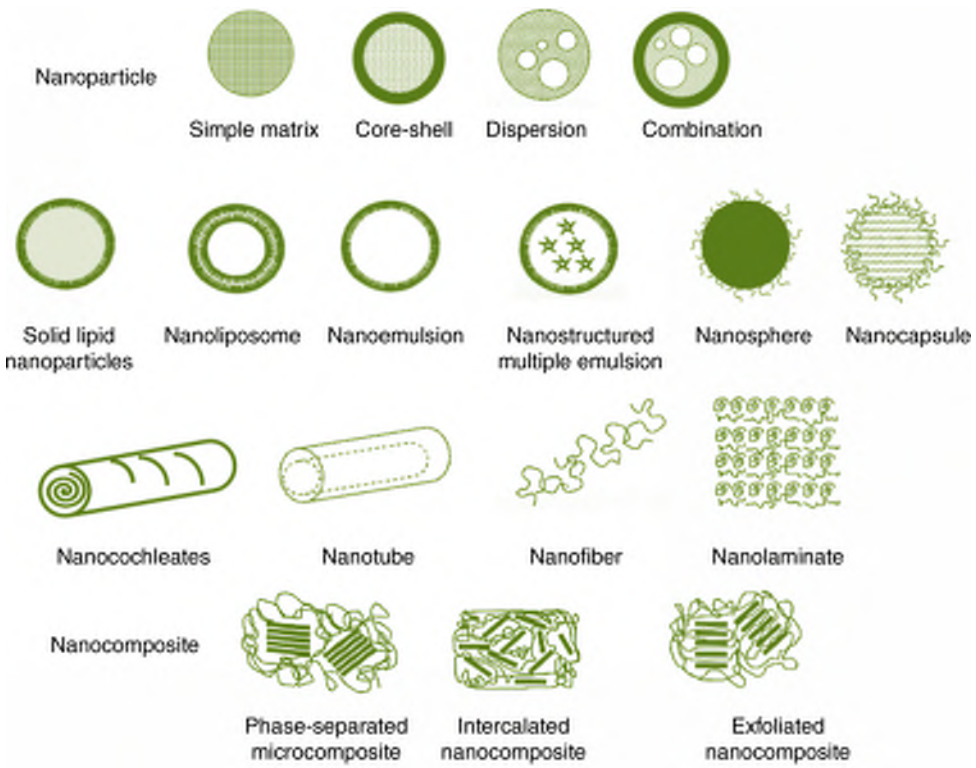

Figure 10.8. Commonly used nanostructures in food technology.

Shimomura and Sawadaishi, 2001; Whitesides and Grzybowski, 2002; Hamley, 2003; Sanguansri and Augustin, 2006; Inkyo et al., 2006; Inoue et al., 2007). It is also possible to combine both bottom-up and top-down approaches to produce nanoparticles (Horn and Rieger, 2001). In food nanotechnology the top-down approach implicates the physical processing of food materials. For example, in the case of green tea the antioxidant activity was enhanced when the size is reduced to 1000 nm. The microfine powder was with a higher ratio of nutrient digestion and absorption with high active oxygen-eliminating potency owing to higher content of superoxide dismutase (SOD), the enzyme eliminating active oxygen (Shibata, 2002). Another example is the dry-milling of wheat into fine wheat flour results. The product has high water-binding capacity than the raw wheat. On the other hand, self-assembly/self-organization is based on fundamental biological processes that have resulted in a bottom-up approach in food nanotechnology. For example, starch, casein micelles, protein results in stable entities to form nanoparticles by self-assembly (bottom-up) (Dickinson and Van Vliet, 2003). In this chapter, the most common methods of top-down (mechanical) and bottom-up (chemical) approaches are discussed (Fig. 10.9).

2.2.1 Approaches for the Preparation of Nanoparticles by Mechanical Methods

The mechanical process signifies procedures that make use of shear force or collision of particles as the energy to break down larger objects into smaller nanoscale particles. The advantage of mechanical processes is that mechanical processes involve least use

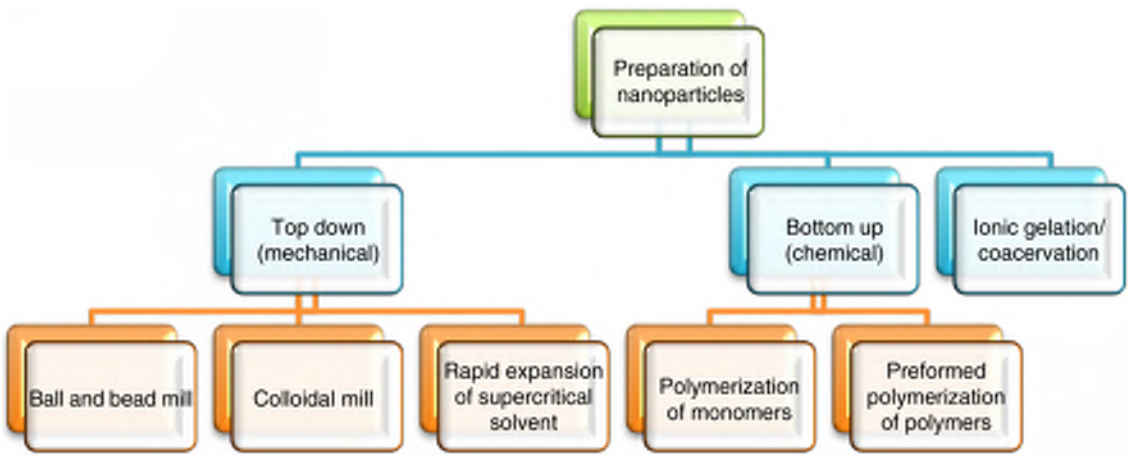

Figure 10.9. Methods for the synthesis of nanoparticles.

of chemicals that diminish the concerns related to the safety and regulations on formulation. Generally, there are two kinds of mechanical processes used for the generation of nanoparticles: mills for the nanonization of solid particles, and microfluidic processes for the nanonization of liquids or melts. Owing to the simplicity and the capability to work with a wide range of materials, ball mill process is considered as one of the promising milling techniques for the production of solid nanoparticles (Inkyo et al., 2006). It is used for producing nanoparticles in the range of 20 nm from micrometer-sized crystalline drugs. However, there are no reports on the use of bead mills on the synthesis of nanoparticles in solid forms of nutrients and nutraceuticals. Bead mills could also be used to assist chemical reactions during the milling process.

Colloid mills or microfluidization processes are associated with liquid-based technologies. It depends on the flow-induced shear of liquids, hot melts, and soft aggregates to produce nanosized material. Flow-induced shear is attained by stimulating large pressure drops through nozzles that are competitively very small. The major challenge in colloid mills is the stabilizing of the product against aggregation. Therefore, several measures to avoid aggregations such as spray drying, rapid cooling, solvent evaporation, coatings with hydrocolloids, and surfactants as stabilizing agents are commonly being used. In food industry microfluidization is a well-recognized technology, especially in the dairy industry and also for the encapsulation of probiotics (Feijoo et al., 1997; Tunick et al., 2002; Olson et al., 2003). Also, microfluidization is the foundation for the fabrication of solid lipid nanoparticles (SLN) (Liedtke et al., 2000).

Another type of the microfluidization technique is the supercritical fluid as the solvent media (Shariati and Peters, 2003; Wright et al., 2010; Reverchon, 1999; Jung and Perrut, 2001; Vemavarapu et al., 2005; Mishima, 2008). Supercritical fluid and dense gas technology offers an exciting and efficient technique of the production of nanoparticles. The two principles for the production of nanoparticles using supercritical fluids are (1) rapid expansion of supercritical solution (RESS) and (2) rapid expansion of supercritical solution into liquid solvent (RESOLV).

In conventional RESS, the solute/active ingredient (hydrophobic component) is dissolved in a supercritical fluid (eg, carbon dioxide) to form a solution, followed by an expansion of the solution across a small orifice or a capillary nozzle. The elevated shear rates across the orifice, the high degree of super saturation, and rapid pressure reduction in the expansion results in homogenous nucleation. It generates a fine mist where the supercritical fluid evaporates rapidly and induces the precipitation

of the solute into nanoparticles (Jung and Perrut, 2001; Tom and Debenedetti, 1991). It has been demonstrated that RESS process results in both nanometer and micrometer-sized particles and final particle size could be controlled by the addition of stabilizing agent. Although RESS resulted in the formation of nanoparticles, majority of the products was microparticles than nanoparticles. To overcome this disadvantage a new supercritical fluid technology by modifying RESS was developed. RESOLV or RESAS is a modification of RESS, where the expansion of the supercritical solution was into a second liquid instead of ambient air (Rao and Geckeler, 2011). In RESOLV the second liquid solvent reduces the particle growth in the expansion jet, thus resulting in nanosized particles (Meziani et al., 2004, 2005; Hutchenson, 2002).

Other methods of precipitation or solidification with mechanical shear force are the spray freezing into a cryogenic liquid (SFL), atmospheric freeze-drying (ATMFD), and the spinning disk processing (SDP) method (Hu et al., 2004; Anantachoke et al., 2006). In the spinning disk processing (SDP), a jet is imposed onto a heated rotating disk. The centrifugal force owing to rotation breaks down the jet to small particles. The heat transmitted from the spinning disk into the liquid results in the rapid evaporation of the solvent, resulting in the formation of small particles. SDP technology also involves the use of surfactants to control agglomeration of particles.

2.2.2 Approaches for the Preparation of Nanoparticles by Chemical Methods

There are several approaches for the preparation of nanoparticles by chemical method. The major methods are

1. Dispersion of preformed polymers
2. Polymerization of monomers
3. Ionic gelation or coacervation

2.2.2.1 Dispersion of Preformed Polymers

Dispersion of drug/active ingredient in preformed polymers is the most common approaches practiced for preparation of biodegradable nanoparticles from synthetic polymers. It can be performed by several methods as described in the next sections.

2.2.2.1.1 Nanoprecipitation Nanoprecipitation is also known as solvent displacement method or interfacial deposition method. It is one of the simplest, efficient, and least energy-consuming approaches for the production of nanosphere from preformed polymers. The formulation of nanopharmaceuticals by this method involves the nanoprecipitation of preformed polymer from

an organic solution. The diffusion of the organic solvent in the aqueous phase (in the presence or absence of surfactant) results in the formation of nanoparticles (Fessi et al., 1989; Barichello et al., 1999; Rodriguez et al., 2004; Ganachaud and Katz, 2005). In other words it is based on the interfacial deposition of a polymer after the displacement of a semipolar solvent, from a lipophilic solution. This method is mainly appropriate for lipophilic drugs due to the miscibility of the solvent with the aqueous phase. The three main components that are involved in nanoprecipitaion are (1) the polymer (natural, synthetic, or semisynthetic), (2) the polymer solvent (soluble in water, and easily removed by evaporation), and (3) the nonsolvent of the polymer. The nonsolvent phase is generally composed of a single or a mixture of nonsolvent of the polymer with or without the addition of surfactant. When the lipophilic polymer solution is added slowly to the nonpolar solvent, the nanoparticles are formed by the rapid diffusion of the aqueous polymer solvent in the nonsolvent phase. The interfacial tension between the two phases leads to an increased surface area and results in the precipitation of polymeric nanoparticles. The formation of nanoparticles by nanoprecipitation depends on several factors, such as the miscibility of the polymer solvent with the nonsolvent phase, the interaction of polymer and solvent, the rate of injection of organic phase, the addition of polymer solution, and also the surfactants, which support the stabilization of the nanoparticles by preventing the aggregation and for long storage periods in suspensions (Fessi et al., 1989; Barichello et al., 1999). Earlier studies with curcumin encapsulated PLGA nanoparticles synthesized by nanoprecipitation has demonstrated enhanced cellular uptake of nanoparticles via active targeting and excellent therapeutic efficiency in killing cancer cells under in vitro conditions (Mathew et al., 2012a,b; Sivakumar et al., 2013, 2014, 2015; Balasubramanian et al., 2014).

2.2.2.1.2 Solvent Evaporation In solvent evaporation method, the polymer is usually dissolved in organic solvents such as ethyl acetate, dichloromethane, or chloroform. A nanoemulsion is formed between solvent (partially water miscible) with polymer and the lipophilic drug/active ingredient, and an aqueous phase along with the stabilizer. The emulsion is then converted into nanoparticle suspension on evaporation of the solvent for the polymer. The main two strategies used for the formation of nanoparticles are the preparation of single-emulsions [oil-in-water (o/w)] or double-emulsions [(water-in-oil)-in-water, (w/o)/w]. The solvent is removed slowly either by reducing the pressure or by continuous stirring that results in the formation of nanoparticles. Ultrasonication or high-speed homogenizer are commonly used to

optimize the size of nanoparticles (Zambaux et al., 1998). The solidified nanoparticles are collected by ultracentrifugation and washed with distilled water to remove the surfactants and the final product is lyophilized until use (Rao and Geckeler, 2011; Pinto et al., 2006). Poly(lactic-*co*-glycolic acid) (PLGA) is a biocompatible, biodegradable, and FDA-approved aliphatic polyester. PLGA nanoparticles have been successfully prepared by solvent evaporation method. Several hydrophobic and hydrophilic drugs are encapsulated in PLGA particles by solvent evaporation method. PLGA degrades slowly via hydrolysis in aqueous environments to lactic acid and glycolic acid, and encapsulated drug are released slowly. There are several factors that affect the size of nanoparticles. For example, dichloromethane as a solvent generally produces larger nanoparticles with a broader size distribution where as ethyl acetate (miscible in water) results in the formation of smaller nanoparticles owing to the reduction in the surface tension of the polymer droplet in the primary emulsion. Other factors include the type of polymer, solvent, solvent volume, solvent–polymer ratio, concentration of stabilizer, sonication intensity, homogenizer speed, and speed/duration of centrifugation.

2.2.2.1.3 Emulsification/Solvent Diffusion Emulsification/solvent diffusion is a modified version of solvent evaporation method. The polymer is dissolved in a polar organic solvent and mixing with aqueous solutions to attain the initial thermodynamic equilibrium. Emulsification occurs rapidly upon addition of water with stabilizer. The polar organic solvent diffuses out of the emulsion drop and to the aqueous phase resulting in the precipitation of the lipophilic polymer and the drug/active ingredient as nanoparticles. The solvent is then removed by evaporation or filtration, based on the boiling point. The advantage of this method includes the high encapsulation efficiencies (>70%), high reproducibility, ease of scale-up, and narrow size distribution. This method is one of the efficient techniques for encapsulating lipophilic drugs (Mohanraj and Chen, 2006). Some of the disadvantages of this method are the large volumes of water to be removed from the suspension and the escape of hydrophilic drug into the saturated-aqueous phase during emulsification thus reducing encapsulation efficiency (Pinto et al., 2006; Ganachaud and Katz, 2005). This method has been used to produce β-carotene encapsulated PLGA nanoparticles (20–80 nm) using acetone as the organic polar solvent and PLGA as stabilizing polymer in the presence of an emulsifier (Ribeiro et al., 2008).

2.2.2.1.4 Salting-Out The salting-out method is a modification of the emulsification/solvent diffusion technique. It involves

emulsification of polymer solvent that is totally miscible with water. Polymer along with drug are dissolved in a solvent and emulsified into an aqueous phase containing the salting-out agents. The salting out agents can be electrolytes (magnesium chloride, calcium chloride, magnesium acetate, etc.) or nonelectrolytes such as sucrose (Allemann et al., 1992; De Jaeghere et al., 1999; Perugini et al., 2002; Nguyen et al., 2003; Zweers et al., 2004). Salting-out can also be induced by saturation of aqueous phase by PVA (Allemann et al., 1992). The emulsion is then diluted with appropriate amounts of water or to an aqueous solution to drop the salt concentration and to enhance the diffusion of solvent into the aqueous phase, resulting in the formation of nanoparticles. This method can be useful for heat sensitive drugs because salting out technique does not require high temperature (Lambert et al., 2001b). However, this method is limited to lipophilic drugs/active ingredients and another disadvantage is extensive washing steps for collecting the nanoparticles (Couvreur et al., 1995).

2.2.2.1.5 Dialysis Dialysis is based on a solvent displacement mechanism. The polymer is dissolved in organic polymer solvent and sealed in a dialysis tube or semipermeable membranes with an appropriate molecular weight cutoff that function as a physical barrier for the polymer (Jeon et al., 2000, 2001; Kostag et al., 2010). Dialysis is carried out against the nonsolvent of the polymer that is miscible with the polymer solvent. There will be a loss of solubility of the polymer when the polymer solvent is displaced through the membrane that results in the formation of nanoparticles in homogeneous suspension. Several polymer and copolymer nanoparticles have been synthesized by this technique and the solvent used in the preparation of the polymer solution influence the particle size distribution and also the shape of the nanoparticles (Jung et al., 2004; Choi and Kim, 2007; Hornig and Heinze, 2007; Heinze et al., 2007; Liu et al., 2007; Park et al., 2007; He et al., 2008; Zhang et al., 2008; Errico et al., 2009; Faheem et al., 2009).

2.2.2.2 Polymerization of Monomers

In this approach, the nanoparticles are formed through polymerization of monomers. Some of the major emulsion polymerization techniques are described in the next sections.

2.2.2.2.1 Emulsion Polymerization Emulsion polymerization is one of the traditional, fastest, and easily scalable methods for the preparation of nanoparticles. It is basically classified into conventional emulsion polymerization and solvent-free emulsion polymerization. The main components in this method are a monomer (low solubility in water), initiator (water soluble), a

surfactant and water. The water-soluble initiator can be either an ion or a free-radical. The process of polymerization is initiated when a monomer collides with the initiator. Other techniques for initiating the radical from the monomer include UV irradiation, ultrasonication, and γ-irradiation. It involves the application of surfactants to prevent the aggregation during the polymerization. The surfactant-free emulsion polymerization is carried out without the application of emulsifier and it is considered as the "green" method for the preparation of nanoparticles. The major disadvantage of use of surfactant is the removal of the same by repeated washing, which is very time consuming. The components in solvent-free polymerization process are water miscible initiator, monomer, and water. Ionizable initiators as well as ionic comonomers are often used to stabilize the nanoparticles. Several improvements and parameters should be optimized to prepare monodisperse uniform sized nanoparticles.

2.2.2.2.2 Miniemulsion Polymerization The formulation used in miniemulsion consists of water, monomer mixture, costabilizer, surfactant, and initiator. The nanoemulsion is formed by the monomer phase stabilized by the adsorbed surfactant. The mechanism of polymerization is based on radical polymerization that is instigated in the emulsions droplets by the integration of the initiator in the continuous phase. The number and size of particles do not consequently vary during the polymerization process. Several factors such as the choice of the initiator as well as its solubility can have great impact in the properties of the nanoparticles formed specially the size of the particle. The major distinction between emulsion polymerization and miniemulsion polymerization techniques are use of a low molecular weight costabilizer and application of high-shear device such as ultrasound (Bardajee et al., 2007; Wang et al., 2007; Yildiz and Landfester, 2008; Rotureau et al., 2008; Crespy and Landfester, 2009; Wu et al., 2009a; Ethirajan et al., 2009; Jiang et al., 2010; Sharon and Margel, 2010). Miniemulsions are stabilized, which may require a high shear and should possess interfacial tension greater than zero (Rao and Geckeler, 2011).

2.2.2.2.3 Microemulsion Polymerization Microemulsion polymerization is another effective approach for preparing polymeric nanoparticles. The major difference between microemulsion and miniemulsion polymerization methods is based on the kinetics of the dispersed phase in the emulsified system. Microemulsion is a thermodynamically stable system maintained with large amount of surfactant. Microemulsion polymerization systems comprise of a water miscible initiator that is added to the aqueous phase of

a thermodynamically stable microemulsion and polymerization initiates spontaneously. The interfacial tension at the oil/water interface in microemulsion is close to zero (Yoo et al., 1999). Polymer chains are formed initially in few droplets because the initiation process could not be accomplished in all microdroplets. After some time, the osmotic and elastic influence of the polymeric chains destabilizes the microemulsions that results in an increase in the particle size, and finally to the generation of empty micelles and secondary nucleation (Puig, 1996; Antonietti and Landfester, 2002). The very high-dilute formulation and large quantity of surfactant is the major disadvantages that limit commercial use of this technique.

2.2.2.2.4 Interfacial Polymerization Interfacial polymerization is one of the commonly used methods for the preparation of polymer nanoparticles especially nanocapsules (hollow polymer nanoparticles) (Lambert et al., 2001a; Charcosset and Fessi, 2006; Gaudin and Zydowicz, 2008; Wu et al., 2009b; Landfester et al., 2010). It is often characterized by the polycondensation of monomers used in the synthesis, at the droplet interface resulting in the formation of nanocapsules instead of nanospheres. There are several approaches in obtaining nanocapsules that depends on the monomer that is dissolved in either the continuous and/or in the dispersed phase and also on the formulation. In one approach, the monomer is introduced to the continuous phase of the emulsified system and it reacts with the emulsion droplets, resulting in the formation of nanocapsules. The emulsions formed in this approach are w/o type owing to the water immiscible nature of the monomer. Solubility of the monomer for the external phase and its appropriate reactivity in the aqueous phase are the important parameters that result in the formation of nanocapsule of uniform size. In another approach, the monomer is introduced in the droplet phase. Polymerization is initiated by the reaction in the continuous phase or by the introduction of initiator in the external phase (Tiarks et al., 2001; Landfester, 2001). Another method is the simultaneous generation of interfacial polymerization and nanoemulsion by solvent diffusion technique. Nanocapsules are formed by the polymerization initiated along with the rapid solvent diffusion to the aqueous continuous phase. The most common approach is in which two reactive species of dissimilar solubilities are introduced respectively in the continuous and dispersed phases and the reaction occurs at the interface of the two liquids.

2.2.2.2.5 Controlled/Living Radical Polymerization The lack of control over the molar mass and molar mass distribution, macromolecular architecture, and the end functionalities are some

of the major disadvantages of radical polymerization owing to the unavoidable fast radical–radical termination reactions. The controlled/living radical polymerization is an alternative approach and it is based on "green chemistry" and engineered specially for biomedical applications with hydrophilic polymers (Zetterlund et al., 2007, 2008; Matyjaszewski and Xia, 2001). Among the controlled/living radical polymerization methods the most extensively studied methods are nitroxide-mediated polymerization (NMP) (Nicolas et al., 2007; Farcet et al., 2000, 2001, 2002; Dire et al., 2009), atom transfer radical polymerization (ATRP) (Oh et al., 2009; Siegwart et al., 2009; Li et al., 2008, 2009; Min and Matyjaszewski, 2005; Min et al., 2006, 2009), and reversible addition and fragmentation transfer chain polymerization (RAFT) (Manguian et al., 2006; Zhou et al., 2007; Rieger et al., 2010). Parameters such as the nature and concentration of the monomer, initiator, surfactant, control agent, and emulsion type influence the size of nanoparticles.

2.2.2.3 Ionic Gelation or Coacervation of Hydrophilic Polymers

Polymeric nanoparticles can also be prepared by using biodegradable hydrophilic polymers (gelatin, chitosan, sodium alginate, etc.) (Hittinger et al., 2004; Reis et al., 2006b). Nanoparticles are formed after nanoemulsification to gelify polymer or crystallize lipid dissolved in the droplets. Nanoemulsions are formed at moderate high temperatures above the melting point and cooling stimulates gelation of the emulsion droplets and conversion into nanoparticles. Gelation can be induced by physicochemical factors such as pH or by addition of divalent cation (generally calcium) (Reis et al., 2006a). Solid lipid nanoparticles are also produced by the same method by crystallization of the lipid under the melting temperature (Hittinger et al., 2004).

2.3 Potential Nanodelivery Systems

There are several formulations designed for the delivery of nanonutraceuticals based on nanoscience and nanotechnology. An efficient nanodelivery system should perform several functions such as an ideal carrier/vehicle for the active ingredient, highly biocompatible, protect the active component from degradation (chemical and biological) while processing and storage, controlled and sustained release of active components or under stimulation (pH, ionic strength, or temperature), and qualitative aspects (texture, taste, shelf life) of the product (Weiss et al., 2006). Nanodispersions and nanocapsules can perform these aforementioned objectives and are ideal means for delivery

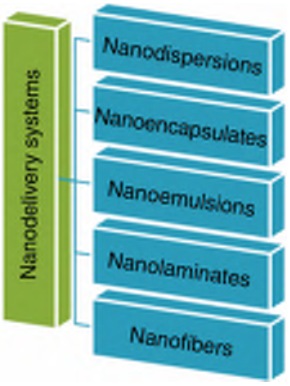

Figure 10.10. Potential nanodelivery systems.

of functional ingredients. These nanostructures include: nanoemulsions, association colloids, and biopolymeric nanoparticles (Fig. 10.10). Each mode of the nanodelivery system has advantages and disadvantages in terms of encapsulation, delivery, and stability of active ingredient, biocompatibility, and biodegradability, cost of production, and regulatory status.

2.3.1 Nanodispersions and Nanoencapsulates

Nanoemulsions are efficient drug delivery systems with mean droplet diameters ranging from 50 to 1000 nm. Nanoemulsions are lipid droplets made from pharmaceutical surfactants that are permitted for human consumption and are "generally recognized as safe" (GRAS) by the FDA. Owing to its small particle size, nanoemulsion remain stable for a longer period upon storage. Nanoemulsions can be prepared either by high-energy methods (high-pressure homogenizer or ultrasound generator) or by low-energy methods (self-emulsification and phase inversion methods) and both of the methods generate stable nanoemulsions. In nanoemulsion, active ingredients can be incorporated within the droplets, the interfacial region, or in the continuous phase (McClements, 2004). Complex nanoemulsions such as nanostructured multiple emulsions, and nanostructured multilayer emulsions provide various encapsulating abilities that can encapsulate different functional components that could be released upon environmental trigger (pH, temperature, etc.) (McClements and Decker, 2000). Apart from these developments, interfacial engineering technology for smart delivery systems by designing nanoshells around droplets has also been successfully demonstrated. Nanoemulsions exhibit distinctive textural characteristics at low oil concentrations that make them efficient for developing reduced

fat products (Padua and Wang, 2012). Nanoemulsions are based on food-grade components such as triglyceride oils, dairy protein emulsifiers. It augments the bioavailability of lipophilic active component by accelerating lipid digestion and its release, enhanced formation of mixed micelles with solubilization capacity and also by the formation of chylomicrons, which transport the active ingredient via lymphatic route. In addition to these mechanisms, by the addition of efflux inhibitors and permeation enhancers to the aqueous phase can enhance the bioavailability of lipophilic active ingredient (McClements, 2013). Sun et al. (2015) has recently synthesized and characterized pterostilbene-enriched nanoemulsions with olive oil or flaxseed oil. They demonstrated that the developed nanoemulsions significantly enhanced bioaccessibility of pterostilbene after digestion and the nanoemulsions increased transenterocyte transport of pterostilbene after digestion.

Association colloids are colloidal systems comprising of polar and nonpolar parts, in which the particle sizes are smaller than that in colloids. The particle size in association colloids ranges between 5 and 100 nm. It includes vesicles, surfactant micelles, reverse micelles, bilayers, liquid crystals, and so on. Association colloid systems are spontaneously formed thermodynamically favorable systems and are transparent solutions. These are used as delivery agents for lipophilic components by entrapping within hydrophobic interiors. Examples include the encapsulation of lycopene, limonene, lutein, omega-3 fatty acids, and DHA/EPA into canola oil for cooking.

Biopolymeric nanoparticles are biodegradable nanoparticles that form stable nanostructures by spontaneous self-assembly. Biopolymer is converted into smaller nanoparticles either by aggregative (net attraction) or segregative (net repulsion) interactions. These nanoparticles are appropriate for encapsulating and delivering lipophilic nutraceuticals and are usually prepared by antisolvent precipitation method. Biopolymers such as zein, chitosan, casein, polylactic acid, and poly-3-hydroxybutyrate are good examples for biopolymeric nanoparticles for encapsulation. The stability of biopolymeric nanoparticles are enhanced by the addition of emulsifiers or thickening agents to the aqueous phase.

2.3.2 Nanolaminates

Nanotechnology offers excellent methods for food scientists to develop nanolaminates suitable for application in the food industry. A nanolaminate is thin food-grade film that comprises two or more layers of materials with dimensions in nanometer scales, which are physically or chemically bonded to each other. A layer-by-layer deposition technique is employed to coat a charged

surface with interfacial film with multiple nanolayers of different materials. This method is used for the preparation of edible coatings or films over conventional casting technique. Several adsorbing materials such as natural polyelectrolytes (polysaccharides, proteins), colloidal particles (micelles, vesicles, droplets) and charged lipids (phospholipids, surfactants) are used for the fabrication of nanolaminates. The thickness and properties of the multilayered nanolaminate can be controlled by several methods such as changing the adsorbing material in the dipping solutions, the number of dipping steps, the order of the material introduced into the dipping solutions, the solution and physicochemical conditions (pH, ionic strength, dielectric constant, temperature, etc.). The adsorption to the surface basically depends also on the nature of the surface and adsorbing material and the possible interactions could be electrostatic, hydrophobic interactive, hydrogen-bonding, thermodynamically incompatible, and so on. Owing to their fragile nature nanolaminates are often used as coatings that are attached to the surface of food. Edible films are used on a wide variety of foods, including fruits, vegetables, meats, chocolate, candies, bakery products, and French fries (Morillon et al., 2002; Cagri et al., 2004). These films serve as barriers for moisture, lipids, and gas. In addition to functioning as a barrier, nanolaminates also improves the texture, color, flavor, nutrients, and antimicrobials. Nanotechnology principles can be used to develop invisible nanolaminate incorporated with value added nutrients with health promoting components, sweetness, various flavors, antimicrobial agents, and so on.

2.3.3 Nanofibers

The use of nanofibers has garnered considerable interest in the food industry for their exploitation as outstanding packaging materials, encapsulating functional ingredients, processing aids, and food quality and safety sensors. Electrospinning is another nanofabrication technique, which is used to develop nanofibers with diameters below 100 nm from polymer solutions especially biopolymers. Nanofibers can be developed by various processing techniques such as chemical vapor deposition, phase separation, self-assembly, drawing, the sol–gel method, template synthesis, thermal oxidation, and electrospinning. Among these techniques, electrospinning has demonstrated to be simple and versatile method (Subbiah et al., 2005; Zhang et al., 2005; Zhao et al., 2011; Lala et al., 2007). Electrospun nanofibers find application in the food industry when the components are composed of edible polymers and GRAS ingredients. Nanofibers can be fabricated with functional ingredients (nutraceuticals, antioxidants,

antimicrobials, and flavors), active packaging materials (edible) or as processing aids (catalytic reactors, membranes, filters) and sensors. In the food industry, rather than as a food component for ingestion, nanofibers play a significant role in novel bioactive packaging strategies. Multiple functionalities can also been assigned to nanofibers by exploiting the encapsulation potential with various active ingredients. The ease of fabrication, high-surface area, the versatility with respect to its composition and nonthermal processing makes electrospun nanofibers an attractive nanodelivery mode for encapsulation as well as controlled release of nutraceuticals. The ability of electrospun nanofibers to encapsulate proteins without compromising its functionalities, the fabrication of multiphase nanofibers either from suspension or emulsion by encapsulating hydrophilic active ingredients into biodegradable hydrophobic biopolymer from emulsion electrospinning or fabrication of nanofibers from hydrophilic polymers via microemulsion with stabilized lipophilic active ingredient, encapsulation of multiple nutraceuticals within single nanofiber, fabrication of nanofibers encapsulated with nanoparticles, the fabrication of core shell nanofibers by coaxial electrospinning set up or by controlling polymer phase separation and also the potential to encapsulate probiotics opens various applications of electrospun nanofibers in the food industry. Electrospun nanofibers developed from edible biopolymers (polysaccharides, proteins) find application in encapsulating nutraceuticals and also in packaging materials. Reports on zein nanofibers incorporating nutraceuticals (curcumin) have demonstrated the release and its in vitro applications (Brahatheeswaran et al., 2012). Another area of interest is the encapsulation of vitamins. It has been reported that vitamin E and C derivatives have been incorporated into electrospun nanofibers by coaxial method for sustained release. Thus, electrospun nanofibers are promising nanodelivery agents for various nutraceutical components without compromising its activity.

3 Function of Nanoscience and Technology for Nutraceuticals

Nanoformulations for the delivery of nutraceuticals are basically established on the principles of nanotechnology. There has been great interest in the generation of nanoscale delivery systems for the efficient encapsulation, protection, and delivery of bioactive lipophilic nutrients, vitamins, and nutraceuticals. The significance of various nutraceuticals based on nanoscale materials is

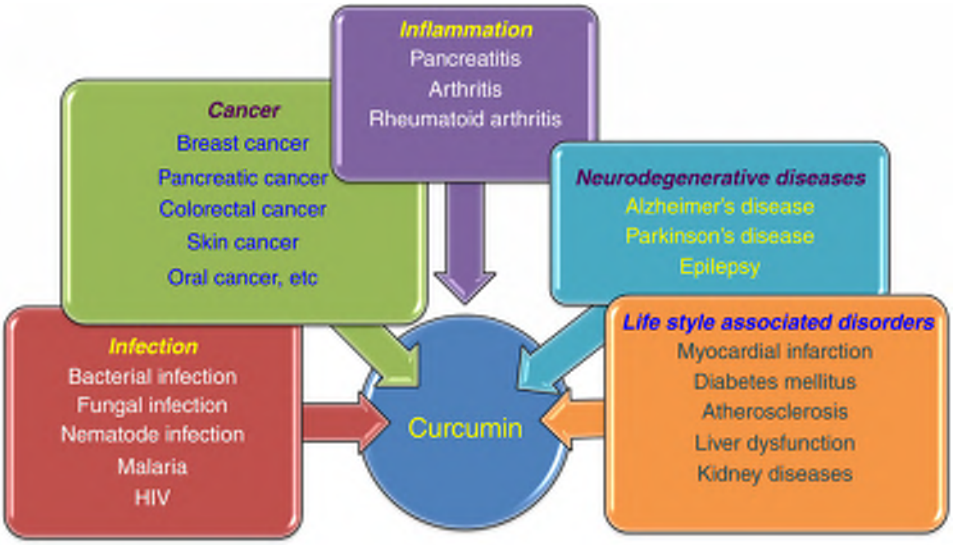

Figure 10.11. Application of curcumin in various clinical disorders.

discussed briefly. Curcumin has been investigated for a variety of disorders owing to its functional properties (Fig. 10.11).

Curcumin in its native form is hydrophobic and hence the bioavailability is extremely low. The nanoformulations developed for curcumin has enhanced the biological activity and its bioavailability. In cancer therapy, it has been demonstrated that at molecular level, curcumin inhibits cell proliferation and induces apoptosis by regulating several pathways. Several studies have demonstrate the successful encapsulation of curcumin in PLGA and was targeted with different moieties including aptamer, transferrin, HER2 for various cancer cells. In some studies magnetic nanoparticles were also encapsulated along with curcumin in the nanocarrier. The synergistic action of curcumin and magnetic hyperthermia enabled the destruction of cancer cells in a rapid fashion than by using curcumin alone in the nanocarrier. The nanoformulations with curcumin showed promising results under in vivo conditions. The sustained release of curcumin from the PLGA-PEG-PLGA micelles resulted in improved pharmacokinetic parameters was reported by Ghahremankhani et al. (2008). The biodistribution analysis of nanoformulation demonstrated higher concentrations of curcumin in brain, lung, and kidney and lower concentrations in spleen and liver than free curcumin. In another study, curcumin was encapsulated into glycerol monooleate based nanoparticles and demonstrated 1000-fold higher

peak concentration when compared to free curcumin after intravenous injection in mice (Mohanty and Sahoo, 2010). Curcumin encapsulated MPEG-PCL polymeric micelles inhibited angiogenesis in a transgenic zebrafish model and it was assessed for its effectiveness in tumor-bearing mice. Intravenous administration of nanoformulation inhibited the subcutaneous LL/2 pulmonary carcinoma in mice than free curcumin (Gong et al., 2013). Babaei et al. (2012) developed dendrosomal nanoformulation of curcumin and it was intraperitoneally injected to tumor-bearing mice. In addition to the significant reduction of tumor, survival rate of mice that received dendrosomal curcumin formulation was prolonged when compared to mice that were administered with free curcumin. NanoCur (curcumin loaded PLGA nanoparticles) was developed by Bisht et al. (2007) and weekly oral administration of for 16 weeks (dose 20 mg curcumin/kg) induced apoptosis in hepatocellular carcinoma (HCC) rats. It provided anticancer effects by preventing the generation of mitochondrial ROS, reducing the level of antioxidant enzyme including superoxide dismutase, catalase, reduced glutathione in hepatic tissues. Histological examination of liver tissues demonstrated the reduction in the formation of hyperplastic nodules and atypical nuclei in the rats treated with nanoformulation than free curcumin. Curcumin-loaded lipid nanocapsules was developed for the treatment of gliomas (Filho et al., 2013). It was demonstrated that the intraperitoneal administration of nanoformulation reduced tumor growth and also the occurrence of necrosis, intratumoral hemorrhages, and lymphocytic infiltration than intraperitoneal administration of a higher dose of free curcumin dissolved in DMSO.

Polyphenols are another category of nutraceuticals that has several functional properties such as scavenging radical oxygen species, complexing properties toward proteins, antiinflammatory, anticancerous. The short half-life, sensitivity to light and heat, lipophilic nature, astringent and bitter taste, low bioavailability limits their application in oral medications. To avoid these disadvantages, nanodelivery systems have been developed for encapsulating polyphenols (Barras et al., 2009). Smith et al. (2010) has promising preclinical results with the use of green tea polyphenol, (−)-epigallocatechin-3-gallate (EGCG) in mouse models with neurodegenerative diseases including Alzheimer's disease (AD) and HIV-associated dementia (HAD). Epigallocatechin gallate (EGCG) immobilized lipid-coated nanoparticles retained 90% of its capability to stimulate the α-secretase in vitro (Smith et al., 2010). The bioavailability of EGCC was increased (twofold) after encapsulation when compared to that of the free EGCC under in vivo studies. The EGCC encapsulated PLGA nanoparticles was

developed and the in vivo antioxidant efficacy of the nanoformulation was evaluated in a rat model with chronic nephrotoxicity. Results suggest that both the intraperitoneal and oral administration of nanoformulation was found to be efficacious in reducing the nephrotoxicity than orally administered EGCG solution. Shutava et al. (2009) reported layer by layer (LBL) technique to develop EGCC nanoparticles of 200 nm. The researchers employed a soft gel-like inner core with or without a surrounding LbL shell of polyelectrolytes with two different combinations of polyanion/polycation pairs (polystyrene sulfonate/polyallylamine hydrochloride, and polyglutamic acid/poly-L-lysine), and polyanion/protein pairs (dextran sulfate/protamine sulfate, carboxymethyl cellulose/gelatin, type A). These nanoparticles preserved its biological activity and blocked intracellular signaling (hepatocyte growth factor induced) in the breast cancer cell line. Recently nanoencapsulation of white tea extract into polymeric nanoparticles based on poly(ε-caprolactone) and alginate was demonstrated by Sanna et al. (2015). The nanoformulation with white tea extract retained its antioxidant activity and nanoparticles protected tea polyphenols from degradation.

Resveratrol is another natural polyphenolic compound present in various food sources (grapes, red wine, peanuts, etc.). It has several biological properties to induce cellular responses such as arresting cell cycle, differentiation, inducing apoptosis, and to enhance the antiproliferation of cancer cells. The nanoformulation of resveratrol is a promising approach for retaining its biological function. Lu et al. (2009) developed nanoformulation with polycaprolactone (PCL) constitutes the hydrophobic core and poly(ethylene glycol) (PEG) is the hydrophilic shell of micelles encapsulating resveratrol. These resveratrol-loaded nanoparticles demonstrated its antioxidant potential in cancer cells. Neves et al. (2013) have developed two novel resveratrol nanodelivery systems based on lipid nanoparticles (solid lipid nanoparticles and nanostructured lipid carriers) to enhance the oral bioavailability of resveratrol as nutraceuticals. The in vitro release studies upon storage revealed a negligible resveratrol release over several hours for both nanosystems. The in vitro simulation of gastrointestinal transit demonstrated that resveratrol remained mostly with the lipid nanoparticles after their incubation in digestive fluids. In another study, solid lipid nanoparticles and nanostructured lipid carriers with resveratrol were used for dermal applications. Both of the lipid nanoparticles had antioxidant properties and resveratrol was determined rat abdominal skin and ex vivo skin studies suggested that nanostructured lipid carriers are more efficient in carrying resveratrol to the epidermis (Gokce et al., 2012). Carboxymethyl chitosan nanoparticles was

used to prepare resveratrol loaded carboxymethyl chitosan. The nanoparticles improved the solubility and exhibited more antioxidant potential than the free resveratrol. Nanoformulation exhibited enhanced in vivo absorption, extended duration of action and increased bioavailability by 3.516 times more than when compared with the raw resveratrol (Zu et al., 2014).

4 Conclusions

There has been significant advancement in the field of delivery of nutraceuticals based on nanotechnology. Nanotechnology based formulations are designed to enhance the bioavailability of lipophilic components. The structure, size, and composition of nanodelivery systems can be efficiently tailored to control their properties such as improved bioavailability, dispersibility, chemical stability, controlled and sustained release. Although nanocarrier is utilized to deliver the active ingredient, the fate of the nanocarrier used for the encapsulation of nutraceuticals is unclear at present situation. Hence effect of carrier agent both the short-term and long-term effects has to be established. Because the properties of nanoregime is entirely different from that of the bulk material, when considering the biological aspects, the biological processes such as absorption, digestion, metabolism, and elimination of nanonutraceuticals in the body may differ differently. More research are highly recommended to examine the toxicity aspects of nanomaterials, specially while considering the size of nanoparticles, some chemical materials in larger size may function as more safer than when the same are in the nanoscale. In addition to these the commercial feasibility of the design and development of nanodelivery systems should also be addressed.

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11

NUTRACEUTICAL ASPECTS OF β-GLUCAN WITH APPLICATION IN FOOD PRODUCTS

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1 Introduction

One seeded dry fruit produced by cereals is known as caryopsis, mostly referred to as kernel or grain. Nutritional constituents obtained from these grains include carbohydrates, proteins, lipids, vitamins, and minerals, and so forth (Evers and Millar, 2002). Among carbohydrates there are two fractions: digestible starch and nondigestible dietary fiber, but it may be partially or completely hydrolyzed by intestinal micro flora. Dietary fiber is generally classified into two groups, water-soluble dietary fiber that dissolves in water and has many physiological benefits and water-insoluble fiber that does not dissolves in water and mainly acts as a bulking agent. Nonstarch polysaccharides, celluloses, arabinoxylans are some examples of dietary fiber but, β-glucan is one of the most important types of dietary fiber (Wood and Webster, 1986), which is found specifically in barley, oats, and some types of mushrooms (Brennan and Cleary, 2005). There is a long history of defining dietary fiber. In earlier eras it has been defined variably by different researchers. Details of these definitions from the last three decades are presented in Table 11.1.

Cereal β-glucan is a polysaccharide (nonstarch) that is found in the cell wall of cereal kernels or caryopsis of oats, barley, and other cereals (Buckeridge et al., 2004; Izydorczyk et al., 2003). Dehulled barley contains 3–7% β-glucan (Lee et al., 1997). Barley endosperm is a rich source in which 75% of the β-glucan is concentrated in cell wall while the remaining 25% β-glucan resides in the aleurone layer (Vasanthan et al., 2002). The amount of β-glucan

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Table 11.1 Developments in Dietary Fiber from Last Three Decades

Sr. No.	Date/Year	Definition/Development	References
1	1985	Methods that were developed in the past disappointed some researchers; method with enzymatic gravimetric was modified and this method was adopted by the Association of Official Analytical Chemists, as an official method of analysis named "AOAC Official method 985.29." In the same year this method was also adopted by AACC under approved method 32-05	Prosky et al. (1985)
2	1991	Official method AOAC 991.42 was adopted for insoluble dietary fiber in food and food products—phosphate buffer, enzymatic gravimetric method	DeVries et al. (1999)
3	1993	AOAC method no. 993.16 was adopted for soluble dietary fiber in food and food products.	DeVries et al. (1999)
4	2001	AACC reported that edible plant parts and similar carbohydrates that possess resistance to absorption and digestion in small intestine but may be partially or completely fermented in the large intestine are referred to as dietary fiber.	Ahmad et al. (2012a,b)
5	2002	According to National Academy of Sciences total fiber is the whole or aggregate functional fiber and dietary fiber, dietary fiber is a complex of nondigestible carbohydrate and lignin obtained from plant while functional fiber is the part of nondigestible carbohydrate that have beneficial physiological effects on humans.	Tungland and Meyer (2002)
6	2003	WHO added the concept of intrinsic and added fiber in the definition of dietary fiber.	Ahmad et al. (2012a)
7	2006	A comprehensive definition was proposed by Codex Alimentarius Commission and Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU). According to this definition carbohydrates polymers having at least about three degrees of polymerization, deprived of ability to be digested or absorbed by small intestine are referred as dietary fiber.	Ahmad et al. (2012a)
8	2008	In Rome, Italy, CAC adopted a new definition that was provisioned by CCNFSDU. The new definition is consistent with previous definition as fiber but has three categories: naturally present carbohydrate polymers; carbohydrate polymers that are obtained from food material by enzymatic, physical, or chemical means; and carbohydrate polymers that are synthetic in nature. But these carbohydrates polymers must have at least three degree of polymerization.	Turner and Lupton (2011)

varies in whole grain rye ranges between 1.3% and 2.2% (Hansen et al., 2003). The naked or dehulled oats contain β-glucan extending from 3% to 7% (Peterson, 1991), while in wheat it is less than 0.5%. Demirbas (2005) reported β-glucan content of cereal crops grown in Turkey. Oat and barley grains appeared as a major source of β-glucan with 3.9–5.7% and 3.2–3.6% β-glucan content respectively (Demirbas, 2005), of which the major content are found in oat caryopsis around the starch layer, which is about 60% (Bhatty, 1992) while the amount of dietary fiber ranges from 60 to 90 g/kg (Mälkki et al., 2001) having 3.0–5.4% soluble fiber (Welch, 1995a) while the main component of soluble fiber is β-glucan, varies from 1.8 to 7.5% of kernels (Bhatty, 1992; Welch, 1995a). Other cereal crops grown in Turkey include rice (0.4–0.9%), millet (0.5–0.1%), maize (0.5–1.3%), spring wheat (0.6–1.1%), rye (0.7–1.5%), which also contain appreciable amounts of β-glucan (Demirbas, 2005). β-Glucan levels in these cereals are regulated by multiple genes interconnected by expression networks, and thus effectively control the metabolism and biosynthesis of β-glucan in these cereal grains (Islamovic et al., 2013)

Some fungi are also good source of β-glucan especially in edible mushrooms as focused by most researchers. In mushrooms the content of β-glucan varies from 0.21 to 0.53 g per 100 g on dry basis (Manzi and Pizzoferrato, 2000). The most prominent source of β-glucan mushrooms include *Pleurotustuberregium* (Zhang et al., 2004), *Agaricusbrasiliensis* (Mizuno et al., 2003), *Grifolafrondosa* (Kodama et al., 2003), *Lentinusedodes* (Chihara, 1993), *Pleurotusostreatoroseus* and *Pleurotuseryngii* (Carbonero et al., 2006), *Pleurotusostreatus*, *Pleurotuseryngii*, *Pleurotuspulmunarius*, *Pleurotusostreatus* (Manzi and Pizzoferrato, 2000). Fungi which are not edible, while having good amount of β-glucan, include *Penicilliumchrysongenum* (Wang et al., 2002), *Termitomyceseurhizus* (Chakraborty et al., 2006). *Sacchromycescerevisiae* yeast is the main source of β-glucan, while other sources include *Zaygsaccharomycesbailii*, *Debaryomyceshansenni*, *Kluyveromycesmarxians*, *Schizosaccharomycespombe,* and *Kluyveromycesmarxianus* (Nguyen et al., 1998). Bacterial source of β-glucan includes Agrobacterium sp. 10C3 and its derivatives, *C. flavigena*, *S. pneumonia* (McIntosh et al., 2005).

Chemically, cereal β-glucan are straight chain homo polysaccharides consisting of glucosyl units connected with (1→4)-β-D-linkage and separated mostly by single unit with (1→3) linkage as shown in Fig. 11.1 (Lazaridou and Biliaderis, 2007). While β-glucan extracted from baker's yeast has different linkage pattern that is, it consists of β-(1→3) along with (1→6) linkage, as shown in Fig. 11.2 (Ahmad et al., 2012). In cereal β-glucan linkage (1→4) is present

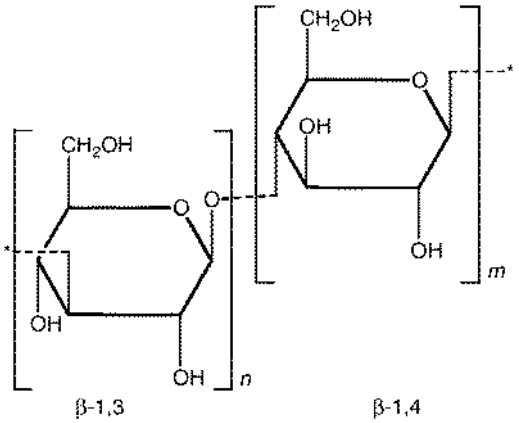

Figure 11.1. Cereal β-glucan structure (*m* = 2 − 4, *n* = 1).

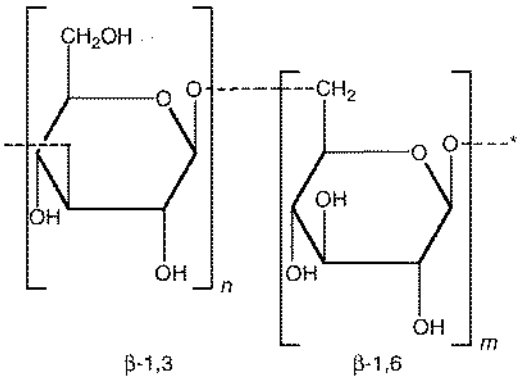

Figure 11.2. Yeast β-glucan structure (*n* = 2 − 4, *m* = 1).

in groups of two to four while linkage (1→3) is present singly. This resulted in a structure that is dominated by linkage β-(1→3) cellotriosyl and cellotetraosyl units, while the remaining structure is made up of longer blocks of (1→4) linked 4–15 units of glucopyranosyl units (Ahmad et al., 2012; Wood et al., 1994). In cereals β-(1→3) cellotriosyl is present about 58–72% while cellotetraosyl is 20–34%. Lichenan is a β-glucan obtained from *Cetrariaislandica,* have structure resembling that of cereal β-glucan, but it is composed of cellotriosyl (78%), cellotetraosyl 4%, and 18% cellulose-like segments (Lazaridou et al., 2004). In recent years, NMR and Raman spectroscopy more clearly elucidated the ratio of 1→4 to 1→3 linkages. It is established that conventional barley may have 70.8:29.2% ratio of 1→4 to 1→3 linkages, while mutant barley have 69.5:30.5% 1→4 to 1→3 linkages. Oats contain slightly lower

1→3 linkages with a ratio of 71.3:28.7% for 1→4 to 1→3 linkages (Mikkelsen et al., 2013) Bacterial β-glucan are of three types, linear chain (1→3), branched side chain (1→3, 1→2) which are main components of capsule and cyclic (1→3, 1→6) linked, found in periplasm and helps to regulate osmotic balance. Curdlan is a linear chain (1→3) β-glucan that can be obtained from agrobacterium (McIntosh et al., 2005). β-Glucans have potential application in medicine and pharmacy, food products, cosmetics, and chemical industries, in veterinary medicine, and in feed production (Du et al., 2014).

A lot of health benefits are associated with the intake of β-glucan, including effect on postprandial blood glucose and insulin level, lowering blood cholesterol level, especially LDL- and total serum cholesterol, antitumor activity of β-glucan and modulation of host immune system against bacterial, fungal, and viral infections. This necessities the need that β-glucan must be included in the diet to promote public health because consumer are becoming more conscious about their health due to increase in obesity, coronary heart diseases, diabetes and so forth, so industrialists can reap the benefits because β-glucan have not only nutritional but also possess several physiochemical and rheological properties as it can contribute good functional properties to the end products like bread, pasta, low-fat yogurt, sausages, mayonnaise, functional drinks, and so forth.

2 β-Glucan Extraction

Dry separation and wet separation are the two major methods used for extraction of β-glucan especially from cereals. Advantage of dry separation is that it does not include the solvent for extraction and disadvantage is that yield from this method is quite low and mostly less than 30% (Zheng et al., 2000). Wood et al. (1989a) first time used the wet method for extraction β-glucan and since that it has been the popular method by many food researchers because of high yield that usually ranges from 30 to 70% (Benito-Román et al., 2011). The extraction method could affect the molecular structure of extracted β-glucan significantly (Bhatty, 1993). β-Glucan in barley and oats along with other nonstarch polysaccharide is found in the endosperm cells that cover the starch, lipid, and protein reserves of the grain that makes the recovery of β-glucan difficult (Tosh et al., 2003). For studying β-glucan physiochemical properties necessitates the procedure to be followed that optimizes purity, yield, and retains the β-glucan molecule integrity. Similarly the β-glucan molecular weight profile is also influenced by the method of extraction (Tosh et al., 2003; Wang

et al., 2003). The extraction method could affect the molecular structure, essentially. The enzymes such as grain's endogenous β-glucanases in the aqueous system, along with shear-induced molecular fragmentation that occurs during mixing and centrifugation steps, can greatly affect the structure of β-glucan. Especially the soluble β-glucan is more affected by such degradation result in reduction of molecular weight and viscosity, which in turn minimizes its effect on cholesterol lowering and glucose attenuating (Bhatty, 1993). This is because β-glucan's cholesterol-lowering and glucose-attenuating effects are mainly attributed to its viscosity, molecular weight, and solubility (Wood, 2007). Its extraction from cereals involves three steps: endogenous enzyme inactivation, β-glucan extraction and β-glucan precipitation. Endogenous β-glucanases must be inactivated, because these can degrade β-glucan, resulting in lower molecular weight and altered functional properties of β-glucan (Irakli et al., 2004). These enzymes are inactivated by treating barley flour with dilute aqueous ethanol above 60°C. If extraction is done above 60°C (gelatinization temperature) starch is also coextracted, so starch must be removed from extract (Wood et al., 1977). Wood developed key procedure for extraction of β-glucan from oats and barley. The effects of particle size, pH, ionic strength, and temperature on yield of β-glucan on the laboratory scale was studied by researcher and on a pilot plant scale oat-bran enzymes were inactivated by 75% ethanol along with sodium carbonate solution at pH 10 to obtain a preparation having 78% β-glucan (Wood et al., 1989b). Although β-glucan was successfully extracted from cereal by this method, but McCleary showed that extraction rate of barley β-glucan has increased to 90% and yield by sequential water extraction at 40, 65, and 95°C (Brennan and Cleary, 2005). Another study shows that enzymatic extraction results in higher yield, as compared to acidic and alkaline extraction, but in this study protein was found to be major impurity (Ahmad et al., 2010). Similarly another study shows that addition of enzymes in the extraction process increases the viscosity of dietary fiber by facilitating β-glucan releasing from food matrix (Gamel et al., 2014).

Response surface methodology is a useful statistical tool that can be used for optimization of extraction conditions for yield and other parameters of β-glucan (Ahmad et al., 2009). In a recent research work, yield and molecular weight were optimized from Irish barley using response surface methodology. Maximum extraction of β-glucan can be achieved with solid to liquid ratio of 1:5 and extraction time of 4 h. During this process extraction at pH 6.6 by keeping the medium at 55.7°C can increase the yield to maximum level (Gangopadhyay et al., 2015).

3 Rheology of β-Glucan

The viscosity of β-glucan interferes in the brewing process of barley (Bamforth, 1985) and limits the feed value for poultry (Campbell and Bedford, 1992). Due to high viscosity of β-glucan, oat gum which consists of 70–80% β-glucan can be used as a thickening agent commercially in food formulations (Doublier and Wood, 1995). At low concentration oat β-glucan forms highly viscous solutions. Hence the viscosity of solution is highly dependent on molecular weight and concentration of β-glucan. Less than 0.2% concentration of β-glucan shows Newtonian solution properties (Autio et al., 1987; Doublier and Wood, 1995). High molecular weight β-glucan at concentration above than 0.2% shows pseudoplastic behavior and this property increases with the increase of concentration (Autio et al., 1987). β-Glucan having low molecular weight forms soft gels at higher concentration while β-glucan having high molecular weight forms pseudoplastic and viscous solutions (Doublier and Wood, 1995).

The most commonly used term for dietary fiber is *apparent viscosity* and is defined by viscosity of non-Newtonian fluid represented as Newtonian fluid (Bourne, 2002) and may be calculated as coefficient from empirical data. As if the fluid being measured shows Newtonian flow and follow Newton's Law, representing a single viscosity value at constant shear rate (McDonald et al., 2001). Less than 0.2% concentration of β-glucan shows a Newtonian solution behavior in which viscosity is not affected by increasing the rate of shear. Above 0.2% concentration the molecules (high molecular weight) of β-glucan start to entangle, resulting in viscous and pseudoplastic solutions (Wood et al., 1994).

The apparent viscosity of β-glucan solutions as a functions of time, temperature, and stirring speed can be measured by Rapid Visco Analyzer (RVA) which is rapid, simple, and reliable measurement, requiring a small amount of sample and providing results in graphical form that shows how the viscosity of solution changes with time. Freshly prepared solution of β-glucan shows high flow viscosity at low concentrations (Yokoyama et al., 2002). While β-glucan having low molecular weight forms soft gel at relatively higher concentrations. Generally β-glucan solution viscosity depends upon molecular weight, structural feature and concentration of the β-glucan (Wood et al., 1994). A big dependency between steady shear flow curves of β-glucan aqueous solutions and molecular size exists that serves a valuable tool for detecting molecular weight differences. Apparent viscosity falls sharply with increasing shear thinning rates, whereas as endogenous

β-glucanases tend to reduce viscosities of β-glucan solution to exhibit almost Newtonian behavior (Lazaridou et al., 2014a).

4 β-Glucan Physiological Effects in Humans

Seeking the importance of β-glucan, the Food and Drug Administration Authority (FDA) allowed it to be used in food products and made it mandatory for labeling requirements to claim health benefits. FDA also recommended that diet having high soluble fiber from whole oats may be used to reduce coronary heart diseases. Consumption of β-glucan leads to reduction of plasma cholesterol level and can be used to control postprandial glucose level in humans as well as animal models (Bhatty, 1999). Animal model studies on rats exhibited that consumption of β-glucan from barley and oats had a significant effect on total cholesterol while comparing with control animals (Fig. 11.3); this reduction in cholesterol was statistically at par with pectin (Ahmad and Anjum, 2010). This was attributed to the presence of soluble fiber that reduces the cholesterol level by a feedback mechanism of bile acid in liver (Jenkins et al., 2002). Several clinical investigations have shown that oat-based product consumption lowers serum cholesterol level, reduces uptake of glucose, control weight, and decreases insulin response in plasma after prolonged satiety (Mälkki et al., 2001; Wood, 2007). These physiological effects of oat-based product are related to increase in viscosity of gastrointestinal tract (Wood, 2007), which in turn is related to β-glucan. The high luminous viscosity increases the amount of bile acid excreted in feces because it lowers the reabsorption of bile acid in the ileum

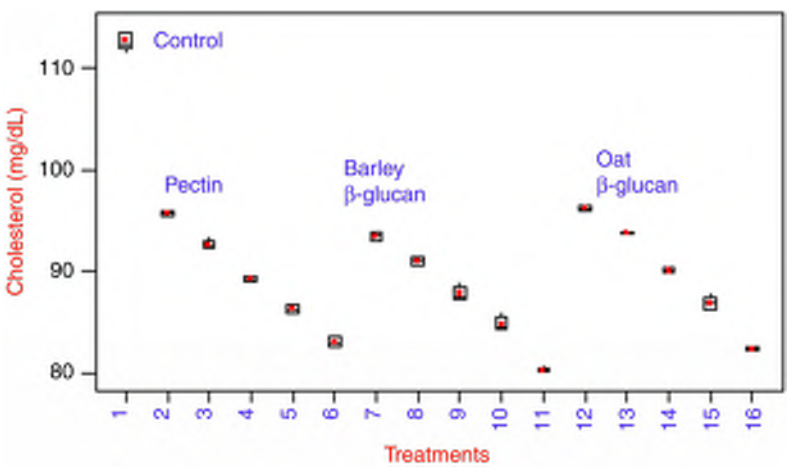

Figure 11.3. Effect of β-glucan on cholesterol.

(Drzikova et al., 2005). For studying whether β-glucan has blood cholesterol lowering properties or some other group these properties, it is necessary to check effect of β-glucan isolate. It is believed that in the gastrointestinal tract, increased luminal viscosity is a key factor responsible for less absorption of sugars, cholesterol, and absorption, and re-absorption of bile acid (BA). Delayed absorption of sugars is often attributed to formation of unstirred layer nearby to the mucosa, which act as a physical barrier for bile acid reabsorption and other nutrient absorption (Schneeman, 1987; Würsch and Pi-Sunyer, 1997). The removal of bile acid from enteroheptic circulation resulted in increased synthesis of bile acid, which in turn is related to higher amount of serum cholesterol conversion into the bile acid and thus lowers the level of serum cholesterol (LaRusso, 1983). Similar lowering effect for triglycerides in animal model (rats 7 weeks old) was noticed through consumption of β-glucan. Consumption of β-glucan at higher levels (>1%) significantly reduces the triglyceride level over control group of animals; this reduction in triglyceride was significantly at part same level of pectin consumption. A trend of triglyceride reduction is shown in Fig. 11.4 (Ahmad and Anjum, 2010). Thus β-glucan reduces the incidence of coronary heart diseases more efficiently because β- glucan lowers both total serum cholesterol and serum triglyceride level. A study on Japanese men and women shows that even low levels of total serum cholesterol and presence of serum triglycerides are associated with coronary heart diseases (Iso et al., 2001).

Anderson and colleagues show for the first time the cholesterol lowering effect of oats, indicated that oat bran in hypercholesterolemia lowers the total serum cholesterol about 23% without

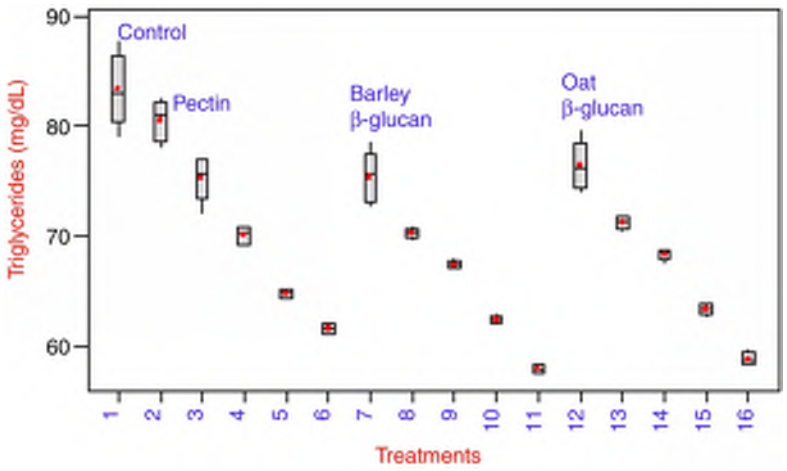

Figure 11.4. Effect of β-glucan on triglycerides.

effecting High Density Lipoprotein (HDL) cholesterol, due to its enrichment in β-glucan (Anderson et al., 1984; Anderson et al., 1990; Anderson et al., 1991). There is inverse correlation between dose of β-glucan, serum cholesterol level, and HDL (Davidson et al., 1991) but this relation was established for eating oatmeal and oat bran. In a study, 6 g of concentrated oat β-glucan taken per day for 6 weeks significantly reduced the total cholesterol and low-density lipoprotein (LDL) in hypocholestrolemic humans in comparison to control groups and thus a practical dose of β-glucan improve colonal health and reduce serum lipids in high-risk populations (Queenan et al., 2007).

In multigastric animals, cereal β-glucans are digested in the upper GI tract and ileum part of small intestine. Digestibility in these animals is dependent on particle size, feed matrix, source of β-glucan, and diet composition (Knudsen et al., 1993). During the digestion process, molecular weight of these glucan molecules often depolymerized during digestion in animal models. This depolymerization mainly occurs in upper GI tract and in the small intestine. Thus, before reaching in the large intestine for the fermentation, its Mw has already been reduced into less than 100 kDa and thus facilitate the action of microbiota in large intestine (Holtekjølen et al., 2014).

5 β-Glucan Immune Modulating Effect

Hot-water extract of tree fungi and mushrooms has been used for centuries in China, Japan, and central Russia as folk remedies. Now they are used for specific therapies and as a stimulator of general health (Kogan, 2000). *Saccharomyces cerevisiae* cell wall crude extract, zymosan was investigated for its drug-like properties during the 1940s in Europe. This zymosan has been shown to modulate immune system and the virucidal and bactericidal activity of serum increased after the administration of zymosan (Fizpatrick and DiCarlo, 1964; Pillemer and Ross, 1955). Since that time there are a number of patents and papers that describe the properties of β-glucan obtained from yeasts and plants. β-Glucan are members of classes of drugs that are known as "biological response modifiers" which helps in the modification of host biological response by stimulating the immune system (Zekovic et al., 2005).

β-glucan immune-pharmacological activities include development of resistance against infection of virus, bacteria, and fungi (Bohn and BeMiller, 1995). The protective effect of β-glucan has been described by nonspecific immunomodulation by using different immune pathways like T-cell stimulation, activation of

macrophage, activation of reticulo-endothelial system, natural killer (NK) cells activation, and higher amounts of production of antibodies. Of these, macrophages are the best-known targets of β-glucan (Cozens et al., 1981). Similarly in CR3primed state is induced by β-glucan that triggers killing of iC3b target cells, which were resistant to cytotoxicity (Vetvicka et al., 1996). Glucan activates macrophages to increase their number and size and thus stimulates lysozyme secretion and tumor necrosis factor, resulting in increased phagocytosis of antigens (Meira et al., 1996). The oat β-glucan provides a high level of strength against bacterial and parasitic infections. This is attributed to cellular and antigen specific humoral immunity (Daou and Zhang, 2012).

Yeast and fungi β-glucan contain 1→3 backbone, having side branches linked through 1→6 bond. This chemical structure is very useful for immune-modulating effect of β-glucan. This immune-modulatory effect has useful applications for cancer patients in which they boost the immune system of patients and reduce the side effects of chemotherapy (Novak and Vetvicka, 2008). In cancer patients β-glucan improves the capacity of immune cells by releasing of cytokines, which in turn transfer signals to other immune cells. Thus a flow of transduction of information system starts that stimulates the whole immune system to respond against unwanted cell growth. This feature of β-glucan is effective in treatment of cancerous growth and malignant tumor (Chan et al., 2009). β-Glucan through binding with macrophage, natural killer cells, and neutrophils produces innate and acquired immune response. In T-lymphocyte system, β-glucan interacts with natural killer cell resulting in cytokine production further leads to lysis of infected cells and triggers innate immune response through macrophage (Brown, 2005).

β-Glucan ingestion trigger human monocytes as well, which stimulates the flow of reactions that give rise to Necrosis Factor-a (TNF-a) and interleukin-1B by a process that is dependent on trypsin sensitive β-glucan receptors (Abel and Czop, 1992). These reactions start with the binding of β-glucan with specific proteins. Fibronectin and vitronectin are two important glucan binding proteins. Complex formed by β-glucan and these proteins results in the release of macrophage cytokines (Olson et al., 1996; Vassallo et al., 2001). β-Glucan can be used in the control of several fungal diseases. Paracoccidioidomycosis is a fungal disease largely spread in Latin America, can be controlled by IL-12 which production is primed by consumption of β-glucan (Pelizon et al., 2005).

Many studies have shown that exhaustive exercise suppresses immune system and increases the chances of infection development. Innate immune system components are compromised during single or repeated stress from exercise (Davis et al., 1997;

Nieman et al., 1998). For example, antiviral alveolar macrophage resistance to herpes simplex virus (HSV) decreased in mice after fatigue due to exercise (Davis, 1997). A study shows that exercise stress is associated with increased morbidity and mortality along with decrease in macrophage antiviral resistance. Consumption of oat-based diet containing β-glucan reduces morbidity and mortality along with increase in macrophage antiviral resistance in rats. No cytotoxicity was observed for NK cells with the ingestion of oat β-glucan (Davis et al., 2004).

6 Skin Health Promotion

6.1 Wound Healing

Wound healing is an intricate process in which the skin (or another organ tissue) repairs itself after injury (Nguyen et al., 2009). Wound healing process consists of three phases including inflammatory phase, proliferative phase, and remodeling phase (Stadelmann et al., 1998). Macrophages played important role in healing of wounds of diabetic mice and trauma patients (Du et al., 2014). Macrophages play a role in healing of wounds either by phagocytizing of the bacteria and damaged tissue or by debriding damaged tissue with help of released proteases (Deodhar and Rana, 1997). β-Glucans improved the wound healing by improving its transportation to the damaged tissue (Browder et al., 1988). β-D-glucans from *Ganoderma lucidum* were reported as potent stimulators of murine and human macrophages in vitro and in vivo (Han et al., 1998). Inflammation is the first biological response of the immune system to infection or irritation (Debnath et al., 2013). There is no clear dividing line between acute and chronic inflammation, but the former generally refers to a response that has an abrupt onset and is of short duration (Zamora et al., 2000). In the inflammatory response, there is an increase of permeability of endothelial lining cells and influxes of blood leukocytes into the interstitium, oxidative burst, and release of cytokines (Miguelemail, 2010). Cytokines are glycoproteins that transmit information from one cell to other and cell respond accordingly. Cytokines that are produced during inflammation represents a series of regulatory proteins of immunologic system. Nitric oxide (NO) is a chemical indicator of inflammatory disease; and inducible nitric oxide synthase (iNOS) when induced in inflamed tissue screens a large amount of NO. The lipoxygenase (LOX) and cyclooxygenase (COX) are enzymes involved in the process of inflammation. Both iNOS and COX-2 stimulate the production of large amounts of proinflammatory mediators (Du et al., 2015). Most of the research

has focused on the secretion of proinflammatory cytokines or mRNA expression of inflammatory mediators such as IL-1, IL-6, IL-8, IL-12, IL-18, and interferon-g (IFN-g). β-Glucans from variety of sources are quite effective as antiinflammatory agents (Du et al., 2014); however, the β-glucans from fungal sources have received considerable attention of the researchers of the present era for said health benefits (Du et al., 2015). Hot-water extract (which was mainly composed of β-glucan) from fruiting bodies of *Cordycepsmilitaris* on lipopolysaccharide (LPS)-stimulated TNF-a and IL-6 release, and NO production as antiinflammatory effect in RAW 264.7 cells (Jo et al., 2010). His findings demonstrated the treatment of macrophages with different concentrations of hot-water extract from *C. militaris*-significantly reduced LPS-induced NO production as well as secretion of TNF-a and IL-6 in a concentration-dependent manner. The above-mentioned result depicted that hot-water extract from *C. militaris* may prevent inflammation by suppressing LPS-induced inflammatory mediators, which was mainly due to presence of β-glucan in significant amounts (Jo et al., 2010). LPS-stimulated NO production in RAW 264.7 cells was reported to be an effective approach for evaluating the antiinflammatory activity of β-glucan (Hida et al., 2009). Polyvinyl alcohol/propyleneglycol/β-glucan mixed hydro-gels could significantly enhance the healing without causing irritation through observing the wound healing of rat skin (Gwon et al., 2011). Berdal et al. (2007) depicted that topical applications of the macrophage-stimulant, aminated β-1,3-D-glucan, could improve wound healing in an animal model with diabetes mellitus in rat model. Toklu et al. (2006) study depicted that both systemic and local administration of β-glucan was quite effective against burn-induced oxidative tissue in mice model. Delatte et al. (2001) concluded that β-glucan collagen matrix (BGC) had the ability to treat thickened skin in children owing to burning. BGC also had the ability to reduce the postinjury pain with affective acceleration of wound healing.

6.2 Cosmetics Development

β-Glucan usage in cosmetic products has been expanded in recent past. The products in which β-glucan have been used include: ointments, suspensions, and powders, and protective creams. The attributes that make it a good choice for cosmetic products as it increases collagen production, and reduces, wrinkles, acnes, age-lines, dermatitis, crow's feet, cellulite, eczema, and other skin conditions including psoriasis. It has been found that β-glucan acts as a promoter of wound healing and film-forming moisturizer (Zhu et al., 2016). Study carried out on skin models to evaluate

the clinical efficacy of oat β-glucan depicted that it has efficacy for reducing the wrinkles and fine lines of skin since it can penetrate deeply into the skin epidermis and dermis. It helps to conclude that β-glucan can be used in the cosmetic treatment of signs of aging and in the care and maintenance of healthy skin (Pillai et al., 2005). Several researchers have reported that β-glucan from cereals and mushroom have skin health promotion and antiaging and revitalizing effects on the skin (Du et al., 2014; Zhu et al., 2016). Chen (2014) found that mushroom glucan has greater moisture retention and can be used in eye drops for alleviating xerophthalmia. Another study concluded that glucan from liquid mycelia culture of *S.commune* can impart skin whitening effect, can postpone skin aging, and have the ability to cure damage skin very effectively (Park et al., 2001). Collage loss is mainly involved in the aging process of skin. If we can increase collagen production in skin, we can overcome or at least slow down the aging process. Ketkeaw et al. (2012) in a case control study reported that β-glucan from *Hevea latex* increased fibroblast collagen production as compared to control. These finding suggested the possible usage of β-glucan from *Hevea latex*, where collagen loss is responsible skin aging. Kanlayavattanakul and Lourith (2008) aimed to study antiwrinkle efficacy of β-glucan. For that purpose he formulated 0.04% CM glucan in O/W emulsion, which was tested on 10 subjects having age above 60 years. The frequency of usage of emulsion was twice a day. The result of intervention depicted that 0.04% CM glucan emulsion improved skin condition of the subjects after 28 days usage with firmer skin and a reduced wrinkle depth around the eyes. Vacharaprechakul, Krisdaphong, and Kanlayavattanakul (Vacharaprechakul et al., 2007) used cosmetics in which they replaced source of CM, that is, they used brewer's yeast CM glucan. The results of clinical study led them to conclude that the emulsion had a soothing effect and good spreadability if its formulation contained 0.1% of CM glucan. In another study, affect β-glucan was explored against the human ulcers and wound healing. The findings depicted that β-glucan significantly enhanced the ulcer healing and added on epithelial hyperplasia, as well as increased inflammatory cells, angiogenesis, and fibroblast proliferation (Medeiros et al., 2012).

7 Effects of β-Glucan on Some Environmental Toxins

In the present era life, the quality of life for human being is badly affected owing to an increase in the prevalence of autoimmune disease. The major reason for such autoimmune diseases is

due to the increase of environmental toxins in our surroundings, water, food, and feed. Along with autoimmune diseases, environmental toxins enhance the chances of a host being susceptible to infections and to have a compromised immune system. Glucan variant sources have been shown to reduce the immunosuppressive effects of a number of factors, including chemo and radiation treatment (Vetvicka, 2014). Since the establishment of the role of β-glucan on bone marrow protection and activation of bone marrow progenitor cells, studies have been carried out to explore the new health-related functions of β-glucan (Vetvicka and Novak, 2013). Now it has been established that glucan strongly stimulates all the facets of the immune system. This led to the hypothesis that glucan either reduces or offsets the immunosuppression caused by toxic agents (Vetvicka, 2014; Vetvicka and Novak, 2013). Among toxic agents, mycotoxins are widely spread in the environment and are a serious danger not only to commercially farmed animals but also to humans. Although many species of *Aspergillus* produce mycotoxins, predominant among those are *Aspergillus flavus* and *Aspergillus parasiticus* (Vetvicka, 2014). High level of aflatoxins exposure causes acute hepatic necrosis that may later result in cirrhosis or carcinoma of the liver. Other negative consequences of high-level aflatoxins include: alteration in digestion and changes to absorption and metabolism of nutrients, edema, and acute hepatic failure manifest as hemorrhage (Khlangwiset et al., 2011; Liu et al., 2012). The first studies depicted that (1-3)-β-D-glucan, particularly with the (1-6)-β-D-glucan side chains, can regulate the presence of aflatoxins. Hydroxyl, lactone, and ketone groups of zearalenone molecules [(aftlatoxins) via hydrogen bonding with glucan single helix via van der Waal's interaction between β-D-glucopyranose moieties and the phenyl (Vetvicka, 2014; Yiannikouris et al., 2004)]. In initial study insoluble β-glucan from *Saccharomyces cerevisiae* was investigated as a decontaminant. In subsequent study β-glucan from *Saccharomyces cerevisiae* was modified to form cross-linked carboxymethy glucan (CM). The modified glucan with modified cross-linkage pattern had greatly influenced the adsorption of mycotoxin T-2 zearalenome (Freimund et al., 2003; Pereyra et al., 2012).

Glucan has been found to protect the bone marrow against the lethal effect of irradiation and chemotherapy. Glucan has received considerable attention to overcome the side effects of cancer treatment. It was concluded from the experimental studies that prophylactic use of glucan-reversed radiation caused changes in MDA levels and SOD activities. It was hypothesized from these findings that glucan actually played a protective role against the oxidative injury induced by the electromagnetic radiation,

which may be attributed to strong antioxidant abilities possessed by the glucan (Ceyhan et al., 2012; Vetvicka, 2014). Vetvicka and Vetvickova (2009) conducted a 2-week study in which β-D-glucan was orally administered to the patients. The result depicted that glucan greatly ameliorated the immunosuppressive action of mercury, including IL-6 and IL-12 production, interantibody secretion, NK cell activity, and phagocytosis of peripheral blood cells. Consequent study further revealed that 7-day exposure to mercury could cause a significant decrease in cellularity in spleen; which was restored when the patient was supplemented with glucan. The case was the same with suppressed proliferation of T lymphocytes that moved to their normal value when the patient took glucan orally.

Glucan can also play a role to overcome the negative effects caused by antibiotics. Amikacin, which is antibiotic, causes significant ototoxicity in which the person loses the ability to hear at most tested frequencies. It was found that oral administration of glucan together with amikacin limited the hearing loss. Glucan also shows profound effect on hearing improvement when used alone. It was speculated by the researchers that glucan inhibited the formation of free radicals that were a possible reason of hearing loss; however, the exact mechanism is still not known (Bayindir et al., 2013; Vetvicka, 2014). Uranium toxicity is also a big problem with persons working in atomic energy institutes in different parts of the world. The main negative impact caused by uranium is that it manifests mostly via mitochondrial dysfunction. A detailed study revealed that glucan attenuated the formation of depleted-uranium-induced mitochondrial reactive oxygen species, lipid peroxidation, and glutathione oxidation. In addition, further mitochondrial dysfunction, including outer membrane damage and release of cytochrome c was prevented.

The mechanism by which glucan supplementation blocks or at least lowers immunosuppression is currently unclear. One option is that exposure of the body to toxins may lower immunity, which may be stimulated by usage of β-glucan.

8 Health Benefits of β-Glucan

The health benefits of β-glucan include:

1. Promote laxation, which helps in curing constipation (Liu, 2010).
2. Used cyclic glucans as wound-dressing material due to cyclic glucans, which have larger inner cavity diameter (Venkatachalam et al., 2013).

3. It has been postulated that these water-soluble sulfated curdlan-enucleotide complexes could find applications in gene technology in medicine (Zhu et al., 2016).
4. Flexible hydroxyapatite/glucan composite showed potential as a bone substituting material (Belcarz et al., 2013).
5. Levitz (2014) used β-glucan as a novel vaccine platform. β-Glucan can be loaded with antigens and immunomodulators such that the "payload" is released, following phagocytosis.
6. The combination of white rice with high β-glucan barley could play a favorable role in averting and treating overweight and other overweight-related metabolic diseases (Aoe et al., 2014).
7. Helps in the prevention of colorectal cancer:
   a. Increases the fecal bulk, which dilutes the concentration of carcinogens in the colon;
   b. Increases the transit rate in colon resulting in reduction of available time for interaction of colon tissue to carcinogens;
   c. Reduction in generation of carcinogen by bacterial micro flora (Hill and Fernandez, 1990).
8. Fermentation of soluble fiber such as β-glucan by micro flora in colon results in production of short chain fatty acids volatiles, which in turn lowers the pH of lumen of colon. The lowering of pH in colon may help in:
   a. Modification of bacterial degradation of bile acids and resulting in decreased risk of colorectal cancer (Hill and Fernandez, 1990);
   b. Enhances mineral absorption such as magnesium and calcium (Cummings, 1981);
   c. Hepatic damage prevention by reduction intaxol-induced hepatic damage (Karaduman et al., 2010);
   d. Growth promotion of gut beneficial micro flora (Crittenden et al., 2002);
   e. Coronary heart diseases prevention (Wang et al., 2002);
   f. Insulin resistance prevention (Brennan and Cleary, 2005).

Some evidences suggested that β-glucan and other dietary fiber possess properties that have protective role against chronic diseases such as diabetes mellitus, colon dysfunction, and cancer (Ahmad et al., 2012; Liu et al., 2000; Truswell, 2002). Consumption of 3 g soluble fiber lowers total cholesterol by 0.041 mmol/L in hypocholestrolemic persons while in nonhypocholestrolemic persons this reduction is about 0.13 mmol/L. In the same way consumption of 2.1 g β-glucan lowers total cholesterol by 9.5%, while other researcher showed that glycemic index decline by 4 units by the consumption of 1 g of carbohydrate per 50 g carbohydrate. To attain such health benefits of β-glucan, the FDA also recommended consumption of 3 g β-glucan (Ahmad et al., 2012).

Viscous dietary fibers including cereal β-glucan are very effective in reducing postprandial plasma glucose levels. The viscous nature of these dietary fibers are responsible for these physiological effects (Regand et al., 2009). Previously, amount of the dietary fiber and rheological properties were considered important parameters for estimation of glucose lowering effects (Holm et al., 1992). Recently Panahi et al. (2014) elucidated the role of food matrix in estimation of glucose-lowering effects. The food matrix, food processing, and storage individually or in combination modulate the viscosity enhancement properties of β-glucans and thus their physiological functionality in bakery products (Lazaridou et al., 2014a).

9 β-Glucan Application in Foods

Beside from nutritional and health benefits of β-glucan, it possesses several other functional properties to be used in food products. For example, it can be used as thickening agent in the modification of appearance and texture in salad dressings, ice cream formulations, and gravies (Lazaridou et al., 2004; Wood and Webster, 1986), or it can be used in formulation of reduced calories food like fat mimetics (Lazaridou et al., 2004). β-Glucan has various physical properties such as stabilizing, emulsification, and gelation (Ahmad et al., 2012b). Food processors can advantageously use β-glucan and other dietary fibers to improve/modify oil holding capacity, controlling synersis, gelling, emulsification, and water-holding capacity. These processes are often required in bakery products, jams, jellies, soups, meat products, dairy products (Ahmad et al., 2012a). Much of the interest in the use of cereal β-glucans has stemmed from their use as a functional dietary fiber (Du et al., 2014). β-Glucan obtained from barley is specifically suitable for such uses, due to its smooth mouth feel imparting properties, and beverage formulated also becomes an excellent source of dietary fiber. Thickening properties of β-glucan makes its suitable source for replacement of thickening agent like alginates, xanthun gum, pectin, gum Arabic, and carboxy methylcellulose (Giese, 1992). Commercial available products of β-glucan include Nature, Viscofiber, Ceapro, C-trim50, C-trim30, C-trim20, Natureal, OatVantage, Cerogen, and Glucagel (Ahmad et al., 2012). Some products in which β-glucan can be used are shown in Fig. 11.5.

9.1 Use of β-Glucan in Pasta Products

Oat flour pasting properties affect textural attributes and the acceptance of food products to consumer. Oat slurries having peak

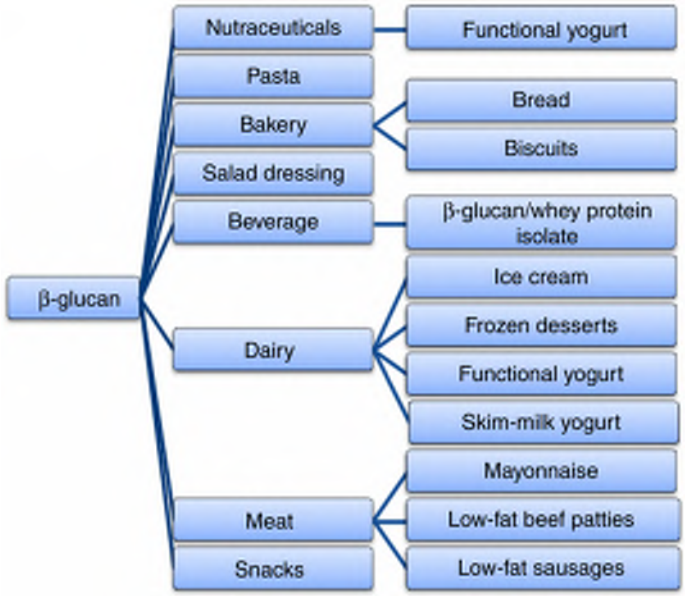

Figure 11.5. Potential of β-glucan for different food products.

viscosity at short time has greater acceptability by Australian consumer. In the same way low peak viscosity and longer time required to attain peak viscosity are unacceptable to consumer. The pasting properties of oat slurries are mainly affected by three components: β-glucan, starch, and protein. Among these three contributors affecting pasting properties, β-glucan is the major contributor, the secondary contributor is starch, and the minimal contributor is protein (Liu, 2010). High water-binding capacity of β-glucan results in high viscosity at low concentrations (Hallfrisch and Behall, 2000; Welch, 1995b). The effect of β-glucan on pasting showed significant decrease in apparent viscosity after degradation of β-glucan with enzyme lichenase (Wood, 2007), indicating that β-glucan improves physicochemical properties of pasta products (Liu, 2010). It is possible to produce β-glucan-enriched spaghetti by partial substitution of durum wheat semolina with barley flour (at levels of 7.5, 15, and 20%), this preparation contained natural β-glucan. β-Glucan content of the resultant pasta product increased from 0.3% to 6% in uncooked and 8% in cooked pasta. Such product was also rich in antioxidant activity that was stable during processing and cooking. Other properties such as pasta loss on cooking, water absorption, sensory properties, and aroma varied nonsignificantly (Aravind et al., 2012).

9.2 β-Glucan as a Bread Ingredient

Bread is an important component of our daily diet, so to overcome the health problems bread may be fortified with specific ingredients like β-glucan. One way to add β-glucan is by mixing oat flour with wheat flour, since oat flour contains β-glucan. Oat flour is a suitable ingredient because it has good water retention capabilities, so the bread appears fresher for a longer period of time (Flander et al., 2007). Similarly, the addition of oat starch or lecithin may be helpful in the retardation of staling rate in bread (Forssell et al., 1998), but this may affect the baking quality (Gormley and Morrissey, 1993). Some processors face the problem of weight loss in bread during storage. This problem can be best tackled by incorporation of barley β-glucan in formulation of bread. Increasing the barley β-glucan in formulation will result in a high number of rounded gas cells. These structures impart enough strength to cells to retain moisture for longer period of time (Foschia et al., 2013).

Bread is a good source of carbohydrates but it does not contain a sufficient amount of dietary fiber. Dietary fiber has been shown to possess many health-promoting effects like hypocholestrolemic and reduction in the chances of development of colon cancer. Seeking the benefits of dietary fiber, many researchers have prepared bread that contains a high amount of fiber, especially β-glucan (Ahmad et al., 2008). In addition to health benefits β-glucan positively effects bread properties such as an increase in water absorption (Crowley et al., 2000).

In a study oat bread was prepared by using 51/100 g oat flour while 49/100 g wheat flour and the effect of gluten content and baking conditions were investigated. The results showed that ingredients mainly affect the sensory crumb properties while baking conditions influence crumb properties and flavor of crumb. The β-glucan content of bread was found to be 1.3/100 g, which shows that a portion of bread (two slices) contain 0.78 g β-glucan, which is in accordance with the specifications of FDA, that is, 0.75 g β-glucan per portion for claiming health benefits (Flander et al., 2007). In another study, β-glucan rusk was prepared through imitation of breadmaking process and resulted in high solubilities of β-glucan during mixing and fermentation. High temperature of baking favors the porous structure of rusks; this is very similar to bread porosity (Lazaridou et al., 2014a).

By fortification of wheat flour with β-glucan, it increases the water absorption capacity of dough along with increasing water activity and moisture content of bread. This effect increases with increase in molecular weight of β-glucan. β-Glucan addition to dough formula increases the development time, resistance to deformation, stability, and darkens the color of bread while it

decreases the firmness of bread (Skendi et al., 2010) The amount of β-glucan has a positive effect on loaf volume of bread and also retention of this loaf volume for about 48 h, which starts declining after 48 h. This increase in loaf volume is due to better gas retention of bread due to β-glucan. Similarly, symmetry of form and evenness of bake are not affected by β-glucan while character of crust—which is related to thickness and toughness of crust—and score of the grain of bread—which include uniformity, holes in the cell wall, and thickness—also increased with increase in the concentration of β-glucan. So β-glucan incorporation into dough of bread is of great perspective for baking industry (Ahmad et al., 2008; Wang et al., 1998). Other dietary fiber sources (wheat bran, resistant starch, and locust bean gum) also have positive influence on crumb color acceptance, crumb appearance acceptance, and texture acceptance. These and other properties depend upon the type and quantity of the dietary fiber source used to manufacture the bread and other bakery products. Response surface methodology can be employed to optimize these dietary fibers for the development of bread products. Wheat bran dietary fiber is often used when high-speed mixing is employed for the production of bread with less specific volume and crumb luminosity, the resultant breads will be high in crumb moisture and crumb chroma. Regarding the preferences of consumers, they always prefer to see bran and fiber particles in dietary fiber-enriched breads, thus higher amounts of bran and dietary fiber additions yielded good results in the sensory evaluation of crumb color and appearance. Such breads will fetch higher prices in the market (Almeida et al., 2013). Kittisuban et al. (2014) studied the impact of hydroxypropylmethylcellulose; a β-glucan produced by yeast and whey protein isolate on physical attributes of gluten-free bread baked from recipes which were based on rice starch only. It was found that said bread formulated with yeast β-glucan and rice starch was acceptable for consumers as per results of sensory analysis. Iranshahi et al. (2014) explored the impact of inulin and β-glucan on barbari bread staling. Bread was prepared by incorporation 3% inulin and 1.5% β-glucan. The result depicted bread made with previously combination has acceptable sensory properties with extended shelf life.

9.3 β-Glucan as Fat Replacer in Mayonnaise

A widely consumed product is mayonnaise, which is oil-in-water emulsion. According to the Codex Alimentarius Commission, mayonnaise must contain total fat 78.5% and pure egg yolk 6% (Codex Alimentarius Commission, 1989). In mayonnaise many

important functions like flavor, texture, appearance, and shelf life are contributed by fats. Due to the apparent relationship between dietary fat and hypertension, cardiovascular diseases and obesity have changed the minds of consumers regarding low-fat products. Light mayonnaise can be produced by substituting fat in the basic formula. Sine β-glucan extracted from brewer's yeast (*Saccharomyces cerevisiae*) possesses high apparent viscosity, oil binding, water holding, and emulsion stabilizing properties, it can be used as a fat replacer in mayonnaise (Thammakiti et al., 2004). A study was conducted in which 25, 50, and 75% fat was replaced with β-glucan (reduced fat) and then rheological, physiochemical, sensory, and microbiological analysis were performed. The results showed that reduced fat mayonnaise has low energy content and but higher water content. No significant difference in pH, firmness and adhesive values was found for full-fat and reduced-fat emulsions. Both reduced-fat and full-fat mayonnaise exhibited shear tinning thixothopic behavior, rheologically classified as weak gels. Throughout the storage period microbial load remained in the acceptable range (Worrasinchai et al., 2006) but incorporation of β-glucan has adverse effects on appearance and color resulting in lower sensory quality as compared to full-fat mayonnaise. The β-glucan enriched mayonnaise has pale and dense appearance while full-fat mayonnaise has shiny yellowish-white appearance. Another study shows that this problem of mayonnaise can be overcome by the addition of colorants like carotenoids, that is, carotene and lutein. Addition of carotenoids do not effect textural characteristics of mayonnaise and their addition improves color and the extent of color varies with concentration of colorants (Santipanichwong and Suphantharika, 2007). β-Glucan and inulin are two potential dietary fibers having application as fat replacers in meat products, where it may impart softer texture, emulsion stability, and binding properties to the product. β-Glucan acts as a valuable fat-replacement ingredient that can make dense matrices, having ability to hold larger volume of water in reduced-fat products and thus improving the textural properties of the product. Combination of β-glucan and inulin gel help to improve stability and texture of low-fat meat products (Álvarez and Barbut, 2013).

9.4 Use of β-Glucan in Beverages

The increasing awareness of consumers about health and role of food in improving the quality of life necessitates that scientists and industries develop new functional foods (Blades, 2000; Bland and Medcalf, 1994). Mostly probiotic foods available at market

are milk- or dairy-based and fewer attempts are made to develop probiotic food using different substrate like cereals. β-Glucan being one of the most important functional ingredients comes from mainly barley and oat is a prebiotic substrate as it stimulates the growth of colon residential beneficial microorganisms such as bifidobacteria (Jaskari et al., 1998; Wood and Beer, 1998). A probiotic drink was prepared by using substrate of whole grains oats that are fermented with lactic acid bacteria to obtain health-promoting effects of probiotic culture in which β-glucan will serve as prebiotic. The concentration of β-glucan [0.31–0.0 (36%)] does not change during fermentation and also during storage of drink. So β-glucan containing fermented drink can be prepared, which has a good effect on health (Angelov et al., 2006). Barone Lumaga et al. (2012) formulated three beverages: the first beverage contained only 3 g β-glucan, the second contained 2.5 g dietary fiber (DF) from fruit, and the third was control (without dietary fiber). The findings of the study depicted that beverage which contained 3 g β-glucan resulted in control food intake, which leads to reduced 24 h energy intake.

Different whey beverages have been produced by using concentrated whey protein and unprocessed liquid whey (Prendergast, 1985). Besides whey, which many industrialists are using as an ingredient because of its nutritional and functional properties, β-glucan is favored due to its health-promoting properties to be used as an ingredient in food products. To use β-glucan as a food ingredient, an orange-flavored β-glucan/whey protein isolate drink was prepared having 0.5% β-glucan and different concentrations of whey protein isolate. No significant difference was observed for intensity of sweetness and all attributes of degree of liking, while sourness and orange flavor decreased with increasing intensity of whey protein isolate. The beverage produced remained stable during the 8 weeks of storage. This study shows a great potential of β-glucan as a beverage ingredient (Temelli et al., 2004).

9.5 Use of β-Glucan in Dairy Products

A great potential of β-glucan lies in the dairy industry for development of new nutraceutical products. Oat β-glucan containing bioactive functional dairy-based product was developed by Bekers et al. (2001). This product was developed on the basis of symbiotic association of prebiotic and probiotic, because dietary fiber is released in the fermentation process. Promising results are shown by the incorporation of β-glucan from oats for functional yogurt development. Addition of β-glucan improved the body and texture of unsweetened yogurts. In combination with sweetener

it also increases the apparent viscosity of the product. Symbiotic relationship between microorganisms and β-glucan stimulate the fermentation process resulting in production of several bioactive compounds (Kontula et al., 1998). β-Glucan also perform well with glucono-δ-lactone (GDL) during fermentation process and modify textural attributes of milk products by controlling phase separation between these polysaccharides and milk proteins. These changes are governed by the molecular weight and dose of β-glucan (Lazaridou et al., 2014b).

Sharafbafi et al. (2014) developed low-calorie and low-cholesterol dairy product by incorporating the high molecular weight oat β-glucan. Subsequently product was examined for rheological properties, microstructure, and phase behavior in relation to oat β-glucan addition. The result depicted that addition of oat β-glucan and developed microstructure governed the behavior of bimodal curve. Additionally, Rinaldi et al. (2015) reported the yogurt which was incorporated with pectin and β-glucan showed higher proportion of free amino acids, faster proteolysis, lower release of large peptides than those with starch or without β-glucan.

9.6 β-Glucan Uses in the Development of Low-Fat Yogurt

Yogurt is a worldwide popular product obtained by milk lactic acid fermentation through the action of yogurt starter culture (Tamime and Robinson, 1999). Yogurt is a healthy food because it contains a high amount of protein and calcium. Traditional yogurt contains 3–4% fat, but in concentrated yogurts fat content increases to 9–10%. For diet-conscious persons yogurt has been produced from skimmed milk, but in the meanwhile consumer demand is for nonfat yogurt having sensory characteristics similar to full-fat yogurt (Tamime and Robinson, 1999). For improvement of texture, alternative materials were used including: pectin (Ramaswamy and Basak, 1992), gelatin (Fiszman et al., 1999), dietary fiber (Fernandez-Garcia and McGregor, 1997), and inulin (Guven et al., 2005). Dietary fiber use in nonfat yogurt has advantages due to their health-promoting effects and prevention of hypercholesterolemia, diabetes, and hypertension. Use of β-glucan hydrocolloid composite as fat replacer in fat-free yogurt did not affect pH, acetaldehyde, gel firmness, water-holding capacity and volatile fatty acids content during the storage period. Tyrosine content and titratable acidity increased throughout the storage time significantly. β-Glucan addition decreased the separation of whey, while increasing the viscosity of yogurt during storage time. Sensory characteristics showed a preference for control yogurt but

yogurt having a low level of β-glucan shows satisfactory sensory scores, that is, 0.25–0.50% β-glucan hydrocolloid composite containing yogurt got similar to control and were acceptable to expert panels (Sahan et al., 2008).

9.7 Uses of β-Glucan in Meat Industry

In the meat industry the chopped mixture of lean meat, spices, fat, and ice is known as sausage batters or emulsions. In the solution of protein-water fat globules are suspended making similar structure as to emulsions, but technically sausage is not emulsion because no phase is in liquid form (Pearson and Gillett, 1996). In sausage a protein network forms which is important in maintaining the structural integrity of the system, and the strength of this protein network depends upon the interacting forces that results in a three-dimensional protein network. Protein molecule binding abilities are affected by fat, so fat molecules contribute to stable sausage formation (Ahmed et al., 1990). Since fat is associated with health problems it needs to be replaced. β-Glucan possesses several properties that can replace fat in sausages. In cooked sausage β-glucan holds more water and minimized the cooking loses as compared to carboxy methylcellulose because β-glucan forms a tighter network in the protein matrix. So β-glucan can be used as a fat substitute in value added products of meats (Morin et al., 2004). Dietary fibers from cereals and other sources are also effective to reduce the calories of meat products through substitution of fats and maintaining a fat-like texture in these meat products. Undigested fibers from cereals have a capacity to replace fats in beef burgers, reducing levels of cholesterol and improving their cooking yield, diameter and texture (Mansour and Khalil, 1997).

Another application of β-glucan is in the manufacturing of low-fat beef patties. The addition of oat fiber having β-glucan 13.45% in low-fat patties, that is, fat less than 10% was compared with high fat (20%) control patties. Addition of β-glucan resulted in significant improvement in cooking yield, moisture, and fat retention. Nonsignificant difference was observed for cholesterol concentration, while microbial quality of both types of patties remained stable during frozen storage for 60 days. Similarly tenderness and appearance are not affected by oat-soluble fiber but likeness decreased significantly. These result shows that β-glucan can be used as a substitute of fat in low-fat patties (Pinero et al., 2008). One important aspect of β-glucan and other dietary fiber is that it imparts texture while replacing fats in meat products. Fat replacement through Inulin gel, inulin powder, and β-glucan has influence on color, emulsion stability, texture, and microstructure of

cooked meat batters. Replacing 5% indigenous fats with these fibers have a better influence on emulsion stability, lightness, hardness, and fracturability of cooked meatballs and reduce cooking losses (Álvarez and Barbut, 2013).

9.8 Use of β-Glucan as Gelling Agent

Gelation is an important phenomenon adopted by several food processors for the production of food products. Different types of gels that have application in food products are hydrogel, xerogel, aerogel, and cryogel, all of these are possible with incorporation of β-glucan. For hydrogel formation, β-glucan and some other dietary fibers cross-linked at several points in a dietary fiber polymer. This cross-linkages among dietary fiber polymers results in a three-dimensional continuous network, that traps and immobilizes liquid in the form of a gel (Glicksman, 1982) and provide resistant against flow (Lazaridou and Biliaderis, 2007). Sometime, β-glucan hydrogels are stronger than conventional starch gels. Burkus and Temelli (1999) prepared a 5% w/w β-glucan hydrogel that are more than four times stronger as compared to corn-starch hydrogel of the same concentration. Apart from β-glucan concentration, temperature, chain length, and molecular weight are important for this kind of gels. Another type of gel is aerogel, which is obtained when the liquid phase of a gel is replaced by a gas in such a way that its solid network is retained, with only a slight or no shrinkage in the gel. It can be best achieved using supercritical conditions. A concentration of 5–7% of β-glucan is ideal for formation of aerogels using supercritical CO_2 system. Resultant β-glucan aerogels find their way for production of edible food products, as well as carriers for drugs and nutraceuticals (Comin et al., 2012).

The linkages ratio in β-glucan is important for gelation process; on average 1→3 to 1→4 ratio exists as 3:7 but this may vary from source to source. In cereal sources, 1→3 linkages occur in isolation and interrupted by 1→4 linkages. These sequences of isolation and interruption makes β-glucan water-soluble (Henriksson et al., 1995). In solution form water-soluble β-glucan act via two modes: either it affects viscoelastic flow when it exists in molecular dispersed form or it forms a thermo-reversible gel network structure. Certain factors such as dissolved solids, low temperature, shear force, and alcohol content tend to reinforce this gel network structure. Consideration of these factors is important in the brewing process, where this kind of gelation is highly undesirable (Böhm and Kulicke, 1999).

Fresh solutions of β-glucan barely show gelation process due to lack of association among polymer layers (Comin et al., 2012).

At this stage β-glucan exist in dispersed form, with passage of time and with application of high-temperature β-glucan sol begins to adopt gel properties. In the end the visco-elastic fluid has turned to an elastic solid. This can be evident with the change in storage modulus, G', accessible via rheological oscillatory time experiments and is considered the most suitable indicator for sol–gel transformations. Plotting logarithm of G' as a function of time results in a sigmoidal curve for barley β-glucans but differs with concentration and molar mass.

Gelation rate of β-glucan solutions also depends on concentration and molar mass, low-molecular-weight β-glucan will have higher mobility for shorter chains, thus having more chance to gel (Böhm and Kulicke, 1999). The concentration determines the segment density in solution and hence the probability of contact between the coils, which is a basic requirement for three-dimensional network formation (Burkus and Temelli, 1999). Higher elasticity increment values (IE) values may influence the gelation rates of barley β-glucan. Broadly dispersed samples show higher gelation rates as compared to narrowly dispersed samples. Based on elasticity increment value, following model: log IE_14.8–3.03log (number of average molar mass) can be used for the prediction of rate of an unknown barley β-glucan sample in 10% (w/w) aqueous solution at 25°C (Böhm and Kulicke, 1999). Another factor that can influence β-glucan gels is molar ratio of DP3/DP4. Micro rheology and the bulk rheology of β-glucan gels revealed that utilizing β-glucan with higher DP3/DP4 molar ratios or β-glucan with low molecular weights can reduce gelation time. In these conditions gelation rate and storage modulus G' may increase to higher values. The pore size increased with use of high molecular weight β-glucan along with lower DP3/DP4 molar ratios (Moschakis et al., 2014).

Cereal β-glucans suspensions behave like a non-Newtonian fluid and show a pseudoplastic or shear thinning behavior, when these polymers completely dispersed in water exhibits time-dependent rheological behavior. Such behavior is often referred to as thixotropic implying that intermolecular networks formed with passage of time. As the time passes, β-glucans solutions show unusual shear-thinning notable at low shear rates (Lazaridou and Biliaderis, 2007).

The cereal β-glucan having linkages 1→3 to 1→4 normally gels in a similar fashion by the application of heat. Smaller differences may exist in turbidity, gel melting temperature, and gelation rate between oats and barley β-glucan. In these gels the turbidity increases as a function of storage modulus G' during gelation process of 1→3 and 1→4 β-glucan. Barley and lichenin β-glucan

produce turbid gels but oat β-glucan tends to make slightly clear gels. Melting gel temperature also vary according to source of β-glucan, lichenan have higher melting temperatures followed by barley and oat β-glucan (Böhm and Kulicke, 1999).

Gels prepared from oat β-glucan have a great influence on postprandial glycemic responses due to its high viscosity. Higher molecular weight (580,000 g/mol) increase the viscosity of oat β-glucan solutions but low molecular weight oat β-glucan has a great capacity to form viscoelastic gels, these low molecular weight (145,000 g/mol) gels also influenced the glycemic response. For oat β-glucan gels, positive correlation between peak blood glucose rise and dose of oat β-glucan was observed (Kwong et al., 2013). These health benefits led the idea of oat β-glucan fortification in viscous and gelled type food products. In this context, acidification and gelation kinetics of fermented probiotic milk was explored. Oat β-glucan causes separation in protein that developed the transient weak gels in initial stages. On later stages of fermentation, protein aggregation was retarded thus forming the weaker gels. Acidification kinetics accelerated at higher fermentation temperature, yielding shorter gelation time, but the storage modulus G' values of milk gels decreased. Although probiotic strain increased the gelation rate but it did not affect gelation time and gel strength (Lazaridou et al., 2014b).

10 Safety of β-Glucan as a Food Ingredient

To get health benefits from β-glucan it is necessary to consume large amount of whole grains (Braaten et al., 1994) because β-glucan concentration is relatively low in whole grains (approximately 5%; Asp et al., 1992). Therefore, oats and barley β-glucan concentrated formulations have been used in food products to deliver β-glucan, which possess beneficial physiological effects. Consumption of β-glucan have been shown to alter hematological character and affect immune response which necessities the β-glucan toxicity to be evaluated. A study was conducted in which β-glucan-enriched diet was fed to mice and their plasma hematological parameters were compared with control group. No adverse effects in immunopathology and organ weight were observed (Delaney et al., 2003a), similarly in another study feeding of β-glucan enriched diet in Wister rats for 28 days shows no toxicity (Delaney et al., 2003b). Genetic toxicity of concentrated β-glucan on mice is also studied. The results showed that consumption of 2000 mg/kg body weight did not affect micro nucleated polychromatic erythrocytes in bone marrow cells (Bryan et al., 2004). The Food and Drug Administration Authority recommends consumption of 3 g

oat β-glucan daily to get clinical decrease in serum cholesterol level, which shows that β-glucan is safe for human consumption (Ahmad et al., 2012b).

11 Conclusions

The trend of the use of nutritional food products having numerous health benefits for different fragments of population is gaining considerable attention from researchers. Among those, β-glucan is at the top due to its various health benefits; wide range of industrial application from food to cosmetics and medicine; and most important available from various sources including plant (cereals), fungus, and bacteria. Health benefits of β-glucan include antidiabetic, anticonstipation, hypolipidemic, anticancer, and many others. In food it has applications ranging from baked goods to dairy to the meat industry. It has been used in filmmaking, medicinal products, and in cosmetics. In a nutshell, we can conclude that β-glucan brings a new dawn in food for mankind and its many good attributes will be further explored by scientists in the future.

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12

NANOTECHNOLOGICAL APPROACH TO IMPROVE THE BIOAVAILABILITY OF DIETARY FLAVONOIDS WITH CHEMOPREVENTIVE AND ANTICANCER PROPERTIES

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1 Flavonoids as Dietary Agents with Chemopreventive and Chemotherapeutic Potential

Plants have been a good source for a wide variety of phytochemicals playing an important role in maintaining and promoting health and well-being in humans, both in ancient as well as modern cultures (Mohan et al., 2013; Qiu et al., 2013; Wang et al., 2014a). Indeed, many plant-derived bioactive compounds have been found to possess therapeutic activities for preventing but also treating some major diseases, including cardiovascular and neurodegererative disorders, diabetes, as well as cancer (Jeetah et al., 2014; Khushnud and Mousa, 2013; Luo et al., 2012; Mohan et al., 2013; Vittorio et al., 2014; Wang et al., 2014a). Among these phytochemicals, polyphenols have emerged in the focus over the past three decades as one of the most promising classes of naturally occurring compounds with immense therapeutic potential (Date et al., 2011; Gao and Hu, 2010; Leonarduzzi et al., 2010; Nichenametla et al., 2006; Tabrez et al., 2013). Phenolic agents are ubiquitous in the plant kingdom with more than 8000 structurally different compounds (Gao and Hu, 2010; Santos

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et al., 2013; Wang et al., 2013d). Among them, flavonoids are identified as the largest and most common group of plant polyphenols making a considerable contribution to avoiding onset and retarding progression of many human chronic diseases, reducing thereby the risk of incidence (Jeetah et al., 2014; Leonarduzzi et al., 2010; Luque-Alcaraz et al., 2012; Passamonti et al., 2009; Wang et al., 2013d). These compounds were first mentioned by Albert Szent Gyorgyi already in 1930 and were originally proposed to be necessary as vitamins. Although the term *vitamin P* was initially introduced for flavonoids, this was later dismissed (Han et al., 2012; Ross and Kasum, 2002). Being nonnutritive plant constituents, flavonoids form an important part of the human diet (Chen and Chen, 2013; Santos et al., 2013; Zhai et al., 2013). Besides various fruits and vegetables, flavonoids are found in nuts, grains, seeds, spices, herbs, and plant-derived beverages, such as tea, coffee, wine, and beer (Goniotaki et al., 2004; Leonarduzzi et al., 2010; Park and Pezzuto, 2012; Passamonti et al., 2009; Wang et al., 2013d). Different from plants, mammalians are not able to produce bioflavonoids and detection of these compounds in animal tissues uniquely indicates ingestion of plants (Park and Pezzuto, 2012; Passamonti et al., 2009). Synthesized as secondary metabolites, flavonoids serve various important functions in plants: coloring the flowers, fruits, and leaves; providing flavor; attracting pollinators and seed-dispersers; protecting from different stressors, including the reactive oxygen species (ROS) produced during photosynthesis and UV radiation; providing resistance to predators and pathogens; being involved in normal growth and reproduction (Jeetah et al., 2014; Leonarduzzi et al., 2010; Mignet et al., 2013; Nichenametla et al., 2006; Park and Pezzuto, 2012; Passamonti et al., 2009; Ross and Kasum, 2002). Formation of these phytopigments is affected by several factors, including environmental conditions and light, extent of ripeness, processing and storage, but also variety of species (Ross and Kasum, 2002).

Numerous experimental and epidemiological studies have consistently reported that high and regular consumption of fruits and vegetables leads to a decrease in the incidence of certain cancers (Chen and Chen, 2013; Gao and Hu, 2010; Haratifar et al., 2014a; Jeetah et al., 2014; Khan et al., 2014; Kondath et al., 2014; Krishnakumar et al., 2011; Leonarduzzi et al., 2010; Mignet et al., 2013; Park and Pezzuto, 2012; Passamonti et al., 2009; Wang et al., 2013d; Yang et al., 2014). It has been estimated that an appropriate diet could prevent approximately 30%–40% of all cancer cases, and people who daily consume at least five servings of fruits and vegetables have about a 50% reduced risk of developing cancer (Rodriguez-Mateos et al., 2014; Wang et al., 2013d).

Polyphenolic flavonoids are thought to be responsible for these beneficial effects and therefore these key compounds have recently attracted increasing attention as dietary agents for cancer management (Gao and Hu, 2010; Wang et al., 2013d; Yang et al., 2014). The consumption of flavonoids as plant-based food constituents varies to a large extent among people from different regions and cultures, being estimated to range from 20 mg to 1 g per day (Mignet et al., 2013; Sak, 2014c; Santos et al., 2013; Wang et al., 2013d).

Almost 40 years ago, the term *cancer chemoprevention* was introduced to describe the strategy for stopping or reversing premalignant cells (Park and Pezzuto, 2012). Nowadays the concept of chemoprevention comprises prevention, suppression, or reversing of carcinogenic processes by using one or more natural agents or synthetic chemicals before the appearance of malignancy, with the aim to lower cancer burden (Majumdar et al., 2014; Muqbil et al., 2011; Park and Pezzuto, 2012; Prasain and Barnes, 2007; Rodriguez-Mateos et al. 2014; Santos et al., 2013; Siddiqui et al., 2010; Siddiqui and Mukhtar, 2010; Tabrez et al., 2013; Wang et al., 2012a). It is probably the most cost-effective and best approach for cancer control (Majumdar et al., 2014; Rocha et al., 2011; Sanna et al., 2011; Wang et al., 2012a). Although the initiation phase of carcinogenesis is very short, both promotion and possibly also progression stages can span over several years, making the intervention by chemopreventing methods applicable (Tabrez et al., 2013). Increasing evidence indicates that certain plant polyphenolic flavonoids are able to interfere with all three stages of carcinogenesis, inhibiting cancer development and propagation, and representing promising candidates for application in prevention and treatment of many cancers (Kale et al., 2006; Mignet et al., 2012; Ragelle et al., 2012; Santos et al., 2013; Seguin et al., 2013; Sulfikkarali et al., 2013; Tabrez et al., 2013; Tan et al., 2012; Walle et al., 2007a; Yang et al., 2014). Indeed, one of the most abundant flavonoids in the human diet, quercetin, has multiple antitumor effects, being cytotoxic to cancer cells, and is currently a phase I clinical trial agent (Gao et al., 2012; Guo et al., 2009; Han et al., 2012; Khonkarn et al., 2011; Long et al., 2013; Sak, 2014b; Sun et al., 2014). Another well-known flavonoid from green tea, epigallocatechin gallate (EGCG), functions as a chemopreventive and chemotherapeutic compound inhibiting tumorigenesis through action on multiple molecular targets (Chen et al., 2014; Haratifar et al., 2014a; Khan et al., 2014; Rodrigues et al., 2013; Wang et al., 2012a). Accumulating evidence also supports the use of the principal soy flavonoid, genistein, as a preventive and therapeutic agent against carcinogenesis, revealing antiproliferative action in various cancer cells and inducing cell death (Phan et al., 2013; Si et al., 2010). In addition, numerous

in vitro and in vivo studies have suggested the potential value of many other naturally occurring flavonoids to be applicable in future chemotherapeutic regimens (Sak, 2014c).

There is no doubt that an ideal chemopreventive compound needs to be nontoxic to healthy cells, applicable for oral administration, economical to use, and have a high efficacy toward cancer cells with known mechanisms of action (Majumdar et al., 2014; Tabrez et al., 2013). Despite their general safety based on a long history as food constituents, biocompatibility, and only minor described side effects at high concentrations, the issue of toxicity and tolerability of flavonoids certainly needs further investigation in designing larger, randomized, and long-term clinical trials within different dose ranges (Bothiraja et al., 2014; Gohulkumar et al., 2014; Khushnud and Mousa, 2013; Kondath et al., 2014; Leonarduzzi et al., 2010; Majumdar et al., 2014). Also, the possible interference with the metabolism and pharmacokinetics of other drugs needs assessment (Leonarduzzi et al., 2010). In addition, the precise molecular mechanisms by which flavonoids influence signal transduction pathways and determine the fate of cancerous cells remains to be clarified for each individual compound in certain cellular systems (Wang et al., 2013d). It is well known that these plant secondary metabolites have the capacity to modulate carcinogenic metabolism, suppress growth and proliferation, induce apoptosis, arrest the cell cycle, and inhibit angiogenesis and metastasis in malignant cells by influencing various signaling cascades (Gao et al., 2012; Kale et al., 2006; Rocha et al., 2011; Sak, 2014a; Sak, 2014b; Sak, 2014c; Sak 2015; Santos et al., 2013; Vittorio et al., 2014). Moreover, it is very likely that consuming flavonoids in different combinations (ie, in plant-based foods) might exert much more important health benefits and anticancer potential than the effects described for individual components separately (Ross and Kasum, 2002; Tabrez et al., 2013; Walle et al., 2007b).

To date, more than 6000 structurally different compounds are recognized to belong to the class of flavonoids subdivided further into six main subclasses: flavonols, flavones, flavanones, flavanols, isoflavones, and anthocyanidins (Mignet et al., 2013; Mohan et al., 2013; Wang et al., 2013d). All these compounds have a generic structure of diphenyl propanes (C6—C3—C6), consisting of two aromatic rings (A and C rings) which are joined by three carbon atoms being usually in an oxygenated heterocycle ring or B ring (Cai et al., 2013; Jeetah et al., 2014; Leonarduzzi et al., 2010; Mohan et al., 2013; Nichenametla et al., 2006; Park and Pezzuto, 2012; Passamonti et al., 2009; Rodriguez-Mateos et al., 2014; Ross and Kasum, 2002). The structures of the most common flavonoids are presented in Fig. 12.1.

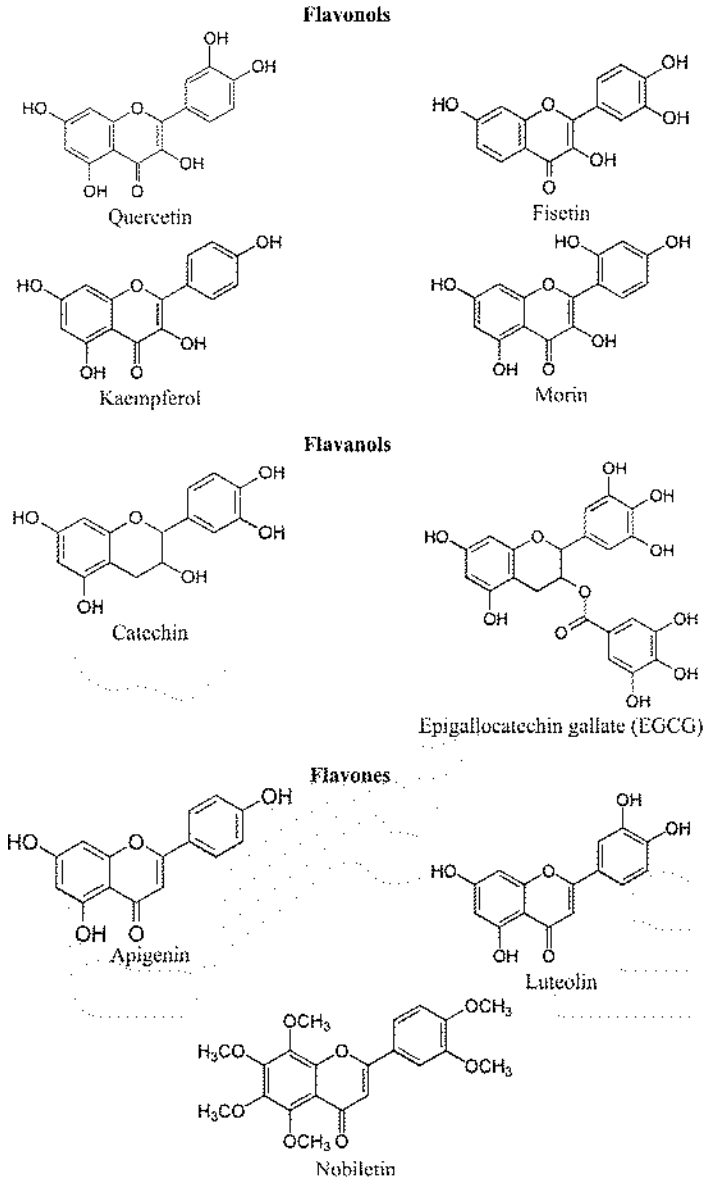

Figure 12.1. Structures of flavonoids used for nanoencapsulation studies.

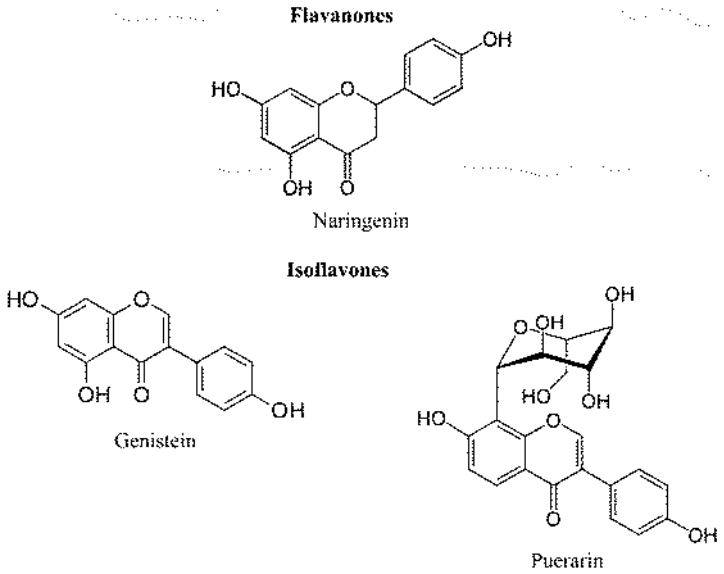

Figure 12.1. (***cont.***)

2 Obstacles to Implementation of Anticancer Potential of Flavonoids

Numerous experimental studies have demonstrated excellent anticancer potential of some flavonoids in preclinical settings, that is, cultured cell lines or animal (rodent) models (Khushnud and Mousa, 2013; Mohan et al., 2013; Muqbil et al., 2011; Park and Pezzuto, 2012). However, when attempting to translate these effects to cancer prevention or therapy in humans, they have failed to prove the expectations and only limited success has been met (Gao and Hu, 2010; Jeetah et al., 2014; Leonarduzzi et al., 2010; Muqbil et al., 2011; Siddiqui et al., 2010; Siddiqui and Mukhtar, 2010; Walle et al., 2007a; Walle et al., 2007b; Wen and Walle, 2006). It means that these laboratorially promising compounds are "lost during translation" without reaching the real life settings, from "bench to bedside," to actually treat the patients (Jeetah et al., 2014; Mohan et al., 2013; Siddiqui and Mukhtar, 2010).

One of the explanations for this discrepancy is the fact that most of the experimental studies have carried out by using concentrations of flavonoids far beyond those achievable in humans through normal dietary intake or even supplementation (Gao and Hu, 2010; Muqbil et al., 2011; Park and Pezzuto, 2012; Walle et al., 2007b). Although flavonoids are largely consumed as food ingredients, their doses in plasma and/or tissues are mostly too

low to exert the expected biological and therapeutic functions (Leonarduzzi et al., 2010; Wang et al., 2013d). Indeed, after normal dietary consumption the doses of flavonoids in blood circulation usually remain at nanomolar, occasionally also at very low micromolar level (Rodriguez-Mateos et al., 2014). For instance, the baseline plasma concentration of quercetin from dietary sources is 50–80 nM and intake of food items rich in this flavonol or supplements can elevate this level up to low micromolar range (~1.5 μM; Tan et al., 2012; Wang et al., 2014a). However, these doses are far below those that are necessary to cause pharmacological activities (Sak, 2014b,c). Similarly, the doses of EGCG in systemic circulation are about 5–50 times lower than concentrations demonstrated to exhibit biological effects in vitro conditions (Gao and Hu, 2010; Khan et al., 2014; Lemarie et al., 2013; Rodrigues et al., 2013). In this way, after drinking two cups of green tea, the plasma concentration of EGCG remains around 0.15 μM (Castillo et al., 2013; Wang et al., 2014a). Also, the serum doses of soy isoflavone, genistein, seldom exceed the 1 μM level; even in the case of the highest intake of soy products by healthy subjects (Phan et al., 2013). The major concern and challenge behind this failure is the very poor oral bioavailability of natural polyphenolic compounds limiting health benefits and restricting the development of flavonoids into chemopreventive or chemotherapeutic agents (Gao and Hu, 2010; Gohulkumar et al., 2014; Khan et al., 2014; Khushnud and Mousa, 2013; Luo et al., 2012; Majumdar et al., 2014; Santos et al., 2013; Siddiqui et al., 2009; Siddiqui et al., 2010; Walle et al., 2007a; Walle et al., 2007b; Wen and Walle, 2006).

Bioavailability is usually defined as the fraction of administered substance that reaches to the systemic circulation or arrives to the site of biological or pharmacological action in the structurally unmodified form (Haratifar et al., 2014b; Park and Pezzuto, 2012; Passamonti et al., 2009; Prasain and Barnes, 2007; Walle et al., 2007b). Concerning dietary flavonoids, the oral route of administration is obvious; furthermore, this is also the most preferred and accepted way of delivery of chemotherapeutic drugs, considering patient compliance and convenience, particularly in nonhospital settings (Khan et al., 2014; Park and Pezzuto, 2012; Passamonti et al., 2009; Siddiqui et al., 2010; Siddiqui and Mukhtar, 2010). The very low oral bioavailability of flavonoids can be illustrated by the respective values of about 17% in rats and only 1%–2% in humans in the case of flavonol quercetin (Date et al., 2011; Jain et al., 2013a; Jain et al., 2013b). For EGCG and catechins, it is even lower as only 0.1%–1.1% of the orally administered agent can enter the systemic circulation in humans, being less than 2% in rats and less than 20% in mice (Castillo et al., 2013;

Dube et al., 2010; Lemarie et al., 2013; Rocha et al., 2011; Wang et al., 2014a). Similarly, the oral bioavailability of flavanone naringenin is only 4% in rabbits (Krishnakumar et al., 2011) and that of luteolin remains 30.4% in rats (Qiu et al., 2013).

The reason of poor oral bioavailability of flavonoids is multifaceted and the limiting factors include low water solubility and stability of polyphenols, their poor absorption and extensive metabolism, lack of site specificity in distribution, and rapid elimination and clearance from the body (Bothiraja et al., 2014; Cai et al., 2013; Gao and Hu, 2010; Jeetah et al., 2014; Leonarduzzi et al., 2010; Luque-Alcaraz et al., 2012; Mignet et al., 2013; Mohan et al., 2013; Park and Pezzuto, 2012; Wang et al., 2014a). The great structural diversity of flavonoids involves different pharmacokinetic profiles and the most abundant compounds in the food cannot necessarily be the ones reaching target sites (Haratifar et al., 2014b; Prasain and Barnes, 2007). In addition, the bioavailability of flavonoids depends on the dietary source of the compound and food matrix interactions (Haratifar et al., 2014a; Haratifar et al., 2014b; Mignet et al., 2013; Ross and Kasum, 2002). Moreover, also the host-related factors, such as gender, age, and health conditions, play important roles in the bioavailability of flavonoids (Mignet et al., 2013; Ross and Kasum, 2002).

Solubility is an important factor for oral bioavailability, as only dissolved compounds can permeate the intestinal epithelium (Gao and Hu, 2010). Therefore, low water solubility of phytochemicals results in poor absorption and low bioavailability (Muqbil et al., 2011; Santos et al., 2013). On the other hand, the hydrophobicity seems to be an intrinsic property of many anticancer drugs, facilitating their penetration through cellular membrane and reaching to intracellular target sites (Muqbil et al., 2011; Tan et al., 2012). Indeed, many flavonoids are only poorly soluble in water (Goniotaki et al., 2004; Khonkarn et al., 2011; Majumdar et al., 2014; Muqbil et al., 2011; Tan et al., 2012; Yang et al., 2014). For instance, the reported solubility of quercetin in an aqueous solution remains in the range of 1–2.15 μg/mL; the same parameter in gastric fluid is 5.5 μg/mL and intestinal fluid 28.9 μg/mL (Cai et al., 2013; Date et al., 2011; Sun et al., 2014; Wang et al., 2014a). This extreme water insolubility is also a major obstacle to the use of quercetin in pharmaceutical field (El-Gogary et al., 2014; Gao et al., 2012; Kale et al., 2006; Long et al., 2013; Men et al., 2014; Sun et al., 2014; Tan et al., 2012; Wang et al., 2012b; Wang et al., 2013b; Wang et al., 2013c; Wong and Chiu, 2010; Wu et al., 2013). Although attempts have been made to dissolve this flavonol in dimethyl sulfoxide, this solvent is harmful to the liver and kidneys and carries the risk of neurological toxicity and vasoconstriction (El-Gogary

et al., 2014; Ghosh et al., 2012; Guo et al., 2014; Yuan et al., 2006; Wang et al., 2012b). Therefore, it is clear that development of novel and water-soluble derivatives of quercetin is an urgent and important task. However, the chemical modifications done so far for increasing the water solubility have also brought about the decrease in drug potency (Tan et al., 2012; Yuan et al., 2006). Similarly to quercetin, the poor aqueous solubility remains a drawback also for many other flavonoids, hindering their potential clinical applications. Thus, the water solubility of fisetin has been reported to be 10.45 μg/mL (Bothiraja et al., 2014; Chen et al., 2015; Ragelle et al., 2012); the same parameter for apigenin is 2.16 μg/mL (Jeetah et al., 2014; Munyendo et al., 2013; Zhai et al., 2013); for luteolin less than 5.72 μg/mL (Jeetah et al., 2014; Qiu et al., 2013); and for genistein only 0.9 μg/mL (Phan et al., 2013).

Another important factor for bioavailability of flavonoids is their stability. In this way, the green tea flavanol, EGCG, is rapidly degraded in physiological fluids and water, being stable at acidic conditions (Castillo et al., 2013; Dube et al., 2010; Khushnud and Mousa, 2013; Rocha et al., 2011; Wang et al., 2014a). Such instability renders the administration of EGCG at cancer preventive and therapeutic doses unrealistic. However, introduction of peracetate groups to EGCG molecule and synthesizing protected prodrugs can significantly improve the stability of this flavanol in physiological conditions (Rodrigues et al., 2013).

It is clear that much work still needs to be done to overcome the bottlenecks related to poor oral bioavailability of flavonoids before these agents can be taken into the therapeutic use in the fight against cancer. Besides chemical modifications, also the design of appropriate drug delivery systems can prove to be successful in achieving these goals.

3 Absorption and Metabolic Bioconversion of Flavonoids

With the exception of flavanols, most flavonoids exist in plants as hydrophilic glycosides (Park and Pezzuto, 2012; Passamonti et al., 2009; Prasain and Barnes, 2007; Rodriguez-Mateos et al., 2014; Ross and Kasum, 2002; Walle et al., 2007b; Wang et al., 2013d). This glycosylation process is usually the final step in the herbal biosynthesis of polyphenolic compounds being necessary for localizing the metabolites into specific compartments, as well as their storage and accumulation (Park and Pezzuto, 2012; Prasain and Barnes, 2007). After human ingestion and passing through the stomach, most flavonoid glycosides

arrive intact in the small intestine (Rodriguez-Mateos et al., 2014; Wang et al., 2013d). However, due to the highly hydrophilic nature of glycosidic flavonoids, their direct penetration through the intestinal membrane is impeded and, for absorption, the hydrolysis to respective aglycones is required (Gao and Hu, 2010; Park and Pezzuto, 2012; Prasain and Barnes, 2007; Walle et al., 2007b; Wang et al., 2013d). This reaction is conducted by a cascade of hydrolases and glucosidases, including lactase phlorizin hydrolase and cytosolic β-glucosidase, and the released aglycones can translocate from the intestinal lumen to enterocytes by passive diffusion (Park and Pezzuto, 2012; Passamonti et al., 2009; Prasain and Barnes, 2007; Rodriguez-Mateos et al., 2014; Walle et al., 2007b; Wang et al., 2013d). Thus, the intestinal wall represents the first biological barrier to be crossed by flavonoids for actual implementation of their cancer chemopreventive and therapeutic potential (Gao and Hu, 2010; Park and Pezzuto, 2012; Passamonti et al., 2009; Prasain and Barnes, 2007).

Once absorbed by the intestinal epithelium, flavonoids undergo extensive enzymatic bioconversion and are conjugated with glucuronic acid, sulfate and methyl groups (Leonarduzzi et al., 2010; Prasain and Barnes, 2007; Rodriguez-Mateos et al., 2014). The respective metabolites pass through the portal vein to the liver and follow further conversions before entering the systemic circulation (Gao and Hu, 2010; Park and Pezzuto, 2012; Prasain and Barnes, 2007; Rodriguez-Mateos et al., 2014). These complex conjugation reactions typically lead to the modified products with changed (usually lower) biological activity than parent flavonoids being responsible for the real bioactive effects (Chen and Chen, 2013; Gao and Hu, 2010; Haratifar et al., 2014a). The spectrum of metabolites may significantly vary between human and animal (rodent) systems and can also reveal interindividual differences depending on the age and gender of subjects (Gao and Hu, 2010; Rodriguez-Mateos et al., 2014; Walle et al., 2007b). Moreover, the fate of dietary polyphenols could also depend on certain food matrixes where the respective flavonoids are incorporated (Haratifar et al., 2014a). Furthermore, besides entering into the bloodstream, parent flavonoids and their metabolites represent also good substrates for different transporters, such as P-glycoprotein, multidrug resistance-associated proteins, and breast cancer resistance protein. As a result, substantial amounts of flavonoids and their conjugates are excreted back to the intestinal lumen limiting thus essentially the overall bioavailability (Gao and Hu, 2010; Leonarduzzi et al., 2010; Park and Pezzuto, 2012; Passamonti et al., 2009; Rodrigues et al., 2013; Rodriguez-Mateos et al., 2014).

Liver is the organ in which a large number of flavonoids are metabolized via numerous different mechanisms and the generated conjugates can be further secreted into bile. These excreted phenols can be reabsorbed in the intestinal lumen, hydrolyzed to aglycones, and enter back to the plasma, thus executing the recycling loop (Gao and Hu, 2010; Leonarduzzi et al., 2010). A fraction of unabsorbed polyphenols could undergo further transformation by the colonic microflora to produce smaller phenolic acids that can be locally absorbed before entering the circulation and being ultimately excreted by urine (Gao and Hu, 2010; Prasain and Barnes, 2007; Rodriguez-Mateos et al., 2014; Wang et al., 2013d).

It is believed that extensive and rapid conjugation of the free hydroxyl groups is one of the major reasons for poor oral bioavailability of dietary flavonoids (Gao and Hu, 2010; Walle et al., 2007a; Walle et al., 2007b; Wen and Walle, 2006). Indeed, blocking these hydroxyl moieties by methyl groups has shown to result in increased intestinal absorption and higher hepatic metabolic stability (Park and Pezzuto, 2012; Walle et al., 2007a; Walle et al., 2007b; Wen and Walle, 2006). Thus, the extensive metabolism is a central contributor to the poor oral bioavailability of flavonoids, limiting the application of these agents in pharmaceutical field. Therefore, novel strategies to bypass these drawbacks are urgently needed to improve and expand our current arsenal of anticancer therapies.

4 Nanotechnological Approach to Overcome the Current Barriers

To circumvent the aforementioned limitations of flavonoids (ie, low aqueous solubility and stability, poor absorption, and extensive metabolism), to improve the oral bioavailability and achieve maximum preventive and therapeutic benefit of these bioactive dietary components development of novel formulations is of great importance. Perhaps the most promising and innovative method for this is nanotechnology (Cai et al., 2013; Chen and Chen, 2013; Date et al., 2011; Luo et al., 2012; Muqbil et al., 2011; Wang et al., 2012b). This emerging and promising strategy encompasses different disciplines, such as biology, engineering, chemistry, and medicine; and its application to medicine and pharmaceutics, known as nanomedicine, probably has a bright future in the design of novel diagnostic and therapeutic tools (Castillo et al., 2013; Hsieh et al., 2012; Jeetah et al., 2014; Luo et al., 2012; Winter et al., 2014). In general, nanotechnology deals with structures in the size range of 100 nm or smaller; however, this scale is not so strictly defined and many particles

larger than 100 nm in diameter are still termed as nanoparticles (Bharali et al., 2011; Castillo et al., 2013; Santos et al., 2013; Wang et al., 2012a; Wang et al., 2014a). Thus, the aim of nanomedicine is to develop nanoscale particles with large surface-to-volume ratios for improving the drug delivery and bioavailability (Khushnud and Mousa, 2013; Leonarduzzi et al., 2010; Muqbil et al., 2011).

Most biological processes, including those that are related to cancer, happen in the nanoscale dimensions (Castillo et al., 2013; Khan et al., 2014; Leonarduzzi et al., 2010; Santos et al., 2013; Siddiqui et al., 2009; Siddiqui et al., 2010). Therefore, nanomaterial-based techniques have recently attracted increasing attention in treatment, detection, and diagnosis of cancer, being commonly referred to as cancer nanotechnology (Bharali et al., 2011; Chen et al., 2014; Majumdar et al., 2014; Santos et al., 2013; Tabrez et al., 2013; Wang et al., 2013c). This approach can offer many novel benefits in cancer research; indeed, strategies that focus on the drug delivery in a tumor-specific manner using nanovehicles are devised for improving the efficacy and safety of chemotherapeutic agents, enhancing also the patient compliance (Ghosh et al., 2012; Leonarduzzi et al., 2010; Mohan et al., 2013; Muqbil et al., 2011; Park and Pezzuto, 2012; Santos et al., 2013). Numerous nanocarrier-based chemotherapeutics are currently in development and several of these formulations, including Doxil, DaunoXome, Abraxane, and Myocet, are already clinically approved and on the market (Bharali et al., 2011; Mohan et al., 2013; Wang et al., 2014a).

Nanoparticles are able to incorporate entities either by means of ionic absorption or covalent binding, rendering them ideal carriers for various anticancer drugs (Hsieh et al., 2011; Luo et al., 2012). These nanoscale structures enable researchers to solve many inherent problems of potential chemopreventive and chemotherapeutic agents, such as stability, solubility, and toxicity, providing also a platform for targeted delivery to tumor sites (Bharali et al., 2011; Bothiraja et al., 2014; Castillo et al., 2013; Han et al., 2012; Jeetah et al., 2014; Leonarduzzi et al., 2010; Qiu et al., 2013; Rocha et al., 2011; Si et al., 2010; Siddiqui et al., 2010; Wang et al., 2014a). Indeed, nanoparticles can enhance the absorption of bioactive compounds, protect them from environmental destructive factors and premature degradation in the body, extend their circulation time, and improve cellular uptake (Castillo et al., 2013; Ghosh et al., 2012; Khan et al., 2014; Leonarduzzi et al., 2010; Rodrigues et al., 2013; Sun et al., 2014; Wang et al., 2012a, 2014a). Sustained release of drugs results in prolonged availability, lowering administration frequency, dose requirement, and likelihood of adverse activities (Bharali et al., 2011; Khushnud and Mousa, 2013; Leonarduzzi et al., 2010; Siddiqui

et al., 2010; Siddiqui and Mukhtar, 2010; Wang et al., 2014a). Moreover, nanoparticles can carry and deliver the loaded drugs exactly into the locations where they need to be, that is, targeting tumors (either passively and/or actively), optimizing further the efficacy and minimizing systemic side effects; hence, patient compliance is also improved (Bharali et al., 2011; Das et al., 2013a; Khushnud and Mousa, 2013; Leonarduzzi et al., 2010; Liang et al., 2014; Majumdar et al., 2014; Snima et al., 2014; Tabrez et al., 2013; Wang et al., 2014a). Different from healthy tissues where endothelial cells are tightly connected, blood vessels in tumor tissues have abnormal and disorganized architecture with leaky endothelial lining that is easily permeable for nanosized particles, contributing to passive targeting (Chen et al., 2015; Men et al., 2014; Seguin et al., 2013; Siddiqui et al., 2010; Siddiqui and Mukhtar, 2010; Sulfikkarali et al., 2013; Wong and Chiu, 2010; Yang et al., 2014). In this way, nanoscale particles can extravasate through the tumor's porous and defective capillary endothelium and accumulate in the cancerous tissue (Chen et al., 2015; El-Gogary et al., 2014; Mignet et al., 2012; Seguin et al., 2013; Siddiqui et al., 2010; Siddiqui and Mukhtar, 2010; Yuan et al., 2006). This process is termed as enhanced permeability and retention (EPR) effect and it is successfully exploited by nanomedicine to improve the delivery of drugs into malignant sites (Chen et al., 2014; Krishnakumar et al., 2011; Leonarduzzi et al., 2010; Sulfikkarali et al., 2013; Wang et al., 2013e). Furthermore, conjugation of nanoparticles with specific ligands can lead to the preferential accumulation and selective cellular binding of encapsulated agents to tumor cells constituting an actively targeted cancer therapy (Men et al., 2014; Tabrez et al., 2013). In addition to all the foregoing, simultaneous encapsulation and delivery of two or several drugs might generate additive or even synergistic responses for cancer management (Jeetah et al., 2014; Leonarduzzi et al., 2010; Siddiqui et al., 2009; Siddiqui et al., 2010; Tabrez et al., 2013).

The nanoencapsulation strategy is a new and important research field for enhancing the bioavailability and optimizing delivery of phytochemicals, including flavonoids (Khushnud and Mousa, 2013; Mohan et al., 2013; Santos et al., 2013; Singh et al., 2011; Wang et al., 2013d; Wang et al., 2014a). An essential improvement in oral bioavailability and therapeutic efficacy of these plant secondary metabolites has been reported in numerous studies (Krishnakumar et al., 2011; Luque-Alcaraz et al., 2012; Santos et al., 2013; Tabrez et al., 2013; Wang et al., 2014a). It is even believed that the full potential of flavonoids in prevention and treatment of cancer will be uncovered in the next few years along with the development of nanotechnological approaches.

However, until now, no clinical trials are introduced to certify the real benefits of nanoencapsulated polyphenols in preventive and/or therapeutic interventions (Mohan et al., 2013).

5 Overview of Nanovehicles Used for Encapsulation of Flavonoids

Incorporation of flavonoids in nanostructure systems is a novel perspective strategy to valorize these plant secondary metabolites (Jeetah et al., 2014). Numerous biocompatible and biodegradable nanobased carriers are currently known and available for encapsulating or loading flavonoids to improve their preventive and therapeutic potential in the fight against cancer (Jeetah et al., 2014; Khushnud and Mousa, 2013; Mignet et al., 2013; Siddiqui and Mukhtar, 2010; Wang et al., 2013d). These drug delivery systems include various different platforms among which lipid-based vehicles (nanoliposomes, nanoemulsions, solid lipid nanoparticles), and polymer-based vehicles (polymeric nanoparticles, polymeric micelles) are the most popular and suitable for delivery of flavonoids to tumor sites (Bothiraja et al., 2014; Ebrahimnezhad et al., 2013; Han et al., 2012; Leonarduzzi et al., 2010; Men et al., 2014; Pooja et al., 2014; Santos et al., 2013; Wang et al., 2013d, 2014a). Besides these widely exploited carriers, inclusion with cyclodextrin has also been successfully applied to enhance the bioavailability of polyphenolic phytochemicals (Cai et al., 2013; Kale et al., 2006; Men et al., 2014; Rodrigues et al., 2013); whereas various metal nanoparticles, such as nanogold particles, are becoming innovative and promising scaffolds for drug delivery (Chen et al., 2014; Hsieh et al., 2011, 2012; Kondath et al., 2014; Rodrigues et al., 2013). Each of these systems has its own advantages and disadvantages that need to be circumvented considering their potential future application in human systems (Wang et al., 2013c).

The overview of nanocarrier formulations used for encapsulation of flavonoids for anticancer studies is presented in Tables 12.1–12.4. General structures of different nanovehicles are depicted in Fig. 12.2.

5.1 Lipid-Based Nanocarrier Systems

Different types of lipid formulations, including liposomes, solid lipid nanoparticles, and nanoemulsions, have been established to improve the availability and therapeutic efficacy of bioactive compounds (Fig. 12.2; Cai et al., 2013; Date et al., 2011; Santos et al., 2013).

Table 12.1 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Flavonols

			Physicochemical Properties*					Anticancer Effects		
Flavonol	**Nanocarrier**	**Formulation**	**Mean Size, nm**	**PDI**	**ζ Potential, mV**	**EE, %**	**DL, %**	**In vitro**	**In vivo**	**Reference**
Quercetin	Liposomes	Egg phosphatidyl-choline	414.1 ± 12.6	0.53	−54.4 ± 7.8	86.0 ± 12.1		Growth inhibitory doses (GI_{50}) in MCF-7 human breast cancer cells 93.1 μM, H460 nonsmall cell lung cancer cells >100 μM, SF268 central nervous system cancer cells 41.9 μM; less active than plain drug		Goniotaki et al. (2004)
Quercetin	PEGylated liposomes		163 ± 10	0.085 ± 0.011	−34.5 ± 2.3	92.53 ± 0.96	4.87 ± 0.05	Inhibition of proliferation, induction of apoptosis and cell cycle arrest in cisplatin-sensitive A2780s and -resistant A2780cp human ovarian cancer cells	Suppression of tumor growth and inhibition of angiogenesis in xenograft nude mouse model subcutaneously injected with A2780s or A2780cp cells	Long et al. (2013)

(*Continued*)

Table 12.1 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Flavonols (*cont.*)

			Physicochemical Properties*					Anticancer Effects		
Flavonol	**Nanocarrier**	**Formulation**	**Mean Size, nm**	**PDI**	**ζ Potential, mV**	**EE, %**	**DL, %**	**In vitro**	**In vivo**	**Reference**
Quercetin	PEGylated liposomes		<150					Inhibition of CT26 mouse colon adenocarcinoma cells, induction of apoptosis	Inhibition of tumor growth, metastasis and angiogenesis in C57BL/6N mice bearing LL/2 Lewis lung cancer and BALB/c mice bearing CT26 colon adenocarcinoma; prolonging the survival of tumor-bearing mice	Yuan et al. (2006)
Quercetin	PEG2000-DPSE-coated liposomes		116.7					Induction of necrotic cell death in C6 rat glioma cells		Wang et al. (2012b)
Quercetin	PEG2000-DPSE-coated liposomes		50–100					Cytotoxicity in C6 rat glioma cells, through ROS-mediated pathway		Wang et al. (2013a)
Quercetin	PEG2000-DPSE-coated liposomes							Induction of cell death in C6 rat glioma cells via JAK2/STAT3 and p53-mediated ROS pathways		Wang et al. (2013a)

Quercetin	PEGylated liposomes	Egg sphingomyelin/ cholesterol/ PEG2000 ceramide				78.3 for quercetin; 78.5 for vincristine	Significant synergism by coencapsulation with vincristine in MDA-MB-231 human breast cancer cells; the doses required to attain 50% cell kill were reduced by about 6 log-fold for quercetin and 2 log-fold for vincristine		Wong and Chiu (2010)
Quercetin	PEGylated liposomes	Egg sphingomyelin/ cholesterol/ PEG2000 ceramide				78.5 for quercetin; 78.3 for vincristine	Significant synergism by coencapsulation with vincristine in JIMT-1 human breast cancer cells; the doses required to attain 50% cell kill were reduced by about 10-fold for quercetin and 1.5-fold for vincristine	Inhibition of tumor growth by coencapsulation with vincristine in JIMT-1 human breast cancer xenograft in SCID mouse model	Wong and Chiu (2011)
Quercetin	Cationic liposomes	LeciPlex	403.1 ± 12.1	0.54	+31.1 ± 1.6	91.3 ± 1.9		Significant increase in antitumorigenic activity in C57BL/6 mice subcutaneously injected with B16F10 melanoma cells as compared to plain drug	Date et al. (2011)

(*Continued*)

Table 12.1 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Flavonols (*cont.*)

			Physicochemical Properties*					Anticancer Effects		
Flavonol	**Nanocarrier**	**Formulation**	**Mean Size, nm**	**PDI**	**ζ Potential, mV**	**EE, %**	**DL, %**	**In vitro**	**In vivo**	**Reference**
Quercetin	Lipid nanoparticles	Triglyceride: phosphatidyl-choline: a-tocopheryl acetate: KolliphorHS15	30	0.059		95	11	Enhanced cytotoxicity in MCF-7 and MDA-MB-231 human breast cancer cells (IC_{50} 15.8 and 14.1 μM, respectively) as compared to plain drug (>50 μM in both lines); induction of apoptosis		Sun et al. (2014)
Quercetin	Lipid nanoparticles	GeluPearl with or without oil	365.41 ± 9.302	0.353 ± 0.040	−21.5 (without oil); −24.1 (with oil)	95.94 (without oil); 92.94 (with oil)			Improvement of antitumor and antimetastatic potential compared to plain drug in C57BL/6 mice subcutaneously injected with B16F10 melanoma cells	Jain et al. (2013b)

Quercetin	Polymeric nanoparticles	PLGA	148.6 ± 1.6 (by DLS); 113.14 (by TEM)	0.088 ± 0.03	−27.0 ± 0.7	41.36 ± 3.4		Significant increase in cytotoxicity in A549 human lung adenocarcinoma cells as compared to plain drug (IC_{50} 21.75 and 45.15 μM, respectively); further enhancement in activity by coencapsulation with etoposide		Pimple et al. (2012)
Quercetin	Polymeric nanoparticles	PLGA	185.3 ± 1.20 (with tamoxifen)	0.184 ± 0.04 (with tamoxifen)		68.60 ± 1.58 for quercetin; 67.16 ± 1.24 for tamoxifen		Increased cytotoxicity by coencapsulation with tamoxifen in MCF-7 human breast cancer cells; about 34.13- and 33.3-fold increase in cytotoxicity as compared to free tamoxifen and free quercetin	Significantly higher tumor suppression by coencapsulation with tamoxifen in DMBA-induced breast tumor bearing SD rats; antiangiogenic potential	Jain et al. (2013a)
Quercetin	Polymeric nanoparticles	PLGA	270						Protection of rat liver from carcinoma mediated by DEN injection	Ghosh et al. (2012)
Quercetin	Polymeric nanoparticles	PEG-PLA	116.0 ± 16.4 (by TEM); 169.8 ± 23.1 (by DLS)			98	3.8	More efficient antiproliferative effect in HepG2 human hepatoma cells compared to plain drug (IC_{50} 85.1 and 439.6 μM, respectively)		Wang et al. (2013c)

(*Continued*)

Table 12.1 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Flavonols (*cont.*)

			Physicochemical Properties*					Anticancer Effects		
Flavonol	**Nanocarrier**	**Formulation**	**Mean Size, nm**	**PDI**	**ζ Potential, mV**	**EE, %**	**DL, %**	**In vitro**	**In vivo**	**Reference**
Quercetin	Polymeric nanocapsules	PLGA	153.0 ± 4.3	0.11 ± 0.01	−46.2 ± 1.5	98.10 ± 0.28		Comparable cytotoxic effects to plain drug in HeLa human cervical cancer and CT26 murine colon carcinoma cells		El-Gogary et al. (2014)
Quercetin	PEGylated polymeric nanocapsules	PLGA	143.1 ± 1.7	0.13 ± 0.01	−32.9 ± 1.8	99.00 ± 0.42		Comparable cytotoxic effects to plain drug in HeLa human cervical cancer and CT26 murine colon carcinoma cells		El-Gogary et al. (2014)
Quercetin	PEGylated polymeric nanocapsules	PLGA, folic-acid targeted	155.0 ± 1.2	0.19 ± 0.01	−40.0 ± 0.9	97.8 ± 0.14		Enhanced cytotoxic effect in folic acid receptor-positive HeLa human cervical cancer cells (in folate free medium), showing active targeting		El-Gogary et al. (2014)

Quercetin	Polymeric micelles	MPEG-PCL	31 ± 2	0.121 ± 0.015	−0.21 ± 0.07	98.70 ± 0.46	14.81 ± 0.07	Stronger antiproliferative, antiapoptotic, antimigratory and antiinvasive activity in 4T1 mouse breast cancer cells compared to plain drug	Improved suppression of tumor growth, metastasis and angiogenesis in BALB/c mice subcutaneously injected with 4T1 cells as compared to plain drug; prolonging the survival of tumor-bearing mice	Wu et al. (2013)
Quercetin	Polymeric micelles	mPEG750-b-OCL with Bz or Np capping groups	14...19	<0.2			10 (with Np); 6 (with Bz)	Effective inhibition of growth of doxorubicin-sensitive and -resistant K562 human leukemia and GLC4 human lung carcinoma cells; induction of G2/M phase cell cycle arrest		Khonkarn et al. (2011)
Quercetin	Polymeric micelles	MPEG-PCL	36.1 ± 3.2 (by DLS); ∼23 (by TEM)	0.13 ± 0.04	−2.69 ± 0.45	98.1	6.9		Effective inhibition of tumor growth in BALB/c mice subcutaneously injected with A2780s human ovarian cancer cells; induction of apoptosis and inhibition of angiogenesis	Gao et al. (2012)

(*Continued*)

Table 12.1 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Flavonols (*cont.*)

			Physicochemical Properties*					Anticancer Effects		
Flavonol	**Nanocarrier**	**Formulation**	**Mean Size, nm**	**PDI**	**ζ Potential, mV**	**EE, %**	**DL, %**	**In vitro**	**In vivo**	**Reference**
Quercetin	Polymeric micelles	PEG-PE	15.4... 18.5	<0.250	−14.8 ± 0.7	≥88.9	4	Significantly improved anticancer activity in A549 human lung cancer cells and MDA-MB-231 human breast cancer cells compared to plain drug	Enhanced anticancer activity in Rag-2M mice subcutaneously injected with A549 cells as compared to plain drug	Tan et al. (2012)
Quercetin	Inclusion complexes	Sulfobutyl ether-7β-cyclodextrin complex						Improvement in antiproliferative activity in K562 human leukemia and SiHa cervix cancer cells compared to plain drug	Improved antitumorigenic and antiangiogenic effect in BDF1 mice subcutaneously injected with B16F10 melanoma cells	Kale et al. (2006)
Quercetin	Metal nanoparticles	Nickel						Synergic antiproliferation in SMMC-7721 human hepatocellular carcinoma cells		Guo et al. (2009)

Quercetin	Metal nanoparticles	Germanium	~33		−36.5			Stronger antiproliferation in MCF-7 human breast cancer cells compared to plain drug (IC_{50} 348.6 and 458.1 μM, respectively); induction of apoptosis and cell cycle arrest in S phase	Guo et al. (2014)
Quercetin	Metal nanoparticles	Magnetite	72		6.14	81.6	6.08 ± 0.3	Significant increase in cytotoxicity in MCF-7 human breast cancer cells compared to plain drug; induction of apoptosis	Kumar et al. (2014)
Quercetin	Nanoribbons		100... 200					Increased inhibition of 4T1 mouse breast cancer cell growth compared to plain drug	Han et al. (2012)
Fisetin	Liposomes	P90G, DODA-GLY-PEG2000	175	0.12		73		Comparative cytotoxicity with plain drug in 3LL mouse Lewis lung carcinoma (IC_{50} for liposomal drug 15.0 μg/mL, free drug 15.5 μg/mL) and CT26 mouse colon tumor cells (IC_{50} for liposomal drug 16.7 μg/mL, free drug 15.7 μg/mL)	Mignet et al. (2012)

(*Continued*)

Table 12.1 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Flavonols (*cont.*)

			Physicochemical Properties*					Anticancer Effects		
Flavonol	**Nanocarrier**	**Formulation**	**Mean Size, nm**	**PDI**	**ζ Potential, mV**	**EE, %**	**DL, %**	**In vitro**	**In vivo**	**Reference**
Fisetin	Liposomes	DOPC, DODA-PEG2000	173.5 ± 2.4	0.181 ± 0.016		58		Cytotoxic effects in mouse LLC Lewis lung carcinoma cells similar to plain drug (IC_{50} 15.0 and 15.5 μg/mL, respectively); induction of apoptosis	Significant delay of tumor growth in C57BL/6J mice subcutaneously implanted with Lewis lung tumor fragments as compared to plain drug	Seguin et al. (2013)
Fisetin	Nanoemulsion	Miglyol 812N/ Labrasol/Tween 80/Lipoid E80/ water	153 ± 2	0.129	−28.4 ± 0.6				6-fold higher antitumor activity in C57BL/6J mice subcutaneously implanted with Lewis lung tumor fragments as compared to plain drug	Ragelle et al. (2012)
Fisetin	Nanocochleates	DMPC, cholesterol, calcium cations	275 ± 4		−13.8	84.31 ± 2.52		1.3-fold higher anticancer effect in MCF-7 human breast cancer cells as compared to plain drug (IC_{50} 11.2 and 14.3 μg/mL, respectively)		Bothiraja et al. (2014)

Fisetin	Polymeric nanoparticles	MPEG-PCL	114.0 ± 1.2	0.13 ± 0.01		97.0 ± 0.8	15.5 ± 0.1	Comparative decrease in viability of LL/2 mouse Lewis lung cancer cells to plain drug (IC_{50} 16.16 and 15.14 μg/mL, respectively)	Higher reduction in tumor volume and metastasis in C57BL/6 mice subcutaneous ly injected with LL/2 cells as compared to plain drug	Yang et al. (2014)
Fisetin	Polymeric micelles	MPEG-PCL	22.4 ± 3.0	0.163 ± 0.032	−3.61 ± 0.24	98.53 ± 0.02	9.88 ± 0.14	Enhanced cytotoxicity in CT26 mouse colon adenocarcinoma cells compared to plain drug (IC_{50} 7.968 and 28.513 μg/mL, respectively); induction of apoptosis	More efficient suppression of tumor growth and prolonging survival of BALB/c mice subcutaneously injected with CT26 cells compared to plain drug; induction of apoptosis, antiangiogenic effect	Chen et al. (2015)
Kaempferol	Polymeric nanoparticles	PEO-PPO-PEO	160 ± 30		1.4 ± 4.2			Significant inhibition of A2780/CP70 and OVCAR-3 human ovarian cancer cells compared to plain drug; not selective to malignant cells		Luo et al. (2012)
Kaempferol	Polymeric nanoparticles	PLGA	210 ± 40		0.1 ± 3.4			Significant inhibition of A2780/CP70 and OVCAR-3 human ovarian cancer cells compared to plain drug; selective to malignant cells		Luo et al. (2012)

(*Continued*)

Table 12.1 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Flavonols (*cont.*)

			Physicochemical Properties*					Anticancer Effects		
Flavonol	**Nanocarrier**	**Formulation**	**Mean Size, nm**	**PDI**	**ζ Potential, mV**	**EE, %**	**DL, %**	**In vitro**	**In vivo**	**Reference**
Kaempferol	Polymeric nanoparticles	PLGA-PEI	220 ± 50		34.2 ± 7.9			No significant reduction in viability of A2780/CP70 and OVCAR-3 human ovarian cancer cells compared to plain drug		Luo et al. (2012)
Kaempferol	Polymeric nanoparticles	Chitosan	230 ± 70		11.7 ± 5.9			No significant reduction in viability of A2780/CP70 and OVCAR-3 human ovarian cancer cells compared to plain drug		Luo et al. (2012)
Kaempferol	Polymeric nanoparticles	PAMAM	250 ± 70		37.2 ± 8.3			No significant reduction in viability of A2780/CP70 and OVCAR-3 human ovarian cancer cells compared to plain drug		Luo et al. (2012)
Morin	Metal nanoparticles	Gold	48 (by DLS); 20 ± 6 (by TEM)		−20.8			Increased cytotoxicity in MCF-7 human breast cancer cells compared to plain drug; induction of apoptosis and necrosis and cell cycle arrest at G2/M phase		Kondath et al. (2014)

* Bz, benzyl; DEN, diethylnitrosamine; DL, drug loading; DLS, dynamic light scattering; DMBA, 7,12-dimethylbenz(a)anthracene; DMPC, dimyristoylphosphatidylcholine; DODA, dioctadecyldimethylammonium chloride; DOPC, l,2-dioleoyl-sn-glycero-3-phoshocholine; EE, encapsulation efficiency; GLY, glycine; MPEG, monomethyl PEG; OCL, oligo(ε-caprolactone); Np, naphthyl; P90G, phospholipon 90G; PAMAM, poly(amidoamine); PCL, poly(ε-caprolactone); PDI, polydispersity index; PE, phosphatidylethanolamine; PEG, poly(ethylene glycol); PEI, polyethyleneimine; PEO, poly(ethylene oxide); PLA, polylactide; PLGA, poly(lactide-*co*-glycolide); PPO, poly(propylene oxide); TEM, transmission electron microscopy.

Table 12.2 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Flavanols

			Physicochemical Properties*					Anticancer Effects		
Flavanol	Nanocarrier	Formulation	Mean Size, nm	PDI	ζ Potential, mV	EE, %	DL, %	In vitro	In vivo	Reference
Tea polyphenols	Polymeric nanoparticles	Chitosan				83	16	Relatively weak inhibitory effect in HepG2 human hepatoma cells compared to plain tea polyphenols; induction of apoptosis		Liang et al. (2014)
Catechin	Metal nanoparticles	Dextran-coated iron oxide (Endorem)	105		−13.9			Enhancement of apoptosis in MIA PaCa-2 human pancreatic cancer cells placed under magnetic field		Vittorio et al. (2014)
EGCG	Liposomes	Phosphatidyl-choline, cholesterol; coated by chitosan	85.0 ± 6.6	0.35 ± 0.02	16.4 ± 2.8	∼90	∼3	Significant decrease in viability of MCF-7 human breast cancer cells as compared to plain drug; induction of apoptosis		Castillo et al. (2013)
EGCG	Liposomes	Egg phosphatidyl-choline, cholesterol						Cytotoxicity in BCC-1/KMC human basal cell carcinoma, B16-F0 mouse melanoma and HT-29 human colon cancer cells		Fang et al. (2006)

(*Continued*)

Table 12.2 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Flavanols (*cont.*)

			Physicochemical Properties*					Anticancer Effects		
Flavanol	**Nanocarrier**	**Formulation**	**Mean Size, nm**	**PDI**	**ζ Potential, mV**	**EE, %**	**DL, %**	**In vitro**	**In vivo**	**Reference**
EGCG	Trensferrin-bearing vesicles		294	0.414	−36			Improved therapeutic efficacy in A431 human epidermoid carcinoma, T98G human glioblastoma, and B16-F10 mouse melanoma cells compared to plain drug	Tumor regression in BALB/c mice subcutaneously implanted with A431 andB16-F10 cells; improving animal survival	Lemarie et al. (2013)
EGCG	Polymeric nanoparticles	PLA-PEG						Over 10-fold dose advantage in antiproliferation in PC3 human prostate cancer cells as compared to plain drug (IC_{50} 3.74 and 43.6 μM, respectively); induction of apoptosis, antiangiogenic effect	Significant decrease in tumor volume in athymic mice injected with 22Rvl cells as compared to plain drug; inhibition of serum PSA	Siddiqui et al. (2009)
EGCG	Polymeric nanoparticles	PLGA-PEG	77.18 ± 16.3			6.18 ± 0.9		Antiproliferative activity in LNCaP human prostate cancer cells		Sanna et al. (2011)

EGCG	Polymeric nanoparticles	PLGA-PEG-DCL (targeting PSMA)	80.53 ± 15.0			9.61 ± 0.7		Improved antiproliferative activity in LNCaP human prostate cancer cells as compared to nontargeted nanoparticles		Sanna et al. (2011)
EGCG	Polymeric nanoparticles	PLGA				~26		Over 20-fold dose advantage in anticancer effects in A549 human lung carcinoma, HeLa human cervical carcinoma and THP-1 human acute monocytic leukemia cells as compared to plain drug		Singh et al. (2011)
EGCG	Polymeric nanoparticles	Carbohydrate matrix of gum arabic and maltodextrin	120 ± 28	0.45 ± 0.23	−12.3 ± 0.8	85 ± 3		Reduction of cell viability, induction of apoptosis in Du145 human prostate cancer cells		Rocha et al. (2011)
EGCG	Polymeric nanoparticles	Chitosan	~150...200				~10		Significant inhibition of tumor growth and serum PSA levels in athymic nude mice subcutaneously implanted with 22Rv1 human prostate cancer cells as compared to plain drug; induction of apoptosis, antiangiogenic effect	Khan et al. (2014)

(*Continued*)

Table 12.2 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Flavanols (*cont.*)

			Physicochemical Properties*					Anticancer Effects		
Flavanol	**Nanocarrier**	**Formulation**	**Mean Size, nm**	**PDI**	**ζ Potential, mV**	**EE, %**	**DL, %**	**In vitro**	**In vivo**	**Reference**
EGCG	Micelles	Casein						Comparative antiproliferation in cancerous transformed counterpart of 4D/WT rat colon epithelial cells as compared to plain drug (IC_{50} 0.02 and 0.012 mg/mL, respectively)		Haratifar et al. (2014a)
EGCG	Micelles	Casein						Comparative decrease in proliferation of HT29 human colon cancer cells as compared to plain drug (IC_{50} 0.02 and 0.01 mg/mL, respectively)		Haratifar et al. (2014b)
EGCG	Metal nanoparticles	Gold	~50		−8			Significant increase in cytotoxicity in MBT-2 mouse bladder carcinoma cells as compared to plain drug; induction of apoptosis	Reduction in tumor volume in C3H/He mice subcutaneously injected with MBT-2 cells; antiangiogenic effect	Hsieh et al. (2012)

EGCG	Metal nanoparticles	Gold			Significant reduction in number of MBT-2 mouse bladder cancer cells as compared to plain drug; induction of apoptosis, cell cycle arrest in G0/G1 phase	Reduction in tumor volume in C3H/HeN mice subcutaneously implanted with MBT-2 cells; antiangiogenic effect, boosting antitumor immunity	Hsieh et al. (2011)
EGCG	Metal nanoparticles	Gold	64.7	−3.36	4.91 times higher cytotoxicity in B16F10 murine melanoma cells as compared to plain drug; induction of apoptosis	Reduction of tumor volume in C57/BL6 mice subcutaneously injected with B16F10 cells	Chen et al. (2014)
EGCG	Metal nanoparticles	Radioactive gold, ^{198}Au				Reduction of tumor volume in SCID mice bearing PC-3 human prostate cancers; selective binding to Laminin67R receptor	Shukla et al. (2012)

* DCL, N-[N-[(S)-1,3-dicarboxypropyl]carbamoyl]-(S)-lysine; PEG, poly(ethylene glycol); PLA, polylactide; PLGA, poly(lactide-*co*-glycolide); PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen.

Table 12.3 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Flavones

			Physicochemical properties*					Anticancer effects		
Flavone	Nano-carrier	Formu-lation	Mean size, nm	PDI	ζ potential, mV	EE, %	DL, %	In vitro	In vivo	Reference
Apigenin	Polymeric nanopar-ticles	PLGA	100.2 ± 0.005	0.262	−12.2	87.7		Stronger antiproliferative potential in A375 human skin melanoma cells as compared to plain drug (IC_{50} 15 and 25 μM, respectively); induction of apoptosis		Das et al. (2013b)
Apigenin	Polymeric nanopar-ticles	PLGA	101.3 ± 0.004	0.258	−12.1 ± 0.001	87.2			Enhanced anticancer effect against UVB and BaP induced skin tumor in Swiss albino mice as compared to plain drug; induction of apoptosis	Das et al. (2013a)
Apigenin	Polymeric micelles	Plururonic P123, Solutol HS 15	16.9	0.046	−5.87	96.36	16.9	Higher cytotoxicity in HepG2 human hepatoma and MCF-7 human breast cancer cells as compared to plain drug (IC_{50} 5.57 and 3.75 μg/mL for nanoencapsulated drug and 20.19 and 16.62 μg/mL for free drug, respectively)		Zhai et al. (2013)

Apigenin	Micelles	Phospholipid, TPGS	137.1 ± 3.4	0.12	−12.94	87.35	12.6	More efficient cytotoxicity in A549 human lung cancer cells as compared to plain drug	Effective tumor inhibition in BALB/cA-nu mice injected with S180 murine carcinoma cells	Munyendo et al. (2013)
Luteolin	Polymeric nanoparticles	PLA-PEG	115					Comparative growth inhibition of H292 human lung cancer and Tu212 human head and neck carcinoma cells as compared to plain drug (IC_{50} 14.96 and 4.13 μM for nanoencapsulated drug and 15.56 and 6.96 μM for free drug, respectively)	Significant inhibition of tumor growth in athymic nude mice subcutaneously injected with Tu212 cells as compared to plain drug	Majumdar et al. (2014)
Luteolin	Polymeric nanoparticles	MPEG-PCL	38.6 ± 0.6	0.16 ± 0.02	−3.54 ± 0.32	98.32 ± 1.12	3.93 ± 0.25	Stronger cytotoxicity in 4T1 mouse breast cancer and C-26 mouse colon carcinoma cells as compared to plain drug (IC_{50} 6.4 μg/mL and 12.62 μg/mL for nanoencapsulated drug and 10.21 μg/mL and 13.06 μg/mL for free drug, respectively)		Qiu et al. (2013)
Nobiletin	Polymeric nanoparticles	Chitosan	∼500			69.1	7.0	More pronounced anticancer activity in RAW 264.7 murine leukemia cells as compared to plain drug (IC_{50} 8.3 μg/mL and >80 μg/mL, respectively)		Luque-Alcaraz et al. (2012)

* BaP, benzo[a]pyrene; MPEG, monomethyl PEG; PCL, poly(ε-caprolactone); PEG, poly(ethylene glycol); PLA, polylactide; PLGA, poly(lactide-*co*-glycolide); TPGS, D-α-tocopherol acid polyethylene glycol 1000 succinate; UVB, ultraviolet B.

Table 12.4 Physicochemical Characterization and Anticancer Activity of Nanoencapsulated Formulations of Other Types of Flavonoids

			Physicochemical Properties*					Anticancer Effects		
Flavonoid	**Nanocarrier**	**Formulation**	**Mean size, nm**	**PDI**	**ζ potential, mV**	**EE, %**	**DL, %**	**In vitro**	**In vivo**	**Reference**
Naringenin (flavanone)	Polymeric nanoparticles	Eudragit E: PVA	~90 (by DLS); <50 (by TEM)					Higher cytotoxic efficacy in HeLa human cervical cancer cells as compared to plain drug; induction of apoptosis		Krishnakumar et al. (2011)
Naringenin (flavanone)	Polymeric nanoparticles	Eudragit E: PVA	~90 (by DLS); <50 (by TEM)			88 ± 2.7			More efficient antitumor effect than plain drug by preventing oral carcinogenesis in DMBA-treated golden Syrian hamsters	Sulfikkarali et al. (2013)
Genistein (isoflavone)	Liposomes	HSPC/ cholesterol/ DOPE	161 ± 6	0.116	−22.6 ± 2.1			5–7-fold higher anticancer activities in 4T1 mouse breast cancer, PC-3 human prostate cancer and OVCAR-3 human ovarian cancer cells as compared to plain drug (IC_{50} 34.1, 22.7 and 28.3 μM for nanoencapsulated drug and 167.2 μM, 144.3 and 169.5 μM for free drug, respectively); induction of apoptosis		Phan et al. (2013)

Genistein (isoflavone)	Metal nanoparti-cles coated by polymer	Magnetite coated by CMCH	~11		9.8	Significantly enhanced growth inhibition of SGC-7901 human gastric cancer cells as compared to plain drug; induction of apoptosis		Si et al. (2010)
Puerarin (isoflavone)	Nanosus-pension	Lecithin/ HPMC	~400...500	~0.20		Significant increase in antiproliferation of HT-29 human colon cancer cells as compared to plain drug; induction of apoptosis	Higher anticancer efficacy in BALB/c nude mice inoculated with HT-29 cells as compared to plain drug	Wang et al. (2013e)

* CMCH, carboxymethylated chitosan; DLS, dynamic light scattering; DMBA, 7,12-dimethyl benz(a)anthracene; DOPE, dioleyl phosphatidylethanolamine; HPMC, hydroxypropyl methylcellulose; HSPC, hydrogenated soy phosphatidylcholine; PVA, polyvinyl alcohol; TEM, transmission electron microscopy.

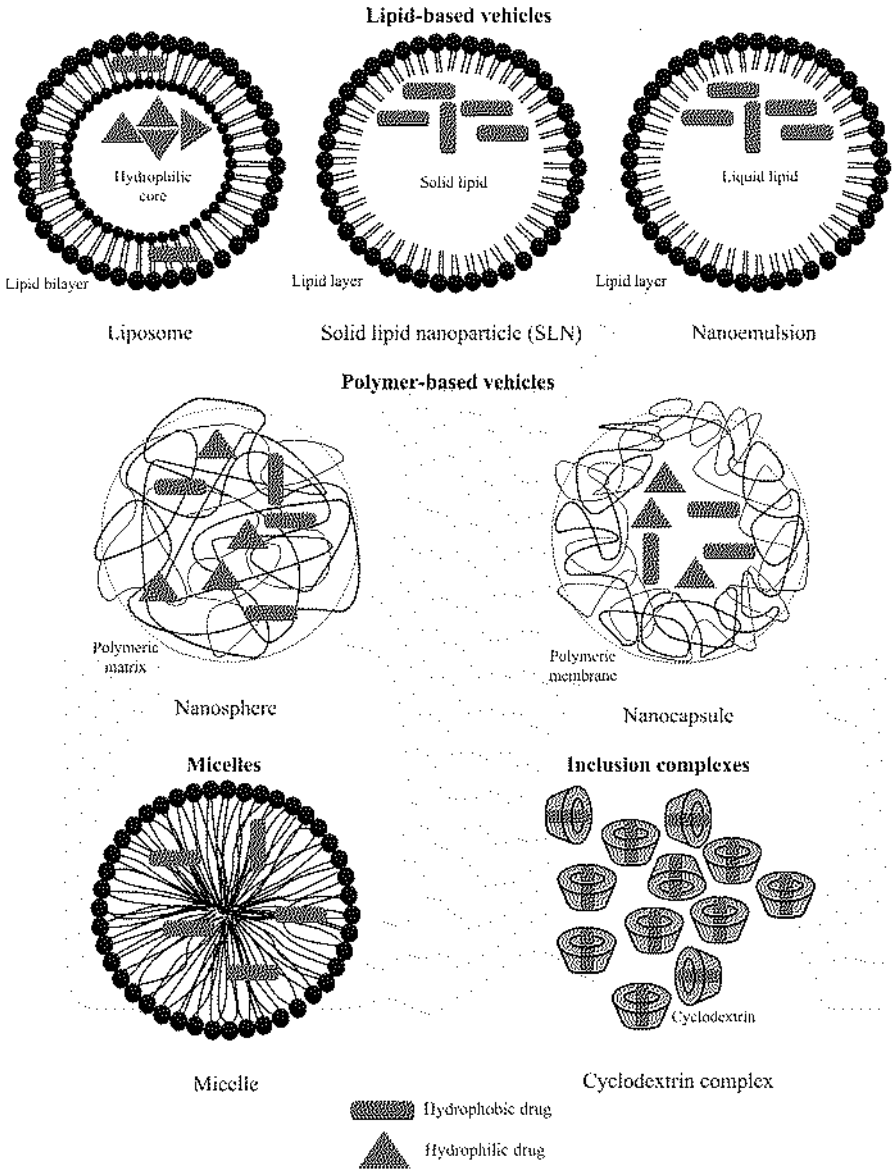

Figure 12.2. Representative schema of nanovehicles transporting flavonoids.

Liposomes represent the first platform employed to drug delivery being currently the most widely studied nanosized vehicle system used to carry flavonoids (Leonarduzzi et al., 2010; Muqbil et al., 2011). These small spherical and concentric vesicles consist of an aqueous core surrounded by a membranous lipid bilayer and are obtained either from naturally occurring phospholipids

or from synthetic mimetic counterparts (Cai et al., 2013; Jeetah et al., 2014; Leonarduzzi et al., 2010; Men et al., 2014; Mignet et al., 2012, 2013; Rodrigues et al., 2013; Santos et al., 2013; Seguin et al., 2013; Wang et al., 2014a; Wong and Chiu, 2011). By virtue of their biphasic character, liposomes can entrap and deliver both hydrophilic (in the central aqueous cavity) as well as hydrophobic (within the lipid bilayers) agents (Castillo et al., 2013; Leonarduzzi et al., 2010; Men et al., 2014; Mignet et al., 2012; Mignet et al., 2013; Muqbil et al., 2011; Rodrigues et al., 2013; Santos et al., 2013; Wang et al., 2014a; Wong and Chiu, 2010). As a result of the EPR effect, these systems can preferentially accumulate at tumor sites (Cai et al., 2013; Seguin et al., 2013; Wang et al., 2014a; Wong and Chiu, 2011; Yuan et al., 2006). Although nanoliposomes can be administered orally, parenterally, intravenously, topically, or nasally, an essential disadvantage of these vesicles is their relatively fast elimination from the bloodstream. Specially, they are recognized in the circulation as foreign particles and rapidly cleared by the cells of reticuloendothelial system (RES; Goniotaki et al., 2004; Leonarduzzi et al., 2010; Men et al., 2014; Wang et al., 2014a). To diminish this drawback, liposomes can be modified by coating their surface with inert and biocompatible polymers, such as polyethylene glycol (PEG), to form a sterically stabilized protective corona, impeding their recognition by opsonins and achieving long-term circulating systems with sustained release of encapsulated drug (Leonarduzzi et al., 2010; Mignet et al., 2013; Phan et al., 2013; Wang et al., 2014a; Yuan et al., 2006). Indeed, liposomes are highly flexible, their composition can be tuned and adapted to the respective drug, and their surface can be modified to improve the targeting properties (Mignet et al., 2013).

Solid lipid nanoparticles (SLNs) possess a solid hydrophobic matrix that is surrounded by a lipid monolayer (Cai et al., 2013; Leonarduzzi et al., 2010; Santos et al., 2013; Wang et al., 2014a). These vehicles can be administered via multiple ways, by oral, intravenous, parenteral, pulmonary, transdermal, and ocular routes (Cai et al., 2013; Leonarduzzi et al., 2010). SLNs are widely used and offer increased stability compared to liposomes; however, some limitations also exist, including the drug leakage during storage (Jeetah et al., 2014; Leonarduzzi et al., 2010; Wang et al., 2014a; Zhang et al., 2013).

Nanoemulsions represent fine dispersions of liquid droplets with the poorly soluble drugs absorbed in the oil core and stabilized by surfactants (Rodrigues et al., 2013; Wang et al., 2013e; Wang et al., 2014a; Wang et al., 2014b). Intravenous injection of these colloidal delivery systems is becoming a promising approach (Men et al., 2014; Zheng et al., 2011).

5.2 Polymer-Based Nanocarrier Systems

Polymeric nanoparticles can be composed of natural or synthetic polymers (Leonarduzzi et al., 2010; Men et al., 2014; Rodrigues et al., 2013). Depending on the shape and organization of these particles, they are divided to nanocapsules and nanospheres (Fig. 12.2; Leonarduzzi et al., 2010). In the case of nanocapsules, the drug is confined in a core surrounded by a polymeric membranous shell (El-Gogary et al., 2014; Jeetah et al., 2014; Leonarduzzi et al., 2010). On the contrary, nanospheres are porous polymeric matrices in which the drug is uniformly dispersed (Jeetah et al., 2014; Leonarduzzi et al., 2010). Different polymeric materials are utilized to encapsulate flavonoids possessing high biocompatibility, biodegradability, and stability, and allowing also modifications of surface properties and functionality (Cai et al., 2013; El-Gogary et al., 2014; Gao et al., 2012; Khushnud and Mousa, 2013; Leonarduzzi et al., 2010; Men et al., 2014; Siddiqui and Mukhtar, 2010; Wang et al., 2013c; Wang et al., 2014a). The most extensively used synthetic polymers for encapsulation of polyphenols are poly(lactic acid) (PLA), poly(glycolic acid) (PGA), their copolymer poly(lactic-*co*-glycolide) (PLGA), and poly(ε-caprolactone) (PCL); whereas widely employed natural polymers include chitosan, starch, alginate, gelatin, and albumin (Das et al., 2013b; Gao et al., 2012; Jain et al., 2013a; Khan et al., 2014; Khushnud and Mousa, 2013; Leonarduzzi et al., 2010; Liang et al., 2014; Luo et al., 2012; Luque-Alcaraz et al., 2012; Majumdar et al., 2014; Men et al., 2014; Pooja et al., 2014; Qiu et al., 2013; Rodrigues et al., 2013; Siddiqui et al., 2010; Siddiqui and Mukhtar, 2010; Tabrez et al., 2013; Wang et al., 2014a; Yang et al., 2014). However, besides many advantages of polymeric nanoparticles, including the possibility to load large amounts of bioactive agents, the ability to control the time and rate of polymer degradation and sustained release of incorporated drug, these nanoparticles possess also some drawbacks, such as being rapidly removed from blood by phagocytic system (Das et al., 2013b; El-Gogary et al., 2014; Leonarduzzi et al., 2010; Luo et al., 2012; Men et al., 2014; Pimple et al., 2012; Siddiqui et al., 2009; Siddiqui et al., 2010; Siddiqui and Mukhtar, 2010; Tabrez et al., 2013; Wang et al., 2013c). This problem can be countered by coating the vehicles with protective moieties, such as PEG, reducing thus the opsonization as well as extending the circulation time of nanoparticles (Bharali et al., 2011; Leonarduzzi et al., 2010; Siddiqui et al., 2009; Tabrez et al., 2013). Moreover, due to their appropriate size and because of the EPR effect, polymeric nanoparticles can efficiently accumulate at the targeted tumor sites in the body, enhancing thus the anticancer efficacy of encapsulated flavonoids (El-Gogary et al., 2014;

Krishnakumar et al., 2011; Sulfikkarali et al., 2013). The two main administration ways of polymeric nanoparticles are the oral and the intravenous routes (Leonarduzzi et al., 2010).

Among the various polymer-based nanosized carriers, micelles have gained a lot of attention showing a great potential in delivering anticancer agents (Men et al., 2014). These particles consist of hydrophobic inner core serving as a carrier for water-insoluble phytochemicals and hydrophilic corona, forming thus a core-shell architecture (Fig. 12.2; Chen et al., 2015; Khoee and Rahmatolahzadeh, 2012; Jeetah et al., 2014; Khonkarn et al., 2011; Men et al., 2014; Muqbil et al., 2011; Wang et al., 2014a; Wu et al., 2013; Zhai et al., 2013). Improved chemopreventive and chemotherapeutic activities can be achieved by sustained release of incorporated bioactive agents, passive targeting to tumor sites as a result of the EPR effect, and through the prevention of being recognized by RES (Chen et al., 2015; Munyendo et al., 2013; Muqbil et al., 2011; Wang et al., 2014a; Wu et al., 2013; Zhai et al., 2013). Micelles can be administered by various routes, including oral, parenteral, nasal, topical, and ocular applications (Wang et al., 2014a).

5.3 Biophysicochemical Properties Impacting Nanosized Drug Delivery Systems

The functional performance and therapeutic potential of nanosized drug delivery systems are primarily determined by their physicochemical characteristics, such as size, shape, physical state, and surface properties (Das et al., 2013a; Gohulkumar et al., 2014; Leonarduzzi et al., 2010; Sanna et al., 2011; Santos et al., 2013; Tabrez et al., 2013; Wang et al., 2014a; Winter et al., 2014). Summary of these features for nanoencapsulated flavonoids used for anticancer studies is presented in Tables 12.1–12.4.

Nanoparticle size is a fundamental parameter that directly influences physical stability, drug loading, cellular uptake and drug release, but determines also biodistribution and biological fate of circulating particles (Das et al., 2013a; Gohulkumar et al., 2014; Leonarduzzi et al., 2010; Tabrez et al., 2013; Wang et al., 2014a). In general, smaller size is preferable to increase the delivery of incorporated agents into tumor sites by passive targeting via the EPR effect; however, too small nanoparticles can also easily move out from the target tissue and rapidly leak into blood capillaries (Hsieh et al., 2012; Gohulkumar et al., 2014; Pimple et al., 2012; Wang et al., 2014a). The range of tumor vascular gap junction size depends on certain cancer type and therefore can vary (Hsieh et al., 2012; Wang et al., 2013e). At the same time, nanostructure systems should be small enough (generally <200 nm) to

surpass the capture by phagocytes of the RES (Castillo et al., 2013; Leonarduzzi et al., 2010; Pimple et al., 2012; Santos et al., 2013; Shukla et al., 2012; Wang et al., 2013d; Zhai et al., 2013). Different methods are used to determine the nanoparticle dimensions, whereas transmission electron microscopy (TEM) measures the size of dry particles and gives usually smaller values than dynamic light scattering (DLS) that detects the hydrodynamic diameter of particles in hydrated state (Gao et al., 2012; Krishnakumar et al., 2011; Kumar et al., 2014; Pimple et al., 2012; Munyendo et al., 2013; Sulfikkarali et al., 2013; Wang et al., 2013c).

The polydispersity index (PDI) reveals the nanoparticle size distribution and allows to prove the homogeneity of formulations (El-Gogary et al., 2014; Wang et al., 2014a; Winter et al., 2014).

Superficial charge or zeta potential reflects the electrical potential of nanovehicles being an important parameter for physical stability (Hu et al., 2012; Gohulkumar et al., 2014; Leonarduzzi et al., 2010; Pimple et al., 2012; Pooja et al., 2014; Wang et al., 2014a). As the surface charge prevents the particles aggregation, the more negative or more positive values point to the higher stability (Hsieh et al., 2012; Leonarduzzi et al., 2010; Pimple et al., 2012). Moreover, negative zeta potentials are generally considered advantageous for prolonging the circulation time and intensifying drug delivery, due to minimization of nonspecific interactions with negatively charged cellular membranes through electrostatic forces (Lemarie et al., 2013; Pimple et al., 2012; Zhai et al., 2013).

For therapeutic efficacy, it is crucial that a large amount of compounds is entrapped in nanoparticles (Sulfikkarali et al., 2013). High encapsulation efficiency (defined as the ratio of incorporated drug mass to total drug mass) proves that the agent is well incorporated and is shown to be substantially dependent on flavonoid structure (Gohulkumar et al., 2014; Goniotaki et al., 2004; Wang et al., 2014a; Xu et al., 2013). Drug loading capacity is estimated by dividing the mass of incorporated agent by the mass of nanoparticles (Wang et al., 2014a).

Encapsulated compounds are typically released from nanocarriers by biphasic profile involving the initial burst release followed by a steady and slower release (Bothiraja et al., 2014; Das et al., 2013b; Krishnakumar et al., 2011; Kumar et al., 2014; Wang et al., 2013c). The first burst is probably related to the rapid release of drug absorbed on the surface layer of nanoparticles, while the continued release can be attributed to the diffusion of the entrapped drug from inner core of nanostructures as well as the degradation of nanoparticulate matrices (Bothiraja et al., 2014; Das et al., 2013b; Khan et al., 2014; Krishnakumar et al., 2011; Qiu et al., 2013; Wang et al., 2013c; Yang et al., 2014; Zhai et al., 2013).

Such a sustained release is important for maintaining the therapeutic concentrations in plasma for a longer period allowing the continuous exposure of tumor cells to flavonoids and enhancement of potential chemopreventive and chemotherapeutic activities (Chen et al., 2015; Date et al., 2011; Gohulkumar et al., 2014; Krishnakumar et al., 2011).

Despite the intensive research and fast progress done in nanoencapsulation of therapeutically interesting agents in recent years, it is clear that nanomedicine is a very new discipline and still in its infancy, possessing many technical and practical limitations and challenges (Wang et al., 2014a). Indeed, nanoparticles can provoke also some negative effects, such as blood cell toxicity, and therefore a systemic approach to study the general safety is certainly required before moving to clinical applications (Bharali et al., 2011; Chen et al., 2014; Tabrez et al., 2013; Wang et al., 2013d; Wang et al., 2014a). It is clear that pharmacokinetic and pharmacodynamic profiles of nanoencapsulated phytochemicals should be thoroughly addressed to avoid any adverse reactions (Wang et al., 2014a). Also, the fate of nanocarrier materials *in vivo* systems needs further clarification (Cai et al., 2013; Tabrez et al., 2013). For instance, the use of cyclodextrin is reported to be associated with the risk of nephrotoxicity (Cai et al., 2013; El-Gogary et al., 2014; Han et al., 2012). Other shortcomings and limitations may include the difficulties in long-term storage and administration of some nanostructure systems; potential damage to DNA after possible entry to cellular nucleus; induction of changes in oxidative balance as well as inflammatory and immune responses; but also the high cost of preparation of nanosized drug delivery systems (Cai et al., 2013; El-Gogary et al., 2014; Han et al., 2012; Leonarduzzi et al., 2010; Mignet et al., 2013; Wang et al., 2014a).

6 Enhanced Anticancer Efficacy of Nanoencapsulated Flavonoids

Over the past 10 years, several nanoformulations have been prepared for incorporation of different flavonoids in nanosized vehicles and testing their anticancer properties in vitro systems and animal (rodent) models. The results of these experiments are summarized for flavonols (quercetin, fisetin, kaempferol, morin) in Table 12.1, for flavanol EGCG in Table 12.2, for flavones (apigenin, luteolin, nobiletin) in Table 12.3, and for some other types of flavonoids (flavanone naringenin, isoflavones genistein and puerarin) in Table 12.4. Compiling the greater part of the respective studies, the current work can be considered as the first

comprehensive overview on the use of nanotechnological strategies for improvement of anticancer properties of flavonoids. These data are promising and show a significant potential for further applications in clinical settings providing new treatment approaches for cancer patients suffering from different tumors, but widening also the possibilities for chemoprevention.

In general, in vitro studies of nanoencapsulated flavonoids in different human and murine malignant cell lines (derived from prostate, breast, cervix, ovary, bladder, colon, stomach, lung, head and neck, brain, and skin cancers as well as leukemia and melanoma) reveal an improved (or similar) antitumor efficacy compared to the respective nonformulated polyphenols. For instance, encapsulation of EGCG in PLA-PEG nanoparticles resulted in a more than 10-fold dose advantage for exhibiting cytotoxic, apoptotic, and antiangiogenic activities in several human prostate cancer cells (Rodrigues et al., 2013; Siddiqui et al., 2009, 2010; Siddiqui and Mukhtar, 2010; Wang et al., 2012a). Similarly, EGCG entrapped into PLGA polymeric nanoparticles revealed an over 20-fold dose advantage compared to its free counterpart in exerting anticancer effects in different human cancer lines (A549 lung carcinoma cells, HeLa cervical carcinoma cells, THP-1 acute monocytic leukemia cells; Singh et al., 2011). A superior antitumor activity was observed also in the case of liposomal formulation of genistein in respect to the plain isoflavone, with an approximately 5–7-fold decrease in cytotoxic parameters (IC_{50} values) in human and murine cancer cell lines (Phan et al., 2013). These and other data characterizing anticarcinogenic efficacy of nanoencapsulated flavonoids are presented in detail in Tables 12.1–12.4. As an exception, some reduction in growth inhibitory activity of liposomal quercetin in three human cancer lines (MCF-7 breast cancer cells, H460 non-small cell lung cancer cells, SF268 central nervous system cells) was described; however, this adverse effect could be related to the large size of liposomes (>400 nm) used in the respective work (El-Gogary et al., 2014; Goniotaki et al., 2004).

The experiments with tumor xenograft models of mice (but in a few cases also in rats and hamsters) typically confirm the enhanced anticancer efficacy of nanoencapsulated flavonoids revealing stronger suppression of tumor growth and lower tumor volumes, decrease in the number of malignant nodules, delay in metastasis and improvement of survival time (Chen et al., 2014; Chen et al., 2015; Das et al., 2013a; Date et al., 2011; Gao et al., 2012; Hsieh et al., 2011, 2012; Jain et al., 2013b; Kale et al., 2006; Long et al., 2013; Majumdar et al., 2014; Munyendo et al., 2013; Ragelle et al., 2012; Seguin et al., 2013; Siddiqui et al., 2009; Shukla et al., 2012; Sulfikkarali et al., 2013; Wang et al., 2013e; Wu

et al., 2013; Yang et al., 2014; Yuan et al., 2006). Moreover, these nanoformulations are usually well tolerated by tumor-bearing animals revealing no significant weight loss (Lemarie et al., 2013; Long et al., 2013; Majumdar et al., 2014; Tan et al., 2012; Wang et al., 2013e; Yang et al., 2014).

Such increase in antiproliferative, apoptotic, antiangiogenic, antiinvasive, and antimetastatic activities by encapsulation of flavonoids into nanostructures can be explained by several factors. First, the aqueous solubility of these polyphenols can be significantly improved. For instance, incorporation of quercetin into nanomicelles made from PEG-derivatized phosphatidylethanolamine (PE) increased its aqueous solubility by 110-fold (Tan et al., 2012). Furthermore, micellar formulation of apigenin attained even up to 1,800-fold improvement in its solubility, that is, from 2.16 μg/mL for free apigenin to 3.9 mg/mL for respective nanoconjugate (Munyendo et al., 2013). Second, the circulation time in blood may be prolonged and thereby exposure of flavonoids to malignant tissues extended. Third, the alterations in biodistribution promote higher accumulation of plant secondary metabolites in tumor sites, allowing sustained release after facilitated cellular uptake (Bothiraja et al., 2014; Castillo et al., 2013; Chen et al., 2015; Das et al., 2013a,b; Guo et al., 2009; Guo et al., 2014; Fang et al., 2006; Han et al., 2012; Hsieh et al., 2012; Jain et al., 2013b; Kale et al., 2006; Khan et al., 2014; Long et al., 2013; Mignet et al., 2012; Phan et al., 2013; Ragelle et al., 2012; Seguin et al., 2013; Si et al., 2010; Siddiqui et al., 2009; Sulfikkarali et al., 2013; Sun et al., 2014; Tan et al., 2012; Wang et al., 2013a; Wang et al., 2012b; Wang et al., 2013c; Yang et al., 2014; Yuan et al., 2006). In addition, other possible mechanisms could also contribute to the increased anticancer activity of nanoencapsulated flavonoids including protection of polyphenols from enzymatic and degradative action in gastrointestinal tract and enhancing the intestinal absorption, or increased adhesion of nanostructures to intestinal mucosa (Bothiraja et al., 2014; Castillo et al., 2013; Fang et al., 2006; Khan et al., 2014; Munyendo et al., 2013; Tan et al., 2012). Furthermore, flavonoids entrapped into lipid-based nanovehicles can undergo also lymphatic distribution, and this is beneficial for access to lymph nodes that can often harbor metastases (Bothiraja et al., 2014; Seguin et al., 2013; Tan et al., 2012).

Altogether, flavonoids-loaded nanoparticles may exhibit enhanced anticancer properties both in vitro and in vivo, showing potential for further biomedical and clinical applications, both in chemoprevention as well as chemotherapy. It is obvious that significantly improved anticancer activities of nanoencapsulated bioactive agents provide evidence for dose reduction without

affecting therapeutic efficacy, diminishing thereby the possible toxicological risks (Cai et al., 2013; Kale et al., 2006).

6.1 Targeted Anticancer Therapy with Nanoencapsulated Flavonoids

Targeted delivery of nanoencapsulated flavonoids, specifically to the tumor cells, could further enhance the anticancer efficacy of phytochemicals, minimize potential adverse effects by preventing exposure to nontarget tissues, and reduce administration dose and frequency (El-Gogary et al., 2014; Khoee and Rahmatolahzadeh, 2012; Khushnud and Mousa, 2013; Men et al., 2014; Muqbil et al., 2011). For active targeting, certain moieties, including small peptides, antibodies, and receptor binding compounds, can be incorporated on the surface of nanoparticles without affecting the integrity of nanovehicles. These ligands can recognize certain molecular signatures unique to cancer cells increasing the cellular uptake and release of encapsulated drugs at specific sites (Khoee and Rahmatolahzadeh, 2012; Men et al., 2014; Muqbil et al., 2011; Rodrigues et al., 2013; Siddiqui and Mukhtar, 2010; Wang et al., 2014a). Moreover, active targeting can also be achieved by using specific carrier materials that are sensitive and responsive to surrounding conditions, such as temperature, pH, or magnetic field, whereas respective environmental stimuli can affect the release rate and tumoricidal efficacy of bioactive agents (Guo et al., 2014; Khoee and Rahmatolahzadeh, 2012; Kumar et al., 2014; Vittorio et al., 2014).

To date, several targeting drug delivery systems have been developed by conjugating nanoencapsulated flavonoids with specific ligands. It is well accepted that a wide variety of malignant cells vastly overexpress folate receptors (Chen and Chen, 2013; Khoee and Rahmatolahzadeh, 2012). Therefore, binding of folic acid to the surface of PLGA polymeric nanocapsules was shown to facilitate the selective uptake of targeted nanocarriers by folate receptor-enriched HeLa cells, improving thus the cytotoxic efficacy of quercetin (Table 12.1; El-Gogary et al., 2014). As a second example, encapsulation of EGCG into transferrin-bearing vesicles could result in a selective drug delivery to different types of cancer cells, which abundantly express transferrin receptors. The increased cellular uptake and improved therapeutic efficacy of EGCG was clearly demonstrated both in vitro cell lines (A431 human epidermoid carcinoma cells, T98G human glioblastoma cells, B16-F10 murine melanoma cells) as well as in established xenograft models in mice (Lemarie et al., 2013). Moreover, EGCG-loaded nanoparticle system, consisting of PLGA-PEG and

functionalized with ligands that target the prostate-specific membrane antigen (PSMA), greatly enhanced the binding to PSMA-expressing prostate cancer cells leading to a significant increase in antiproliferative activity in in vitro assays with LNCaP human prostate cancer cells. PSMA is a well-known transmembrane protein that is highly expressed in malignant prostatic epithelial cells and therefore this method could open new avenues for treatment of prostate cancer (Table 12.2; Rodrigues et al., 2013).

6.2 Coencapsulation of Flavonoids with Standard Chemotherapeutics

In principle, nanostructure systems can be designed to encapsulate single or multiple agents inside a single carrier; nevertheless, single loaded flavonoids are mostly investigated (Jeetah et al., 2014; Siddiqui et al., 2010). Very recently, a few examples about the dual loading with conventional chemotherapeutic drugs have been also described; however, tumoricidal activity of these nanoconstructs is still available only for flavonol quercetin (Table 12.1; Jeetah et al., 2014).

It is well accepted that combination therapy administering multiple drugs with different mechanisms has a crucial role in cancer chemotherapy to exert multitargeted action and achieve superior therapeutic effects (Narayanan et al., 2014). However, due to the differences in pharmacokinetic profiles, these drugs might not maintain the synergistic ratio after in vivo administration, while reaching tumor sites (Wong and Chiu, 2010; Wong and Chiu, 2011). Therefore, rationally designed drug delivery systems to transport desired combinations of different agents in regulated manner to the tumor tissues would be an appealing method. Several nanoformulations coencapsulating quercetin and conventional chemotherapeutic drugs have been engineered and characterized (Table 12.1). At that, liposomal constructs coincorporating quercetin and vincristine prolonged the plasma circulation time and showed a coordinated release of both agents exerting significant synergistic anticancer action both in vitro assays with human breast cancer cells, as well as in xenograft mouse model (Wong and Chiu, 2010, 2011). A superior cytotoxic activity in human lung adenocarcinoma cells was observed also by coencapsulating quercetin and etoposide into polymeric PLGA nanoparticles (Pimple et al., 2012). PLGA nanoparticles with quercetin and tamoxifen were also found to exhibit remarkably higher cytotoxicity than free compounds, either alone or in combination, in human breast cancer cells in both in vitro and in vivo systems (Jain et al., 2013a). Such coencapsulation strategy allows administering much lower

doses of chemotherapeutic drugs to attain similar tumoricidal activities compared to the monotherapeutic regimens and this advantage has a great clinical significance reducing the incidence of adverse side effects (Wong and Chiu, 2010; Wong and Chiu, 2011).

Taken together, coencapsulation of flavonoids and standard anticancer drugs could provide a significantly improved chemoresponse. However, there are still no reports available about the anticancer activities of nanoconstructs incorporating two different flavonoids, although these phytochemicals usually exist in various combinations in food and could also act synergistically.

7 Summary and Further Perspectives

Nowadays, in vitro anticancer action of flavonoids is widely acknowledged and well confirmed harboring no further doubts. However, attempts to translate these promising results to human systems have mostly defeated the expectations, due to the poor bioavailability, low intestinal absorption and extensive biotransformation of these plant seconday metabolites in humans. To overcome this disturbing bottleneck, technologically novel strategies for drug delivery are highly needed. Application of nanotechnological approach and encapsulation of flavonoids into structurally different nanosized systems have opened new avenues for implementation of anticancer potential of plant polyphenols in real life.

The concept of nanochemoprevention is already introduced to outline the application of nanotechnological methods for enhancing the effects of chemoprevention (Muqbil et al., 2011; Santos et al., 2013; Siddiqui et al., 2010; Siddiqui and Mukhtar, 2010). This discipline includes delivery of bioactive dietary agents entrapped into nanoconstructs to control carcinogenesis process in interfering with one or more of its stages, that is, initiation, promotion and progression phases, in order to keep cancer from forming, growing, or recurrence (Hu et al., 2012; Mignet et al., 2013). It is clear that oral consumption is the most convenient and acceptable route to deliver chemopreventive agents and therefore design and development of flavonoid-loaded nanostructured systems suitable for oral administration is crucial for further progress (Siddiqui et al., 2010; Siddiqui and Mukhtar, 2010; Wang et al., 2013d). Besides prevention, rationally engineered nanoformulations of flavonoids have attracted a great attention also for promising clinical applications in chemotherapy, whereas conjugation of these nanoconstructs with actively targeting ligands or coencapsulation of phytochemicals and conventional chemotherapeutic agents can further broaden our current prospects. As a consequence, nanoencapsulation and targeted delivery of

cytotoxically bioactive agents can lead not only to enhancement of anticancer efficacy but provide also some options to improve the life quality of patients and prolong their survival times.

It is clear that nanomedicine is in its infancy and much still needs to be done before achieving an ideal nanovehicle for delivery of flavonoids for chemopreventive and chemotherapeutic purposes, in preclinical and clinical settings. Cost-effectiveness and long-term safety are only a few aspects among many that need to be carefully addressed. Also, no human studies are yet reported for nanoencapsulated flavonoids and all current works are performed either by using established cell lines or experimental animals. Nevertheless, all these studies allow us to consider nanotechnology as a powerful novel approach to overcome the recent obstacles associated with the use of flavonoids, that is, their very low bioavailability.

In the future, intense laboratory research has to be continued to improve the composition and properties of phytochemical-loaded nanoformulations. In conjunction, human clinical trials are expected to be introduced to gain more perspective about the actual potential of nanoencapsulated flavonoids in the field of cancer control and management.

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13

IMPROVING BIOAVAILABILITY OF NUTRACEUTICALS BY NANOEMULSIFICATION

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1 Introduction to Nutraceuticals

1.1 Definitions

The term *nutraceutical* was coined by combining "nutrition" and "pharmaceutical" in 1989 by Dr. Stephen DeFelice, MD, founder and chairman of the Foundation for Innovation in Medicine (FIM), Cranford, New Jersey. DeFelice defined nutraceuticals as a wide variety of compounds which can be considered as a food or part of a food providing specific medical or health benefit including the prevention and treatment of disease or improving the physiological performance (Brower, 1998; Kalra, 2003).

Nutraceuticals include isolated nutrients, dietary supplements, and diets, as well as genetically engineered "designer" foods, herbal products, and processed products such as cereals, soups, and beverages (Andlauer and Fürst, 2002; Dureja et al., 2003). Several other terms like *functional foods*, *pharmaconutrients*, and *food of specified health use* (Foshu), *biochemopreventatives*, and *dietary integrators* are used synonymously in various countries (Krohn et al., 2008). However, in Japan, a new term, *physiologically functional foods*, was introduced for any food or ingredient with nutritional value that has additional beneficial effects on health, physical performance, or state of mind (Hardy, 2000). Lockwood (2007) further emphasized that nutraceuticals may be purified or concentrated, and used for improvement of health, by preventing or treating a disease. Kalra (2003) redefined functional food and nutraceuticals on the basis of using anaemia as the clear distinct

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separating feature. Functional food is the food being cooked, processed, or prepared using "scientific intelligence" with or without knowledge of how or why it is being used. Functional food thus provides the body with the required amount of vitamins, fats, proteins, carbohydrates, and so forth, needed for its healthy survival. When functional food aids in the prevention and/or treatment of disease(s) and/or disorder(s) other than anaemia, it is called a nutraceutical. Therefore, a functional food for one consumer can act as a nutraceutical for another consumer (Kalra, 2003).

The Dietary Supplement Health and Education Act of 1994 (DSHEA) of United States Food and Drug Administration (FDA) specifies dietary supplement as follows (http://www.fda.gov (a), Kalra, 2003):

- The product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients; like vitamin, mineral, a herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing the total dietary intake, or a concentrate, metabolite, constituent, extract, or combination of these ingredients.
- The product intended for ingestion in pill, capsule, tablet, or liquid form and is not represented for use as a conventional food or as the sole item of a meal or diet.
- The product is labelled as a "dietary supplement."
- Dietary supplement also includes products such as an approved new drug, certified antibiotic, or licensed biologic that was marketed as a dietary supplement or food before approval, certification, or license (unless the Secretary of Health and Human Services waives this provision).

1.2 Nutraceuticals and Their Impact on Health

With industrial revolution and modernization, the lifestyle and human diet have undergone fundamental changes. High-calorie junk food, sedentary lifestyle, and lack of exercise have led to higher incidences of diseases, including coronary heart disease, obesity, hypertension, type 2 diabetes, epithelial cell cancers, autoimmune diseases, and osteoporosis (Carrera-Bastos et al., 2011; Das et al., 2012; Magrone et al., 2013). These diseases are no longer limited to the developed countries, as millions of people in developing countries also are adopting a Westernized lifestyle and are being affected by these diseases. In recent years, a new diet health paradigm is evolving and giving more prominence to the positive aspects of diet. Due to longevity, these chronic age-related diseases, along with neurodegenerative diseases and several types

of cancer, known to be related to dietary habits, continue to expand. Epidemiological studies on the relationship between dietary habits and disease risk have shown that food has a direct impact on health (Espín et al., 2007). Therefore, usage of natural dietary supplements has steadily increased worldwide, since the past two decades. Dietary supplements are becoming more popular among the Western populations (Gurley, 2011). The nutritional components, supplements, and herbal products that provide therapeutic or physiological benefits beyond basic nutritional needs, have been regarded as an emerging method for preventing chronic diseases (Braithwaite et al., 2014). Apart from modern medical systems that target immediate relief of symptoms and diseases, diet regimens encourage a moderate long-term process for illness prevention or treatment. Being of natural origin as a nutritional source, nutraceuticals possess comparatively less toxicity and fewer side effects than drugs used to treat similar illnesses and their symptoms (Ting et al., 2014). Treatment of chronic diseases requires long-term therapy, and chronic use of synthetic drugs may cause severe irreversible adverse effects. The use of plant products as medicine for the safe treatment of various chronic diseases is supported by many traditional systems of medicine. WHO, in support of natural remedies, encourages use of traditional medicines of natural origin. In addition to plant products, marine biomaterials also contribute to nutraceuticals with a rich source of structurally diverse compounds with various biological activities (Zhang et al., 2012).

1.3 Nutraceuticals Market Share

The well-established relationship between dietary bioactive constituents and health promoting functionalities have made nutraceuticals a rapidly growing area of biomedical, food, and cosmetic, as well as pharmaceutical research (Andlauer and Fürst, 2002; Braithwaite et al., 2014). A decade back, in June/July 2002, Jim Wagner, editor of *Nutritional Outlook* predicted the bright future of nutraceuticals by saying that "Nutraceuticals are in their formative years. But make no mistake, the nutraceutical boom is coming and it will be worth billions to the companies who define it" (Wagner, 2002). Annual sales of the core nutraceuticals are approximately $150 billion, almost one fifth of the size of the global pharmaceutical industry turnover (Kearney, 2014). The market size of allied nutraceutical products like infant nutrition, food intolerance products, diabetes control, medical nutrition, and weight management solutions, is about $420 billion with an expected 7% rise rate for the next few years (Fig. 13.1; adapted from Kearney, 2014).

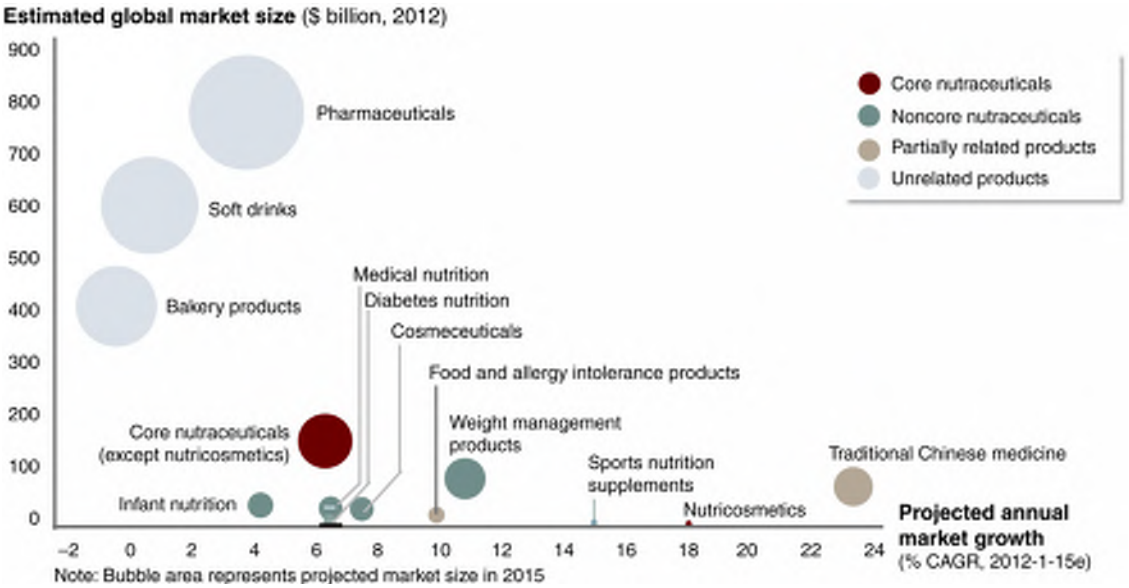

Figure 13.1. Nutraceutical market size and projected growth. Reprinted with permission from Kearney (2014).

2 Categorizing Nutraceuticals

2.1 Nutraceuticals Classification Based on Therapeutic Constituent

Nutraceuticals can be classified in various classes based on the therapeutically important constituent responsible for the health benefit (Table 13.1).

Nutraceuticals are also classified broadly as either potential nutraceuticals or established nutraceuticals (Pandey et al., 2010). A potential nutraceutical becomes an established one only after demonstrating beneficial health effects clinically. Many nutraceutical products are still in the developmental stage and are therefore regarded as potential nutraceuticals.

It is well understood that suitable biopharmaceutical properties of a drug substance are pivotal for successful development into a formulation (Kuentz, 2012). Taking into consideration this fact, Amidon et al. (1995) developed a classification system for drugs, designated as Biopharmaceutic Classification System (BCS; Fig. 13.2).

The BCS correlates in vitro drug product dissolution and in vivo bioavailability considering that drug dissolution and gastrointestinal permeability are the fundamental parameters controlling rate and extent of drug absorption. This classification is based

Table 13.1 Classification of Nutraceuticals Based on the Nutritive Constituent

Nutraceutical	Examples
Micronutrients	1. Vitamins[a]: antioxidative vitamins like Vitamin C, E, and carotenoids 2. Microminerals/trace elements[a]: iron, cobalt, selenium, chromium, phosphorous, seaweed[b] (Rich source of iron and iodine)
Polyunsaturated fatty acids[a,c] (ω-3 and ω-6 fatty acids)	Linseed oil contains α-linolenic acid (ω-3 fatty acid). Evening primrose oil contains γ-linoleic acid (ω-6 fatty acid).
Natural products[c] Phytochemicals and marine products	1. Phytochemicals: Flavanol (quercetin), flavone (apigenin), flavanone (naringenin), isoflavone (genistein), anthocynidines, phytosterol, carotenoids, and so forth. 2. Marine[d,e]: Lectin, bioactive peptides, phorotannins, chitooligosaccharide derivatives, polyphenols, carotenoids, prebiotics and sulfated polysaccharides.
Proteins/peptides/amino acids[a]	Lectin, milk protein, soyprotein hydrolysate
Dietary fiber[a,c]	Nonstarch polysaccharides like gums and pectins, lignin, celluloses, hemicelluloses, resistant dextrins and resistant starches, mushrooms[f]
Prebiotics[c]	Live microbial feed like 1. *Lactobacilli*, such as L. acidophilus, L. brevis, L. cellobiosus. 2. Gram-positive cocci such as *Lactococcus lactis*, *Enterococcus faecium* 3. Bifidobacteria such as *B. bifidun, B. adolescentis, B. infantis, B. longum*
Probiotics[c]	Fructo-oligosaccharides present in banana, tomato, chicory roots
Spices[c,g]	Garlic, onion, red paper/capsaicin, turmeric/curcumin, ginger/gingerol, clove/eugenol, pepper/piperine

[a] Andlauer and Fürst (2002)
[b] Mišurcová et al. (2011)
[c] Das et al. (2012)
[d] Vidanarachchi et al. (2012a)
[e] Zhang et al. (2012)
[f] Cheung (2013)
[g] Srinivasan (2005)

on primary physicochemical and biological properties, that is, solubility and permeability respectively (Dahan et al., 2009).

Since its introduction in 1995, the BCS has made a significant impact on the global pharmaceutical sciences arena, in drug discovery, development, and regulation. The BCS is effectively implanted by drug regulatory agencies around the world in setting bioavailability/bioequivalence standards for immediate-release oral drug product approval (http://www.fda.gov

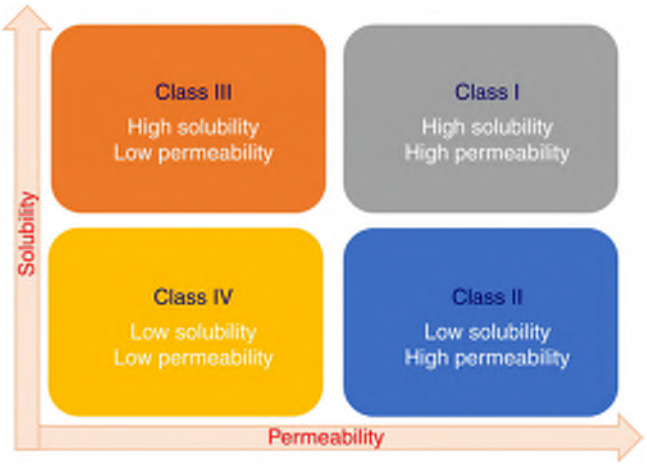

Figure 13.2. Biopharmaceutics Classification System (BCS) as defined by Amidon et al. (1995). From Dahan et al. (2009).

(b); Dahan et al., 2009). The BCS is applicable to drugs and is useful for the development of delivery systems with improved bioavailability of drug. However, in the case of nutraceuticals, numerous complicating factors influence their bioavailability within food, and, therefore limit the applicability of the BCS for nutraceuticals. Understanding the importance of BCS for drugs, McClements et al. (2015) recently introduced a new classification scheme for nutraceuticals: The NuBACS.

2.2 The Nutraceutical Bioavailability Classification Scheme (NuBACS)

NuBACS scheme is analogous to BCS and categorizes the nutraceuticals into three main classes, depending on the main factors limiting the oral availability of nutraceuticals in food matrices, namely, bioaccessibility (B*), absorption (A*), and transformation (T*). McClements and Xiao (2014) modified and extended the BCS to make it apt for classification of nutraceuticals, considering the factors limiting their bioavailability. NuBACS is based on the three specific physicochemical or physiological mechanisms that influence bioavailability and nutraceuticals are designated accordingly (McClements et al., 2015; Fig. 13.3).

More than 75% of B* and A* is considered as “high” and designated with sign (+), and less than 75% is considered as “low” and designated with sign (–), and vice versa for T*. Further subclass affecting the bioavailability will be added as suffix (Fig. 13.3a,b,c; McClements et al., 2015).

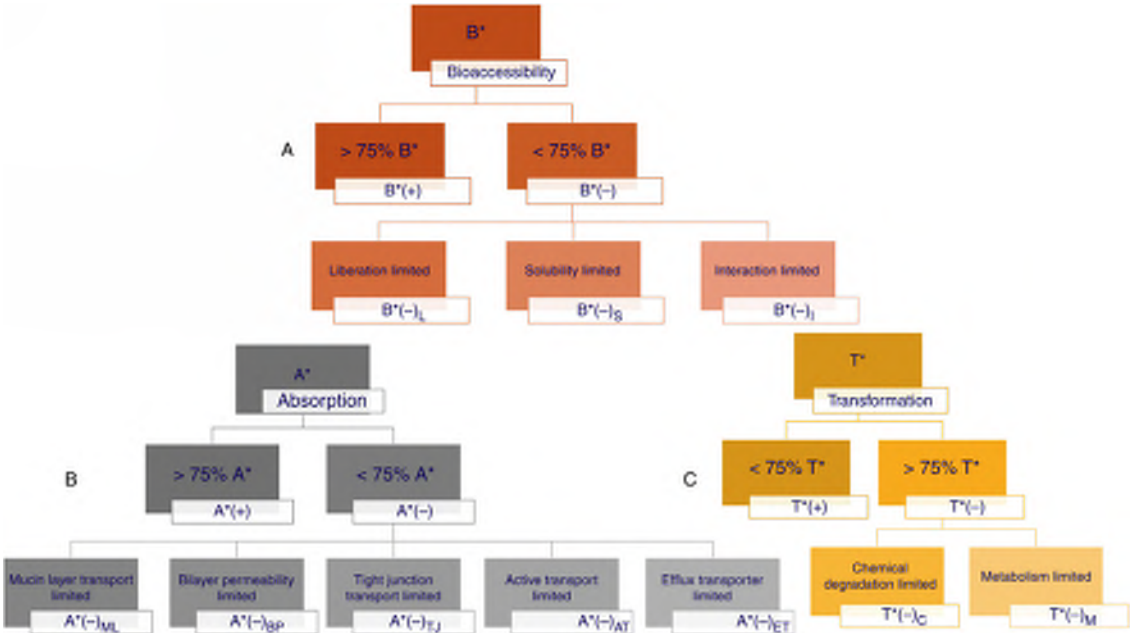

Figure 13.3. The Nutraceutical Bioavailability Classification Scheme (NuBACS) developed by McClements et al. (2015). A, B, and C represent the further classification and designations of nutraceuticals based on the specific factor affecting the bioavailability namely: (A) bioaccessibility; (B) absorption; and (C) transformation. From McClements et al. (2015).

*2.2.1 NuBACS B**

Before a nutraceutical gets absorbed from the food matrix, it has to be liberated from the food item and remain dissolved in the contents of intestinal lumen. Hydrophilic nutraceuticals simply get dissolved, whereas lipophilic nutraceuticals get solubilized via micelle formation. Bioaccessibility is further classified into three subclasses namely, L, S, and I, based on factors that affect bioavailability (Fig. 13.3a).

Liberation limited bioaccessibility (*L*): Release of nutraceutical trapped in the cellular structure of the food item into the gastrointestinal lumen takes into consideration the mechanical processing factors responsible for rapid diffusion, for example, cooking, homogenization, duration of mastication, and changing the physicochemical properties of food. Food processing improves bioavailability of nutraceuticals by breaking down cell walls which weaken the bonding force between the nutraceutical and the tissue matrix and also increases the surface area available for diffusion (Srivastava and Srivastava, 2013); for example, lycopene bioavailability is greater in processed tomato products as compared to unprocessed fresh tomatoes (Shi and Le Maguer, 2000).

Solubility limited bioaccessibility (*S*): Prior solubilization in the gastrointestinal content is a prime requisite before the absorption can take place. This becomes a rate limiting step for lipophilic nutraceuticals that need micellar solubilisation. Several nutraceuticals exhibit poor aqueous solubility and therefore poor bioaccessibility, for example, carotenoids.

Interaction limited bioaccessibility (*I*): Liberated nutraceutical may interact with the components present in the gastrointestinal lumen. This interaction may be beneficial as in the case of micellar solubilization of hydrophobic nutraceuticals due to amphiphatic bile surfactants such as sodium taurocholate (Avdeef, 2001; Srivastava and Srivastava, 2013) or may be adverse such as the formation of insoluble chelates (McClements et al., 2015). For example, chitosan, a cationic polyaminosaccharide derived from chitin and widely used in fabrication of nanoparticles is actually a lipid binder (Wydro et al., 2007). Being cationic in nature, it binds with several free anionic fatty acids like oleic, linoleic, and α-linolenic acid and decrease their availability (Wydro et al., 2007).

2.2.2 NuBACS A*

The solubilized chemical entity gets absorbed into systemic circulation across the epithelial layer either by paracellular or transcellular pathway (González-Mariscal et al., 2005). A nutraceutical with relatively high permeation (>75%) through epithelium cells will be designated as A*(+). This absorption class is further divided into five subclasses, namely, ML, BP, TJ, AT and ET, according to the specific factors limiting bioavailability (Fig. 13.3b) (McClements et al., 2015).

Mucin layer transport limited (*ML*)*:* Mucus is a hydrogel that covers the epithelial surface of gastrointestinal tract and protects the underlying tissues against the extracellular environmental insults and the effects of enzymes or other chemical agents (Khanvilkar et al., 2001). It is complex mixture of glycoproteins, lipids, salts, DNA, enzymes, and cellular debris (Sigurdsson et al., 2013). It acts as a barrier for permeation of several lipophilic drugs (Larhed et al., 1998) especially with log $P > 1$ (Behrens et al., 2001). Similar way, nutraceuticals permeability is also influenced by electrostatic or hydrophobic interactions with mucus (McClements et al., 2015).

Bilayer permeability limited (*BP*)*:* Passive transport of nutraceutical molecules by crossing the nonpolar phospholipid bilayer of epithelial cells is also a rate limiting factor for bioavailability. The phospholipid bilayer is relatively hydrophobic, and therefore nonpolar molecules with a relatively high log P can easily penetrate through it, whereas polar molecules with lower log P cannot. Therefore, if log $P < 1$, then the bioavailability of a bioactive

compound is limited by its bilayer permeability (McClements et al., 2015). Piperine increases the cell membrane permeability and therefore acts as a bioenhancer for several coadministered bioactive agents (McClements and Xiao, 2014).

Tight junction transport limited (*TJ*)*:* Epithelial cells are pivotal for the maintenance of homeostasis in the body by acting as a biological barrier that separates the inside of the body from the outside environment. Intercellular space between the adjacent epithelial cells is tightly sealed by tight junctions, which prevent solutes from freely moving across the epithelial cell (Takahashi et al., 2011). Tight junctions consist of narrow channels that allow sufficiently small molecules or particles to travel through them. Macromolecules cannot cross these tight junctions. However, size of channels in these tight junctions can be modulated to increase the absorption by use of some biopolymers, surfactants, minerals, and chelating agents (Deli, 2009; Hochman and Artursson, 1994; Kondoh and Yagi, 2007; Saaber et al., 2014; Takahashi et al., 2011)

Active transport limited (*AT*): This is a transcellular absorption mechanism involving a membrane bound transporter protein. It is also referred as carrier-mediated transport. There are more than 400 membrane transporters, which belong to two major super-families of membrane transporters: the ATP binding cassette and the solute carrier families. If the transport process directly or indirectly consumes energy in the form of hydrolysis of ATP, it is said to be active and may not require a concentration gradient of a permeant (Sugano et al., 2010). This mechanism is responsible for uptake of glucose, amino acids, bile salts, and several pharmaceuticals (Dobson and Kell, 2008; Tsuji and Tamai, 1996). It is saturable, cell type specific mechanism, subject to inhibition (Sugano et al., 2010), and therefore rate of absorption slows at higher concentration of a permeating drug or a nutraceutical (McClements et al., 2015).

Efflux transporter limited (*ET*)*:* After absorption by intestinal epithelium cells, some nutraceuticals are transported back into the gastrointestinal lumen by membrane bound efflux transporter proteins (Liu and Hu, 2007; McClements and Xiao, 2014; McClements et al., 2015). Several nutraceuticals are substrate for efflux transporters and exhibit low bioavailability (An et al., 2011; Luo et al., 2015; Nait Chabane et al., 2009). for example, quercetin and rutin (Yu et al., 2011)

The absorption of a nutraceutical across the gastrointestinal mucosa may be limited by one or more factors, depending on its molecular and physicochemical characteristics. Accordingly, nutraceutical will be classified as $A^*(-)_{ML}$, $A^*(-)_{BP}$, $A^*(-)_{TJ}$, $A^*(-)_{AT}$, or $A^*(-)_{ET}$ (Fig. 13.3b).

2.2.3 NuBacs T*

The bioavailability of many nutraceuticals is limited due to chemical transformation either in vitro or in vivo after administration. If a nutraceutical is relatively stable to molecular transformations within the GIT (>75% remaining in a bioactive form or < 25% transformation), then it will be designated as T*(+). The molecular transformations further categorises nutraceutical into two subclasses, C and M, depending upon the site of transformation (Fig. 13.3c).

Chemical degradation limited (*C*)*:* Nutraceuticals may get destabilized chemically within the food matrix in vitro or even after ingestion of food item, in vivo. Due to this chemical degradation the bioavailability of nutraceutical gets reduced or biological effect may get altered. Examples of chemical instabilities are oxidation, reduction, or hydrolysis. Oxidation prone chemicals are omega-3 fatty acids, carotenoids, tocopherols, and water soluble polyphenols. Addition of antioxidants may help to enhance the shelf life of the food matrix (Augustin and Sanguansri, 2015). Proteins or peptides get hydrolyzed in the acidic environment of stomach. Vitamin C, anthocyanins, and polyphenols are chemically unstable nutraceuticals (McClements, 2015)

Metabolism limited (M): Enzyme-catalyzed metabolism of nutraceuticals by Phase I Functionalization and Phase II Conjugation reactions in the liver lowers the bioavailable fraction. Curcumin (Dempe et al., 2013; Grill et al., 2014; Vareed et al., 2008), chrysin (Galijatovic et al., 1999), lycopene (Srivastava and Srivastava, 2013; Wang, 2012), resveratrol (Wenzel and Somoza, 2005) and many more nutraceuticals undergo extensive intestinal and hepatic metabolism.

Utilization of NuBACS will help food process industries as well as phytotherapeutic manufacturers to rationally design food matrices or nutraceutical dosage forms with improved oral bioavailability (McClements et al., 2015).

3 Bioactive Phytoconstituents as Nutraceuticals

Natural products as herbal medicines are as old as human civilization and constitute a comprehensive storehouse of remedies to cure acute and chronic diseases (Dureja et al., 2003). The knowledge of herbals has accumulated over thousands of years (Balunas and Kinghorn, 2005; Dureja et al., 2003). Initially, plant-derived medicines were used in the form of processed crude drugs such as extracts, tinctures, teas, poultices, powders, and

multiherbal formulations (Balunas and Kinghorn, 2005). Research on herbal medicines in early 19th century was evolved as isolation of active constituents from plants, for example, isolation of morphine from opium, or quinine from cinchona bark. During the last half of the 20th century, science of herbal medicine evolved further and shifted focus of drug research to combinatorial chemistry and cell and molecular biology (Cragg and Newman, 2013; Kinghorn, 2001). A great deal of successful examples of natural product that provided significant value to health care is evident (Rishton, 2008). Examples of those natural products that have been transformed to pharmaceuticals are salicylic acid, artemisin, codeine, digoxin, and many more (Balunas and Kinghorn, 2005; Mousa et al., 2007). The enormous structural diversity of herbal medicines and their medicinal significance provides a library of new "lead" compounds as chemical templates (Houghton, 1995). The structural analogs with enhanced therapeutic value by optimization of pharmacokinetics can be generated from such lead compounds by use of combinatorial chemical and biosynthetic technology (Chen et al., 2015; Cragg and Newman, 2013; Khazir et al., 2013; Szychowski et al., 2014).

Rapidly increasing knowledge on plant biotechnology, medicine, and nutrition has revolutionized the understanding about food and health. It has resulted in cultivation of plants not only for food but also for medicinal purposes. The concept of the use of plants for promoting health has emerged into a new generation of botanical therapeutics that include phytopharmaceuticals, nutraceuticals, dietary supplements, and functional foods (Raskin et al., 2002). Nutritional therapy and phytotherapy have emerged as new healing systems (Zhao, 2007). Since the natural sources are recognized as safe for human use, the active ingredients extracted from the natural sources have also attracted the attention of the food industry for the development of novel nutraceuticals. Plant-derived bioactive constituents have been considered as the main source of nutraceuticals (Vidanarachchi et al., 2012b).

The health benefits and importance of nutraceuticals has been well documented (Braithwaite et al., 2014; Dillard and German, 2000; Espín et al., 2007; Gosslau and Chen, 2004; Pandey et al., 2010; Tripathi et al., 2005). Now nutraceuticals are used not only for prophylaxis, but also prescribed and recommended as complementary or alternative medicine for healthy ageing and the treatment of various chronic diseases (Braithwaite et al., 2014; Ferrari, 2004; Houston, 2013; Morgan and Baggott, 2006; Sung et al., 2012).

Plant based nutraceuticals are actually bioactive secondary plant metabolites and are considered as products of biochemical

'side tracks' in the plant cell which are not needed for daily functioning of the plant (Bernhoft, 2008). These products provide almost all the categories of chemically diverse nutraceuticals, for example, minerals, polyunsaturated fatty acids, bioactive phytochemicals, proteins, dietary fiber, vitamins, and probiotics. Though they have several beneficial effects on human health, the potential of these compounds is not optimally utilized (McClements and Xiao, 2014; McClements et al., 2015; Rein et al., 2013). The poor solubility, low permeability, and variable oral bioavailability is limiting their bioefficacy and successful clinical application (Huang et al., 2010; Rein et al., 2013; Shakeri and Sahebkar, 2016; Yao et al., 2014). Apart from solubility, many nutraceuticals have restricted liberation from the matrix, extensive metabolism, form insoluble complexes, and are substrates for efflux transporters (McClements and Xiao, 2014; McClements et al., 2015; Ting et al., 2014).

In order to modulate the in vivo pharmacokinetic parameters of a nutraceutical, its physicochemical characteristics like solubility, partition coefficient, crystallinity, and others needs to be improved. This can be achieved with the applications of nanotechnology in the formulation design. Nanotechnology-based formulations provide distinct advantages for delivery of nutraceuticals, for instance, from improving solubility and stability to controlling the release and targeting of the nutraceutical for enhanced functionality (Borel and Sabliov, 2014). There is now considerable evidence to support the application of nanotechnology for improving the bioavailability of nutraceuticals (Ghaderi et al., 2014; Huang et al., 2010; Ting et al., 2014; Yao et al., 2014). There exists considerable interest in the use of oil-in-water nanoemulsions amongst various nanotechnology-based formulations, to encapsulate, protect, and deliver lipophilic pharmaceuticals. The nanoemulsification technology has been successfully applied to several pharmaceuticals and cosmeceuticals and is now extending its applicability for delivery of nutraceuticals, too (Garti and Yuli-Amar, 2008; McClements, 2012; McClements, 2013; Pol and Patravale, 2009). Preparation of nanoemulsion involves solubilization of the bioactive component in a carrier lipid phase by either dilution and/or heating and then the carrier lipid and water phases are homogenized to form an emulsion consisting of small oil droplets dispersed in water.

4 Nanoemulsion Fabrication

A nanoemulsion is defined as kinetically stable system having a mean droplet radius < 100 nm (diameter < 200 nm). Emulsions with milky appearance and globule size up to 500 nm are also

Figure 13.4. Advantages offered by nanoemulsions.

considered as nanoemulsions (Bilbao-Sáinz et al., 2010; Bouchemal et al., 2004; Jafari et al., 2006). For a transparent commercial product, globule size of the emulsion needs to be < 50nm. Various advantages offered by nanoemulsions are shown in Fig. 13.4 (McClements, 2013).

Nanoemulsions offer several advantages due to small droplet size over other types of particle-based delivery systems for encapsulation and delivery applications. However, nanoemulsions are thermodynamically unstable systems, susceptible to breakdown due to several physicochemical mechanisms, like gravitational separation, coalescence, flocculation, and Ostwald ripening. Therefore, for designing a physically and chemically stable nanoemulsion, excipients and fabrication technique need to be judiciously selected.

4.1 Excipients for Nanoemulsion Formulation

Formulation of nanoemulsion consists of water along with excipients namely, oil, emulsifier which are generally recognized as

Table 13.2 Examples of Various Excipients Used for Oral Nanoemulsion Formulation

Ingredient	Examples[a,b,c,d,e,f]
Oil	Fatty acids (oleic acid, stearic acid), Fatty acid esters (ethyl oleate), short-, medium-, long-chain triglycerides, phospholipids (lecithin), propylene glycol esters, mineral oil, stearin-rich milk fat, tetradecane, α-tocopherol special food grade oils with nutritional attributes (almond, flax seed, olive, sunflower, safflower, soybean, algae, and fish oil).
Emulsifier/surfactant	Spans® (sorbitan esters) and their ethoxylated derivatives (polysorbates, Tweens®), natural proteins, phospholipids, Brijs®, citric acid esters of monoglycerides, Polyoxyethylene hydrogenated castor oil, hydrogenated l-a-phosphatidyl choline, polyglyceryl fatty acids (Labrasol), pluronic F68, soyabean lecithin, polyoxyethylene 4-lauryl ether, bile acids, vitamin E TPGS. Special food-grade anionic surfactants (Citrem, Datem, sodium stearoyl lactylate), cationic (lauric alginate), nonionic (sugar esters), and zwiterrionic (lecithin).
Cosolvent/costabilizer	Ethanol, glycerol, sorbitol, propylene glycol, Polyethylene glycol, glycofurol, triacetin, Diethylene glycol monoethyl ether.
Water	Aqueous phase with or without buffer
Miscellaneous additives	Oil-soluble antioxidants (α-tocopherol, butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate), buffers, preservatives, sweetening agents. Food-grade nanoemulsions include texture modifiers; biopolymers (egg, milk, and vegetable protein), sugars (sucrose, high fructose corn syrup), polyols (sorbitol, glycerol), and polysaccharides (starch, pectin, and gums), weighting agents (bromovegetable oil, sucrose-acetate isobutyrate), ripening retarders (long-chain triglyceride, mineral oil, or ester gum).

[a] Desai et al. (2012)
[b] McClements (2011)
[c] McClements and Rao (2011)
[d] McClements (2013)
[e] Pouton and Porter (2008)
[f] Silva et al. (2012)

safe (GRAS). Addition of cosolvents facilitate the formation of nanoemulsions with small droplets, or to produce systems with high physical stability (McClements, 2013). Commonly used GRAS-listed nanoemulsion excipients along with food grade chemicals are tabulated in Table 13.2.

The nature of the oil, water and stabilizer has significant impact on type of fabrication technique used for making a stable nanoemulsion product. The various fabrication techniques along with their pros and cons are discussed later.

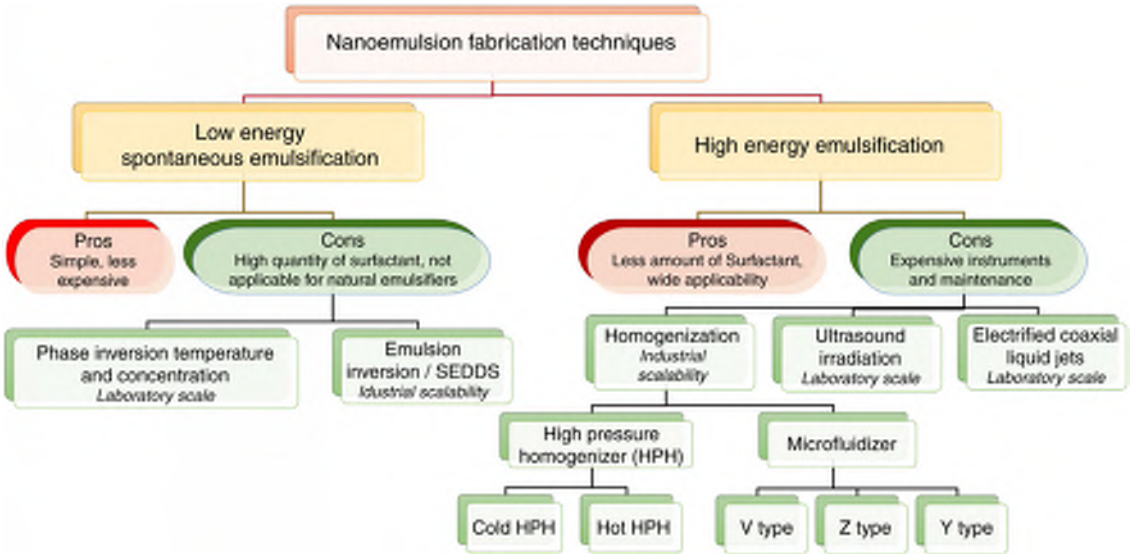

Figure 13.5. Classification of various techniques of nanoemulsion fabrication based on energy requirement.

4.2 Nanoemulsion Fabrication Techniques

Nanoemulsions can be prepared using low-energy methods and high-energy methods. The systematic classification of several methods is represented in Fig. 13.5.

The low-energy methods include spontaneous emulsification such as membrane emulsification, phase inversion temperature (PIT) method, phase inversion composition (PIC) and emulsion inversion point method (Jafari et al., 2006; Saberi et al., 2013). High-energy methods require specialized mechanical devices that provide energy required for emulsification. for example, high-shear homogenization, high-pressure homogenizer (HPH), microfluidizer, ultrasonicator, and electrified coaxial liquid jets (Anton et al., 2008; Huang et al., 2010; Leong et al., 2009; Qian and McClements, 2011). In both high-energy and low-energy techniques, first the bioactive component is usually dissolved in the oil phase, and then the nanoemulsion is formed by combining the oil and aqueous phases together in the presence of a stabilizer.

4.2.1 Low-Energy Spontaneous Nanoemulsification

4.2.1.1 PIT and PIC

Spontaneous emulsifications are catastrophic phase inversions, less expensive and energy-efficient techniques that utilize the chemical energy stored in the system (Bilbao-Sáinz et al., 2010). Membrane emulsification requires less surfactant

and involves formation of a dispersed phase (droplets) through a membrane into a continuous phase (Sanguansri and Augustin, 2006). PIT method involves heating a surfactant–oil–water mixture to a temperature around the PIT, and then rapidly cooling with stirring. An emulsion is formed spontaneously at that specified temperature. PIT method is based on the changes in solubility of polyoxyethylene-type nonionic surfactants with temperature (Herrera, 2012), colloidosomes, cubosomes, and microfluidic channels (Huang et al., 2010). Typically, nonionic surfactants that undergo extensive head-group dehydration during heating are used to form nanoemulsions using this approach, such as Tweens®, Spans®, and Brijs® (McClements, 2013). The movement of a hydrophilic surfactant from the organic phase into the aqueous phase results in spontaneous emulsification (Anton and Vandamme, 2009). The droplet size of the emulsion in spontaneous emulsification can be varied by changing the formulation composition and process parameters.

The technique is simple and does not require expensive machinery. Simple low-energy laboratory mixers like magnetic stirrer or low shear overhead stirrer can be used (Fig. 13.6a,b). However,

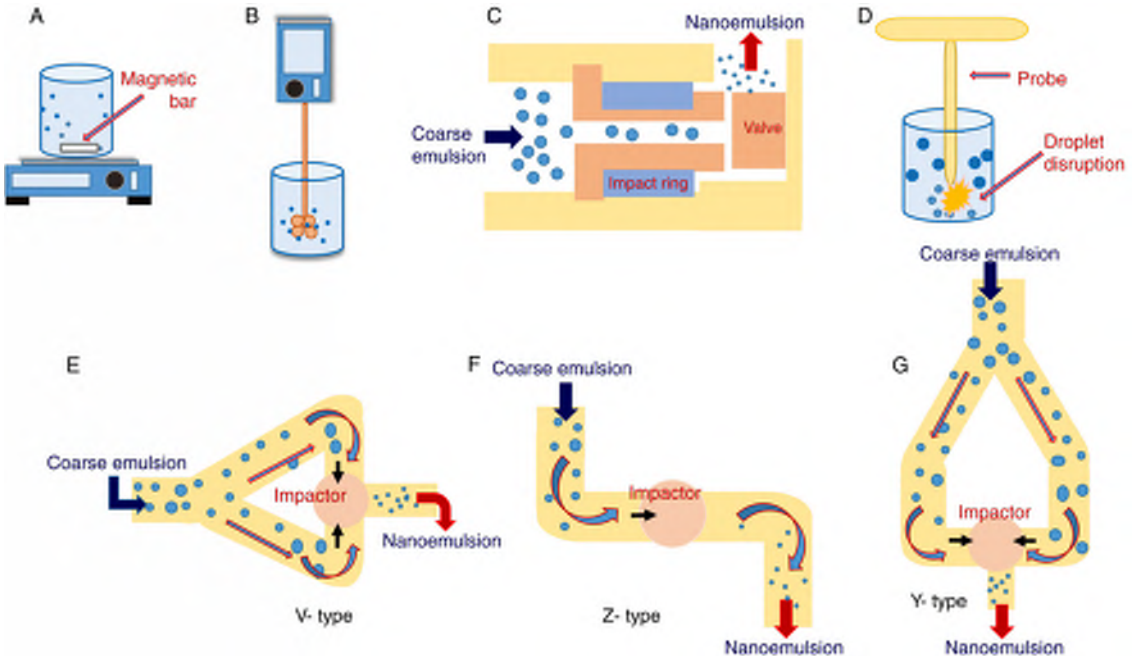

Figure 13.6. Nanoemulsion fabrication techniques. Low-energy emulsification techniques: (A) magnetic stirrer, (B) overhead stirrer. High energy emulsification techniques: (C) high pressure homogenizer, (D) ultrasonic probe sonicator. E, F, and G are microfluidizer V-type, Z-type, and Y-type, respectively.

the technique has limitations, such as the need of comparatively large amounts of surfactant (Yang et al., 2012) and a careful selection of surfactant–cosurfactant combination, and is not applicable to large-scale industrial productions (Jafari et al., 2007a). It is also difficult to produce nanoemulsions using highly nonpolar oils or ionic surfactants using the PIT method since the required temperature for phase inversion may not be practically in an appropriate range (McClements, 2013). Low-energy emulsification cannot be used to prepare nanoemulsions with natural emulsifiers like proteins or polysaccharides (McClements, 2013).

Another low-energy nanoemulsification technique, PIC, involves changes in the solution conditions in a mixture of surfactant, oil, and water like a change in pH, electrolyte concentration, or ionic strength. This change results in spontaneous phase inversion from a W/O to an O/W system (or vice versa).

4.2.1.2 Emulsion Phase Inversion or Self-Emulsifying Drug Delivery System (SEDDS)

The emulsion phase inversion method involves titrating water with an anhydrous mixture of oil and surfactant with low shear mixing to get nanoemulsion. Such anhydrous mixtures devoid of water and containing bioactive substance constitute a novel delivery system. In this, nanoemulsions is spontaneously formed inside the human body. Such delivery systems are isotropic mixtures and may additionally contain cosolvent and are known as SEDDS. SEDDS or self-microemulsifying drug delivery system (SMEDDS) have gained more attention and importance as a promising dosage form in recent years (Porter et al., 2008; Pouton, 2006). Upon mild agitation or digestive motility that would be encountered in the gastrointestinal tract followed by dilution in aqueous media, such as gastrointestinal fluids, SEDDS form fine O/W emulsions. SEDDS is a broad term, typically producing emulsions with an emulsion globule size, 100 to 250 nm in case of SMEDDS and less than100 nm in the case of self-nanoemulsifying drug delivery (SNEDDS) (Dokania and Joshi, 2015; Wei et al., 2012; Xi et al., 2009). The basic difference between SEDDS and SMEDDS is that SEDDS typically produce opaque emulsions with a droplet size above 300 nm while SMEDDS form transparent microemulsions with a droplet size of less than 250 nm (Dokania and Joshi, 2015). The fine droplets of this dosage form have the advantage of presenting the drug in a dissolved form with a large interfacial surface area for drug absorption, which results in more uniform and reproducible bioavailability. Moreover, the drug is maintained in a dissolved state throughout the gastrointestinal tract, which helps in enhancing the bioavailability of drugs with poor aqueous solubility.

In addition, the fine droplets offer a large surface area for pancreatic lipase to hydrolyze the lipids, and thereby enhance the rate of drug release and/or generation of mixed micelles containing the drug (Chakraborty et al., 2009). SEDDS also reduces the slow and incomplete dissolution of a drug, facilitates the formation of its solubilized phase, improves intestinal lymphatic transport, and bypasses the P-gp efflux, thereby enhancing the drug absorption from the gastrointestinal tract (Singh et al., 2009).

Because of anhydrous nature, SEDDS can be easily filled into soft or hard gelatin capsules making them convenient for handling and oral administration. Supersaturable SEDDS are now emerging as a dosage form for the delivery of a drug with limited solubility; for example, Singh and Pai (2015) developed supersaturable SNEDDS for improving the absorption of resveratrol. Anhydrous SEDDS, after suitable dilution with water, can be used as a binder for wet granulation or extrusion-spheronization. SEDDS can also be adsorbed onto inert porous carriers like Aerosil 200 or Neusilin US2 for converting them into powder form, which can be filled in capsule or can be tabletted (Desai et al., 2012). Onoue et al. (2012) used hydroxylpropyl cellulose for adsorbing SEDDS of coenzyme Q10.

4.2.2 High-Energy Nanoemulsification

High-energy emulsification utilizes specialized kinds of equipment that are expensive to purchase and maintain. However, they can produce nanoemulsions from a wide range of different oil phases and emulsifiers and with relatively low amounts of surfactant (McClements, 2013). Nanoemulsification using highly viscous oils and slow adsorbing emulsifiers can be difficult to achieve through high-energy techniques.

4.2.2.1 HPH

HPH is the most popular method of formulating nanoemulsions. It involves two steps of processing. First, coarse emulsion is formulated by mixing all the constituent phases together using a high-shear mixer. A coarse emulsion is fed into HPH, which consists of a high-pressure plunger pump with a subsequent relief valve (homogenizing valve). The plunger pump provides the energy level required for the relief. The relief valve consists of a fixed valve seat and an adjustable valve. These parts form an adjustable radial precision gap (Fig. 13.6c). The gap conditions, the resistance, and thus the homogenizing pressure vary as a function of the force acting on the valve. The instrument is available in two types, batch and continuous. The continuous version is suitable for optimizing the various parameters of the homogenization process. Use of the discontinuous version is sensible if the drug is very costly or of

limited availability. HPH operates at pressures from 100 to 1,500 bars. In a few HPH, a maximum pressure of 2000 bars is used for processing (Patravale et al., 2004). The pressure is responsible for nanonization due to various principles, such as cavitation, disintegration, and shearing (Patravale et al., 2012). Droplet size and the polydispersity index can be reduced effectively by increasing the homogenization pressure, number of homogenization cycles, emulsifier concentration, and processing temperature (cold or hot) (Patravale et al., 2004; Qian and McClements, 2011; Sanguansri and Augustin, 2006; Walstra, 1993). HPH offers ease of scale-up, minimum batch-to-batch variation, aseptic processing of sterile products, and flexibility in handling a wide range of volumes (Patravale et al., 2004). However, prehomogenization or micronization of the product is essential before processing in HPH to avoid any damage to the homogenizing valve. Several nutraceuticals are formulated as nanoemulsions using HPH, for example, resveratrol (Pangeni et al., 2014; Sessa et al., 2011), and curcumin (Ahmed et al., 2012). Donsì et al. (2010) utilized HPH for preparation of sunflower-oil nanoemulsion using a natural emulsifier, pea protein.

4.2.2.2 Microfluidization

Microfluidizer consists of a fixed-geometry interaction chamber in which two jets of crude emulsion from two opposite channels collide with one another at supersonic speed (Jafari et al., 2007a; Jafari et al., 2007b). In this high-shear interaction chamber, pressure energy is converted into shear and impact forces, resulting in size reduction of the dispersed phase. The interaction chamber may be V-, Y-, or Z-shaped, either single or multislotted (Fig. 13.6e,f,g). The process stream is delivered by a pneumatic pump, capable of pressurizing the in-house compressed air (150–650 kPa) up to about 150 MPa creating tremendous shearing action that results in size reduction and exceptionally fine emulsion. The inertial forces in turbulent flow along with cavitation are predominantly responsible for droplet disruption in microfluidizer (Jafari et al., 2007b). The main problem with this technique is overprocessing, which results in coalescence of the newly formed droplets during extreme emulsification conditions. Therefore, pressure and the number of cycles need to be optimized (Jafari et al., 2007a). The difference between microfluidizers and traditional HPH is the presence of interaction and auxiliary chambers with microchannels in microfluidizers. These extra chambers can provide cavitations and shear forces to facilitate emulsion formation in microfluidizers (Sanguansri and Augustin, 2006). Since emulsification occurs in extremely short period of time in the

interaction chamber of microfluidizer, the technique cannot be used for encapsulation using biopolymeric stabililizers like starch and proteins, as natural biopolymers are slow adsorbing (Jafari et al., 2007b). Several nutraceuticals were nanoemulsified using microfluidization, for example, lutein (Vishwanathan et al., 2009), coenzyme Q10 (Cho et al., 2014), and quercetin (Pool et al., 2013).

4.2.2.3 Ultrasound Irradiation

Two immiscible liquids, the oil phase and aqueous phase, are subjected to high-frequency sound waves in the presence of a stabilizer. Emulsion droplets are formed by acoustic cavitation, that is, the formation and subsequent collapse of microbubbles by the pressure fluctuations of a simple sound wave (Fig. 13.6d). Each bubble collapses (an implosion on a microscopic scale) causing highly localized intense turbulence. The turbulent microimplosions are effective in reducing the size of primary droplets of dispersed oil into submicron size (Kentish et al., 2008; Leong et al., 2009). Ultrasound can produce nanoemulsions with comparable droplet size as that of microfluidized emulsions, with a benefit of no occurrence of overprocessing. However, ultrasound irradiated nanoemulsions may have broader size distribution resulting into high polydispersity index (Jafari et al., 2007b). Droplet size can be decreased in an ultrasonic homogenizer by increasing the amplitude or duration of the applied sonication (McClements, 2013), however, increasing the sonication time beyond the optimum level do not reduce droplet size significantly. The limitation of this process is less efficient for industrial-scale applications (Silva et al., 2012).

4.2.2.4 Electrified Coaxial Liquid Jets

Loscertales et al. (2002) reported a method utilizing electrohydrodynamic forces for generating coaxial jets of immiscible liquids with nanosize range. The aqueous and oily phase are injected at appropriate flow rate through two concentric needles. The outer needle is connected to an electrical potential of several kilovolts relative to a ground electrode. The inner needle is kept to an electrical potential that depends on the conductivity of the outer liquid and can be varied. By controlling the diameter of the needles, flow rate, and the applied voltage, the size of emulsion droplets can be varied (Loscertales et al., 2002). This technique has been limited to laboratory use and is not suitable for production of nanoemulsion on large scale (Huang et al., 2010).

Applications of these methodologies of nanoemulsification for improving the bioavailability of nutraceuticals is tabulated in Table 13.3.

Table 13.3 Common Nutraceuticals Encapsulated in Nanoemulsion: Literature at a Glance

Formulation	Fabrication Methodology	Therapeutic Benefit and Principal Application	Reference
Curcumin			
Nanoemulsion	Ultrasonication	Improved permeability and oral bioavailability	Yu and Huang (2012)
Nanoemulsion	Low-shear mixing	Increased physical stability and enhancement of solubility of curcumin in aqueous solution	Kumar et al. (2012)
SEDDS	Low-shear mixing	Improved antiinflammatory activity and bioavailability	Young et al. (2014)
		Enhanced oral bioavailability	Zhongfa et al. (2012)
SMEDDS: liquid and solid	Low-shear mixing	Increased absorption and stability	Setthacheewakul et al. (2010)
SNEDDS	Low-shear mixing	Prolonged plasma exposure and bioavailability, enhanced efficacy in experimental diabetic neuropathy	Joshi et al. (2013)
SMEDDS	Low-shear mixing	Enhanced dissolution and oral bioavailability	Wu et al. (2011)
		Enhanced oral absorption	Cui et al. (2009)
SEDDS: liquid and solid	Low-shear mixing (liquid), spray drying (solid)	Improved stability and oral bioavailability	Yan et al. (2011)
Nanoemulsion	Thin film hydration followed by sonication	Increased absorption, antiinflammatory and antiallergic effects	Onodera et al. (2015)
		Improved the stability and prolonged the cytotoxic effect	Anuchapreeda et al. (2012)
Mucoadhesive nanoemulsion	Low-shear mixing	Higher flux and permeation across nasal mucosa for the treatment of Alzheimer's disease	Sood et al. (2014)
Nanoemulsion	Low-shear mixing	Improved topical delivery for local therapeutic effects in inflammatory arthritic disorders	Naz and Ahmad (2015)
Nanomulsion	High-speed mixer and HPH	Improved oral availability and improved antiinflammatory activity	Wang et al. (2008)
Nanoemulsion	High-speed mixer and microfluidization	Excipient emulsion with increased bioaccessibiliy	Zou et al. (2015)

(*Continued*)

Table 13.3 Common Nutraceuticals Encapsulated in Nanoemulsion: Literature at a Glance (*cont.*)

Formulation	Fabrication Methodology	Therapeutic Benefit and Principal Application	Reference
Resveratrol			
Nanoemulsion	Spontaneous emulsification	Low-energy emulsification with improved physical and photochemical stability	Davidov-Pardo and McClements (2015)
Nanoemulsion	Sonication and HPH	Increased bioavailability by decreasing metabolism	Zhou et al. (2015)
Supersaturable SNEDDS	Low-shear mixing	Improved rate of permeation and extent of absorption	Singh and Pai (2016)
SEDDS	Low-shear mixing	Improved antioxidant activity	Amri et al. (2014)
SMEDDS	Low-shear mixing	Improved solubility and physical stability in hard gelatin capsules	Bolko et al. (2014)
Nanoemulsion	Spontaneous emulsification and HPH	Increased antioxidant activity and enhanced nose-to-brain delivery for treatment of Parkinson's disease	Pangeni et al. (2014)
SNEDDS	Low-shear mixing	Enhanced antiangiogenic activity	Pund et al. (2014a)
SMEDDS	Low-shear mixing	High solubilisation capacity with reduced intestinal presystemic metabolism	Seljak et al. (2014)
Lutein			
Nanoemulsion	Phase inversion	Useful for the clinical treatment of neuro- and hepatoxicity	Schwingel et al. (2014)
Nanoemulsion	Microfluidization	Higher lutein bioavailability and improved antiinflammatory efficacy of lutein nanoemulsion.	Vishwanathan et al. (2009)
SNEDDS	Low-shear mixing	High retinal availability	Shanmugam et al. (2011)
		Affect bioavailability of coadministered warfarin	Yoo et al. (2013)
		Enhanced solubility and dissolution of lutein	Yoo et al. (2010)

Coenzyme Q10			
Nanoemulsion	Ultrasonication	Food grade product improved oral bioavailability	Thanatuksorn et al. (2009)
	High-shear mixer followed by spray drying on solid carrier	Improved solubility and oral bioavailability	Onoue et al. (2012)
	HPH	Improved stability and oral bioavailability	Zhou et al. (2014a)
	Ultrasonication followed by HPH	Food grade product with improved stability and oral bioavailability	Belhaj et al. (2012)
	High-shear mixing followed by microfluidization	Improved oral bioavailability	Cho et al. (2014)
Quercetin			
Nanoemulsion	High speed homogenization	Optimization studies	Karadag et al. (2013)
	Spontaneous emulsification	Enhanced permeation flux though skin for effective topical delivery	Fasolo et al. (2009).
	Spontaneous emulsification and ultrasonication	Optimization studies	Ebrahimi and Salmanpour (2014)
	Ultrasonication	High encapsulation efficiency, less toxicity, controlled delivery with enhanced transdermal drug permeation	Bennet and Kim (2013)
	High-speed mixing followed by microfluidization	Enhanced solubility and bioaccessibility, can be added to food products	Pool et al. (2013)
SNEDDS	Low-shear mixing	Improved transport across Caco2 cells and oral bioavailability	Tran et al. (2014)
Capsaicin			
Nanoemulsion	Low-shear mixing	Self-assembly biopolymericnanoemulsion with mproved physicochemical properties and stability, suitable for functional food	Choi et al. (2011)
Betulinic acid			
Nanoemulsion	Ultrasonication	Small globule size and stable toward aggregation	Cavazos-Garduño et al. (2015)
	Microfluidization	Improved delivery and antiangiogenic efficacy	Dehelean et al. (2011)

(*Continued*)

Table 13.3 Common Nutraceuticals Encapsulated in Nanoemulsion: Literature at a Glance (*cont.*)

Formulation	Fabrication Methodology	Therapeutic Benefit and Principal Application	Reference
Rutin			
Nanoemulsion	Spontaneous emulsification and HPH	Increased in vitro release and ex vivo permeability, improved antioxidant and antiinflammatory activity	Sharma et al. (2015)
	High-shear homogenization followed by HPH	High encapsulation efficiency and sustained release	Macedo et al. (2014)
Naringenin			
Submicron emulsion	Vortex mixing	Improved stability, enhanced safety and permeation through skin for topical application	Tsai et al. (2015)
SNEDDS	Low-shear mixing	Improvement in drug release and bioavailability	Khan et al. (2015)
β-carotene			
Nanoemulsion	High-speed mixing followed by microfluidization	Food-grade nanoemulsion with improved stability	Jo and Kwon (2014)
	Emulsification–evaporation, microfluidization	Stable formulation for incorporation in food products	Tan and Nakajima (2005)
	High-speed mixing followed by solvent evaporation	Improvement in physical as well as chemical stability of β-carotene	Silva et al. (2011)
	High-speed homogenization followed by HPH	Improvement in stability of β-carotene	Yuan et al. (2008a,b)
		For protection of carotenoids	Liang et al. (2013)
		Effect of different emulsifiers for improving the stability	Mao et al. (2009)
α-tocopherol			
Nanoemulsion	High-speed homogenization followed by HPH	Emulsification and stabilization using natural stabilizers for application in food and other industries.	Ozturk et al. (2014, 2015)
		Improvement in the stability of α-tocopherol	Relkin et al. (2008)
	High-speed homogenization and microfluidization	Improved oral bioavailability and antioxidant activity	Hatanaka et al. (2010)
	High-shear homogenization	Stable nano-cosmeceutical	Teo et al. (2010)

Genistein			
Nanoemulsion	Spontaneous emulsification	Topical antiherpetic	Argenta et al. (2014)
		Enhanced skin permeation for topical delivery	de Vargas et al. (2012)
		Slow skin permeation for prolonged topical delivery	Silva et al. (2009)
Ursolic acid			
Nanoemulsion	Spontaneous emulsification	Dermal application with improved safety and antiinflammatory activity	Alvarado et al. (2015)
Berberine			
SNEDDS	Low-shear mixing	Improved antiinflammatory and antiangiogenic activity	Pund et al. (2014a,b)
		Rapid dissolving formulation	Ke et al. (2015)
SMEDDS	Low-shear mixing	Improved oral bioavailability	Zhu et al. (2013)
Oleanolic acid			
SNEDDS	Low-shear mixing	Improved dissolution and oral bioavailability	Xi et al. (2009)
Eugenol			
Nanoemulsion	Ultrasonication	Antibacterial against *Staphylococcus aureus* and for preservation of fruit juice against microbial spoilage	Ghosh et al. (2014)
		Protection of cotton seed from *Fusarium* wilt infection, efficient nanofungicide in plant disease control	Abd-Elsalam and Khokhlov (2015)
Nanoemulsion	HPH	Cytotoxic to human colon and liver cancer cell lines	Majeed et al. (2014)
Apigenin			
SMEDDS	Low-shear mixing	Enhanced solubility and dissolution	Zhao et al. (2013)
Ellagic acid			
SEDDS	Low-shear mixing	Improved solubility and permeation	Avachat and Patel (2015)
Ursolic acid			
Nanoemulsion	Spontaneous emulsification	Dermal application with improved safety and antiinflammatory activity	Alvarado et al. (2015)
Thymoquinone			
Nanoemulsion	High-speed homogenization followed by HPH	Improved stability	Tubesha et al. (2013)

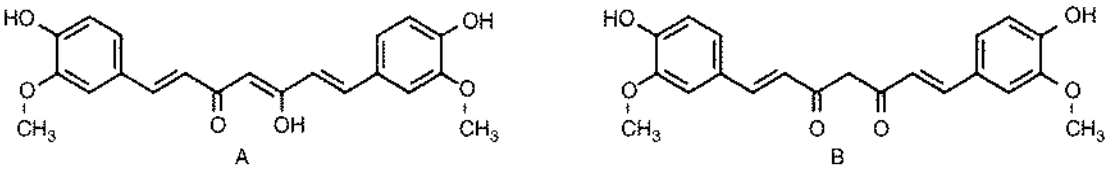

Figure 13.7. Curcumin: (A) enol form; (B) Keto form.

Special emphasis is given on plant-based lipophilic nutraceuticals, namely curcumin, resveratrol, and lutein, and are explained further in detail.

5 Examples of Nutraceuticals Encapsulated in Nanoemulsion

5.1 Curcumin

Curcumin, the Indian solid gold (Aggarwal et al., 2007), is chemically a diferuloylmethane [1, 7-bis (4- hydroxy-3-methoxypheny–) -hepta-1, 6-diene-3, 5-dione]. It is mixed with its two derivatives, demethoxycurcumin and bis-demethoxycurcumin (Heger et al., 2014; Siviero et al., 2015; Fig. 13.7).

Curcumin is the active ingredient in rhizome turmeric (*Curcuma longa*) and is utilized in numerous food products as a spice and pigment because of its characteristic flavor and yellow color (Prasad et al., 2014; Syed et al., 2015). Curcumin and its two demethoxy derivatives exist in the trans-trans keto-enol form. The aromatic groups provide hydrophobicity, and the linker gives flexibility. The tautomeric structures also influence the hydrophobicity and polarity. The hydrophobicity of curcuminoids makes them poorly soluble in water. Three acidity constants (pK_a), 8.38, 9.88, and 10.51 are reported for curcumin (Bernabé-Pineda et al., 2004).

Curcumin is a prominent candidate for treating inflammation, cancer, cystic fibrosis, Alzheimer's and malarial diseases (Maheshwari et al., 2006). In addition, curcumin has also been used for the treatment of numerous ailments, such as anaemia, bacterial infections, colds, coughs, eczema, fevers, jaundice, liver diseases, skin diseases, urinary diseases, viral infections, and wounds (Syed et al., 2015; Wilken et al., 2011). Curcumin is a highly pleiotropic molecule with antioxidant, chemosensitization, and radiosensitization activities. The pleiotropic activities attributed to curcumin come from its complex molecular structure and chemistry, as well as its ability to influence multiple signalling molecules (Gupta et al., 2011). Curcumin modulates various signalling

molecules, transcription factors, enzymes, protein kinases, protein reductases, carrier proteins, cell survival proteins, drug resistance proteins, adhesion molecules, growth factors, receptors, cell-cycle regulatory proteins, chemokines, DNA, RNA, and metal ions (Gupta et al., 2012; Huang et al., 1991; Sandur et al., 2007; Sarkar et al., 2008; Shehzad et al., 2010; Shishodia et al., 2007; Siviero et al., 2015; Yallapu et al., 2012; Zhou et al., 2014b). It has also been shown that curcumin is capable of binding to DNA and can cause strand scission (Ahsan and Hadi, 1998).

Due to potential health benefits, there has been considerable interest in incorporating curcumin into functional food products as a nutraceutical that may promote human health and wellness through diet. According to a joint FAO/WHO report on food additives, the recommended daily intake of curcumin is 3 mg/kg body weight with no adverse effects (EFSA, 2010). Chronic toxicity studies on curcumin showed that it can be administered safely at oral doses of up to 8 g per day (Cheng et al., 2001). However, the poor aqueous solubility and high melting point of curcumin makes it difficult to incorporate into many aqueous-based food products (Ahmed et al., 2012; Heger et al., 2014). A further challenge is that curcumin is highly susceptible to chemical degradation in aqueous environments, especially around neutral pH, which further reduces its bioactivity (Fu et al., 2014). Curcumin is a BCS class II drug (Kasim et al., 2004) indicative of poor permeability. An extremely low P_{app} value of less than 0.1×10^{-6} cm/s was obtained for the apical to basolateral permeation of pristine curcumin, while its metabolites exhibited a considerably higher permeation of around 1×10^{-6} cm/s in CaCo-2 cells (Dempe et al., 2013). Curcumin undergoes both phase I (reduction) and phase II (conjugation) metabolism (Grill et al., 2014; Ireson et al., 2002; Pan et al., 1992). Hence, due to its rapid intestinal and hepatic metabolism, approximately 60% to 70% of an oral dose of curcumin gets eliminated in the feces (Pan et al., 1992). Consequently, the oral bioavailability of curcumin in humans is about 1% (Siviero et al., 2015). Therefore, despite much evidence of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent (Siviero et al., 2015).

Hydrophobic nature of the curcumin presents a challenge for its incorporation into clear aqueous foods and beverages like juices. For wider applicability in diet, the bioactive nutraceutical must be stabilized in a liquid environment, which is completely different from stabilization in a solid environment (Hosseini et al., 2015). Many attempts have therefore been made to improve the water solubility and oral bioavailability of curcumin using food-grade nanoemulsions as a delivery approach (Aditya et al., 2015; Bergonzi et al., 2014; Sari et al., 2015).

Anhydrous self-emulsifying systems improve the bioavailability of curcumin by protecting it from chemical degradation throughout storage and during passage through the gastrointestinal tract, and by increasing its solubility within the gastrointestinal fluids. Therefore, Zhongfa et al. (2012) prepared curcumin self-emulsifying preconcentrate using Cremophor EL as oily solubilizer, Tween 80 as surfactant, and polyethylene glycol 600 as cosolvent. Cremophor EL is macrogolglycerol ricinoleate; a castor oil; derivative and also acts as nonionic emulsifier, as it has hydrophilic-lipophilic balance, 12–14. After dilution with water, the resultant curcumin nanoemulsion containing up to 20% curcumin (w/w) was stable at room temperature for 2 m and showed emulsion globule size 68.7 nm. Pharmacokinetic studies of oral administration in mice exhibited a 10-fold increase in bioavailability when calculated as area under plasma concentration-time curve and more than a 40-fold increase in the C_{max} as compared to pure curcumin.

Anuchapreeda et al. (2012) prepared a curcumin nanoemulsion by thin film hydration method in a bath type sonicator. The composition was soybean oil, hydrogenated L-α-phosphatidylcholine from egg yolk and cosurfactants (Tween 80 and polyoxyethylene hydrogenated castor oil 60, Cremophor-RH60). The resultant nanoemulsion had a mean particle diameter of 47–55 nm and with a concentration of curcumin of 0.9 mg/mL. This formulation was stable for 60 days at 4°C. Further, 25% of the loaded amount was released from these nanoemulsions in 72 h when dispersed in phosphate buffer saline, pH 7.4, containing 25% human serum (Anuchapreeda et al., 2012). Cytotoxic effect was analyzed on B16F10 (mouse melanoma cell line) and four types of leukemic cell lines, namely HL60 (promyelocytic leukemia), K562 (chronic myelocytic leukemia), Molt4 (lymphoblastic leukemia), and U937 (monocytic leukemia). Curcumin nanoemulsion was capable of inhibiting cell growth of all the cell lines tested. The nanoemulsion improved the stability and prolonged the cytotoxic effect of curcumin by delaying the release (Anuchapreeda et al., 2012; Ucisik et al., 2013).

Curcumin has pleiotropic therapeutic effect in refractory cancer. Therefore, Ganta and Amiji (2009) utilized a multimodal therapeutic strategy to overcome tumor drug resistance by efficiently delivering the drug to the tumor and lowered the apoptotic threshold by modulation of the intracellular signaling mechanisms. In this study, augmentation of therapeutic efficacy upon coadministration of flaxseed oil nanoemulsion of paclitaxel and curcumin was analyzed. Curcumin is an inhibitor of nuclear factor kappa B (NFκB) as well as a potent down-regulator of ABC transporters.

Flaxseed oil containing nanoemulsion was prepared by coarse homogenization followed by high-energy ultrasonication using lecithin as emulsifier. The prepared nanoemulsion had a particle size of around 133 nm and was stable over a period of 3 m. Intracellular drug delivery, down-regulation of P-gp, and inhibition of NFκB pathway, enhancement of cell-kill efficacy, and the apoptotic response following treatment with single and combination therapy in aqueous solution and nanoemulsion formulations was evaluated. The combination therapy of paclitaxel and curcumin as a nanoemulsion was very effective in enhancing the cytotoxicity in wild-type and resistant cells by promoting the apoptotic response.

Later, Ganta et al. (2010) evaluated the effect of curcumin on oral bioavailability and therapeutic efficacy of paclitaxel in nanoemulsion to SKOV3 tumor-bearing *nu/nu* mice. Oral administration of the mice with curcumin showed down regulation of intestinal P-gp and cytochrome P450 3A2 protein levels. Bioavailability of paclitaxel was 5.2-fold higher resulting in 3.2-fold higher paclitaxel accumulation in the tumor tissue (Ganta et al., 2010).

Curcumin exhibits beneficial role in several neurodegenerative disorders such as dementia of Alzheimer type (Agrawal et al., 2010). Brain delivery of curcumin via nasal route using mucoadhesive nanoemulsion was attempted by Sood et al. (2014). Chitosan was used as mucoadhesive agent, Capmul MCM and Captex 500 as oil phase, and PEG 400 and Transcutol as surfactant and cosurfactant. The nanoemulsion was optimized using Box–Behnken design. The cytotoxicity studies of the optimized curcumin mucoadhesive nanoemulsion was carried out in SK-N-SH cells, a human neuroblastoma cell line along with nasal ciliotoxicity histopathological study. The developed formulations did not show any toxicity and was safe for intranasal delivery for brain targeting. Additionally, curcumin mucoadhesive nanoemulsion exhibited higher flux and permeation across sheep nasal mucosa (Sood et al., 2014).

For efficient delivery of curcumin nanoemulsion for local and transdermal application for scleroderma, psoriasis and skin cancer was analyzed (Rachmawati et al., 2015). Nanoemulsion was prepared by self-emulsification method, using glyceryl monooleate as oil, Cremophor RH40 as emulsifier and polyethylene glycol 400 as cosolvent. The mean droplet diameter, polydispersity index, and zeta potential of optimized nanoemulsion were found to be 85 nm, 0.18 and –5.9 mV, respectively. Nanoemulsification significantly improved the stability as well as permeation flux of curcumin from the hydrophilic matrix gel, suggesting suitability of curcumin nanoemulsion for topical therapy (Rachmawati et al., 2015).

Improved oral availability of curcumin from nanoemulsion has also resulted in improved antiinflammatory activity of curcumin (Wang et al., 2008). Curcumin nanoemulsion was prepared using high-speed and high-pressure homogenization using medium-chain triacylglycerols as oil and Tween 20 as emulsifier. The nanoemulsion with mean droplet size 618.6–79.5 nm was successfully prepared. Antiinflammatory activity was analyzed in 12-O-tetradecanoylphorbol-13-aceta–e -induced edema of mouse ear. There was 43% and 85% inhibition of edema with nanoemulsion with 618.6–79.5 nm droplet size. The oral administration of 1% curcumin in Tween 20 water solution shows little or no inhibition effect of edema of mouse ear. Antiinflammatory effect was found to be inversely proportional to the globule size in nanoemulsion (Wang et al., 2008).

To overcome the instability of curcumin during processing and to improve bioavailability, curcumin was nanoemulsified by ultrasonication and the efficacy was evaluated by simulated digestion study. Medium-chain triglyceride was used as oil and mixture of whey protein concentrate-70 and Tween 80 were used as emulsifiers. The encapsulation efficiency of the emulsion was 90.56% with average particle diameter 141.6 nm and zeta potential of –6.9 mV. In vitro release in simulated gastrointestinal fluid showed that the curcumin nanoemulsion was relatively stable to pepsin digestion, however, pancreatin triggered the release of curcumin. This modulated slow release of curcumin from the nanoemulsion could help to increase its bioavailability (Sari et al., 2015). The nanoemulsion was stable to pasteurization, different ionic strengths and over pH 3 to 7, indicating its wider commercial applicability.

Ahmed et al., 2012 studied the influence of different lipid-based formulations on curcumin encapsulation and bioaccessibility. Physically stable curcumin-loaded nanoemulsions were prepared using a variety of different lipid phases: long-, medium-, and short-chain triacylglycerols (LCT, MCT, and SCT). The maximum loading of curcumin was observed with lipid with less molecular weight and loading was found to decrease as molecular weight increased: SCT > MCT > LCT. The rate and extent of lipid digestion also depended on lipid type. The initial rate decreased in the following order: SCT > MCT > LCT. The bioaccessibility of curcumin, assumed to be equal to the fraction of curcumin incorporated into the micelle phase after digestion, decreased in the following order MCT > LCT > >SCT. The bioaccessibility of curcumin appeared to be slightly higher in conventional emulsions than in nanoemulsions, but nanoemulsions had much better physical stability (Ahmed et al., 2012).

Curcumin has beneficial effects in the treatment of various diabetes-related complications; however, its limited bioavailability affects its antidiabetic activity. Hence, Joshi et al. (2013) formulated SNEDDS of curcumin, to enhance its bioavailability and therapeutic efficacy in experimental diabetic neuropathy in male Sprague Dawley rats. SNEDDS of curcumin provided better protection against functional, behavioral, and biochemical deficits in experimental diabetic neuropathy, when compared wilive curcumin. Further, Western blot analysis confirmed the greater neuroprotective action of SNEDDS curcumin and the potential of formulation in diabetic neuropathy (Joshi et al., 2013).

Metabolic clearance of curcumin was significantly reduced by coadministration of natural UGT inhibitors; silibinin as SMEDDS of curcumin (Grill et al., 2014). It was evident from increased curcumin glucuronide concentration in plasma without change in curcumin plasma concentration. Similarly, coencapsulation of curcumin and etoposide in nanoemulsion improved pharmacokinetic profile of both the drugs and increased cytotoxicity 1.5 times in PU3 prostate cancer cell line in comparison to control (Shukla et al., 2014).

Indeed, nanoemulsions are an attractive approach for better utilization of the therapeutic potential of lipophilic nutraceutical–like curcumin for various chronic and acute diseases. Nanoemulsions of curcumin showed significant potential for delivery of curcumin with higher bioavailability and stability. However, extensive human clinical trials have to be conducted to establish their safety, especially after chronic and repeated use, and effectiveness for treatment of cancer and other diseases.

5.2 Resveratrol

Resveratro' (3,5,4′-trihydroxy-*trans*-stilbene) is a stilbenoid and a nonflavanoid, natural phenolic compound (Fig. 13.8). It is largely found in peanuts and skin of red grapes, mulberries, blueberries, as well as a variety of other plant sources (Amri et al., 2012; Sanders et al., 2000). Resveratrol was isolated from the roots of plants *Polygonum cuspidatum* and *Veratrum grandiflorum,* used in traditional Chinese and Japanese medicine (Kimura and Okuda, 2001; Vastano et al., 2000). It is one of the natural compounds that has been intensively investigated in recent years for its health-beneficial properties and for potential applications in the fields of pharmaceutics, nutraceuticals, and functional foods (Sessa et al., 2011).

Resveratrol is well-known for its high antioxidant activity. It is considered as an important constituent of red wine for the

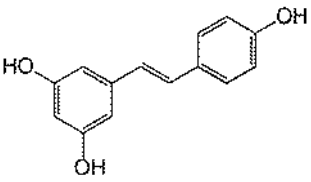

Figure 13.8. Resveratrol.

health benefits, which has popularized the concept "French paradox." The French paradox concept was proposed by French epidemiologists in the 1980s. It is the observation of low incidence of coronary heart disease and related mortality in France despite high intake of dietary cholesterol and saturated fat. It was correlated to the higher consumption of red wine by French people (Ferrières, 2004).

Trans-resveratrol has several pharmacological activities, like antiinflammatory and antioxidant (Baur and Sinclair, 2006), inhibition of platelet aggregation, antiatherosclerotic effects (Olas and Wachowicz, 2005), and protective effects on the cardiovascular system (Das and Das, 2007; Hung et al., 2004). The pharmacological effect of resveratrol is due to the inhibition of oxidation of human low-density lipoprotein. It also suppresses cyclooxygenase-2 and inducible nitric oxide synthase activities leading to its antiinflammatory and antioxidant effects (Baur and Sinclair, 2006). Resveratrol is also classified as a phytoestrogen because of its ability to interact with estrogen receptors. It is structurally related to the synthetic estrogen diethylstilbestrol (Fulda, 2010). Resveratrol is a potential chemopreventive and chemotherapeutic agent and its activities have been demonstrated in all three stages of carcinogenesis (initiation, promotion, and progression), in both chemically and UVB-induced skin carcinogenesis in mice, as well as in various murine models of human cancers (Athar et al., 2007; Gusman et al., 2001). The anticarcinogenic effects of resveratrol are closely associated with its antioxidant and antiinflammatory activity (Athar et al., 2007) In addition, resveratrol also inhibits angiogenesis and maintains vascular fitness through antioxidant activity, thus exhibiting dual antitumoral action (Kasiotis et al., 2013).

Despite potential pharmacological activities, the in vivo biological effects of resveratrol appear strongly limited due to poor bioavailability. This has restricted the therapeutic applications of resveratrol for health beneficial effects in practice (Amri et al., 2012). The poor in vivo efficacy is attributed to its poor physicochemical characteristics, namely, aqueous solubility and stability. Aqueous solubility of resveratrol is ~30 mg/L. It has more solubility in DMSO ~160 mg/L, in ethanol 500 mg/L, and in coconut oil 1.8 mg/L (Amri et al., 2012; Hung et al., 2006). Its aqueous solubility categorises it as practically insoluble substance. However, higher value of log P (3.1) is responsible for its lipid solubility and permeability across the biological membranes. This classifies resveratrol as BCS Class II drug (low solubility, high permeability) (Amri et al., 2012; Seljak et al., 2014). Resveratrol undergoes rapid and extensive intestinal presystemic metabolism after oral administration, resulting in almost nil bioavailability (Marier

et al., 2002; Maier-Salamon et al., 2008; Seljak et al., 2014; Wenzel and Somoza, 2005; Yu et al., 2002).

Resveratrol exists as two structural isomers: cis- (Z) andIans- (E). The trans-isomer is superior in stability and activity to the cis isomer (Filip et al., 2003; Fulda, 2010; Orallo, 2006). The difference in activity of the isomers was attributed to the nonplanar conformation of cis isomer (Mérillon et al., 1997). Cis-resveratrol is unstable and is therefore not available commercially. Resveratrol is also a photosensitive chemical (Trela and Waterhouse, 1996).

Sessa et al. (2011) developed an efficient food-grade nanoemulsion of resveratrol by high-pressure homogenization. Objective of the study was to formulate physically and chemically stable nanoemulsion using natural excipients without affecting its antioxidant efficacy in vivo. Emulsion formulation was based on peanut oil and the combination of emulsifiers. Lipophilic emulsifiers were soy lecithin Solec IP, soy lecithin Lecinova, and glycerol monooleate and hydrophilic emulsifiers were sugar ester P1670, defatted soy lecithin Solec FS-B, and polysorbate Tween 20. Ethanol was used as cosolvent to solubilize resveratrol. Primary emulsion was prepared using high-speed homogenizer at 24,000 rpm for 4 min. It was later processed on bench-top HpH at 300 MPa for 10 cycles. Resultant nanoemulsion was physically as well as chemically stable, in terms of mean droplet size (<180 nm) and resveratrol loading, during both accelerated aging and gastrointestinal digestion. These formulations also exhibited the highest chemical and cellular antioxidant activities, which were comparable to unencapsulated resveratrol dissolved in DMSO. This implies that nanoencapsulated resveratrol, not being metabolized in the gastrointestinal tract, can be potentially absorbed through the intestinal wall in active form. Sessa et al. (2014) further extended their work for bioavailability assessment of resveratrol through nanoemulsion. Lecithin-based nanoemulsion showed faster transport of resveratrol through Caco-2 cell monolayers, in characteristically shorter times than those required for the metabolism. Encapsulation of resveratrol in the inner core of the nanoemulsion, as confirmed using fluorescence spectroscopy, was responsible for the increased chemical stability. Release profile was analyzed using dialysis bags and was found to be sustained in comparison to the unencapsulated compound.

SEDDS of resveratrol was developed (Amri et al., 2014). Formulation containing 20% Miglyol® 812, 70% Montanox® 80, and 10% ethanol 96% v/v was found to be optimal atoxic to bovine aortic epithelial cells and cellular uptake studies showed significantly increased intracellular concentrations of resveratrol. Improved endothelial cell protection from hydrogen peroxide induced injury

was observed with cells treated with self-emulsifying composition in comparison with resveratrol dissolved with ethanol.

Pund et al. (2014a) developed self-nanoemulsifying delivery system of resveratrol using Acrysol K 150 as a lipid. Labrasol and Transcutol HP were used as surfactant and cosolvent. The developed systems showed rapid emulsification with an average globule diameter; 85–120 nm and slight negative zeta potential. The nanocompositions were stable to heat as observed from cloud point above 55°C and also exhibited gastrointestinal stability for variations in the pH. Nanoemulsification has significantly increased the in vitro cytotoxicity in MCF-7 breast cancer cells. Enhanced antiangiogenic potential of higher lipid composition supported the therapeutic application of nanoemulsion of resveratrol for breast cancer therapy.

A kinetically stable, rapid-dissolving nanoemulsion of resveratrol was formulated using vitamin E:sefsol (1:1) as the oil phase, Tween 80 as the surfactant, and Transcutol P as the cosurfactant for the better management o' Parkinson's disease (Pangeni et al., 2014). The nanoemulsion was prepared by a spontaneous emulsification method, followed by high-pressure homogenization. The homogenized formulation containing resveratrol 150 mg/mL, showed spherical globules with an average globule diameter of 102 nm, with narrow size distribution and optimal zeta potential of –35 mV. The antioxidant activity determined by using a DPPH assay showed high scavenging efficiency. Pharmacokinetic studies showed the higher concentration of resveratrol in the brain (brain/blood ratio: 2.86 ± 0.70) following intranasal delivery. Histopathological studies showed decreased degenerative changes in the resveratrol nanoemulsion administered groups. The levels of glutathione and superoxide dismutase were significantly higher, and the level of malonaldehyde was significantly lower in the resveratrol nanoemulsion treated group.

Resveratrol is susceptible to intestinal glucuronidation and use of labrasol inhibits the intestinal glucuronidation and thus improved bioavailability of resveratrol 560%. Nanoemulsion containing Labrasol as coemulsifier, showed significant and dose-dependent increase in the transport of resveratrol in an in vitro absorption study with everted sacs (Zhou et al., 2015).

Resveratrol is a potential phytochemical and can be a pharmacological drug for prevention and treatment of many diseases. However, till date its role is still a dietary nonnutritional bioactive phytoconstituents due to its poor bioavailability. Nanoemulsification has certainly improved its bioavailability and biological efficacy, which will reduce its dose and can assist to become a commercially viable nanotherapeutic.

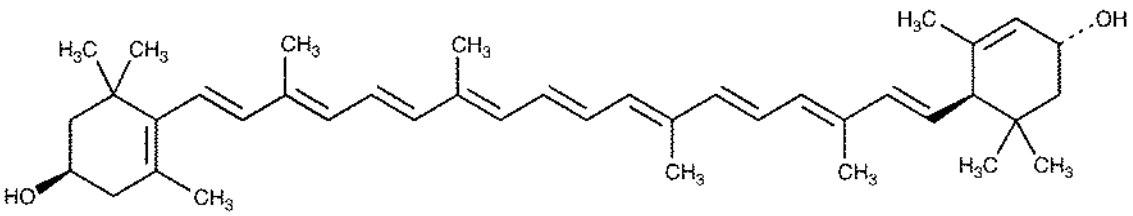

Figure 13.9. Lutein.

5.3 Lutein

Lutein is an important xanthophyll type carotenoid organic natural pigment (Qian et al., 2012), mainly found concentrated in the macular region of human eye (Shegokar and Mitri, 2012; Fig. 13.9). Lutein is beneficial to age-related macular degeneration and cataract (Stringham and Hammond, 2005).

Carotenoids are synthesized only by plants, not by animals. Their presence in animal tissues is entirely of dietary origin. Although animals cannot synthesize carotenoids de novo, they can convert them into other carotenoids or can metabolize them (Granado-Lorencio et al., 2010; Hencken, 1992). There are two categories of carotenoids: (1) Carotenes comprised entirely of carbon and hydrogen, for example, α-carotene, β-carotene, and lycopene; and (2) xanthophylls comprised of carbon, hydrogen, and oxygen, for example, lutein and zeaxanthin (Qian et al., 2012).

Only lutein and zeaxanthin, amongst all carotenoids, cross the blood-retina barrier and become concentrated in the macular region (Johnson, 2012). Lutein and zeaxanthin are stereoisomers of each other and are referred as macular pigments or macular carotenoids because of their location in macular region (Dasch et al., 2005; Krinsky et al., 2003). Lutein absorbs high-energy blue light (Johnson, 2014; Krinsky et al., 2003) and provide protection against age related maculopathy (Landrum and Bone, 2001) and age-related macular degeneration (Abdel-Aal et al., 2013; Carpentier et al., 2009; Huang et al., 2015; Liu et al., 2014). Lutein consumption also reduces the incidence if cataract (Manayi et al., 2015). Cataract, an age related disorder is one of the most leading cause of blindness. Photo-induced oxidative stress is responsible for the initiation and progression of the disease. Lutein gets concentrated in the macula, suppresses the oxidative stress in the eye tissues, and thus reduces the risk of cataract (Arnal et al., 2009; Delcourt et al., 2006; Manayi et al., 2015; Moeller et al., 2000).

Lutein also plays important role in cognitive development. The greater proportion of lutein, amongst all carotenoids, in the pediatric brain suggests valuable role of lutein during neural

development. In adults, higher lutein status is related to better cognitive performance, and lutein supplementation improves cognition (Johnson, 2014). Vishwanathan et al. (2014) correlated macular pigment optical density and the cognitive function in older people. Relatively higher concentration of macular pigments in multiple central nervous tissues, like cortex and neural retina is associated with better global cognition, verbal learning, and fluency, recall, processing speed and perceptual speed. Serum levels of xanthophylls were significantly related to only verbal fluency' Alzheimer's disease patients show significantly less macular pigments and poorer vision (Nolan et al., 2015). Supplementation with lutein and zeaxanthin carotenoids improves macular pigment levels and visual as well as cognitive function (Nolan et al., 2014; Nolan et al., 2015).'Huntington's disease is also a cognitive and motor disorder and lutein has been proved as a promising candidate for the management of Huntington's disease (Binawade and Jagtap, 2013).

Lutein exhibits antiinflammatory and antitumor properties and has shown to modulate the expression of growth and survival-associated genes in prostate cancer cells (Rafi et al., 2015). Lutein was investigated for its antimutagenic activity in vitro. The strong antioxidant activity of lutein and inhibition of carcinogen metabolism enzymes were responsible for the inhibition of mutagenicity (Sindhu et al., 2012). Lutein is hepatoprotective (Sindhu et al., 2010), nephroprotective (Sindhu and Kuttan, 2013) and beneficial in reducing the risk of breast cancer (Wang et al., 2014). Lutein protects the skin from UV-induced damage and help reduce the risk of cardiovascular disease (Alves-Rodrigues and Shao, 2004).

Despite promising therapeutic benefits, poor and variable bioavailability of lutein is still a concern. Inherent extremely poor aqueous solubility, 1.3×10^{-9} M of lutein is responsible for its low bioavailability (Liu et al., 2015; Shanmugam et al., 2011). However, lutein is soluble in fats and lipids and gets absorbed via chylomicrons (Yeum and Russell, 2002). Formulation composition (Evans et al., 2013) and dissolution of lutein are responsible factors for lutein bioavailability (Bowen et al., 2002). Bioavailability of lutein gets enhanced by use of lipids in the formulation (Baskaran et al., 2003; Gorusupudi and Vallikannan, 2012; Lakshminarayana et al., 2006; Mamatha and Baskaran, 2011).

Shanmugam et al. (2011) developed lipid-based nanoemulsifying formulation using Phosal® 53 MCT, 53% phosphatidylcholine in medium-chain fatty acid triglycerides as a new carrier. Phosphatidylcholine is an endogenous lipid in bile secretion that undergoes natural digestion process by phospholipase and forms

lyso-phosphatidylcholine. Phosphatidylcholine as well as lyso-phosphatidylcholine are efficient emulsifiers and help the drug to stay in solubilized form, thereby improving the absorption of lutein. Bioavailability studies in beagle dogs after oral administration showed almost 11-fold increase in C_{max} and relative bioavailability, 473% in comparison to control formulation (devoid of phospholipid). T_{max} was reduced by almost 6.5 h indicative of rapid permeation of the nanoemulsified lutein in presence of phospholipid. Retinal accumulation of lutein was analyzed in rats by administration of single oral dose daily for 14 days. Lutein concentration in the eye of the rats was ~500-fold higher for phospholipid nanoemulsifying formulation as compared to control formulation.

Vishwanathan et al. (2009) formulated a stable nanoemulsion of lutein by microfluidization using food-grade soyabean oil as an oil phase. The nanoemulsion was stable with globule size 150 nm. In vivo bioavailability studies were carried out in healthy human volunteers. Two different lutein supplements were compared to the nanoemulsion formulation in two separate pilot cross-over studies. Study 1 utilized a 6 mg lutein supplement daily for one week whereas study 2 consisted of a multivitamin containing 2 mg of lutein. After a one-week baseline phase, subjects consumed a lutein supplement pill followed by a lutein nanoemulsion added to orange juice for one week each with a 2-week washout phase between treatments. In study 1, mean serum lutein concentrations was increased by 104% and 167% after treatment with 6 mg supplement and nanoemulsion phases, respectively. In study 2, mean serum lutein concentrations increased by 37% and 75% after the 2 mg lutein supplement and nanoemulsion phases, respectively. The nanoemulsions resulted in 31% and 28% increased serum lutein concentrations in study 1 and study 2 compared to the supplement. This study demonstrated the significant greater bioavailability of lutein when administered as nanoemulsion.

Vishwanathan et al. (2011) extended their work on lutein nanoemulsion. Comparative analysis was carried out for digestive stability, micellization efficiency, and cellular uptake of lutein by Caco-2 cells, from egg yolks and three different nanoemulsions. The nanoemulsions were prepared using three different emulsifiers, Phospholipon 85G (N1), Polysorbate 80 (N2), and Lipoid S45 (N3), respectively. Digestive stability of lutein from the egg yolks and the three nanoemulsions did not differ significantly. The efficiency of micellarization of lutein during intestinal digestion was significantly greater from N1 and N2 as compared to egg yolks. Cellular uptake of lutein micelles obtained from the egg yolks was

found to be maximum and therefore egg yolk matrix was considered suitable for lutein uptake by Caco-2 cells despite the significantly smaller globule size of the nanoemulsions.

Murillo et al. (2015) studied effect of lutein nanoemulsion (globule size ~250 nm) in reducing the hepatic steatosis and liver inflammation in guinea pigs with diet-induced liver injury. Hypercholesterolemic diet was fed to guinea pigs (control group, 0.25 g/100 g dietary cholesterol), and lutein (3 mg/d) and lutein nanoemulsion (3 mg/d) was administered to treatment groups along with 0.25 g/100 g dietary cholesterol. Plasma lutein concentration was found to be 3 times higher in the nanoemulsion treated group as compared to the lutein group. Lutein concentrations in liver, adipose, and eyes were greater in the nanoemulsion-treated group, indicating easy and effective absorption and delivery to the target tissues by the nanoemulsion. Hepatic steatosis, inflammatory cytokines, interleukin-1β beta and monocyte chemo-attractant protein-1 were significantly lower in nanoemulsion treated group. This study by Murillo et al. (2015) demonstrates the higher lutein bioavailability and improved antiinflammatory efficacy of lutein nanoemulsion.

These several studies on nanoemulsification of lutein suggest the bioenhancing potential of nanoemulsion for effective and targeted delivery.

6 Concluding Remarks

Nanoemulsion is a now a cutting-edge encapsulation strategy for improving the solubility, bioaccessibility, and bioavailability of lipophilic nutraceuticals. Several studies of nanoemulsified nutraceuticals signify the enhanced pharmacological effects due to nanoemulsification, thereby reducing the dose required for a wide range of safe and nontoxic therapeutic applications. However, there are several challenges and obstacles in realizing successful translation of nanosized nutraceuticals from laboratory to food as well as the pharmaceutical market. First and foremost, short-term as well as long-term safety of these nanotechnology-based methods of sdelivery of nutraceuticals must be established in preclinical and clinical settings. Various other factors such as cost of the materials and advanced instrumentation, feasibility of developing a palatable emulsified product, regulatory status, ease of manufacture and scale-up, reproducibility of in vitro physicochemical properties and in vivo therapeutic effectiveness, and cost to benefit ratio for nanonutraceuticals are also major factors affecting commercialization. However, spontaneously emulsifying systems have emerged as a novel approach to

rejuvenate lipophilic nutraceuticals. These systems havegreat potential to overcome these obstacles and to succeed in reaching food and pharmaceutical markets. A combination of a variety of oils, emulsifiers, and/or costabilizers that are generally recognized as safe, and new methodologies for emulsification, have extended the applicability of nanoemulsion for a wide variety of nutraceuticals. To summarize, the nanoemulsions of nutraceuticals have the potential to having profound effects on the new era of fortified food products and novel drug delivery.

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14

BIOAVAILABILITY AND DELIVERY OF NUTRACEUTICALS BY NANOPARTICLES

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1 Introduction

Nanotechnology is of substantial importance to the food industry. Nanotechnological approaches promise new amenities to create novel structures that enhance organoleptic attributes and nutritional quality of foods (eg, foods with improved mouthfeel; stable encapsulated ingredients [eg, probiotics, omega-3 oils, vitamins] during storage and processing), new devices (eg, nanotechnology-based packaging materials with improved mechanical, heat resistant and barrier properties; nanotracers), encapsulation systems that allow the delivery of nutraceuticals through foods, and new processes that have the potential to significantly influence the processing (eg, nanofiltration, nanoscale enzymatic reactors), safety and security (eg, nanobiosensors, nanoparticles having the ability to selectively bind and remove chemicals from food) of foods (Augustin and Oliver, 2012; Chaudhry et al., 2008; Farhang, 2007; Sanguansri and Augustin, 2006; Sozer and Kokini, 2009).

In fact, food being "Nature's own nanotechnology" is not a novel conception (Leser et al., 2003). For example, milk fat globule membrane has a nanostructured trilayer, mainly composed of proteins, phospholipids, polar lipids, glycolipids, glycoproteins, enzymes, and other minor components. The constitution and organization of molecular building blocks designates their natural function. For example, the casein micelle is appropriate for being a natural carrier for calcium, and the native milk fat globule membrane stabilizes the fat globules in milk, preserves the milk fat from lipolysis, and has got antibacterial activity. These examples assert the importance of the organization of matter and its

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role in defining function. It is not surprising, therefore that structures in nature have inspired the architectural design of functional nanostructured materials (Augustin and Oliver, 2012; Lopez et al., 2010; Wendell et al., 2006). It is also known that many food proteins, folding into their stable globular structure, are formed on a wide range of length scales changing between 10 and 100 nm in size, and most polysaccharides and lipids are linear polymers less than 2 nm in thickness (Chaudhry et al., 2008). In other words, most of nutritional elements (eg, lycopene, lipoproteins, whey proteins, lactose, amino acids) are already in nanoscale. For example, the structure of muscle in meat and fish shows a complex and highly organized nanostructure. The nanostructural characteristics of pectins are associated with fruit firmness. Biological nanoparticles are generally biocompatible and have reproducible structure. Thus, nanotechnologists are hopeful of the development of enhanced quality and safety in engineered food products, since targeted delivery of bioactive compounds will be successful when carried through foods that are already familiar to the human digestive system. Besides, processing of foods may result in losing some or all of the nutrients under severe heat treatments of pasteurization or canning; or processed foods may not naturally contain nutrients like antioxidants at all. Furthermore, adding antioxidants or other valuable bioactive molecules to those products with conventional methods may encounter some difficulties because of the heat sensitivity of those nutrients. Thereby, nanoengineered products give an opportunity to protect valuable nutrients against degradation during manufacturing and storage (Kaya-Celiker and Mallikarjunan, 2012; Magnuson et al., 2011; Sekhon, 2014; Takhistov, 2006; Zhang et al., 2008a). Currently, greater fundamental understanding of polymer-polymer and polymer-nutraceutical interactions at the molecular level and their potency on functional properties of the delivery systems is needed to provide design of superior nutraceutical carriers for use in the food industry (Bouwmeester et al., 2009).

In this chapter, some applications of nanonutraceuticals are described and the development of nanodelivery systems for improving the bioavailability of many functional compounds is briefly discussed.

2 Nutraceuticals and Functional Foods

The term *nutraceuticals* has combined the words “nutrition” and “pharmaceuticals” that reflect that these products have potential as treatment and need to be treated similar to pharmaceuticals

(Pathak, 2010). There is no worldwide definition of nutraceuticals and/or functional foods as it varies across countries and markets (Aryee and Boye, 2015). Health Canada (1998) defines a nutraceutical as a "product isolated or refined from foods that is generally marketed in medicinal forms not normally related with foods. A nutraceutical is evidenced to have a physiological benefit or provide protection against chronic disease."

Although there is no clear-cut distinction between functional foods and nutraceuticals, the basic difference between them is in the form in which they are presented. Functional foods are "foods" similar in appearance to conventional foods, or they may even be conventional foods, consumed within a usual diet, evidenced to have physiological benefits and/or to reduce the risk of chronic disease beyond basic nutritional functions. Nutraceuticals, on the other hand, are dietary supplements that supply a concentrated version of an admitted bioactive agent extracted from a food, and are presented in a "nonfood matrix," often in the form of capsules or tablets and utilized with the aim of promoting health in dosages that exceed those naturally present in foods (Clydesdale, 1997; Espin et al., 2007; Hsieh and Ofori 2010). The importance given to the role of foods has changed from substances consumed only to quench hunger or to provide needed nutrients for normal cellular function to substances that can potentially promote health and wellness and, especially, reduce risk of disease. These foods are referred to as nutraceuticals and/or functional foods with various reported bioactive functions (eg, immunomodulators, antihypertensives, osteoprotectives, hypocholesterolemics, antioxidatives, antiestrogenics, anticarcinogenics, and antimicrobials). Today's consumers are interested in preventing and/or slowing the progression of illness and disability before they become irreversible and expensive, to promote the quality of life. In return for this demand, food companies are developing technologies for processing health and wellness products that will improve the effectiveness of these products, maximize the potential benefits to consumers, and be cost-effective for the industry's survival in a competitive marketplace (Aryee and Boye, 2015; Cencic and Chingwaru, 2010). With the exception of modern medicinal systems that target urgent relief of symptoms and diseases, diet regime supports a moderate long-term process for illness prevention or treatment. Being isolated from natural nutritional sources, nutraceuticals are anticipated to reveal relatively less toxicity and less secondary side effects than drugs used to cure similar symptoms (Bernal et al., 2011; Chauhan et al., 2013; Ting et al., 2014).

Nutraceuticals and dietary supplements can be broadly classified into five categories, which are named vitamins, minerals,

botanical substances, herbal extracts, and miscellaneous or specialty components. The vitamins category includes fat and water-soluble vitamins and nutritional factors. The minerals category comprises mineral chelates, salts, single and trace elements, and multiple minerals consisting of amino acids mixes. Some mixed and single whole herbs, essential oils, tea mixtures, and traditional formulas are counted as botanical substances. The specialty components include antioxidants, carotenoids, omega-3, and omega-6 fatty acids, phytosterols, anthocyanins, flavonoids, probiotics, lecithins, and digestive acids (Koomer, 2010).

Bioactive components in functional food and nutraceutical products are naturally found when ingested alive in plants, animals, bacteria, fungi, and microalgae, and their primary and secondary metabolites. Primary metabolites including amino acids, nucleic acids, and fatty acids, are required for normal healthy growth and development, while secondary metabolites, such as phenolics, carotenoids, terpenoids, and alkaloids, are synthesized in specialized cell types under specific conditions. Except from their role when ingested alive in dairy and nondairy products to improve the quality of intestinal microflora and gastrointestinal (GI) health, some generally recognized as safe (GRAS) microorganisms may be indirect sources of high-yielding nutraceutical and functional ingredients (eg, conjugated linoleic acid, bioactive peptides, and vitamins liberated during fermentation). Probiotic microorganisms may further provide useful beneficial effects such as the prevention of food intolerance and/or sensitivity, and they may further decrease food allergies by degrading and decreasing allergenic epitopes required to induce an inflammatory response (Aryee and Boye, 2015; Champagne et al., 2005; Di Crisco et al., 2010; Gibson, 2004; Vasudha and Mishra, 2013).

The delivery of nutraceuticals is a major challenge as their effectiveness depends mainly on their stability and bioavailability. For this purpose, two groups of factors should be considered: (1) effect of processing of the food matrix and storage conditions on nutraceuticals, for example, heating conditions, oxygen, and light, which results in significant losses of the loaded bioactive constituents; (2) protection of bioactive molecules from degradation during their passage through the gut, which depends on gastric residence time, permeability, and/or solubility, pH, and compatibility with other food constituents. Generally, a small proportion of nutraceuticals consumed in the free form remains available. On the other hand, addition of bioactive constituents may have some undesirable effects on sensory properties of food matrix. For this reason, the objectives of food manufacturers and

nutritionists have been to maximize the availability of administered nutraceuticals without compromising consumer acceptability. Several strategies have been developed to maintain the stability of the active principle throughout processing, handling, and passage through the gut until it reaches their target in the body. Also, the used approach should minimize the deleterious effect of the added free nutraceutical molecules on the quality of the food matrix (Abd El-Salam and El-Shibiny, 2012).

2.1 Routes of Nutraceutical Delivery

Nowadays, many different delivery systems are available to deliver bioactive components in nutraceuticals and functional foods. However, clear in vitro or in vivo evidences of their biological effectiveness are still limited (Huang et al., 2010).

2.1.1 Oral Delivery

Nutraceutical delivery through oral route is considered to be the most acceptable, cost-efficient, and preferred route as it follows the same natural process of food and nutrient consumption in the body. But this route is often influenced by dietary factors that may either enhance or impede nutraceutical bioavailability. For example, lutein shows a significantly increased absorption profile when consumed with a high fat meal, and lycopene absorption improves with concomitant intake of β-carotene (Alves-Rodrigues and Shao, 2004; Khachik et al., 2002). Fat-soluble vitamins are preferentially absorbed with a meal, yet fiber interferes with the oral absorption of some antioxidants. In the gastrointestinal tract (GIT), the efficiency of any formulations depends on the physiology at the administration site, on the composition and the thickness of the membrane barriers present at those sites. Among the various membrane barriers in the GIT, the first one that a drug and/or functional food encounters is the mucus layer. This viscoelastic translucent layer secreted throughout the GIT and covering the majority of the epithelium is a potential issue for drug and nutrient absorption, since it prevents direct adhesion to the epithelial cells and retards the transport of active molecules. Food and/or drug delivery is completed when the active molecules reach the target cells. This means that molecules must reach the blood stream and consequently should not only adhere to the mucus layer but they must diffuse through it. The various components of the mucus layer can affect the diffusion of the controlled-release formulation through this membrane (Lafitte, 2008).

Nutraceuticals delivered via the oral route include, glucosamine, chondroitin, lycopene, resveratrol, coenzyme-Q10

(CoQ10), creatine, melatonin, green tea extract, acetyl L-carnitine, S-adenosyl methionine, lipoic acid, and water and fat soluble vitamins, to name but a few. Despite the assumed benefits of nutraceuticals, many have reportedly low oral bioavailability, and studies have shown wide variations in serum levels and inconsistent pharmacokinetics following oral dosing. These anomalies are the result of extreme GIT conditions that the bioactive is exposed to: low stomach pH, degradative and metabolic enzymes, and alkaline pH in the intestine, and so forth (Walle et al., 2004).

According to the in vitro evaluations, the concentration required for nutraceuticals to produce meaningful bioactivities is usually in the micromolar (μM) range, which is at least an order of magnitude higher than the plasma concentration (<1 μM) obtained from normal dietary intake (Scalbert and Williamson, 2000; Scheepens et al., 2010). To obtain the desirable functionalities, an appropriate concentration of the bioactive compound must reach the target site of action. To achieve sufficient system bioavailability, oral dosing concentration of nutraceuticals must be selected to accommodate the fraction that will be eliminated by physiological barriers (Ting et al., 2015). Novel technologies and formulation strategies play a vital role in the success of orally administered nutraceutical agents by protecting them from rapid elimination and degradation and enhancing solubility and permeation through GIT membranes (Braithwaite et al., 2014).

2.1.2 Dermal Delivery

A wide variety of nutraceuticals, including, but not limited to, carotenoids, melatonin, proanthocyanidins, curcumin, ferulic acid, linoleic acid, gingerol, CoQ10, and silymarin, have been claimed to have beneficial effects in various skin diseases or disorders, such as melanoma and nonmelanoma cancers, age-related skin diseases, acne, dermatitis, psoriasis, skin rash, inflammation, and immunomodulation. In addition, nutraceuticals are also used in skin care to improve skin texture, glow, and smoothness (Kunnamakkara et al., 2008; Yendapally, 2010). Nutraceuticals used topically with positive cosmetic benefits include CoQ10, genistein, curcumin, *N*-acetylcysteine, gluconolactone, and fucose-rich sulfated polysaccharides (Rabe et al., 2006; Schwarz et al., 2013; Wijesinghe and Jeon, 2012). The poorly soluble antioxidants rutin, apigenin, and hesperidin were formulated as nanosuspension for application in skin-protective, antiaging cosmetic products (Al Shaal et al., 2010; Mauludin et al., 2009; Shegokar and Müller, 2010). The chronic topical use of dermal products containing nutraceuticals such as CoQ10 and vitamin C may result in noticeable clinical results including reductions in wrinkle depth in elderly aged

skin (Rabe et al., 2006). It is therefore not surprising that more patients are searching for treatments to impede and reverse the age-associated changes in the skin, and more demands have been placed on industry to develop natural dermal products (Braithwaite et al., 2014; Rabe et al., 2006; Schwarz et al., 2013; Wijesinghe and Jeon, 2012). Nutraceutical derived antioxidant effects may be enhanced by the concomitant oral and dermal application of bioactives, such is the case with lutein (Palombo et al., 2007). Although oral dosing more often achieves higher, more consistent plasma levels, dermal application may result in an accumulation of nutraceuticals in the skin. This often superior reservoir effect observed after dermal application is advantageous for long-term storage and repeated provision of nutraceuticals to the body even after cessation of treatment due to the unique buffering effect of the skin (Meinke et al., 2010). The dermal route is a favoured alternative to the oral route, as it by-passes the GIT milieu and reduces hepatic and renal inactivation (Walle et al., 2004). However, some nutraceuticals, when applied topically to the skin, may become unstable on exposure to light or heat due to photo degradation (Braithwaite et al., 2014).

2.1.3 Ophthalmic Delivery

Many nutraceuticals (eg, CoQ10, vitamin E, lutein) when administered ophthalmically have disease-modifying effects on pathologies of the eye due to their antioxidant, antiinflammatory and anticataract properties. Intraocular treatment success is however, largely dependent on the residence time and permeability of the topically administered drop or ointment. Further, this success is often blocked by the body's defense mechanisms, which make it difficult to sustain an effective concentration of drug at the site of action, and thus the bioavailability of an instilled active is often low (De la Fuente et al., 2010). Advanced delivery devices have therefore been developed for prolonged rate-controlled intraocular delivery of drugs (Choonara et al., 2011). Simple inclusion of selected nutraceutical agents dosed locally and concurrently with allopathic agents may extend intraocular retention time, provide synergistic clinical benefits, and prove to be safer alternatives for long-term ophthalmic therapy (Braithwaite et al., 2014; Martinez-Sancho et al., 2006; Peng et al., 2010; Zhang and Wang, 2009).

2.2 Bioaccessibility, Bioavailability and Bioactivity of Nutraceuticals

The concept of bioaccessibility can be defined as the quantity or fraction that is released from the food matrix in the GIT and

becomes available for absorption (Heaney, 2001). This includes digestive transformations of food into material ready for assimilation, the absorption/assimilation into intestinal epithelium cells, and last, the presystemic metabolism (both intestinal and hepatic). For some nutrients, beneficial effects of unabsorbed nutrients (such as binding of bile salts by calcium in the tract) would be missed by absorption-based definitions. Bioaccessibility is usually evaluated by in vitro digestion procedures, generally simulating gastric and small intestinal digestion, sometimes followed by Caco-2 cells uptake (Carbonell-Capella et al., 2014; Courraud et al., 2013). A nutraceutical needs to be solubilized within the GIT for being bioaccessible to enterocyte absorption. Carotenoids and curcuminoids, highly lipophilic nutraceuticals, have low bioaccessibility owing to their poor solubility in aqueous GI fluids (Qian et al., 2012; Salvia-Trujillo et al., 2013).

The term *bioavailability*, which refers to the body's ability to fully or partially absorb ingested bioactives, describes a function that is essential to the ability to exert beneficial effects. It includes also in its definition the utilization of a nutrient and therefore can be defined as the fraction of ingested nutrient or compound that reaches the systemic circulation and is utilized (Wood, 2005). Overall, bioavailability includes GI digestion, absorption, metabolism, tissue distribution, and bioactivity. Consequently, in terms of bioavailability, when a claim is made, it must be demonstrated that the component analyzed is efficiently digested and assimilated and then, once absorbed, exerts a positive effect on human health. However, practical and ethical difficulties are found when measuring bioactivity, so the term "bioavailability" is usually defined as the fraction of a given compound or its metabolite that reaches the systemic circulation, without considering bioactivity (Holst and Williamson, 2008). According to this definition, bioavailability of a compound is determined in vivo in animals or humans as the area under the curve (AUC) (plasma-concentration) of the compound obtained after administration of an acute or chronic dose of an isolated compound or a compound-containing food (Rein et al., 2013). Bioactivity is the specific effect upon exposure to a substance. It includes tissue uptake and the consequent physiological response (such as antioxidant, antiinflammatory). It can be evaluated in vivo, ex vivo, and in vitro (Carbonell-Capella et al., 2014; Fernandez-Garcia et al., 2009).

The bioavailability and adequacy of active ingredients in nutraceuticals and functional foods are important considerations in their formulation. For example, the bioavailability of active ingredients may be changed depending on the specific compound or isomer formed during formulation (Benakmoum et al., 2008;

Kurzer and Xu, 1997; Rao et al., 1998; Xaplanteris et al., 2012). Besides, the fate, characteristics, and behavior of bioactive components subjected to varying conditions of processing and storage (eg, high or low temperature) and their intrinsic properties (eg, high heat stability or lability, pH tolerance, shear stress tolerance) and the possible alterations that could occur following ingestion, digestion, and absorption may diversely affect their potential health benefits. Knowledge of these properties and susceptibilities is important to mitigate any adverse effects during processing and storage. Other factors that need to be determined include appropriate dosage, mode of delivery, possible interactions, toxicology, fate of carrier materials, and short- and long-term side effects based on age, sex, and health status (Aryee and Boye, 2015; Grooms et al., 2013; Paiva and Russell, 1999; Patisual and Jefferson, 2010; Setchell et al., 2003).

The nutrient composition of a food as well as the location of the nutrient within the food structure may influence its bioavailability in vivo. Process-induced changes to the structure of food materials lead to the creation of new microstructures, which can alter release, transformations, and absorption of nutrients in the body (Parada and Aguilera, 2007). Hence, by structuring foods through appropriate processing techniques, the physiologically induced breakdown of food and absorption of nutrients might be controlled. Depending on the pH of gelation, globular proteins can adopt fine-stranded or particulate structures, and these gelation characteristics can be capitalized upon to carry nutrients. For example, whey protein gels with a filamentous structure showed an enhanced ability to transport and release iron in the intestine under in vitro conditions compared to particulate gels (Remondetto et al., 2004). The authors suggested that filamentous gels are potential matrices to improve the bioavailability of iron. Complexes of proteins and polysaccharides can be used to carry nutrients. An example is using complexes of whey protein and low methoxy pectin to carry thiamine in low-acid environments. The optimum entrapment efficiency of thiamine by whey protein-low methoxy pectin complexes occurred at pH 3.5. However, the entrapment efficiency of thiamine differed depending on whether the complex was prepared by preblending or postblending acidification (Bédié et al., 2008). Altering the physicochemical properties of fat-containing foods by remodeling emulsions is an approach that may be used to manipulate the digestion of lipids (Lairon, 2009). For example, the structure of fat globules may be altered by homogenization, where both size and interfacial properties of the fat globule membrane may be changed as a result of the process. The surface area of the fat globule is a primary determinant of the

rate of lipolysis. Therefore, an increase in the surface area accessible to lipase via decreasing the size of the fat globules may be expected to promote lipolysis. Armand et al. (1999) reported that a lower initial fat droplet size accelerated fat digestion by gastric lipase and pancreatic lipase. However, the authors found that fat assimilation in healthy individuals was not affected by fat droplet size although the peak in the serum triacylglycerols was delayed in a finer emulsion. Michalski et al. (2006) showed that changes in the supramolecular structure of milkfat (eg, unemulsified fat with skim milk, emulsions containing fat globules with natural milkfat globule membranes, and finely homogenized fat droplets) in dairy products with the same composition results in altered kinetics of fat digestion (Augustin and Oliver, 2012).

Despite promising results in preclinical settings, the applicability of nutraceuticals to humans has met with limited success largely due to inefficient systemic delivery and poor oral bioavailability of the health-promoting active agents (Siddiqui et al., 2009; Yao et al., 2015). The major challenge of dietary nutraceuticals is their poor oral bioavailability, and one key barrier to the absorption of nutraceuticals is intestinal epithelium because it is difficult for many nutraceuticals to diffuse across the cells through the lipid-bilayer cell membranes. In addition, insufficient gastric residence time, slow absorption from the GIT, poor solubility within the gut, as well as chemical instability under conditions encountered in food processing (temperature, oxygen, light) or GIT (pH, enzymes, presence of other nutrients) also limit the activity and potential health benefits of nutraceutical molecules (Hu and Huang, 2013; Yao et al., 2015).

3 Nanotechnological Approaches for Enhancing Nutritional Quality and Stability of the Nutraceuticals

3.1 Nanoparticles (NP)

Nanomaterials displaying various sizes, shapes, structures, and properties can be formed depending on the process of formation, manufacturing conditions, environmental conditions, properties of the component(s) and any pretreatments (eg, microfluidization, high-pressure processing, extrusion, ultrasonication) performed to modify the properties of the components (Augustin and Oliver, 2012). A description of various nanostructures is provided in Fig. 14.1.

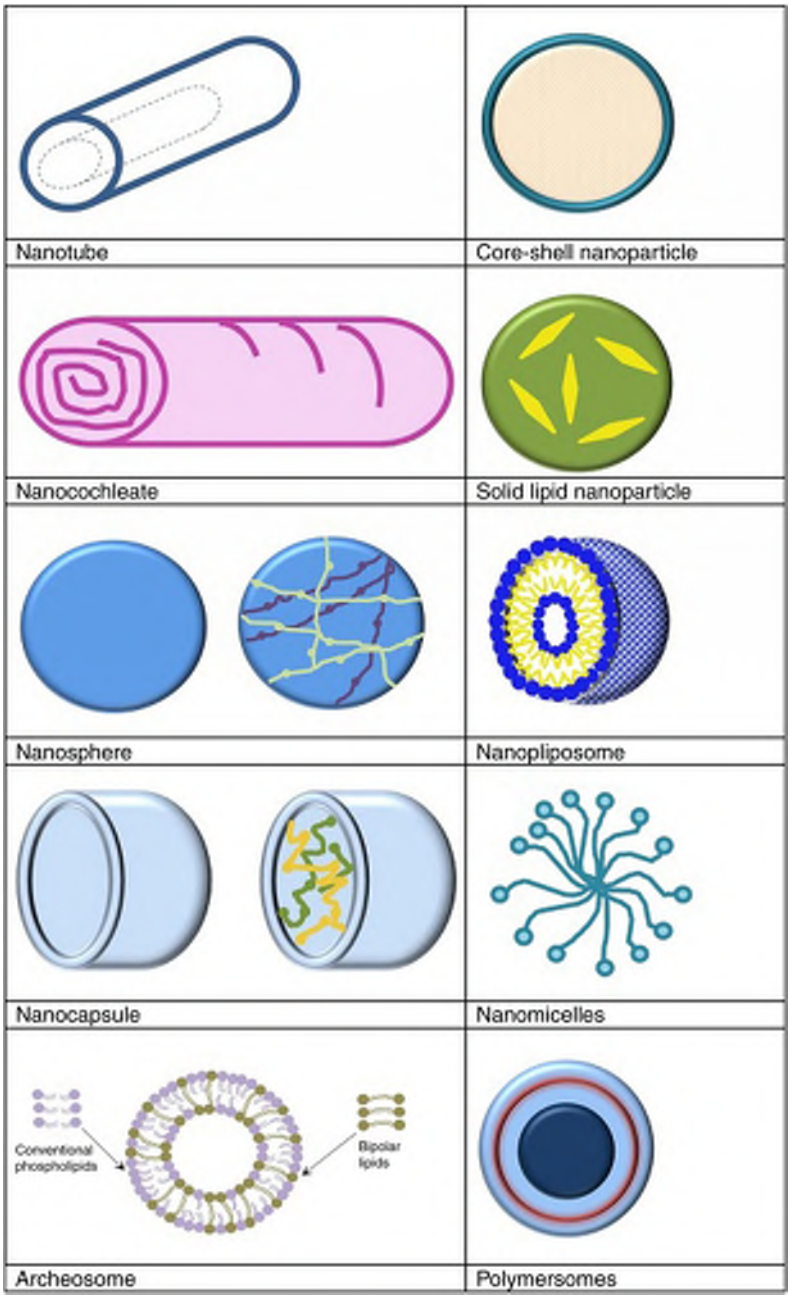

Figure 14.1. Schematic description of several nanostuctures.

Nano- and microtechnologies are applied in the formulation and production of nutraceuticals and are beneficial in many aspects, such as improving the taste, color, flavor, texture, and consistency of food ingredients, as well as increasing the absorption and bioavailability of food ingredients and health supplements. Moreover, research is in progress on the development of food antimicrobials, which are new food packaging materials with improved mechanical, barrier, and antimicrobial properties, and monitoring the condition of the nutraceutical products during transport and storage, and encapsulation of food ingredients or additives (Chaudhry et al., 2008; Wen et al., 2014).

NP comprise of systems with a carrier material such as solid lipid NP, polymeric NP, and from pure compounds.

Solid lipid NP (SLNs) refer to nanoscale-size particles prepared using lipids that remain solid at room temperature (or/and body temperature). The lipid component may comprise a broad range of lipid and lipid-like molecules such as triacylglycerols or waxes (Mehnert and Mader, 2001; Wissing et al., 2004). SLNs have frequently been used to enhance the bioaccessibility of lipophilic nutraceuticals (Qian et al., 2012; Salvia-Trujillo et al., 2013). The main lipid-based nanoencapsulation systems that can potentially be used in food and food supplements are nanoliposomes, archaeosomes and nanocochleates (Mozafari et al., 2006).

Nanoliposomes are defined as bilayer lipid vesicles (<30 or 30–100 nm). Because of their unique properties, for example, hydrophilic and hydrophobic regions, they can entrap, deliver, and release both water-soluble and lipid-soluble material (Mozafari et al., 2006). These may release their contents into cells upon, for example, encountering specific cellular enzymes, due to pH or thermo-sensitivity or after antigen-binding when antibody-tagged (Taylor et al., 2005). Liposomes can be used for delivery of nutraceuticals, antimicrobials, and flavors to foods (Hentschel et al., 2008; Hsieh et al., 2002; Laridi et al., 2003; Were et al., 2003). Lee and Martin (2002) reported that degradation of retinol entrapped in liposomes was decreased by the addition of α-tocopherol. Researches show that the use of liposomes can increase the protection of bioactivity of nutrients against degradation in food (Rashidi and Khosravi-Darani, 2011). Retention of 50% antioxidant activity was shown in liposomal-encapsulated vitamin C after 50 days at refrigerated storage while unprotected ascorbic acid lost all activity after 19 days (Taylor et al., 2005). Mozafari et al. (2006) reported that encapsulation of antioxidant glutathione (GSH) in nanoliposomes delivery system increased protection and residency period of GSH in the body (Kuan et al., 2012). Natural dipeptide antioxidants such as L-carnosine have been applied as biopreservatives,

but their direct application in food is impossible as they are unstable and may induce proteolytic degradation and a potential interaction of peptide with food components. In order to overcome the restraints related to the direct application in food, Maherani et al. (2012) encapsulated these natural antioxidant peptides by nanoliposomes (Dasgupta et al., 2015).

Archaeosomes, which are liposomes made from Archaeobacteria, are even more stress resistant and thermostable when compared to normal liposomes. In general, archaeosomes demonstrate relatively higher stabilities to oxidative stress, alkaline pH, action of phospholipases, bile salts, and serum proteins. For this reason, these are considered as ideal candidates to protect, for example, antioxidants during food processing (Patel, 2000; Patel and Sprott, 1999).

Nanocochleates consist of a continuous, solid, lipid layer sheet rolled up in a spiral fashion with little or no internal aqueous space (Mozafari et al., 2006). Nanocochleates have been used to deliver proteins, peptides, and DNA for vaccine and gene therapy applications. They are resistant to degradation in the GIT, which makes them ideal candidates for oral delivery (Zarif, 2003). Crystalline nanocochleates of about 50 nm in size derived from soya bean can protect micronutrients and antioxidants from degradation during processing and storage (Neethirajan and Jayas, 2011).

Polymeric NP are colloidal particles prepared using natural polymers such as chitosan, gelatin, albumin, alginate, milk protein α-lactalbumin, collagen, and so forth, or synthetic polymers, poly lactic-*co*-glycolic acid (PLGA), and poly lactide (PLA) as carrier stabilized using a surfactant (des Rieux et al., 2006; Graveland-Bikker and de Kruif, 2006; Patel et al., 2008; Soppimath et al., 2001; Zwiorek et al., 2004). They can be broadly divided into reservoir systems like core-shell nanocapsules where the bioactive compound is encapsulated in the core surrounded by polymeric wall, and matrix systems wherein the bioactive molecules are embedded in the polymeric matrix. The loading of functional ingredients in the carrier matrix further leads to prevention of degradation. The release of functional ingredients from carriers occurs through one of the following process (Soppimath et al., 2001; Patel and Velikov, 2011): (1) dissolution; (2) desorption of the surface-bound/adsorbed functional ingredient; (3) diffusion through the matrix; (4) matrix erosion including enzyme degradation; and (5) a combination of these processes.

Nanoencapsulates based on polymers are obtained by the polymerization of more than one type of monomer, typically one hydrophobic and one hydrophilic, so that the resulting molecule is composed of regions that have opposite affinities for an

aqueous solvent. Protein-based nanoencapsulates are comparatively effortless to prepare and can form complexes with lipids, polysaccharides, or other biopolymers. A great variety of nutrients can be integrated (Chen et al., 2006). In addition, to date numerous copolymers have been synthesized, leading to the formation of micelles, nanospheres, polymersomes, and nanocapsules (Kabanov, 2006; Letchford and Burt, 2007).

Micelles are characterized by a core-shell architecture in which the inner core is composed of the hydrophobic regions of the amphiphilic molecules creating a cargo space for the lipophilic bioactive compound (Chen et al., 2006). Nanospheres can be defined as solid colloidal particles in which bioactive compounds are dissolved, entrapped, encapsulated, chemically bound, or adsorbed to the polymer matrix. However, the central core can become more or less solid-like depending on the copolymer composition, which makes it difficult to have a clear distinction between micelles and nanospheres (Chen et al., 2006). Nanocapsules and polymersomes are colloidal-sized, vesicular systems in which the bioactive compound is trapped within a cavity surrounded by a polymer membrane or coating. If the core is an oily liquid and the surrounding polymer a single layer, the vesicle is referred to as a nanocapsule; these systems have found utility in delivery of hydrophobic compound. If the core of the vesicle is an aqueous phase and the surrounding coating is a polymer bilayer, the particle is referred to as a polymersome. These vesicles are analogous to liposomes and find utility in delivery of encapsulation of water-soluble compound, but they differ from liposomes in that the external bilayer is composed of amphiphilic copolymers. Variation in composition, molecular geometry, and relative monomer lengths results in various physico-chemical properties and morphologies of the resulting nanoencapsulates (Letchford and Burt, 2007).

A nanotube is a discrete hollow fiber entity, which has two dimensions in the nanoscale. Self-assembly of hydrolysed calcium binding milk protein α-lactalbumin into nanotubes is another recent development (Momin et al., 2013). These food-protein derived nanotubes demonstrate good stability and offer potential applications in food, nutrients, and pharmaceutics. α-Lactalbumin has an important role in lactose formation, which is essential for milk production; it is already used as a food ingredient in infant formula (Handford et al., 2014; Lien, 2003).

Within the agro-food chain, metal or metal-oxide NP (eg, nano-Ag, nano-ZnO, nano-Cu, nano-TiO_2) are applied. These particles have different structures and shapes. They can be spherical, tubular, irregularly shaped, or can exist in fused aggregated or agglomerated forms (Bouwmeester et al., 2009). The recent inventory of

nanomaterials in food, feed, and agriculture implemented under the European Food Safety Authority (EFSA) shows a tendency from inorganic materials to organic nanomaterials such as nanoencapsulates and nanocomposites (Peters et al., 2014; Smolkova et al., 2015).

3.2 Nano-Delivery Systems

In order to enhance the oral efficacy, many edible delivery systems have been developed to augment the bioavailability of nutraceuticals by means of various mechanisms. Depending on the properties of active compounds, different strategies, such as solubility enhancement, controlled and continuous-release of ingredients (eg, biopolymer-based hydrogels and encapsulation technologies), attenuated interaction between ingredients within a food system, and protection from enzymatic reaction, and so forth, are employed when designing specific delivery systems (Magnuson et al., 2011; Ting et al., 2014).

In NP delivery system, nanocarriers are utilized to encapsulate and deliver bioactive compounds to either enhance their absorption in the GIT by active endocytosis or improve bioactivity in body circulation by specific targeting (des Rieux et al., 2006; Li et al., 2015; Müller et al., 2000).

Several benefits of the advance techniques of nanobased delivery systems compared to current traditional systems are depicted in Fig. 14.2 (Aklakur et al., 2015).

3.2.1 Nanoemulsions and Nanosuspensions

Nanoemulsions (NEs) have emerged as one of the most effective colloidal delivery system hitherto and are increasingly being used in the food and pharmaceutical industries to encapsulate, protect, and deliver lipophilic bioactive ingredients (Bernardi et al., 2011). The numerous potent functional attributes of a NE depend on its polydispersity index, droplet size, surfactants used in reducing the interfacial tension between the two immiscible liquid phases (which contributes to the particle stability, rheology, appearance, color, texture, shelf life), and its resistance against Ostwald ripening, a major factor contributing to the stability concern of NE (Qian and McClements, 2011).

NEs are not thermodynamically stable because the emulsions are nonequilibrium systems and tend to undergo phase separation (Sole et al., 2010). However, NE remain stable during long-term storage due to their nanoscale-sized droplets (<100 nm). This type of NE which are translucent or transparent can be readily incorporated into clear beverages, cosmetics, and medicinal formulations. The important properties of NE are their high kinetic

Figure 14.2. Several benefits of the advance techniques of nanobased delivery systems compared to current traditional systems.

stability against aggregation, creaming or sedimentation and the ability to increase the solubility of lipid bioactive compounds and improve their bioavailability (Abbas et al. 2013; Huang et al., 2010; Lee and McClements, 2010; Lee et al., 2011; Lee and Wong, 2014; McClements, 2010; McClements, 2011; Silva et al., 2011).

NEs have also been used to effectively deliver micronutrients such as carotenoids and fat-soluble vitamins within liposomes via the oral route (Gonnet et al., 2010). Researchers investigated the influence of the carrier oil composition of a NE-based delivery system on β-carotene bioaccessibility using an in vitro model to simulate the GIT (Qian et al., 2012). The researchers reported the β-carotene NE-formulation to possess good physical stability with resistance against chemical degradation in neutral and acidic gastric environments. Additionally, the bioaccessibility of β-carotene was observed to be higher when formulated in long chain triglyceride (LCT)(corn oil)-NE than in medium chain triglyceride

(MCT)-NE or orange oil. An oil/water NE of hyaluronic acid has further been formulated and investigated as a carrier of lipophillic bioactives for transdermal delivery (Kong et al., 2011). An optimized hyaluronic acid formulation resulted in desirable stratum corneum permeability of vitamin E, used as model bioactive with an efficient partitioning and diffusion into the deeper skin layers compared to the control (Braithwaite et al., 2014).

Nanoscale emulsion system enhanced the oral bioavailability of lipophilic compound through improving the aqueous solubility, increasing passive diffusion rate, and facilitating direct uptake of intestinal lymphatic system. The oral bioavailability of lipophilic nutraceuticals, such as berberine from the Chinese herb *Rhizoma coptidis* (Gui et al., 2008), puerarin found in roots of *Pueraria lobaota* (Yu et al., 2011), curcumin from *Curcuma longa L.*, and α-tocopherol (Hatanaka et al., 2010) showed significant improvement in bioavailability when compared to nonemulsion based oral formulations (Ting et al., 2014).

Successful applications of NE include bottled drinking water and milk fortified with vitamins, minerals, and antioxidants (Gu et al., 2005). These functional compounds are incorporated without affecting the organoleptic properties of the final products, yet with controlled release bioactivities (Kuan et al., 2012; Sanguansri and Augustin, 2006).

Silymarin is a mixture of flavolignans (eg, silybin, silydianin, and silychristin) extracted from milk thistle (*Silybum marianum*). The use of silymarin to treat liver diseases, for example, cirrhosis, hepatitis, alcoholic liver disease, and toxin exposure has been well documented (Flora et al., 1998; Fraschini et al., 2002). These biological effects are attributed to the antioxidant, antifibrotic, antiinflammatory, antilipid-peroxidative, and anticarcinogenic activity of silymarin components (Basaga et al., 1997; Luper, 1998; Yang et al., 2004). Clinical application and therapeutic efficiency of silymarin flavolignans are limited due to their poor bioavailability. The latter is mainly due to the crystalline state and low water solubility of silymarin flavonolignans at ambient temperature, as well as to their poor enteral absorption (Gazak et al., 2004). These limitations have been overcome by developing lipid-based delivery systems with increased silybin bioavailability (Javed et al., 2011; Jia et al., 2010; Li et al., 2010; Parveen et al., 2011). Calligaris et al. (2015) evaluated the potential of NE-delivery systems to carry silybin from silymarin extract. The effect of oil type (sunflower oil, extra virgin olive oil and castor oil) on the silybin solubility and in vitro bioaccessibility was determined. Futhermore, the changes in particle size, silybin concentration, oxygen consumption, and hydroperoxide concentration were investigated in NE during storage

at 20°C. According to their results, silybin can be successfully incorporated into physically stable NE prepared with these oils. Although the oil type in the formulation slightly influenced the in vitro bioaccessibility of the silybin, it affected the NE particle size as well as silybin stability during storage. Especially, silybin underwent degradation, exhibiting lower stability in extra virgin oil and sunflower oil than in castor oil.

In another study by Wang et al. (2008), the effect of NE system on the antiinflammation activity of curcumin was evaluated using the mouse ear inflammation model. While curcumin suspension showed no inhibition effect, orally dosed curcumin emulsion (618.6 nm) or nanoemulsion (79.5 nm) exhibited significant reduction in induced mouse ear edema by 43 and 85%, respectively.

5-Demethyltangeretin (5DT) (5-hydroxy-6,7,8,4′-tetramethoxyflavone) is a lipophilic flavonoid that is mainly found in citrus peel (Hirata et al., 2009; Li et al., 2006; Wang et al., 2007). It has been shown to exhibit a range of biological activities, including antiinflammatory, anticarcinogenic, antiviral, antioxidant, antithrombogenic, and antiatherogenic properties. But low water-solubility and poor oral bioavailability may restrict application of 5DT as a nutraceutical. Zheng et al. (2014) investigated the possibility of encapsulating 5DT within food-grade emulsions to improve its intracellular uptake and bioactivity. They utilized emulsion-based delivery systems to increase the bioavailability of 5DT and its uptake by intestinal cancer cells. Oil-in-water emulsions with different mean droplet radius (r = 67, 125, and 203 nm) were fabricated using high-pressure homogenization. The nature of the delivery system had a strong influence on the cellular uptake and bioactivity of 5DT. There was poor uptake of 5DT from both bulk water and bulk oil, while cellular uptake of 5DT was much greater for all three emulsions, with the amount of 5DT taken up increasing with decreasing droplet size. The viability of intestinal cancer cells was inhibited to a much greater extent by 5DT encapsulated in the emulsion systems with smaller droplet sizes. This is presumably because of a greater amount of 5DT that was taken up by the cells where it could demonstrate its inhibitory effect against the cancer cells. These results suggest that NE may increase the cellular uptake and bioactivity of highly lipophilic bioactive components.

Cho et al. (2014) investigated the influence of droplet size and oil digestibility (corn oil versus mineral oil) on the bioavailability of a model long chain fatty acid (heptadecanoic acid) and lipophilic nutraceutical (CoQ10) using a rat feeding study. Heptadecanoic (C17:0) acid was selected as a model fatty acid since it is not normally found in the animal's body, and therefore an increase in its concentration in small intestine tissues is a measure of its

absorption. CoQ10 was used as an example of a model lipophilic nutraceutical. This compound is known to play an important role in the mitochondrial respiratory chain and is an important biological antioxidant (Barshop and Gangoiti, 2007). A number of chronic diseases have been associated with deficiency of CoQ10, including congestive heart failure (Folkers et al., 1985; Quinzii and Hirano, 2010). Consequently, increasing the intake of CoQ10 by fortification or supplementation is of great interest (Belardinelli et al., 2008; Littarru et al., 2011). In summary, the size and composition of the droplets in emulsion-based delivery systems influenced the rate and extent of lipid digestion, as well the bioavailability of CoQ10 and heptadecanoic acid. An in vitro digestion model exhibited that the rate of lipid digestion increased as the droplet size decreased, which was attributed to the increase in surface area of lipid exposed to intestinal juices containing lipase. An in vivo digestion model showed that the bioavailability of heptadecanoic acid and CoQ10 in small intestine tissues was the highest when they were encapsulated with digestible (corn oil) droplets with the smallest size.

Lutein is one of the bioactive food compounds found as lipophilic pigment in various vegetables (Mitri et al., 2011). A beneficial association between consumption of lutein and a lower incidence of ocular diseases, including age-related macular degeneration was reported (Khalil et al., 2012; Liu and Wu, 2010). Moreover, lutein is able to quench singlet oxygen (Palombo et al., 2007) and plays an important role in maintaining skin health by reducing UV-induced inflammation (Stahl and Sies, 2002). Despite these biological activities, lutein is an unstable molecule that has a very low bioavailability caused by its poor solubility in the aqueous media. Mitri et al. (2011) prepared lutein by high-pressure homogenization to produce ~400 nm sized particles within a nanosuspension. The nanosuspension was lyophilized and incorporated in creams and gels for dermal application and modified into pellets to fill into hard gelatine capsules for oral dosing. The nanoformulation provided enhanced penetration due to improved solubility and larger surface area. The permeation of the lutein nanocrystal formulation across a 0.1 μm synthetic cellulose nitrate membrane was reported to be 14 times greater compared to a coarse lutein powder. In the case of the oral formulation, the dissolution profiles demonstrated a clear advantage in terms of improved dissolution and bioavailability.

3.2.2 Solid Lipid Nanoparticles (SLNs)

Solid lipid NP (SLNs) are a class of submicron emulsions containing a solid or semisolid lipid core structure. Due to the reduced

mobility of lipid crystalline structure, SLNs are strong protective mechanism against GIT degradation activities for unstable compounds, such as retinol, CoQ10, citral, and peptides (Almeida and Souto, 2007; Müller et al., 2000; Yang et al., 2011). Furthermore, due to the slow digestion rate of crystalline lipid, SLNs are characterized as a perfect controlled-release system that is capable of hindering burst release and extending the gastric retention time of bioactives (Ting et al., 2014). Moreover, SLNs are more difficult to digest by lipases than liquid oil droplets, which implies that they can be used to develop low-calorie foods (Bonnaire et al., 2008; McClements and Li, 2010). A big advantage of the solid particles is that particles can melt at a preferred temperature, and controlled release of the nutrients incorporated is then obtained (Mao and Miao, 2015).

Some phytochemicals that have been encapsulated within SLNs using various carrier lipids with different melting points include curcuminoids in trimyristin, tristearin, and glyceryl monosterate as solid lipids and MCT as liquid lipid (Nayak et al., 2010), curcuminoids in glyceryl monostearate and stearic acid (Tiyaboonchai et al., 2007), curcuminoids in glyceryl behenate (Kakkar et al., 2011), β-carotene in canola stearin (Malaki Nik et al., 2012), β-carotene in tripalmitin and MCT (Helgason et al., 2009), β-carotene and α-tocopherol in stearyl ferulate or stearic acid (Trombino et al., 2009), and quercetin in glyceryl monostearate (Li et al., 2009). These studies suggested that the water solubility and bioavailability of core materials as well as the stability against degradation can be improved by using their inclusion in SLNs. The results also showed that the encapsulation efficiency and capacity, stability, size, and properties of SLNs can be affected by various parameters, including type and concentration of emulsifier, type of carrier lipid, the ratio of lipid to core material, temperature, and homogenization pressure (Lee and Wong, 2014).

Quercetin, a phytochemical, has attractive properties that lend to its potential use in the treatment of neurological and cardiovascular diseases and cancer therapy and has further advanced to clinical trial evaluation; however, the poor water solubility has limited its further commercialisation (Li et al., 2009; Braithwaite et al., 2014). Li et al. (2009) showed through animal studies that the bioavailability and absorption of quercetin were enhanced significantly when it was encapsulated in SLNs stabilized with Tween 80 and lecithin. The significant enhanced absorption shown in the study was suggested to be due to several factors: (1) the size of SLN being 20–500 nm allows uptake into the intestine; (2) the surface properties of SLN increase the permeability through the intestinal membrane or improve the affinity or adhesion of SLN to the GIT

wall membrane; and (3) quercetin is protected from the degradation during the absorption process (Lee and Wong, 2014).

Yi et al. (2014) prepared protein-stabilized SLNs through a homogenization-evaporation technique. The uptake of β-carotene was significantly improved through nanoparticle delivery systems by 2.6-, 3.4-, and 1.7-fold increase, respectively, for sodium caseinate, whey protein isolate, and soy protein isolate as compared to the free β-carotene. The author proposed that the difference in particle size of the different particles was mainly responsible for the different uptake. The nanosized particles could be directly transported across the epithelium cell layer. As the surface charge of NP greatly affected the particle permeation into the cells (negatively charged), the magnitude of the uptake could be modulated by modifying the surface charge of the particles (eg, using different proteins as emulsifiers).

Compared to emulsions with liquid disperse phase, SLNs are more difficult to be broken down by gastric enzyme activities. Thus, the pharmacokinetic profiles of bioactives delivered in SLNs generally have a delayed peak time and a more stable plasma concentration trend than liquid-lipid emulsion systems. One interesting SLNs system developed by Yu and Huang (2012) included a semisolid organogel formulation. Organogel is a nonpolar semisolid gel composed of gelator and nonpolar solvent (usually lipid in oral formulation). The gel structure allows better physical stability and loading capacity of entrapped compounds. Curcumin, the major pigment of the turmeric root, is a potent bioactive phytochemical was included into the organogel-derived emulsion which showed 9.8-fold increment in the AUC of the pharmaceutical curve when compared to curcumin water suspension. Overall, SLNs systems, regardless of variation in core materials, are effective carriers to enhance and control the bioavailability of lipophilic bioactive compounds (David et al. 2015; Ting et al., 2014).

Teskac and Kristl (2010) used up to 180 nm SLNs as a carrier for resveratrol. Resveratrol solubility, stability, and intracellular delivery were all increased by loading it into SLN. Shao et al. (2009) showed that resveratrol-loaded NP caused significantly higher glioma cell death compared to an equivalent dose of free resveratrol.

Triptolide is a principal bioactive ingredient of the *Tripterygium wilfordii* Hook F, which has been used in traditional Chinese medicine for treating autoimmune and inflammatory diseases for centuries (Brinker et al., 2007). In addition to its immunosuppressive, antifertility, antineoplastic and antiinflammatory properties, triptolide has attracted extensive research interest in its antitumor effects. Previous studies have shown that triptolide is highly effective against a variety of cancer types, including melanoma, breast

cancer, bladder cancer, and gastric cancer (Chen, 2001; Ding et al., 2014; Liu, 2011; Yang et al., 2003). Mei et al. (2003) showed that SLNs prepared for transdermal delivery increased triptolide penetration into the skin and its antiinflammatory activity. This strategy improves the drug's bioavailability at the site of action, reduces the required dose, and reduces dose-dependent side effects like irritation and stinging (Nair et al., 2010).

3.2.3 Nanoencapsulation

Nanoencapsulation involves the incorporation, absorption, or dispersion of bioactive compounds in/at or on small vesicles with nano diameters (Bouwmeester et al., 2009). This technology is the process by which core materials enriched with bioactive compounds are packed within wall materials to form capsules. It helps to protect many functional core compounds, such as antioxidants, enzymes, polyphenol, and micronutrients, to deliver them to the controlled target site and to protect them from an adverse environment (Gouin, 2004; Lee et al., 2013). Based on the capsule size, the name and the technology of the encapsulation are different: the capsules that range from 3 to 800 μm in size are called microcapsules and the technology is called microencapsulation technology (Ahn et al., 2010). If the particle size ranges from 10 to 1000 nm, these are called nanospheres and the technology involved to encapsulate the bioactive compounds within the nano size range is termed *nanoencapsulation technology* (Lopez et al., 2006). Nanocapsules consist of a liquid center surrounded by a polymer membrane, while nanospheres are composed of a dense polymer matrix (Mora-Huertas et al., 2010; Quintanar-Guerrero et al., 1998). In the case of food applications, submicron systems should be made with GRAS substances, including biodegradable polymers (Galindo-Pérez et al., 2015). Nanocapsules differ from nanospheres when the bioactive systems are dispersed uniformly (Couvreur et al., 1995; Kwak, 2014). Reducing the size of the encapsulates into the nanoscale offers opportunities related to extended GI retention time caused by bio-adhesive improvements in the mucus covering the intestinal epithelium (Chen et al., 2006; Medina et al., 2007). Modulations of surface properties (eg, coatings or bio-molecular flags) can enable targeted delivery of compounds. The latter field of application is however, more developed in relation to biomedical applications (Bouwmeester et al., 2009). Nanoencapsulation offers advantages that are similar to, but better than, those of microencapsulation, in terms of conserving the ingredients during processing and distribution, masking unpleasant tastes and flavors, preserving the active substance

for improvement of uptake into the body, as well as the better dispersion of water-insoluble ingredients for the processing of foods (Choi and Kwak, 2014).

Nanocapsules have recently generated much interest in controlled release with the availability of biocompatible and biodegradable polymers. Nanocapsules are submicroscopic colloidal drug carrier systems composed of an oily or an aqueous core surrounded by a thin polymer membrane. The interfacial polymerization of a monomer or the interfacial nanodeposition of a preformed polymer are the two technologies used to create such nanocapsules. The application of shearing, pressure, or heating during food processing can cause the shell or the reservoir type of encapsulates to break, and thus to release its contents (Choi and Kwak, 2014).

Nanocapsules have been used to mask the taste and odor of tuna fish oil (source of omega-3 fatty acids), which is added into bread. The nanocapsules break open only when they reach the stomach and hence the unpleasant fish oil taste can be avoided. Nanocapsules have been used for the protection and controlled release of beneficial live probiotic species to promote healthy gut function. The viability of probiotic organisms including *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, and Bifidobacterium* spp. within freeze-dried yogurt can be improved by nanoencapsulation with calcium alginate (Kailasapathy and Rybka, 1997). Nanoencapsulated *Bifidobacteria* with starch by spray coating exhibited an affordable and industrially convenient encapsulation process (Neethirajan and Jayas, 2011; O'Riordan et al., 2001). Nanoencapsulated live probiotic microorganisms can be incorporated into various foods, including fermented milk, yogurts, cheese, puddings, and fruit-based drinks for the promotion of GI health (Alfadul and Elneshwy, 2010; Handford et al., 2014).

NP are constituted of hydrophobic groups inside and polar groups on surface of particles (Li and Gu, 2014). For this reason, NP are able to remain stable in dispersion system owing to their interparticle repulsions and hydration. Phenolics can be involved in an interaction with hydrophobic sites of NP via hydrogen bonds and hydrophobic interactions. Adequate surface charges and appropriate hydration property maintain phenolic encapsulated NP stable in aqueous system, which improves the water solubility of phenolic compounds. For instance, by using a desolvation method, procyanidin were successfully encapsulated into zein NP and the resultant procyanidine-zein NP increased procyanidin solubility in aqueous system (Zou et al., 2012). PLGA NP, chitosan NP, and protein NP were all stated to encapsulate curcumin to increase its water solubility (Anand et al., 2010;

Das et al., 2010; Duan et al., 2010; Kim et al., 2011; Li et al., 2015; Luz et al., 2012; Mukerjee and Vishwanatha, 2009; Rejinold et al., 2011; Teng et al., 2012).

Epigallocatechin-3-gallate (EGCG) found in green tea has long been documented to provide an array of health benefits including neuroprotection, antitumor effects and cardiovascular protection. Both low acidic and neutral conditions cause rapid degradation of EGCG (Kim and Hong, 2010; Sang et al., 2005; Zhu et al., 1997). Onoue et al. (2011) reported that after encapsulation, the stability of EGCG in simulated stomach fluid (pH 1.2) was about two times higher than free EGCG. Because interaction was established between EGCG and human serum albumin via hydrogen bonds and hydrophobic interactions, EGCG demonstrated much higher stability in neutral and slightly alkaline pH conditions with human serum albumin. Sulfhydryl groups in human serum albumin served as antioxidant against EGCG degradation (Bae et al., 2009; Ishii et al., 2011). The rapid degradation of EGCG observed in soft drinks leaves behind deterioration/degradation products that discolor the beverage and limit the health potential of the remaining compound as most of the activity is lost after long storage times. Coassembled nanovehicles was synthesised for the protection and enhanced delivery of EGCG and other antioxidant polyphenols (Braithwaite et al., 2014). Shpigelman et al. (2010) used thermally modified β-lactoglobulin (βlg) to which EGCG complexed optimally resulting in nanosized coassembles of βlg-EGCG particles that conferred strong protection to EGCG against oxidation. In addition, the nano βlg-EGCG particles were in a size range less than 50 nm providing ideal transparency and suitability of the nanosystem for inclusion in clear beverages.

Recently, several researches have suggested that white tea presents higher levels of antioxidants than green tea because it contains the most pharmacologically active catechin derivatives (Azman et al., 2014; Dias et al., 2013; Dias et al., 2014). Sanna et al. (2015) formulated the white tea extract into novel polymeric NP based on poly (ε-caprolactone) (PCL) and alginate for nutraceutical applications to control the tea polyphenols release in GI fluids and to preserve the antioxidant activity. Interestingly, NP released 20% of the polyphenols in simulated gastric medium, and 80% after 5 h at pH 7.4, showing a good capacity to control the polyphenols delivery. Furthermore, DPPH assay confirmed that white tea extract retained its antioxidant activity and NP protected tea polyphenols from degradation. Moreover, the encapsulation of the white tea extract into NP significantly increased stability, thus preventing the losses of total polyphenol content and catechins over 30 days of storage.

Vitamin D_3 was entrapped in whey protein isolate (WPI) NP prepared by different calcium concentration and aggregation pH. Its stability was investigated in presence of air for 7 days. Residual of vitamin D_3 in NP was higher compared to control samples (water, native WPI, and denaturized WPI). Presence of calcium in composition of particles resulted in formation of compact structure and inhibition of oxygen diffusion in particle. WPI NP containing vitamin D_3 could be used for enriching of clear or cloudy drinks such as herbal beverages, fruit drinks or low-fat food (Abbasi et al., 2014).

Encapsulation of chlorogenic acid (CGA) into chitosan was successfully carried out by ionic gelation method (Nallamuthu et al., 2015). The prepared NP showed a controlled release profile and a preserved antioxidant activity under in vitro conditions. In vitro ABTS assay indicated that the radical scavenging activity of CAG was retained in the nanostructure and further, the release kinetics study revealed the burst release of 69% CGA from NP at the end of 100th hours. They also showed a considerable heat stability demonstrating its usage in various types of thermally processed foods. The synthesized NP with increased bioavailability could be a suitable carrier for better delivery of CGA in food and pharmaceutical applications.

Resveratrol is a polyphenolic compound which has been shown to have several potential benefits for human health, can be extracted from wine industry by-products (Alves, 2012; Catalgol et al., 2012; Neves et al., 2013; Walle et al., 2004). On the other hand, utilization of resveratrol as a nutraceutical ingredient within the food industry is currently limited owing to its poor aqueous solubility, low oral bioavailability, and chemical instability (Hung et al., 2006). Given the favorable prophylactic and therapeutic effects of trans-resveratrol, protection from premature metabolism and from degradation, with increase of its lifetime within the body, are of utmost importance, particularly during the process of intestinal absorption. For this reason, nanodelivery systems have been developed for the encapsulation of trans-resveratrol, which protect the compound during its transport inside the organism while enhancing its bioavailability after oral administration (Neves et al., 2013). The development of resveratrol-loaded NP is essential to further applications as nutraceuticals to supplement juices, yogurts, milk, or cheese with health benefits similar to those attributed exclusively to red wine consumption (Neves et al., 2015).

Davidov-Pardo et al. (2015) evaluated the effect of encapsulating resveratrol in biopolymer NP or biopolymer complexes on its physicochemical stability and bioaccessibility. Resveratrol was encapsulated within biopolymer complexes or within

biopolymer NP. The biopolymer complexes were formed by binding the resveratrol to either caseinate or caseinate-dextran. The biopolymer NP were formed by coating a zein core with either a caseinate or a caseinate-dextran shell. The caseinate-dextran complexes were formed by heating the protein and carbohydrate together under suitable conditions to induce the Maillard reaction. In order to establish the potential effectiveness of these two delivery systems, the physicochemical stability and bioaccessibility of the encapsulated resveratrol were investigated. Both the biopolymer NP and complexes protected trans-resveratrol from isomerization when exposed to UV light, with the NP being more effective. NP coated by caseinate-dextran were more stable to aggregation under simulated GI conditions than those coated by caseinate, probably due to greater steric repulsion. When resveratrol was encapsulated in both biopolymer NP and biopolymer complexes, its bioaccessibility was improved. As a consequence, both delivery systems have the potential for application in functional food and beverage products. The biopolymer complexes are easier to form and can be incorporated into transparent products, whereas the biopolymer NP are more difficult to form and can be used solely in cloudy or opaque products, but are more effective.

Penalva et al. (2015) prepared and characterized casein NP for the oral delivery of folic acid. The encapsulation of this vitamin in these carriers could be of interest to minimize the adverse effects and deteriorative reactions induced during food processing and cooking as well as to improve the oral bioavailability. These NP were prepared by a coacervation process, stabilized with either lysine or arginine and, finally, dried by spray drying. For some batches, the effect of a supplementary treatment of NP (before drying) with high hydrostatic pressure on the properties of the resulting carriers was also evaluated. The resulting NP displayed a mean size close to 150 nm and a folic acid content of around 25 mg per mg nanoparticle. From the in vitro release studies, it was observed that casein NP acted as gastroresistant devices and, thus, folic acid was only released under simulated intestinal conditions. For the pharmacokinetic study, folic acid was orally administered to laboratory animals as a single dose of 1 mg/kg. Animals treated with folic acid-loaded casein NP displayed significantly higher serum levels than those observed in animals receiving an aqueous solution of the vitamin. As a consequence, the oral bioavailability of folic acid when administered as casein NP was calculated to be around 52%, a 50% higher than the traditional aqueous solution. The treatment of casein NP by high hydrostatic pressure modified neither the release profile of the vitamin nor its oral bioavailability.

In another study, electrospraying and nanospray drying were evaluated for the encapsulation of folic acid using both a whey protein concentrate (WPC) matrix and a commercial resistant starch. Results showed that spherical nano-, submicro- and microcapsules were obtained through both techniques, although electrospraying led to smaller capsule sizes and to an enhanced control over their size distribution. Greater encapsulation efficiency was determined using WPC as encapsulating matrix, probably related to interactions between the protein matrix and folic acid which favored the incorporation of the bioactive within the capsules. The best results in terms of bioactive stabilization in the different conditions assayed were also obtained for the WPC capsules, although both materials and encapsulation techniques led to improved folic acid stability, especially under dry conditions (Pérez-Masiá et al., 2015).

3.2.4 Nanostructured Lipid Carriers (NLC)

This new generation of lipid "nano-sized" structures comprising of a lipid matrix with special nanostructures hold potential for optimum nutraceutical delivery via the dermal, oral and topical routes (Teeranachaideekul et al., 2007).

SLNs immobilize the incorporated active compound within the fat lattice (not always), and may increase its stability; they provide the possibility of controlled release, and have high encapsulation efficiency (Mehnert and Mäder, 2012). However, main deficiencies of SLN are low drug loading capacity, and drug expulsion after polymorphic transition of the lipid core (eg, α-crystal → β′-crystal → β-crystal) during storage (Mehnert and Mäder, 2012; Müller et al. 2002), that make SLN unsuitable for many food applications. Nanostructured lipid carriers (NLC) or oil loaded-SLN are modified SLNs in which the lipid phase consists of a biocompatible mixture of solid and liquid lipids (Müller et al., 2002). SLNs are comprised of a solid lipid at room and body temperature; while NLCs vary from SLNs by the incorporation of a liquid lipid into their solid structure (Muchow et al., 2008; Müller et al., 2000; Neves et al., 2013). NLC have an amorphous solid structure or a less-ordered crystalline structure which is not fully crystallized. The incorporation of oil into the core of a solid lipid leads to a higher loading capacity and controlled drug release as the drug is dissolved in the oil and simultaneously encapsulated in the solid lipid (Varshosaz et al., 2010). With respect to the nanoscale of dispersed phase, SLNs and NLC (as well as nanoemulsions) scatter light weakly and so can be incorporated into optically transparent products, have high Brownian motion and may therefore be very stable to particle aggregation and

gravitational separation, and they may increase the bioavailability of incorporated lipophilic compounds. It can be concluded that NLC have some advantages in certain circumstances compared to other colloidal carriers. NLC are widely used to encapsulate lipophilic drugs in pharmaceutical researches. NLC can be produced from food-grade or GRAS ingredients on industrial scale, and hot homogenization technique is the most feasible method for production of these NP (Tamjidi et al., 2013). Currently, NLC containing active compounds are increasingly introduced as ingredients for food applications (Fathi et al., 2013; Hejri et al., 2013; Hentschel et al., 2008; Liu et al., 2012a; Liu and Wu, 2010). MCT and oleic acid (as liquid lipids), stearic acid, glycerol monostearate, glyceryl palmitostearate and glyceryl behenate (as solid lipids), and Tween 80 and lecithin (as emulsifiers), are the most commonly used food-grade ingredients in NLC formulation (Tamjidi et al., 2013; Tamjidi et al., 2014).

Liu and Wu (2010) developed optimized NLC's of lutein for oral delivery that protected the entrapped nutraceutical from simulated gastric fluid and achieved a sustained release.

In order to enhance oral bioavailability of resveratrol for further usage in medicines, dietary supplements, and nutraceuticals, Neves et al. (2013) improved new resveratrol nanodelivery systems, which were solid lipid NP (SLNs) and nanostructured lipid carriers (NLCs) loaded with resveratrol. The in vitro release studies in shelf conditions of storage evinced a insignificant resveratrol release over several hours for both nanosystems. Due to this reason, it could be concluded that both lipid NP were highly stable systems. The in vitro simulation of GI transit showed that resveratrol remained mostly associated to the lipid NP after their incubation in digestive fluids. Thus, it was reported that both nanodelivery systems could be considered suitable carriers for oral administration, conferring protection to the incorporated resveratrol and allowing a controlled release after uptake.

Lacatusu et al. (2013) explored the behavior of fish oil enriched with omega-3 fatty acids in order to obtain stable NLCs with improved characteristics as effective delivery systems for lutein. For this aim, different fish-oil-based lipid nanocarriers with various lutein loading were synthesized and their physicochemical properties were investigated. The in vitro characterization of lutein-lipid nanocarriers showed that the fish oil plays important roles in improving the antioxidant capacity. The free- and lutein-loaded NLCs demonstrated the ability to develop a high blocking effect, with a potential to scavenge up to 98% the oxygen free radicals generated into the chemiluminescence system. The in vitro release profile indicated that NLCs were able to ensure a better, in

vitro sustained release of lutein as compared to conventional nanoemulsions.

Chaurasia et al. (2015) developed and optimized curcumin loaded lipopolysaccharide nanocarriers (C-LPNCs) for oral bioavailability enhancement as well as explored anticancer potential of curcumin for the effective treatment of colorectal cancer. Pharmacokinetic studies revealed ~130-fold increase in oral bioavailability and cytotoxicity studies showed ~23-fold reduction in 50% cell growth inhibition when treated with optimized C-LPNCs as compared to aqueous suspension of pure curcumin. In vivo anticancer study performed with optimized C-LPNCs demonstrated significant increase in efficacy compared with pure curcumin.

NLC's have also been used for the intravenous delivery of the antihepatotoxic herbal nutraceutical, silybin, as an improved application to treat liver disease (Braithwaite et al. 2014). Jia et al. (2010) developed a silybin-NLC that had superior pharmacokinetic properties that included a higher AUC and a biphasic drug release. The novel silybin-NLC formulation achieved a prolonged residence time in the serum and targeted delivery to the liver for improved action.

3.2.5 Self-Emulsifying Drug Delivery Systems

Self-emulsified drug delivery systems (SEDDS) are isotropic mixtures of oils, surfactants and sometimes including cosolvents. Since aqueous phase was not part of the system matrix, SEDDS are considered incomplete emulsion systems that retain their isotropic structure until they are in contact with aqueous solutions. Upon the addition to aqueous environment, SEDDS are simultaneously emulsified into thermodynamically stable emulsions with particle sizes ranging from 100 to 300 nm. When digested orally, GI motility is sufficient to induce the transformation of SEDDS into emulsion. While retaining many advantages of most emulsion systems, SEDDS are a convenient and physically stable alternative for the delivery of nutraceuticals (Chopraa et al., 2011). Following oral administration, SEDDS were reported to provide a twofold increase in the bioavailability compared to a powder formulation (Ljusberg-Wahren et al., 2005; Souto et al., 2013). Since SEDDS are composed of only the isotropic organic phase, they have been proven as an exceptional carrier system for poorly water soluble compounds like curcumin (Chopraa et al., 2011). Unlike single isolated compounds, some nutraceuticals may appear as crude extracts that contain mixtures of multiple bioactive compounds. Compared with single isolated bioactive compound, the encapsulation of extracts has been limited by many factors such as differences in solubility, distribution, and potential interaction

among compositional bio-actives. SEDDS formulations provide a physically stable environment to homogeneously contain the mixture of bioactives during storage while allowing rapid emulsification upon contact with aqueous phase. Crude extracts from *Ginkgo biloba* (Tang et al., 2007) and *Frutus Schisandrae chinensis* (Shao et al., 2010) were encapsulated into the SEDDS and showed significant improvement in oral bioavailabilities. Self-microemulsified drug delivery systems (SMEDDS) are an advanced variation of SEDDS system that form nanosized (<100 nm) emulsion droplets. Smaller emulsion droplet size provides extra benefit to the SEDDS through increasing intestinal absorption rate and lymphatic uptake of lipophilic molecules. Several researchers have utilized SMEDDS as carrier system to enhance bioavailability of bioactive compounds and extracts (Liu et al., 2012b; Ting et al. 2014; You et al., 2005; Zhang et al., 2008b).

Onoue et al. (2015) determined the physicochemical and nutraceutical properties of self-nanoemulsifying particles of coenzyme Q10 (CoQ10/SNEP). Under accelerated conditions, the CoQ10/SNEP was found to be stable in terms of appearance, amorphous nature, and self-nanoemulsifying performance, as long as moisture protection was maintained. Storage of the CoQ10/SNEP under high humidity resulted in slight deliquescence and transition of physicochemical properties, so suitable storage conditions should be considered carefully on the basis of further detailed stability testing. The application of the self-nanoemulsifying strategy to CoQ10 led to significant improvement in both dissolution behavior and the hepatoprotective effects of CoQ10 compared with those of crystalline CoQ10. From these observations, the self-nanoemulsifying formulation might be a promising dosage option for CoQ10 to improve its nutraceutical value.

In another study, Taha et al. (2007) compared the bioavailability of vitamin A SEDDS, vitamin A optimized in capsule, and free vitamin A. Bioavailability was assessed after a single oral dose. The bioavailability increased approximately 2-fold and 1.4-fold for SEDDS and optimized formulation, respectively. It was revealed that SEDDS formulations enhanced the rate and extent of drug absorption compared to the free vitamin A.

4 Nanoparticles in Foods for Improved Nutritional Quality

Food systems are composed of macro- and micronutrients that are needed for metabolism and physiological functions of human tissues and organs. These compounds cover a broad spectrum of

physicochemical and biological properties, and their fate during ingestion, digestion, and absorption in the GIT is important to nutrition and health. The digestion system resembles a biological reactor series with several unit operations. The end result includes macroscopic structure changes by mechanical forces (during mastication and shear forces in the stomach and intestines), dilution by fluids (saliva and digestive fluids), microscopic structure de/reformation due to mass transfer with surrounding fluids and subsequent actions by corresponding compounds, and molecular structure changes due to chemical (eg, acid) and biological (digestive enzymes) catalysts and microorganisms. The degree of these changes is a function of residence time, compositions of digestive fluids, and characteristics of food components (Aguilera, 2006; Aguilera et al., 2000; Parada and Aguilera, 2007). Food matrix microstructures are changed by processing, and this could cause both improved and reduced absorption of nutrients. Eventually, food components are transformed into structures that can be absorbed at the absorption sites. The fraction absorbed through the intestinal walls is commonly referred to as "uptake," and only the fraction uptaken at the intestines and entering the bloodstream is referred to as "bioavailability" of orally ingested compounds (Acosta, 2009). The small intestine is the place where most nutraceuticals are absorbed after their oral ingestion (Oehlke et al., 2014). Generally, compounds or structures are uptaken by the active transport, which involves access to the surface of the epithelial cells on and transportation through channels in the intestinal wall, or passive transport that results from simple diffusion through the epithelial tissues (Acosta, 2009). The eventual aim of engineering food nanostructures is to incorporate them in food systems to utilize their functions. As a part of food systems, the impact of nanostructures on nutritional quality also is critical. Nanostructures from food molecules supply macro- and micronutrients that are critical to human nutrition and health (Zhong and Shah, 2012).

Various NP have been fabricated and tested for their potential use as delivery systems for nutraceuticals with the purpose of improving their health benefits through encapsulation, protection and/or controlled release of nutraceuticals (McClements, 2013; McClements and Xiao, 2014; Yao et al., 2014). Engineered NP can be used to enhance or reduce the absorption of food components by both active and passive transport. Passive transport is improved by reduced dimensions of structures, while the active transport can be engineered based on balancing physical and biological properties of channels in the epithelial tissues and those of materials carrying bioactive compounds (Lesmes and McClements, 2009;

McClements et al., 2007; McClements et al., 2009; Zhong and Shah, 2012).

The oral bioavailability (F) of a nutraceutical encapsulated in NP can be calculated by the following equation (Joyea et al., 2014; Yao et al., 2014; Yao et al., 2015).

$$F = F_B \times F_A \times F_M$$

Hereby, F_B is the fraction of an ingested nutraceutical that survives passage through the upper GIT and that is released from the food matrix/NP into the GI fluids, accordingly becoming bioaccessible for absorption by enterocytes. F_A is the fraction of the bioaccessible nutraceutical that is substantially absorbed by the enterocytes and then transported to the portal blood or lymph (and into the systemic circulation). F_M is the fraction of absorbed nutraceutical that is in an active form after first-pass metabolism in the GIT and liver (and any other forms of metabolism) (Fig. 14.3).

Following digestion of different engineered nanoparticles, nutraceuticals may be released (1) to (A) in the lumen, (2) to (B) trapped in the undigested particles, or (3) to (C) solubilized in mixed micelles. Potential ways of absorption involve: immediate absorption by enterocytes, paracellular uptake via tight junctions,

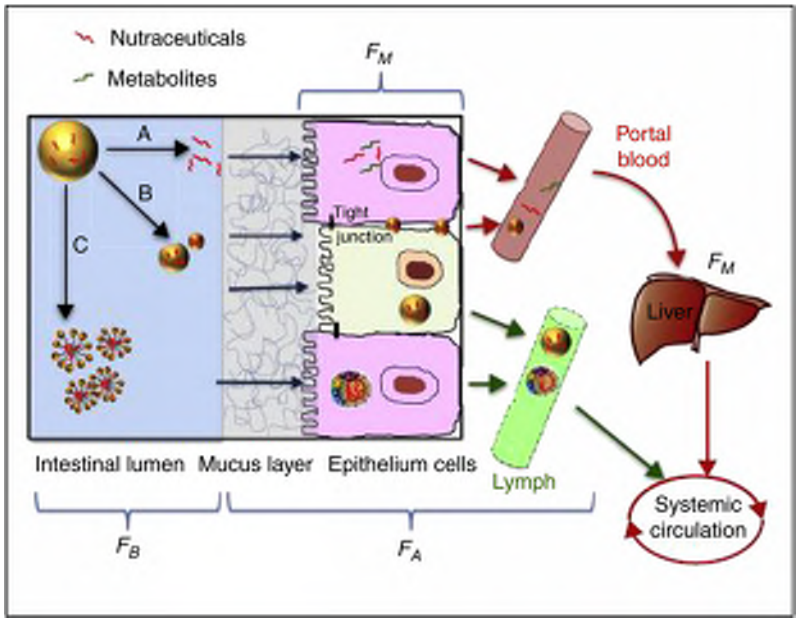

Figure 14.3. The gastrointestinal fate of nutraceuticals encapsulated in engineered nanoparticles. Courtesy of Elsevier. Used with permission.

uptake by *M* cells via Peyer's patches, and chylomicron-mediated transport. Afterwards, nutraceuticals are carried to either portal blood or lymph. Portal blood goes through the liver before accessing systemic circulation, while lymph reaches the systemic circulation without passing through the liver. The enterocytes and the liver are both responsible for the first-pass metabolism of nutraceuticals (Yao et al., 2015).

NP have been developed to protect nutraceuticals from adverse GI conditions. For example, nutraceuticals can be encapsulated in SLNs and biopolymer-based NP that can be designed to protect nutraceuticals from premature degradation and improve their stability in the GIT (Harde et al. 2011; Xu et al. 2013). Dissolution in the stomach of the NP may or may not be desirable depending on the stability of the active ingredients in the acidic pH. If the NP require protection against the acidic environment of the stomach, they can be microencapsulated using enteric coatings (Lee et al., 2003). As the digested food leaves the stomach and enters the duodenum, it mixes with the bile salts that emulsify the fats and other hydrophobic compounds present in the suspension (Acosta, 2009).

A nutraceutical needs to be solubilized within the GIT for being bioaccessible to enterocyte absorption. Lipid-based NP, such as NE and SLNs, have frequently been utilized to improve the bioaccessibility of lipophilic nutraceuticals. The nature of the carrier oil used to solubilize lipophilic nutraceuticals within lipid-based NP has been indicated to influence their loading capacity and bioaccessibility (Qian et al., 2012; Salvia-Trujillo et al., 2013). After ingestion, the compositions, structures and physicochemical properties of nutraceutical-loaded NP may be altered noticeably due to their exposure to different GIT conditions, for example, their physical state, size, charge, and aggregation state. The presence of protein, lipid, and surfactants also plays an essential role in determining the biological fate of lipid-based NP in the GIT, which in turn has a significant effect on the bioaccessibility of nutraceuticals (Yao et al., 2014; Yao et al., 2015).

Lipases hydrolyze digestible carrier oils in NP in the GIT to produce monoacylglycerols and free fatty acids. These lipid digestion products interact with bile salts and phospholipids in the lumen of the small intestine to form "mixed micelles" with complex structures (Yao et al., 2014). Nutraceuticals encapsulated within NP are transferred to the mixed micelles during the digestion process, which greatly enhances their bioaccessibility (Porter and Charman, 2001; Salvia-Trujillo et al., 2013). The type of carrier oils used in NP is crucial for the bioaccessibility of lipophilic nutraceuticals. NE containing mainly LCT produced much higher

bioaccessibility of vitamin E, β-carotene and CoQ10 than those containing mainly MCT (Cho et al., 2014; Qian et al., 2012; Yang and McClements, 2013). In contrast, NE containing MCT has been indicated to produce higher bioaccessibility of curcumin than those containing LCT (Ahmed et al., 2012). These findings bring forward that the effects of different carrier oils on bioaccessibility are specific to different nutraceuticals; therefore NP-based delivery systems should be specifically engineered for particular nutraceuticals in order to effectively improve their bioaccessibility (Yao et al., 2015).

4.1 Effects of the Size and Surface Characteristics of Nanoparticles on Bioavailability

When a compound diffuses from a carrier system before being uptaken in the small intestine, a larger surface area of NP (resulting from smaller particles) increases the rate of mass transfer that in turn may also increase the absorption of the compound (Zhong and Shah, 2012). NE with smaller particles have been declared to give a higher bioaccessibility of β-carotene than those with larger particles (Salvia-Trujillo et al., 2013). A potential explanation for this phenomenon is that smaller lipid particles generate mixed micelles more quickly than larger particles during lipid digestion, which can augment the rate of transfer of the nutraceuticals from the particles to the mixed micelles (Speranza et al., 2013). NP may also have an elongated retention time in the small intestine, when compared to larger structures (Hussaın et al., 2001). Smaller particles can enter intestinal mucous layers and go through structures therein, while bigger particles only go directly straight through allowable bigger channels (Lai et al., 2007). Active transport is the dominant mechanism for highly water-soluble compounds that have poor permeability in the epithelial tissues. In contrast, lipophilic compounds are absorbed by both passive and active transports because of their good permeability through the intestines (Acosta, 2009). NP with size below 20 nm may reversibly disrupt the tight junctions among the epithelial cells and liberate bioactive compounds to capillary blood. After delivery, tight junctions return to normal state for function (Acosta, 2009; Jevprasesphant et al., 2003; Zha et al., 2008). Chitosan, chitosan derivatives, and polyacrylate derivatives (tight junction mediators) were reported to fabricate NP or coating on NP to improve paracellular transport (Bravo-Osuna et al., 2008; des Rieux et al., 2006; Kudsiova and Lawrence, 2008; Li et al., 2015; Martien et al., 2008; Sadeghi et al., 2008; Vllasaliu et al., 2010). In addition, surfactants present in the stomach and small intestine are fully

capable of emulsifying/dissolving water-insoluble compounds into nanoscale structures by the intense agitation therein. For example, bile salt micelles and vesicles have average sizes of 4 and 60 nm, respectively (Hernell et al., 1990). Studies also showed that carotenoids have poor bioavailability when directly ingested without a delivery formulation but have much improved bioavailability when consumed together with food or dissolved in lipids (Faulks and Southon, 2005), possibly resulting from the inability of bile salts to solubilize some compounds (Acosta, 2009). For hydrophilic compounds with poor permeability, preparing them into NP may enhance the uptake (Acosta, 2009). Presence of other food components, particularly surface active compounds, may also complicate the uptake of compounds. In addition, increasing interactions between NP and the mucus layer of the intestine also may enhance the bioavailability of bioactive food components. This aspect is a great opportunity to design nanostructures to improve bioavailability of bioactive compounds and thus nutritional quality of food products (Zhong and Shah, 2012).

The rational design of NP is very important for altering the pharmacokinetics of the encapsulated drug. The shape and size of the particles are key determinants in governing the biodistribution and bioavailability of the cargo (Nair et al., 2010).

The surface properties of NP determine their interaction with the local environment. Sterically stabilized NP exhibit minimal self and nonself interactions (Davis et al., 2008). These particles keep slightly high or low negative or positive charges on their surfaces, which leads to an increased reticuloendothelial clearance; therefore minimizing nonspecific interactions and controlling surface charge by steric stabilization helps to prevent NP loss in undesired locations to a certain extent. NP have high surface-to-volume ratios, which can be manipulated by rational design. The surface properties of the NP will determine their solubility, stability, and clearance. It has been shown that polymer drug or antibody conjugates have superior half-lives, which can improve the pharmacokinetics of the drug (Duncan et al., 2005). Increased opsonization associated with NP's surface during circulation can stimulate substantial hepatic agglomeration. PEGylation has been shown to reduce protein absorption. In general, PEGylated NP have longer circulation times and higher levels of tumor accumulation than nonPEGylated NP (Nair et al. 2010; Pasche et al., 2005).

4.1.1 Nanostructures for Uniform Delivery in Food Matrices and Improved Chemical Stability

In industrial settings, an appropriate mechanism is needed to uniformly disperse bioactive compounds in food matrices and

ensure this distribution is not changed during production and storage. For products involving mostly solid ingredients only, for instance cereal products, micronization or encapsulation of compounds into powdered form can be used, and the uniform distribution in foods can be enabled by a proper mixer. Structure design from this perspective may be to ensure the uniformity is achieved throughout production and storage. Lipophilic compounds can also be incorporated in NP of food biopolymers for delivery in dispersions, as demonstrated for curcumin in micelles of hydrophobically modified starch (Yu and Huang, 2010) and plant essential oil in whey protein-maltodextrin conjugate. In addition to physically dispersing lipophilic compounds, strategies are needed to protect compounds that are degraded by environmental factors during processing and storage, notably thermal degradation and oxidation. Many food products are thermally processed, and this causes molecular structural changes of some bioactive compounds. For proteins, slight changes in secondary and/or tertiary structures may result in loss of bioactivity and are usually worsened by presence of water and other compounds present in food matrices. Polyunsaturated fatty acids (PUFA) and many carotenoids such as lycopene and β-carotene are easily oxidized, and oxidation causes not only undesirable sensory profiles but bioactivity. The extent of lipid oxidation is impacted by catalysts such as cationic metal ions of copper, cobalt, and iron present in food, and environmental factors such as oxygen, light, water activity, and temperature. In this regard, a protective nanolayer(s) can be created around particles/droplets of bioactive compounds to reduce the presence or access of structure-changing compounds. One successful example is layer-by-layer deposition technology applied on droplets of PUFA, where multiple layers of molecules on oil droplet surfaces drastically increase the stability against environmental stresses such as temperature (heat, freeze/thaw), pH, and oxygen and the cationic molecule layer provides electrostatic repulsion against cationic metal ions during emulsion preparation (McClements et al., 2007). Building a thick interfacial layer using NP is another approach that can be used to protect chemical stability of emulsion-based delivery systems for bioactive compounds to improve nutritional quality (Zhong and Shah, 2012).

4.1.2 Nanoparticles for Controlled Enteric or Colonic Delivery

One of the most well-known systems is enteric delivery where compounds are enclosed in capsules or tablets made of an enteric polymer that is insoluble at acidic conditions but soluble at intestinal pH. Enteric delivery is used in the pharmaceutical industry to reduce the degradation of drugs at low pH and by digestive

enzymes present in the stomach. For food applications, micro- and nanocapsules may be developed for incorporation in food products. Enteric delivery systems based on GRAS polymers also were reported for resistant starch from high amylose cornstarch (Dimantov et al., 2004). Similarly, some compounds are to be delivered to function or be absorbed in the colon. Probiotics are other candidates that are desired to maintain activity in the colon. Appropriate carrier materials can be selected to form structures to resist physical, chemical, and biological actions in the mouth, stomach, and small and large intestines so that these compounds/microorganisms can be released in the colon. Pectin and its complex with corn zein have shown promise as carrier materials for colon delivery (Liu et al., 2007). Complex of carboxymethyl high amylose starch and chitosan also is promising (Calinescu and Mateescu, 2008). Once carrier polymers are selected, research can then be focused on technologies producing nanocapsules such as coacervation and precipitation in an antisolvent. Alternatively, carrier polymers can be used to coat solid particles of bioactive compounds or probiotic cells to form a nanolayer using processes such as spray drying or fluidized bed (Zhong and Shah, 2012).

4.1.3 Mucoadhesive Particles to Enhance Bioavailability

Once reaching absorption sites, one common practice to improve bioactivity of compounds is to utilize certain mechanism(s) to "adhere" delivery systems onto the mucosal surface. Mucus layer is considered a physical barrier to protect and lubricate the epithelial surfaces. It inhibits the residence between NP and epithelial cells. The mucoadhesive particulate system improves uptake and increases retention time and thus possibly bioavailability of the delivered compound. Mucoadhesive properties result from either nonspecific or specific interactions between a delivery system and the mucosal surface (Ensign et al., 2012; Ponchel and Irache, 1998). Under physiological conditions, the mucosal surface is negatively charged, and positively charged particles likely adsorb onto the surface by electrostatic attraction (Acosta, 2009). NP may immediately penetrate the mucus by electrostatic interactions and load on small intestinal epithelial cells for cellular uptake (Hariharan et al., 2006; Norris et al., 1998; Roger et al., 2010). Positively charged PLGA NP had an increased cellular uptake as compared with negatively and neutrally charged NP (Hariharan et al., 2006). Too strong electrostatic attraction, however, reduces bioavailability because of the difficulty to diffuse through the intestinal wall (Acosta, 2009). Electrostatic interactions between mucus and NP may result in the cessation of NP in mucus layers, which decreases the cellular uptakes of NP

by epithelial cells. It was reported that hydrophobic polystyrene NP got anchored in the mucus, induced to the lower association with epithelial cells (Behrens et al., 2002). NP with negative surface charges can be repulsed by the mucus layer, lowering resistance to epithelial cells (Hariharan et al., 2006). Hydrogen bonding and van der Waals forces also may be responsible for adhering particles on the mucosal surface. Some hydrophobic polymers, for example, gliadins, may also develop enough affinity for adhesion (Arangoa et al., 2001). Many food biopolymers can be used to deliver bioactive food components, and nanoscale particulate delivery systems may further enhance bioavailability. Some biopolymers also have excellent surface activity applicable for developing emulsion-based delivery systems. Particles with specific mucoadhesive properties are mostly due to specific ligand-receptor type interactions. The receptors are glycoproteins of mucus and/or components of epithelial cells, and the ligand is a part of particle structure with affinity to receptors. Lectins, a large group of glycoproteins naturally existing in plants, animals and microorganisms, are the most-studied ligands of specific mucoadhesive properties that can be used to conjugate particles (Ponchel and Irache, 1998). Excess consumption of some native lectins, however, may present toxicity issues (Vasconcelos and Oliveira, 2004; Zhong and Shah, 2012).

NP loaded with phenolic compounds with controlled shapes, sizes, have been widely studied to improve the bioavailability of phenolic compounds with poor absorption characteristics. Also they are capable of overcoming biological barriers, accumulate preferably in tumors and in particular recognize single cancer cells for detection and treatment (Liang et al., 2011). Chitosan offers absorption enhancement properties, and an increase of the permeation of drugs throughout the paracellular route, and reversible process through cavity of tight epithelial junctions. Moreover, its muco-adhesive properties can allow the NP adhere to the intestinal mucosal surfaces through ionic relations between the groups positively charged (amine) and the natural existing negatively charged groups that exist on intestinal surfaces. This latter property is helpful in the increase of the time of residence in the intestine of the phenolic compounds after oral administration. Since the chitosan NP have normally a medium size under 500 nm, these NP can pass through the mucous barrier of intestinal tissue and interact with the underlying absorptive epithelial cells. When the zeta potential is positive, the NP adhesion to intestinal surfaces is also facilitated. Hence, these conditions will lead to high drug concentrations at the sites of absorption (Dube et al., 2010; Madureira et al., 2015).

4.1.4 Nanoparticles Decrease First-Pass Metabolism of Nutraceuticals

The nutraceuticals are metabolized by a wide variety of enzymes existing mainly in the gut and liver during first-pass metabolism. As a result of first-pass metabolism, only a fraction of the ingested nutraceutical can access the systemic circulation and remain unchanged. That causes a decreased oral bioavailability. NP can be designed to assist nutraceuticals to bypass first-pass metabolism and hereby increase their bioavailability. Lipid-based NP, such as NE, have been utilized to bypass liver metabolism by promoting intestinal lymphatic transport of lipophilic nutraceuticals (Yao et al., 2014). NP can also preserve nutraceuticals from first-pass metabolism in enterocytes. For instance, NP can enhance paracellular transport of nutraceuticals by modulating the integrity of tight junctions (Hu et al., 2008; Kondoh et al., 2012; Sun et al., 2015). Paracellularly absorbed nutraceuticals may have higher bioavailability. Because they are not subject to metabolism by intracellular enterocyte enzymes, NP that support chylomicron-mediated transport of nutraceuticals, such as NE, may also reduce first-pass metabolism in the enterocytes. This is because that nutraceutical associated with the chylomicrons may have less opportunity to interact with metabolizing enzymes within the cell compared to nutraceuticals that freely exist in the cytoplasm of enterocytes (Porter and Charman, 2001). Furthermore, the carrier oil type of nanoemulsions plays a key role in the first-pass metabolism of nutraceuticals in the enterocytes. Pterostilbene is an important phenolic nutraceutical found in blueberries. While olive-oil-based NE resulted in a minimal metabolism of pterostilbene in enterocytes, flaxseed-oil-based NE led to an extensive metabolism of pterostilbene (Sun et al., 2015).

In order to reveal guiding principles for rational design of NP to decrease first-pass metabolism, more mechanistic studies are needed to identify the relationship between the different features of nanoparticles and their effects on first-pass metabolism of specific nutraceuticals.

It is well known that a healthy digestive system only allows absorption of nutrients from the gut after digestion of foodstuffs. The gut wall is designed to ensure the passage of dietary nutrients, and prevent the passage of larger or foreign materials. At the cellular level, the transport of conventional forms of nutrients (and metabolites) is also well regulated. Because of very small size, and depending on the surface charge and coatings, etc., nanomaterials may “override” these mechanisms and end up in other nonintended tissues and organs, potentially posing a health risk to the

consumer. It is worth highlighting that a naturally occurring or synthetic nanomaterial that is not bio-persistent, for example, it is either solubilized, assimilated in the gut or at cellular level, or excreted from the body, is not likely to pose any different health risk from the conventional bulk equivalent. On the other hand, the use of insoluble/indigestible NP in food applications may raise certain health concerns. The presence in food of such NP, such as silver, titanium dioxide, or silica, to name a few, poses the likelihood of translocation of potentially large reactive surfaces to different parts of the body that could have health implications. It is, therefore important to understand how insoluble particulate materials are handled by the digestive system. It is known that many food substances exist naturally, or metabolized in the body at nanoscale (Chaudhry and Groves, 2010).

After ingestion, the fate of food nanostructures has to be studied in the dynamic human digestion system. This includes a consequence as simple as malnutrition due to the reduced absorption resulting from engineered nanostructures that do not allow digestive enzymes to decompose substrates to absorbable compounds, for example, proteins to peptides and amino acids. The opposite of this scenario is the much faster digestion rate of food nanostructures in the human digestion system, which may increase the absorption of energy-providing compounds compared with conventional structures. The excess energy intake as a result of this may create problems such as obesity. It is also important to study seriously micronutrients and nutraceuticals delivered by food nanostructures. Many of these compounds supply important functions for nutrition and health if they are available to human body metabolism at the right amount/range. Excess absorption of these compounds may result in negative impacts, such as toxicity to the liver and other vital organs due to overintake, improper metabolism, or prolonged clearance of fat-soluble vitamins leading to hypervitaminosis or vitamin poisoning (Penniston and Tanumihardjo, 2006; Zhong and Shah, 2012).

5 Conclusions

Nowadays, tremendous attention is being paid to developments in the field of engineered nanomaterials. Many efforts have been devoted to the design and development of several delivery systems to enhance the bioavailability of nutraceuticals through various mechanisms in order to improve the oral efficacy. In addition to the potential technological impact of NP delivery systems in the food industry, there are also concerns about unforeseen side effects of the technology. For this reason, well-designed

studies focused on human health impacts are required. Recently, substantial progress has been made in understanding the potential response of NP to GI conditions, but more clinical trials are definitely needed. Valid studies should be carefully utilized by the food industry to formulate foods and dietary supplements preventing any adverse effect on health issues.

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15

BIOAVAILABILITY ENHANCEMENT OF CURCUMIN NUTRACEUTICAL THROUGH NANO-DELIVERY SYSTEMS

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1 Introduction

Nutraceutical is an amalgamation of the two terms *nutrition* and *pharmaceutical*. Nutraceutical refers to food or a food constituent that gives medical and health benefits. Numerous nutraceuticals have been known for the past decade (Giri et al., 2016; Gupta et al., 2010) to offer defensive action against chronic diseases or to have physiological benefit. The idea normally refers to dietary supplements that include a bioactive substance derived from food. The examples of nutraceuticals with claimed profit comprise resveratrol from red grape as an antioxidant, soluble dietary fibre such as psyllium seed husk for dropping hypercholesterolemia, sulforaphane from broccoli as a cancer preventive, and isoflavonoids from soy or clover that improve arterial health. Various nutraceutical components obtained from the natural source are presented in Table 15.1.

Curcumin is one of the constituents of curry and an accepted dietary spice worldwide. It is the main active component of turmeric, obtained from the rhizome (root) of the *Curcuma longa*, a perpetual herb belonging to the ginger family. Turmeric is comprised of a cluster of three curcuminoids, that is, curcumin, bisdemethoxycurcumin, and demethoxycurcumin, with volatile oils, proteins, sugars, and resins. It is a bis-α,β-unsaturated β-diketone, which exhibits keto–enol tautomerism, having a major keto form in acidic and neutral solutions and stable enol form in alkaline medium. It is a hydrophobic polyphenol that is practically insoluble in water and has inadequate systemic bioavailability owing to its

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Table 15.1 Potential Benefits of Nutraceutical with Functional Components from Natural Sources

Sl. No.	Functional Components	Source	Potential Benefits
1.	Alpha-linolenic acid	Flax seeds	Prevent heart attacks
2.	Beta-carotene	Marigold petals	Prevent cancer and heart disease
3.	Anthocyanins	Berries	Use as pH indicator
4.	Yellow mustard oil	Mustard plant	Anticancer properties
5.	6-[Gingerol]	Ginger	Use in rheumatoid arthritis
6.	Lycopene	Tomato	Uses in cataracts and asthma
7.	Curcumin	Turmeric	Uses in headaches and bronchitis
8.	Resveratrol	Grape	Lower cholesterol level
9.	Genistein	Soya beans	Anticancer properties
10.	Capsaicin	Chilli peppers	Relieve pain
11.	Diallyl sulphide	Garlic	Anticancer properties
12.	Myricetin	Walnuts	Uses in diabetes
13.	Honoliol	Magnolia plant	Anticancer properties
14.	Apigenin	Onions	Exerts anxiety-reducing effects

fast metabolism, mainly through conjugation to sulfates and glucuronides (Anand et al., 2008; Shishodia et al., 2007). In humans, this metabolism most probably occurs in the gastrointestinal tract and to a certain extent in the liver (Ireson et al., 2002).

The pharmacological protection and effectiveness of curcumin makes it a prospective compound for prevention and treatment of a variety of diseases. Despite its protection and efficacy, curcumin has not until now been accepted as a therapeutic agent, and the ineffective bioavailability of curcumin has been mentioned as a chief reason for this. Curcumin was difficult to incorporate into the conventional dosage form owing to poor water solubility, which diminishes the bioavailability of the drugs. There are various approaches that can be used to overcome the problems associated with poor solubility and poor bioavailability of low water soluble drugs (Badwaik et al., 2013; Bhoyar et al., 2012; Giri et al., 2013a; Giri et al., 2013b). Numerous approaches that are used to augment solubility comprise solubilization using cosolvents, oily solutions, micronization, surfactant dispersions, use of permeation enhancers, and salt formation and precipitation techniques (Ajazuddin

et al., 2013; Behra et al., 2012; Giri et al., 2013c; Giri et al., 2012a). The majority of these methods for solubility enhancement has advantages in addition to some restrictions and consequently has limited use for solubility enhancement. Additional methods used for solubility improvement comprise emulsions, solid dispersion, microparticles, microemulsions, and inclusion complexes demonstrate reasonable accomplishment but they lack in general applicability to all drugs (Agrawal et al., 2012; Ajazuddin et al., 2012; Badwaik et al., 2011; Giri et al., 2010; Giri et al., 2012b; Giri et al., 2013d). These methods are not suitable if the drugs are not soluble in both organic and aqueous media. This remains a challenge for the pharmaceutical industry to improve the efficacy and therapy of poorly water-soluble drugs. In the past few years nanotechnology has been developed and applied in the pharmaceutical industry (Giri et al., 2015a). The variety of problems described previously can be solved by the use of nanotechnology. Nanotechnology is the field of engineering and science investigating the production of nanoscale particles, that is, particles of the size 10^{-9} m.

The pharmaceutical applications of nanotechnology have presented recent possibilities for solubility, permeability, and stability enhancement of challenging nutraceuticals (Yallapu et al., 2010; Yallapu et al., 2012; Yallapu et al., 2013). Recently, nanoscale systems in the presence or absence of carriers have emerged for nutraceuticals. Improvement of wettability and dissolution of nutraceuticals occurred through nanonization in the absence of a carrier. Micelles, nanocapsules, and nanoparticles are examples of nanonization of nutraceuticals prepared from carriers. Nevertheless, biodegradability and biocompatibility of the carriers are important. Polyesters are biodegradable polymers approved by the FDA and are used for the preparation of nanoparticles loaded with nutraceuticals. Nanoparticles prepared from biodegradable polymer alter the release of encapsulated drug, shield them from degradation, modify their biodistribution and change their transport across biological membranes from a passive diffusion process to endocytosis one.

In polymer conjugates biologically active substances are chemically linked to a polymer. The conjugates change the physicochemical, therapeutic, and pharmacokinetic properties of the bioactive agent. The curcumin polyconjugates showed changed biodistribution and superior anticancer efficacy as it combines the double advantage of improved polymer mediated drug internalization and aqueous solubility (Safavy et al., 2007). Food compounds and phytoconstituents triggered the look for novel formulation of nutraceutical products to surmount poor bioavailability and achieve better therapeutic efficacy.

2 Curcumin

Curcumin is obtained from the rhizome of *Curcuma longa*, a spice that is familiar to India and the neighboring regions. It is the chief curcuminoid with antioxidant potential, and used in Alzheimer's disease, inflammation, chemoprevention, and chemotherapy. The antioxidant prospective of curcumin is well recognized with the capability to requisition the carcinogenic reactive oxygen species (Leu and Maa, 2002). Curcumin also has various pharmacological targets in varied cancers where it has been shown to be effective. Accordingly, there is extensive concentration in the clinical development of curcumin as a cancer chemotherapeutic agent. That is evidenced by the growth of phase I clinical trials and enrollment in phase II clinical trials.

2.1 Curcumin Chemistry

The alcoholic extract of turmeric primarily contains three curcuminoids, namely curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin (Fig. 15.1).

Its molecular weight is 368.38 and melting point is 179–183°C. The chemical formula of curcumin is $C_{21}H_{20}O_6$. Curcumin is present in keto–enol equilibrium form in biological environment (Fig. 15.2).

Curcumin is poorly soluble in water but readily soluble in acetone, ethanol, and dimethylsulfoxide. The keto form of curcumin

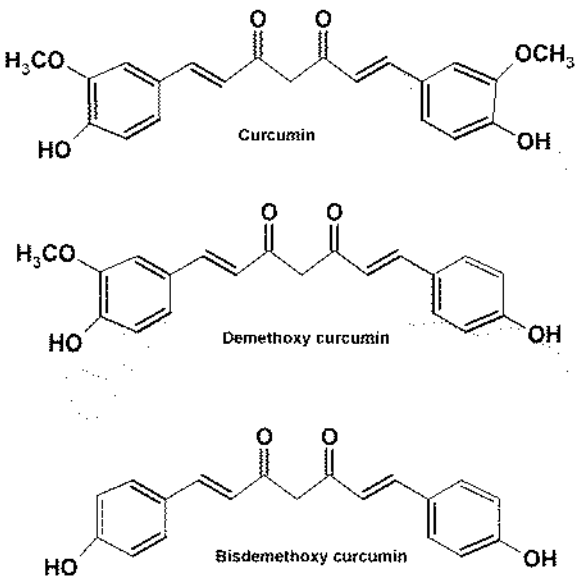

Figure 15.1. Structure of three major curcuminoids in turmeric.

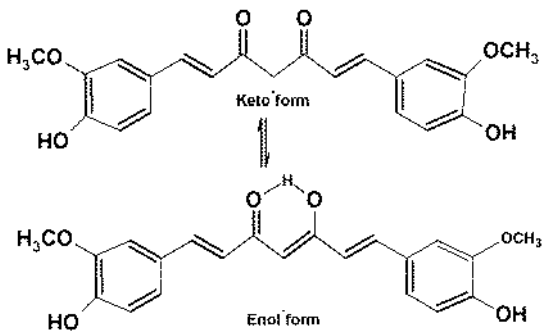

Figure 15.2. pH dependent keto- and enol- tautomeric form of curcumin.

predominates and it donates H-atoms strongly in neutral and acidic solutions. The enolic form of curcumin predominates in alkaline solutions (≥pH 8) and the phenolic part of curcumin plays the main role as an electron donor (Jovanovic et al., 1999).

2.2 Physico-Chemical Properties of Curcumin

Curcumin is an oil-soluble pigment, almost insoluble in water at acidic and neutral pH, and soluble in alkali. Preparations of water-soluble curcumin by inclusion into different surfactant micellar systems have been reported (Humphrey, 1980; Tonnesen, 2002). In solutions the chief coloring constituents of curcumin show keto–enol tautomerism and, depending on the solvent, up to 95% are in the enol form.

The chief coloring components of curcumin are comparatively stable at acidic pH; however, they quickly decompose at pHs above neutral. In a study of alkaline degradation of curcumin (Tonnesen and Karlsen, 1985), products of decomposition at pH 7–10 were determined by HPLC. The primary degradation products are created after 5 min and the chromatographic pattern obtained after 28 h at pH 8.5 is representative for alkaline degradation. Ferulic acid and feruloylmethane are produced first. Feruloylmethane quickly forms colored (generally yellow to brownish-yellow) condensation products. Degradation products produced by hydrolysis of feruolylmethane are vanillin and acetone and their amount is enhanced with incubation time.

In another study (Wan et al., 1997), curcumin was incubated in 0.1 M phosphate buffer, pH 7.2 at 37°C, and about 90% was decayed within 30 min. Trans-6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexenal was predicted as key degradation product and vanillin, ferulic acid, feruloyl methane were identified as minor degradation products.

Regardless of the polarity of the flanking functional groups and the central dicarbonyl moiety, curcumin in general is relatively lipophilic. This is evidenced by its reduced solubility in aqueous solvent and superior solubility in organic solvents in addition to by its log P value, which is a measure of the degree to which a solute prefers the lipophilic phase (typically octanol) over the aqueous phase in a biphasic, immiscible solvent system. The log P of curcumin has been reported in the range of 2.3 (Jankun et al., 2006) to 2.6 (Fujisawa et al., 2004). This comparatively high degree of lipophilicity, which is attributable to the methine-rich segments that connect the polar regions (Balasubramanian, 2006), has numerous significant implications on intermolecular interactions.

The poor aqueous solubility of curcumin bears numerous imperative implications for in vitro and in vivo research. First, curcumin should always be dissolved in solvents that are miscible in water, which comprise acetone, methanol, butanone, 1,2-dichloroethane (up to 8.7 g/l w/w), ethanol, DMSO, and 2-propanol. Of these solvents, the least toxic choice should, if possible, be used in experiments, which is best judged by their lethal 50% dose values, given here for the oral administration route in rats, except noted otherwise (obtained from the personal material safety data sheet): 2-butanone, 2.7 g/kg ; acetone, 9.8 g/kg; ethanol, 7.1 g/kg; methanol, 5.6 g/kg; isopropanol, 5.0 g/kg; 1,2-dichloroethane, 0.41 g/kg (mouse); DMSO, 18.0 g/kg. Consequently, DMSO constitutes the most appropriate solvent for in vitro and in vivo studies, though proper controls (solvent alone) should always be employed in all assays.

2.3 Curcumin Safety and Toxicity

Curcumin is a permitted food-coloring agent as well as a dietary item. Oral administration of curcumin each day 1.2–2.1 g to a patient with rheumatoid arthritis for 2–6 weeks did not produce any toxicity (Deodhar et al., 1980). Similarly, oral administration of curcumin each day 0.5–8 g for 3 months to a patient with premalignant circumstances did not produce any objectionable effects (Cheng et al., 2001). Curcumin capsules containing 2% bisdemethoxycurcumin, 23% demethoxycurcumin, and 75% curcumin were administered to a healthy volunteers with increasing doses from 0.5 to 12 g. Seven healthy subjects developed adverse effects including rashes, diarrhea, yellowish stools, and headaches among 24 enrolled. All the adverse effects were observed to be not related to the doses. The highest dose of curcumin could not be determined because greater than 12 g is regarded as bulky (Lao et al., 2006). Various clinical trials recommended that curcumin doses range between 4 and 8 g to get the utmost therapeutic effects based on the toxicity and safety profile.

2.4 Clinical Studies on Curcumin

In pilot study, tropical pancreatitis patients were treated with curcumin to established antioxidant activity. Either 0.5 g of curcumin in combination with 5 mg piprine or placebo was given to 20 patients for 6 weeks orally (Durgaprasad et al., 2005). Erythrocyte levels of glutathione and malondialdehyde and clinical pain patterns were evaluated. The glutathione and malondialdehyde levels are common indicators of antioxidant potential and lipid peroxidation, respectively. Patients treated with piperine and curcumin had significantly lower malondialdehyde levels in comparison to the placebo group. Conversely, pain severity and levels of erythrocyte glutathione was unaltered by the use of curcumin. No side effects and toxicities were observed before and after the treatment course (Durgaprasad et al., 2005).

Clinical studies in a variety of patient groups on inflammatory disease were performed to investigate the efficacy of oral curcumin. In a pilot study, Crohn disease of five patients and ulcerative proctitis disease of five patients were given curcumin orally (Holt et al., 2005). 80% of Crohn disease patients showed enhanced inflammatory profiles and all the patients with proctitis showed progress. A double-blind placebo-controlled study was conducted on ulcerative colitis in 89 patients (Hanai et al., 2006). Half of the patients were given 1 g of curcumin orally twice a day in combination with mesalazine or sulfasalazine and the other half of the patients was given aminosalicylate plus placebo. It was observed that the curcumin group patients had a significantly decreased rate of relapse during 6-month treatments.

In another study with 25 patients, every 1 treated with 8 g curcumin each day orally for 2 months observed 22–41 ng/mL peaked plasma level (Dhillon et al., 2008). One had continuing recognized disease for more than 18 months; amusingly, one extra patient had a short, but noticeable, tumor degeneration accompanied by considerable increases in serum cytokine levels. Curcumin was down-regulated at the appearance of COX-2, NF-κB, activator of transcription 3 and phosphorylated signal transducer. Even if this molecule is inadequately absorbed, biological action is evident. These clinical trials emphasize the level of existing translational curiosity in studying the biologic prospective of curcumin in the management of premalignant environment and established malignancies.

2.5 Bioavailability and Pharmacokinetics of Curcumin

Low oral bioavailability of curcumin is well documented. Generally, required serum levels are reached when 3600 mg of curcumin are used. The piperine has been used to evaluate the

modulation of curcumin bioavailability that is obtained from *Piper species*, which is a known inhibitor of glucouronidation in the liver and small intestine (Atal et al., 1985; Shoba et al., 1998). 2000 mg of curcumin was given to ten healthy volunteers with and without 20 mg of piperine in a restricted manner with randomized (Shoba et al., 1998). 2000% of bioavailability was enhanced with a piperine and curcumin combination compared to curcumin only. Liposomal drug delivery system improved the bioavailability of poorly soluble drug, which is proved by in vitro and in vivo studies (Li et al., 2005). Oral administration of curcumin undergoes widespread gastrointestinal and hepatic metabolism. Metabolites produced by Phase I reaction is tetrahydrocurcumin, hexahydrocurcuminol, and hexahydrocurcumin. Phase II metabolism produced curcumin glucuronide and curcumin sulfate and rapidly excreted through glucouronidation and sulfation by O-conjugation.

Although curcumin has multiple pathways of action that lead to enhanced therapeutic activity, the main drawbacks connected with its oral administration are the high metabolic unsteadiness and low aqueous solubility that restrict its systemic bioavailability (Anand et al., 2008; Shishodia et al., 2007). Additionally, considerable side effects and low patient conformity may prohibit the use of oral curcumin at the high doses (>8 g/day) desirable to attain a therapeutic activity. To conquer these problems, novel strategies for effectual delivery of curcumin are being investigated (Yallapu et al., 2012). Among these methods, there are liposomal curcumin formulations and encapsulation in diverse polymeric nanoparticles (Bisht et al., 2010; Wang et al., 2008a,b).

Oral administration of 0.4 g of curcumin showed below 5 μg/mL concentration in portal blood and no curcumin in heart blood (Ravindranath and Chandrasekhara, 1980). In another study using tritium-labeled curcumin, the similar group showed noticeable amounts of curcumin in blood per animal with doses ranging from 10 to 400 mg (Ravindranath and Chandrasekhara, 1981). A very recent study showed that intravenous administration in rats of 10 mg/kg of curcumin gave 0.05 μg/mL concentration of curcumin in serum. On the other hand, oral administration of 50 times high curcumin dose gave merely 0.01 μg/mL utmost serum level in rat (Yang et al., 2007). Uptake and distribution of curcumin in body tissues is evidently essential for its biological activity. Only traces of unchanged drug were detected in the kidney and liver following 0.4 g curcumin administered orally to rats. In the stomach and small intestine 90% of curcumin was found at 30 min but 1% was found at 24 h (Ravindranath and Chandrasekhara, 1980). When rat intestinal everted sacs were placed in incubation medium (10 mL) containing 50–750 μg of curcumin, no curcumin was detected in serosal side and 30–80%

of the curcumin was missing from the mucosal side. Less than 3% of the curcumin was detected in tissues at high dose (Ravindranath and Chandrasekhara, 1981).

Metabolism of curcumin was evaluated through a variety of studies in humans and in rodents. The maximum amount of orally administered curcumin to rats was metabolized through biodistribution study (Wahlstrom and Blennow, 1978). Curcumin was mainly metabolized in the liver (Garcea et al., 2004). The main billiary metabolites of curcumin are glucuronides of hexahydrocurcumin and tetrahydrocurcumin in rats (Holder et al., 1978). Insignificant biliary metabolite was traces of ferulic acid with dihydroferulic acid (Holder et al., 1978). Glucuronide conjugates as well as sulfate conjugates were detected in the urine of rats treated with curcumi (Ravindranath and Chandrasekhara, 1980). Curcumin present in plasma was almost 99% as glucuronide conjugates that are revealed by hydrolysis of plasma with glucuronidase. This study also discovered curcumin–glucuronoside, dihydrocurcumin–glucuronoside, tetrahydrocurcumin–glucuronoside, and tetrahydrocurcumin are main metabolites of curcumin in vivo (Pan et al., 1999). These outcomes are in conformity with other investigator who examined curcumin metabolites in human and rat (Ireson et al., 2001). Absorption and metabolism of curcumin through oral administration in rats was evaluated (Asai and Miyazawa, 2000). Glucuronides and sulfates were the main metabolites in plasma following oral administration. The highest conjugated curcuminoids concentration was reached after 1 h administration. Orally given curcumin is absorbed through the gastrointestinal tract and reaches into the systemic circulation. Then the drug is metabolized to glucuronide and sulfate conjugates through conjugative enzyme action (Asai and Miyazawa, 2000).

Systemic removal of curcumin from the body is significant aspects, which determine its comparative biological action. 75% of curcumin was excreted in the feces and an insignificant amount was detected in the urine after oral administration of 1 g/kg curcumin (Holder et al., 1978). Biliary elimination of curcumin was observed from cannulated rats when given by intravenous and intraperitoneal route (Holder et al., 1978).

3 Nano-Drug Delivery System

The absorption, distribution, metabolism, and excretion studies of curcumin have, unfortunately, revealed merely poor absorption, fast metabolism, and excretion of curcumin as main reasons for poor bioavailability of curcumin. Nanoparticles, micelles, liposomes, and phospholipid complexes are prospective novel

formulations, which seem to provide longer circulation, resistance to metabolic processes, and improved permeability. Nanotechnology is progressively a more popular technology of the future. Solubility and bioavailability of poorly water-soluble compound curcumin is increased by the use of nanoparticles. Recently, application of nanoparticles gained tremendous popularity owing to their potential development of delivery system by shielding drugs from enzymatic degradation, alter their pharmacokinetics, providing controlled release and extended blood circulation, restraining their nonspecific uptake, and declining their toxicity (Freitas, 2005). Various emphases have been specified to lengthen the biodistribution of natural curcumin. Conversely, it is merely just in latest times that the application of the field of nanotechnology has significantly improved its therapeutic activity. Nanoparticles such as liposomes, micelles, polymeric nanoparticles, nanogels, cyclodextrins, niosomes, dendrimers, solid lipids, and silvers are promising as one of the valuable alternatives that have been revealed to deliver therapeutic amount of curcumin. Nanoparticles loaded with curcumin improved various problems such as instability, low solubility, rapid metabolism, and poor bioavailability in cancers, Alzheimer's disease, wound healing, ischemia diseases, epilepticus, inflammatory diseases, and so forth.

3.1 Polymeric Nanoparticles

Nanoparticle-based delivery systems will most likely be appropriate for extremely hydrophobic agents like curcumin, eliminating the drawbacks of poor aqueous solubility. Nanoparticles are prepared by using natural polymers and synthetic polymers. Natural polymers are advantageous due to their nontoxic, biocompatible, and biodegradable nature (Giri et al., 2015b; Giri et al., 2015c; Giri et al., 2015d). Conversely, a small number of studies have been available for curcumin nanoparticles. A recent study reported the synthesis, cancer-allied application and physicochemical characterization of a polymeric nanoparticle of curcumin with less than 100 nm size (Karikar et al., 2007). Similar in vitro activity was shown by nanocurcumin as that of free curcumin in pancreatic cell lines. Nanocurcumin also reduces steady-state levels of pro-inflammatory cytokines like interleukins and TNF-R, and diminished activation of the transcription factor NFκB (Karikar et al., 2007). However, the authors did not determine the in vivo effect and biodistribution of curcumin (Karikar et al., 2007). Curcumin-loaded nanoparticles were developed and characterized for topical application (Tiyaboonchai et al., 2007). Nanoparticles having 450 nm sizes were found to be stable for 6 months at room

temperature and gave prolonged release. Moreover, curcumin encapsulated nanoparticles diminished light and oxygen sensitivity of curcumin. The enhanced efficiency of a topical application cream containing curcuminoid-loaded nanoparticles over that containing free curcuminoids was observed by in vivo study with healthy volunteers (Tiyaboonchai et al., 2007).

Shaikh et al. (2009) prepared curcumin-loaded nanoparticles by using emulsion technique. In vivo study showed that curcumin-loaded nanoparticles achieved a 9 times augment in bioavailability in comparison to the administration of curcumin with absorption enhancer piperine (Shaikh et al., 2009). Curcumin nanoformulation prepared from polylactic-*co*-glycolic acid administered by oral route resulted 22 times more bioavailability in rats in comparison to the curcumin alone (Tsai et al., 2011). Curcumin nanoparticles prepared from chitosan and dextran sulphate showed improved killing of cancer cells than normal cells (Anitha, 2011). Pharmacokinetic of nanoparticles containing curcumin in rats showed significant improvement of bioavailability in comparison to solubilized curcumin (Kakkar et al., 2011). Transferrin-mediated curcumin nanoparticles were prepared and applied in breast cancer cells (Mulik et al., 2010). Promising results were found in breast cancer cells. Curcumin nanoparticles were also well applied topically (Tiyaboonchai et al., 2007). Curcumin nanoparticles were evaluated in rat model and showed more antiinflammatory and antiangiogenic behavior (Yadav et al., 2009).

The curcumin-polylactic-*co*-glycolic acid nanoparticles are extremely soluble in water. The elevated solubility character of curcumin-polylactic-*co*-glycolic acid nanoparticles may be a effect of compatibility between polymer nanoparticle and curcumin compounds. The drug release from polylactic-*co*-glycolic acid nanoparticles occurs by diffusion followed by degradation and is molecular weight/copolymer ratio dependent (Shaikh et al., 2009). Curcumin-loaded polylactic-*co*-glycolic acid nanoparticles were considered to progress the oral bioavalability of curcumin. The encapsulated curcumin into polylactic-*co*-glycolic acid nanoparticles exhibited 9 times increase in bioavailability compared with administration of curcumin and absorption enhancer piperine. The plasma concentration of curcumin from suspension formulations diminished quickly, representing fast metabolism of curcumin, while a continuous release of curcumin over 48 h was observed in the nanoparticle formulation.

Human serum albumin is another outstanding nanoparticle carrier since it is nontoxic and nonimmunogenic. Additionally, albumin-bound nanoparticle technology does not necessitate surfactants or polymeric materials for preparation. Consequently,

it is assumed that human serum albumin nanoparticles are expected to be well tolerated in vivo. Curcumin-human serum albumin nanoparticles showed much superior water solubility than free curcumin. Amounts of curcumin in tumors following treatment with curcumin-human serum albumin-nanoparticles were about 14 times higher at 1 h after injection than that achieved by curcumin (Kim et al., 2011).

Curcumin-loaded chitosan nanoparticles when delivered orally enhanced the bioavalability of curcumin in the plasma and red blood cell. Furthermore, curcumin bound to chitosan nanoparticles can advance its chemical stability studied in mouse plasma (Akhtar et al., 2012). A novel formulation of curcumin-loaded poly(ε-caprolactone) plus chitosan was developed recently to get cationic nanoparticles of curcumin (Liu et al., 2012). Poly(ε-caprolactone) as a nontoxic degradation product has been accepted by FDA for various biomedical applications. There are various literatures on the synthesis of poly(ε-caprolactone) micro/nanoparticles for drug delivery applications. Curcumin-poly(ε-caprolactone) nanoparticles with suitable surface modifications with chitosan to obtain cationic nanoparticles can progress the cell uptake of curcumin as compared to unmodified curcumin (Liu et al., 2012).

Nanoparticles of chitosan and dextran sulfate are biocompatible materials that can be used for oral, intravenous, and controlled release system. Cellular uptake of chitosan and dextran sulfate containing curcumin was determined by a spectrophotometric method in MCF-7, L929, MG 63, and PC3 cells (Anitha, 2011). Furthermore, anticancer activity of the nanoparticles is high in MCF-7 in comparison to the other cancer cells, showed by a fluorescence study and cytotoxicity study. Intravenous administration of polycaprolactone nanoparticles containing curcumin analog to rats established higher intracellular levels in liver cells (Anuradha and Aukunuru, 2010). Multidrug resistance cancer cells were more proficiently treated by polymer nanoparticles containing doxorubicin and curcumin (Misra and Sahoo, 2011). Nanoparticles downregulated BCL-2 and MDR1 appearance and also subdued the nuclear efflux mechanism.

3.2 Solid-Lipid Nanoparticle

Generally solid lipid nanoparticles are formulated by solid lipids, water, and emulsifiers. The solid lipids comprise triglycerides, fatty acids, partial glycerides, waxes, and steroids. To stabilize the lipid dispersion different emulsifiers have been used. Solid lipid nanoparticles were prepared by emulsification

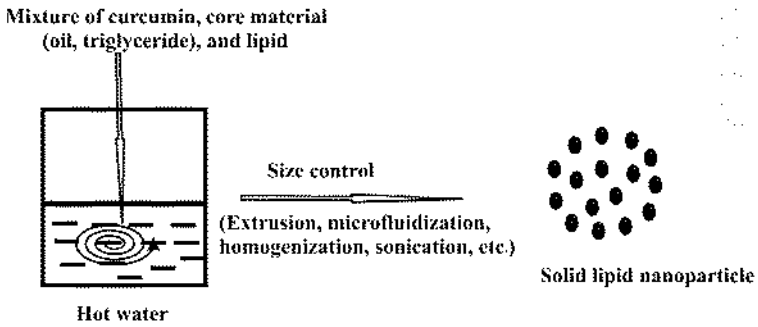

Figure 15.3. Preparation of solid lipid nanoparticles.

process with temperature above the melting point of the lipids. The combination of emulsifiers, lipid melt, and drug is injected into the water and homogenized (Fig. 15.3). The homogenization can be carried out beyond the hot homogenization or beneath of cold homogenization of the lipid melting temperature. To reduce the size of nanoparticles to the submicron size range, the water phase is mechanically stirred. Various homogenization methods can be use such as high-shear, high-pressure, and ultrasound homogenization.

Tiyaboonchai et al. (2007) prepared curcuminoids loaded solid lipid nanoparticles by a microemulsion method at 70–75°C temperature. The size and entrapment efficiency of curcumin-loaded solid lipid nanoparticles were found to depend on the concentrations of the lipids, other additives and emulsifiers. Less than 450 nm particle size and up to 75% entrapment efficiency of curcumin was obtained by this method. Surface modified solid lipid nanoparticles with transferrin were prepared to enhance anticancer activity (Mulik et al., 2010).

Oral administration of solid lipid nanoparticles containing 2–4 g of curcumin in osteosarcoma patients reported that blood concentration reach 31.42–41.15 ng/mL within 4 h without unfavorable side effects (Gota et al., 2010). Curcumin-loaded solid lipid nanoparticles prepared with 134 nm size and entrapment efficiency of 84% showed controlled release of drug over a period of 1 week (Kakkar et al., 2011). This nanoparticle resulted higher bioavailability in plasma of rats. Oral administration of solid lipid nanoparticles containing curcumin at a dose of 50 mg/kg achieved 14.29 μg/mL concentration of curcumin in serum. However, oral administration of free curcumin at a dose of 50 mg/kg achieved 0.292 μg/mL concentration of curcumin in serum. The higher concentration of curcumin in blood through the administration of nanoparticles is the improved curcumin absorption.

Transferrin-solid lipid nanoparticles retained maximum 83% of curcumin for 6 months and targeted to breast cancer cells (MCF-7) and promotes cell death through transferrin mediated endocytosis (Mulik et al., 2010). Structural integrity of curcumin facilitated nanoparticles of lipid bilayer structure and that is stabilized by apolipoprotein (Ghosh et al., 2011). Anticancer properties of nanoparticulate lipid formulation were significant in comparison to the free curcumin.

Dadhaniya et al. (2011) studied the effects of curcumin-loaded solid lipid particle in rats. Conjugated curcumin administration showed no toxicologically considerable changes in the preclinical study include weight gain, behavioral observations, food consumption, ophthalmic examinations, and organ weights. Similarly, there are no changes in clinical parameters include serum chemistry, hematology, and urinalysis. Furthermore, no histopathology or treatment related gross findings were observed. Solid-lipid nanoparticles containing curcumin was prepared for experimental prototype of cerebral ischemia in rats. There was a growth of 90% in cognition and 52% reticence of acetylcholinesterase against cerebral ischemic and neurological scoring (Kakkar et al., 2013). Levels of catalase, superoxide dismutase, glutathione, and mitochondrial complex enzyme behavior were also considerably augmented, while lipid peroxidation, acetylcholinesterase, and nitrite levels diminished following curcumin-solid lipid nanoparticles administration. Gammascintigraphic studies showed 16.4 and 30 times improvement in brain bioavailability upon oral and intravenous administration of curcumin-solid lipid nanoparticles versus curcumin-silver. Outcome indicated the protecting role of curcumin-solid lipid nanoparticles in opposition to cerebral ischemic insult, suggesting that it is packaged suitably for improved brain delivery (Kakkar et al., 2013).

Yadav et al. (2009) developed a colon specific drug delivery system for treating colitis in the rat model. They prepared solid lipid microparticles containing curcumin using palmitic acid, stearic acid, soya lecithin, and poloxamer 188 (Yadav et al., 2009). Then, the developed formulation was studied for their antiinflammatory and antiangiogenic actions by using rat colitis and chick embryo models. Chorioallantoic membrane study showed the angioinhibitory activity of solid lipid microparticles containing curcumin. Rats administered with curcumin-loaded solid lipid microparticle showed quicker weight gain in comparison to the control rats administered with dextran sulfate solution. Furthermore, mast cell numbers were decreased through the administration of curcumin-loaded solid-lipid microparticles. Curcumin is used for the treatment of inflammatory bowel disease. Curcumin-loaded

solid lipid nanoparticles were prepared to augment its activity in an ovalbumin induced rat to treat asthma (Wang et al., 2012). In vitro study was performed to verify physiochemical properties of nanoparticles and release of curcumin from this formulation (Wang et al., 2012). The in vivo pharmacokinetics and tissue distribution were performed in mice. The curcumin is amorphously distributed in the encapsulated matrix that is confirmed by X-ray diffraction analysis. The plasma concentrations of curcumin contained in the nanoparticle were significantly better than free curcumin. The tissue concentrations of curcumin increased after administration of solid lipid nanoparticle containing curcumin, mainly in lung and liver. Additionally, solid lipid nanoparticles containing curcumin significantly masked airway hyper responsiveness and inflammatory cell penetration. It is also reticent the expression of T-helper-2-type cytokinesin bronchoalveolar lavage fluid widely than free curcumin. These observations indicate that solid lipid nanoparticles containing curcumin can be a potential candidate for asthma therapy (Wang et al., 2012).

3.3 Nanogel

Hybrid nanogels containing curcumin were developed for intracellular delivery (Wu et al., 2011). The hybrid nanogel consists of several layers and provides efficient cytotoxicity in opposition to B16F10 cells. Cross-linked copolymer nanogels containing curcumin were synthesized via polymerization of *N*-vinyl-2-pyrrolidone and *N*-isopropylacrylamide in the existence of monoacrylate PEG (Yallapu et al., 2008). Bisht et al. (2007) prepared nanohydrogel formulation containing curcumin and applied on pancreatic cancer cell lines. Nanohydrogel formulation containing curcumin repressed interleukin (IL)-6 syntheses, blocked the formation of NF-κB, and downregulated transcripts of multiple inflammatory cytokines. Nanohydrogel formulation containing curcumin administered through parenteral route widely repressed tumor growth in both orthotopic and subcutaneous xenograft models of pancreatic cancer in mice (Bisht et al., 2010). Additionally, nanohydrogel formulation containing curcumin significantly suppressed tumor growth. Bioavailability and therapeutic activity of curcumin was enhanced through this nanoformulation. Nanoformulation containing curcumin showed dose-dependent antiproliferative activity in glioblastoma neurosphere cell lines and in embryonal tumor-derived cell lines (Lim et al., 2011). Another group described production of curcumin-encapsulated chitosan-poly(vinyl alchohol) silver nanocomposite, poly(acrylamide)-poly(vinyl sulfonic acid) silver nanocomposite,

and poly(acrylamide)-carboxymethyl cellulose magnetic nanocomposites to improve the therapeutic activity of curcumin (Varaprasad et al., 2011). Nanogels containing curcumin using hydroxyl propyl methylcellulose, pluronic F68, and poly (vinyl pyrolidone) have been prepared via a solvent emulsion–evaporation method (Dandekar et al., 2010). The developed nanogels have confirmed oral and cellular protection in a study using mice.

Goncalves et al. (2012) developed a dextrin nanogel containing curcumin and evaluated by using fluorescence measurements and dynamic light scattering. The encapsulation efficiency and stability of curcumin-loaded nanogel depends on the nanogel and curcumin ratio. The in vitro drug release study showed that nanogel is an appropriate carrier for the controlled release of curcumin (Goncalves et al., 2012). Nanogels were prepared and preferred properties can be obtained by changing the stimuli-responsive and surface-active elements, chemical functional groups, and cross-linking density (Yallapu et al., 2011). Nanogels exhibited exceptional prospective for systemic drug delivery. It should have some common features including a lesser particle size, prolonged half-life, biodegradability, and/or biocompatibility, high stability, molecules protection from immune system, and more amount of drug loading (Yallapu et al., 2011). Chitin nanogel containing curcumin was prepared and analyzed by scanning electron microscope, dynamic light scattering, and Fourier transform infrared spectroscopy (Mangalathillam et al., 2012). The cytotoxicity of nanogel was analyzed in human melanoma cells and human dermal fibroblast. Higher amounts of curcumin were released in acidic condition in comparison to neutral condition and nanogel showed correct toxicity on cancer cells, but less toxicity on normal cells. A high quantity of nanogel was taken by human melanoma cells, which was confirmed by confocal analysis. A higher concentration of nanogels shows analogous cell death compared to free curcumin. Nanogels containing curcumin showed a four times augment in transdermal diffusion of curcumin compared to free curcumin. Loosening of the horny layer of the epidermis was facilitating infiltration of nanogels containing curcumin with no signs of inflammation showed by histopathology study (Mangalathillam et al., 2012). These consequences suggested the formulated nanogels explicit advantage for the management of melanoma by successful transdermal penetration.

3.4 Nanoemulsion

Nanoemulsions are stable thermodynamic dispersions of oil and water within 100 nm sizes stabilized by surfactant and

cosurfactant. Nanoemulsion is produced readily without high-energy. O/W nanoemulsions containing curcumin was prepared and evaluated ear inflammation model of mice and showed improved antiinflammatory activity in comparison to free curcumin (Wang et al., 2008b).

3.5 Liposomes

Liposomes are the most studied nanoparticles for drug delivery. Generally, physically stable liposomes are prepared with numerous types of lipid. The first step is shown in Fig. 15.4, representing the preparation of the mixed lipids**.** Methanol, ethanol, t-butyl alcohol, chloroform, and their combination are used as solvents to dissolve the lipids. The lipids and curcumin are dissolved in organic solvent and then evaporated to remove the solvent. Additionally, spray dryers are used to get the fine powder. Then the film was kept in a freezer for succeeding use. Hydration of the film with aqueous medium produced liposomal vesicle.

Liposomes are outstanding drug delivery systems as they can carry both lipophylic and hydrophilic molecules. Antitumor activity of curcumin-loaded liposome was studied against pancreatic cancer cells (Li et al., 2005). Curcumin-loaded liposome inhibits cancer growth and also exhibits antiangiogenic effects.

Cellular uptake of curcumin and albumin-loaded liposome was studied (Kunwar et al., 2006). Liposome is able to load more additional curcumin into cells than either human serum albumin or aqueous-DMSO. Lymphoma cells showed higher uptake of curcumin to lymphocytes.

Wang et al. (2008a) developed liposomal formulation of curcumin that is able to repress the development of squamous cell carcinoma in dose-dependent manner and also proficient to suppress the activation of NF-κB. The appearance of MMP-9COX-2, cyclin D1, Mcl-1S, Bcl-xL Bcl-2, and Mcl-1L were condensed. Takahashi et al. (2007) prepared liposome containing curcumin with maximum

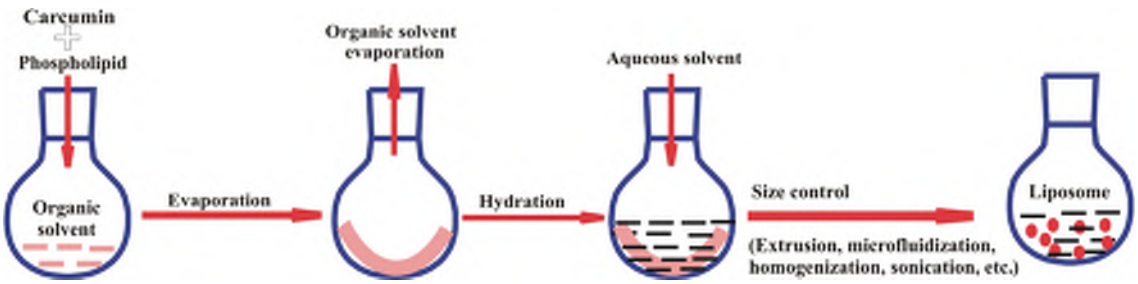

Figure 15.4. Preparation of liposomal curcumin system.

entrapment efficiency of 68% for oral administration. Absorption of curcumin from liposomal formulation after oral administration to rats was more in comparison to non liposomal formulation. Intravenous administration of liposome containing bis-demethoxy curcumin analogue showed enhanced protective activity of liver in comparison to free curcumin (Aukunuru et al., 2009). Narayanan et al. (2009) prepared liposomal formulation containing resveratrol and curcumin and in vivo study showed significant decrease in adenocarcinoma. In vitro studies also showed that liposomal formulation significantly induced apoptosis and suppressed cell growth. These results suggested that liposome containing combined phytoconstituents may decrease prostate cancer occurrence (Narayanan et al., 2009). Liposomal formulations of curcumin are promising therapeutic agents for the management of a variety of cancers (Kurzrock et al., 2004). Li et al. (2007) determined an antiangiogenesis effect of curcumin-loaded liposome administered through intravenous route, which suppressed pancreatic cancer growth in murine xenograft models (Li et al., 2007).

A comparison of the stability of free curcumin and liposomal curcumin in phosphate buffered saline was studied and the effect found that liposomal curcumin can augment curcumin stability in phosphate buffered saline. The high lipo-curcumin formulation can protect curcumin (100%) after incubation in phosphate buffered saline (pH 7.4) at 37°C for 180 min (Chen et al., 2009). Liposomal encapsulation improved the gastrointestinal absorption of curcumin. Takahashi et al. (2009) prepared liposome-encapsulated curcumin from commercially available lecithin. Pharmacokinetic parameters following oral administration of liposome-encapsulated curcumin were compared to curcumin and a combination of lecithin and curcumin. Faster rate of absorption and high bioavailability were observed from curcumin nanoformulation in comparison to the other forms (Takahashi et al., 2009).

Sou et al. (2008) have effectively prepared a lipid formulation of curcumin. Intravenous administration of curcumin-loaded lipid formulation in rats showed no susceptible response in blood cells and maximum amount of curcumin concentrated in spleen tissues and bone marrow. Recently, curcumin was efficiently incorporated in liposomes prepared from egg phosphatidylcholine. A two-times increase in curcumin concentration was found in rat plasma when 100 mg/kg b.wt administered through oral route in comparison to the free curcumin (Pandelidou et al., 2011).

Rahman et al. (2012) prepared liposomes incorporated with inclusion complex of β-cyclodextrin-curcumin (Rahman et al., 2012). In vitro cell culture study showed liposomal formulations inhibit cell growth.

Some studies revealed that the drugs incorporated in liposomes are likely to be transported devoid of fast degradation and result in the least side effects and demonstrate extra recipients stability. Karewicz et al. (2011) bonded curcumin to cholesterol, egg yolk phosphatidylcholine, and dihexadecyl phosphate, and then in order to institute binding of curcumin to liposomes they used fluorescence and absorption measurements (Karewicz et al., 2011). The egg yolk phosphatidylcholine, cholesterol, and dihexadecyl phosphate stabilized after drug entrapment. Conversely, the egg yolk phosphatidylcholine and dihexadecyl phosphate system destabilized after drug loading. Liposomes containing three lipid compositions appear to be the most proficient system for curcumin delivery. Additionally, an interaction of free and liposomal curcumin with mixed monolayers and egg yolk phosphatidylcholine was studied by means of Langmuir balance measurements. It was recognized that egg yolk phosphatidylcholine liposomes containing curcumin are more stable upon interaction with the model lipid membrane than the unloaded ones.

The therapeutic action of liposome-containing artemisinin or curcumin and artemisinin has been studied extensively (Isacchi et al., 2012). It was observed that artemisinin only reduced the parasitaemia levels after 7 days of the start of action. Fluctuation of blood concentration was also observed and that is reflected in effectiveness of antimalarial activity (Isacchi et al., 2012). Artemisinin-loaded liposomes appeared to have an instant antimalarial effect which cured all mice infected with malaria within the same post inoculation period of time. Liposome containing curcumin and artemisinin give the most distinctive and statistically significant therapeutic effect. Higher concentration of drug was obtained through the administration of curcumin and artemisinin-loaded liposome and suggests the suitable application of these systems in parasitic infections (Isacchi et al., 2012). The effects of curcumin-loaded liposome were assessed on different types of seizures in mice (Agarwal et al., 2013). Curcumin-loaded liposome diminishes the length of seizures in status epilepticus and augmented the latency to the onset.

3.6 Micelles

Amphipathic compounds forming micelles have a big hydrophilic head group in association with their hydrophobic regions. So, water soluble polymers are frequently used for the big hydrophilic head group, while lipids, block polymers, or cholesterol are used for the hydrophobic regions. Structurally, micelles are composed of a single hydrophobic core enclosed in hydrophilic groups.

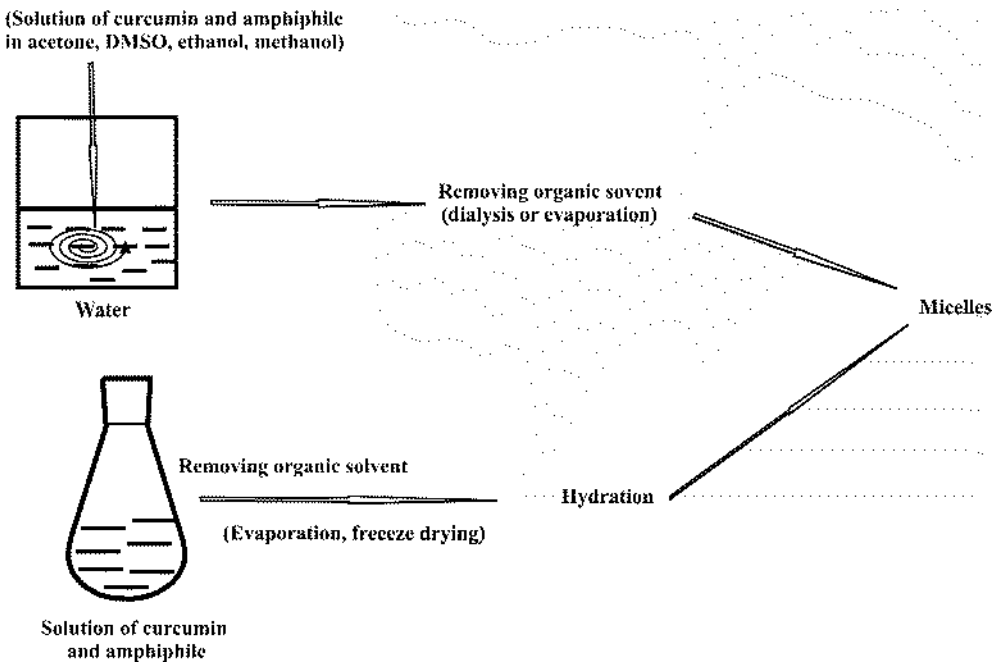

Figure 15.5. Procedure for preparation of curcumin micelles.

A preparation of micelles containing curcumin is based on an impulsive self-assembly procedure in the aqueous phase (Fig. 15.5). The curcumin and amphipathic compounds are dissolved concurrently in acetone, DMSO, or alcohol and then the solution containing curcumin is injected into an aqueous medium and then organic solvent is removed through evaporation or dialysis. The obtained dried mixture of amphipathic compounds and curcumin is hydrated by the aqueous media.

The intestinal absorption of curcumin and curcumin-loaded micelle with bile salt and phospholipid was studied by an in vitro model composed of everted rat intestinal sacs. Biological conversion of curcumin takes place during absorption. In vitro absorption of curcumin was increase from 47 to 56% when the curcumin was present in micelles (Suresh and Srinivasan, 2007). Curcumin-loaded micelle gave a 60-fold better half life in rats in comparison to the solubilization of curcumin in DMA, dextrose, and PEG mixture (Ma et al., 2007). Yu et al. (2011) showed the structure of modified ε-polylysine micelles and their application in augmenting cellular antioxidant activity of curcuminoids (Yu et al., 2011). Outcome of their study discovered that modified ε-polylysine micelles were capable to incorporate curcuminoids and augment their antioxidant activity and aqueous solubility compared with

curcumin. It was established that these micelles may be used for delivering poorly soluble drugs such as curcumin.

Podaralla et al. (2012) delivered curcumin through a natural protein-core-based polymeric micelle. They synthesized recyclable curcumin-loaded micelles by using methoxy polyethylene glycol and zein. The release of curcumin extended up to 24 h in vitro and significantly enhanced its stability and aqueous solubility with the three-fold decrease in IC50 value of curcumin. As the curcumin is secluded from potential inactivation by their micellar environment, its preservation and bioavailability can be improved.

Three types of micelles with different surfactants were used to study the hydrolysis of curcumin in alkaline conditions (Leung et al., 2008). At pH 13, fast breakdown of curcumin occurred in the micellar system containing dodecyl sulfate. Conversely, breakdown of curcumin repressed close to 90% in the presence of either cetyltrimethylammonium bromide or dodecyltrimethylammonium bromide micelles. Curcumin remains present in dodecyltrimethylammonium bromide and cetyltrimethylammonium bromide micelles at pH 13 that is shown by fluorescence spectroscopic studies. The absence of incorporation and stabilization in the dodecyl sulfate micellar solution resulted in quick hydrolysis of curcumin. Wang et al. (2006) introduced the determination of curcumin in mixed micelle by predisposed fluorometric method. Mixed micelle of sodium dodecyl benzenesulfonate and cetyltrimethyl ammonium bromide significantly enhanced the fluorescence of curcumin. Fluorescence quantum yield of curcumin in micelle was about 55 times superior to that of ethanolic solution containing curcumin.

In brief, curcumin-loaded micelles can increase the drug's effectiveness by targeting exact cells. Results demonstrated that minimum amount of drug was built up in healthy tissues and a minimizing of toxicity. Micelles containing curcumin can be used in different circumstances due to their much slower and extended release of drug. The preservation and bioavailability of curcumin could be improved since curcumin is sheltered by its micellar environment. Micelles containing curcumin increase habitation time and half-life and reduce total clearance leading to persistence of acting time of curcumin. Micelles containing curcumin can be predisposed by physicochemical features including their electrical charges and size, location within the micelles, and concentration.

3.7 Phospholipid Complexes

Liu et al. (2006) showed a considerable enhancement in curcumin bioavailability owing to curcumin–phospholipid complex

development. Curcumin and curcumin–phospholipid complex were given orally to rats. Phospholipid complex containing curcumin showed a maximum plasma curcumin level of 600 ng/mL after 2.33 h oral administration. However, free curcumin having maximum plasma concentration of 267 ng/mL following 1.62 h of oral administration. Half life of curcumin in rats was increase about a 1.5-fold for the phospholipid complex containing curcumin compared to free curcumin. These sequences indicate that the phospholipid complex containing curcumin can considerably augment circulating levels of apparently active curcumin in rats (Liu et al., 2006). Another study showed a 3-fold enhancement in water solubility and an improved hepatoprotective effect for a phospholipid complex containing curcumin over free curcumin. Phospholipid complex containing curcumin significantly protected the liver from carbon tetrachloride induced liver damage in rats by restoring enzyme levels of liver glutathione system and that of superoxide dismutase, thiobarbituric acid reactive substances, and catalase (Maiti et al., 2007).

3.8 Nanocrystal

Curcumin is an extremely crystalline compound. To produce pharmaceutical grade nanocrystals containing curcumin, highly developed nanotechnology has been applied. Nanocrystals containing curcumin have been reported by a few authors (He et al., 2010; Onoue et al., 2010). They studied the arrangement of nanoprecipitation containing curcumin from a micromixer. The nanoprecipitate first formed as amorphous nanoparticles then amorphous aggregates and lastly produced needle-shaped curcumin crystals (He et al., 2010). Nanocrystal containing solid dispersed curcumin was obtained by a wet-milling process (Onoue et al., 2010). The size of the nanocrystal containing solid dispersed curcumin resulting from subsequent wet-milling was as low as 250 nm. The creation of curcumin crystal in alcoholic solution is a time-dependant procedure and takes 90 min time (He et al., 2010). The crystals of curcumin begin to aggregate and precipitate after 90 min. Curcumin molecule stabilized by micelles composed of surfactants include Tween 80, sodium dodecyl sulfate, cetyltrimethylammonium bromide, Triton X-100, and pluronic polymers at a critical micelle concentration (Wang et al., 2010).

3.9 Conjugates

Polymer-drug conjugates are one of the nanoscale therapeutic drug delivery system. Curcumin contain active methylene groups and two phenolic rings and those are the sites for conjugation of

biomcaromolecules. Vareed et al. (2008) study the pharmacokinetics of curcumin in human volunteers after administration of single oral dose. Blood samples were analyzed for determination of free curcumin and its sulfate and glucuronide conjugate. Curcumin was absorbed through oral administration in humans and can be detected as sulfate and glucuronide conjugates in plasma. Pandey et al. (2011) anticipated novel polyethylene glycosylated curcumin analogs that control the antioxidant protection system and also act as modifiers for inflammatory diseases. These analogs improved the solubility of curcumin. Polyethylene glycosylated conjugates containing curcumin inhibited the cell growth of several human pancreatic cancer cell lines (Li et al., 2009). The improved activities were observed through Jun activation domain-binding protein-1 suppression by curcumin conjugates. Curcumin analogs-loaded polyamine conjugates were competently transported to mitochondria through intracellular uptake (Simoni et al., 2010). Nanosize micelle was prepared through conjugation by hydrophobic interactions of curcumin and hyaluronic acid in aqueous solution (Manju and Sreenivasan, 2011). Conjugate containing 13 μg of curcumin significantly kill 80% of the L929 cells, representing its prospective in therapeutics.

3.10 Niosome

Niosomes are nonionic surfactant vesicle composed of cholesterol and alkyl or dialkyl polyglycerol ether group. Niosomes is a container for drug molecules with an extensive range of solubilities owing to existence of hydrophilic, lipophilic, and amphiphilic moieties in the constitution. They can be used as an effective replacement to liposomal drug carriers. The properties of niosome depend on the component of the bilayer in addition to the technique of their manufacture.

Proniosomes of curcumin were manufactured by incorporating the drug in a mixture of Span 80, diethyl ether, and cholesterol to examine transdermal drug delivery system (Kumar and Rai, 2011). The designed systems eminent among sizes, repose angle, drug entrapment, vesicular stability, and hydration rate under diverse storage settings. Outcome showed that proniosomes are extremely stable and capable extended delivery systems for curcumin (Kumar and Rai, 2011). Mandal et al. (2013) also developed a comparative study with various microenvironments for photophysical properties of curcumin within niosomes by steady state, dynamic light scattering techniques, and time resolved fluorescence spectroscopy. Results showed that added stiff and restricted microenvironments of niosomes improve the fluorescence

lifetime of curcumin and the steady state fluorescence intensity. It was observed that niosomes are a better tool for delivery system to repress the degradation of curcumin (Mandal et al., 2013). In order to improve the skin penetration of curcumin different nonionic surfactant were used to prepare niosome containing curcumin and evaluated through snake skin (Rungphanichkul et al., 2011). The fluxes of curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin were consistent with the hydrophobicity of curcumin, bisdesmethoxycurcumin, and desmethoxycurcumin, respectively. The study revealed that curcuminoids can be productively manufactured as niosomes, and such formulations have better properties for transdermal drug delivery system (Rungphanichkul et al., 2011).

3.11 Dendrimers

Dendrimers are a group of branched polymers that are produced with structural control and are referred to as synthetic protein. Dendrimers are polymeric structure with different surface and chemical properties. They have single molecular weight relatively than a distribution of molecular weights in association with the conventional linear polymers. The dendrimer consisting of a core, various surface functional groups, and branched interiors. This nanospace represents secluded surroundings, thus lessening toxicity connected with the payload. The distinct organization, size, dense spherical form, controllable surface functionalities, and monodispersity dendrimers create brilliant applicants for evaluation as drug delivery services.

Dendrimer curcumin conjugates were prepared to test both water solubility and cytotoxicity against breast cancer cell lines (Debnath et al., 2013). Conjugates were significantly inducing cytotoxicity against BT549 and SKBr3 breast cancer cells and effectively induced cellular apoptosis measured by caspase-3 activation. Interaction between curcumin and dendrimer was determined by using molecular modeling and fluorescence spectroscopy techniques (Cao et al., 2013). Entrance of curcumin into the interface of dendrimer was taking place in five binding sites by hydrophobic bonds, van der Waals forces, and hydrogen bonds. Binding constants with large values represented that dendrimer holds the curcumin robustly. Moreover, in another study, telomerase activity was repressed by the incorporation of polyamidoamine in breast cancer cell line (Mollazade et al., 2011). Dendrimers have no cytotoxicity in breast cancer cell line that is shown by in vitro study. Polyamidoamine incorporating curcumin concentration augmented while relative telomerase activity decreased. These

outcomes recommended that polyamidoamine-incorporated curcumin had a dose-dependent cytotoxicity effect on breast cancer cell line through inactivation and down regulation of telomerase. So, polyamidoamine can be measured as a excellent carrier particularly for hydrophobic agents.

4 Future Developments

Nanotechnology and nanoscience will be critically essential in facilitating the applications of curcumin. In the past 10 years the application of curcumin nanoformulations has increased drastically and in the near future more numbers of patents are being required. The accessible volume of intellectual property should help the development of curcumin-based pharmaceutical products. The author expects the result of preclinical and clinical tests of curcumin-based nanoformulations will be made accessible in the near future.

Besides the advantage of nanoformulations of curcumin, the danger of the nanoformulations containing curcumin should be evaluated cautiously. A recurrent undesirable reaction to the liposomal injection is harsh allergic reactions. Liposomes containing lipid component should be considered cautiously since the occurrence of the allergic reactions is dependent on the component of the liposomes. Though the oral curcumin is supposed to be secure, intravenous nanoformulations that could augment the bioavailability of curcumin may augment the side effects of curcumin, too, as well as its efficacy. Targeted delivery of drug nanocarriers may shows potential and diminishes the risk of treatment. The existing curcumin-loaded nanoformulations dispersions in aqueous medium provide a diversity of administration alternatives such as oral intake, intravenous injection and others. The aspects of profit cost ratio should be impartially analyzed for projected nanocarriers versus free curcumin orally.

5 Conclusions

The field of food nanotechnology is experiencing considerable growth owing to the convergence of interests of industry, academia, and government. In the area of nutrient and nutraceutical delivery there have been significant advances made in nanoparticle formulations intended to progress the bioavailability of poorly water-soluble ingredients. Most researchers have worked under the postulation that enhancement in bioavailability comes from enhancement in noticeable solubility and have ignored the impact

that mass transfer issues and direct nanoparticle uptake play in improving bioavailability. More basic studies on nanoparticle-mediated nutrient and nutraceutical transportation are desired to understand this technology and engineer new nanoparticle delivery systems. Curcumin resulting from turmeric has been used for centuries as a therapy for many ailments. Extensive research has shown the aptitude of this compound to alter multiple cellular targets and consequently offer defensive and therapeutic value in opposition to an extensive diversity of diseases. Structural modification and new formulations of curcumin have been developed to improve in vitro and in vivo efficacies. On the contrary, improvements in curcumin bioavailability disclose that the curcumin bioavailability improvement has not gained significant consideration. So far, novel delivery strategies including those of liposomes, nanoparticles, and phospholipid complexes present considerable promise and are worthy of further investigation in attempts to augment the bioavailability, medicinal value, and application of this attractive molecule from Mother Nature.

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16

MICROENCAPSULATION OF PROBIOTIC CELLS: APPLICATIONS IN NUTRACEUTIC AND FOOD INDUSTRY

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1 Introduction

For a long time microorganisms have played a predominant role in human life, as they take part in all the rutinary ecosystems playing an important role in our organism. In 1908, Elia Metchnikoff was the first to establish a relationship between the consumption of *Lactobacillus* fermented food and the longevity of Bulgarian farmers from the Balkans, by the substitution of putrefactive bacteria in the intestinal microbiota by these other bacteria found in fermented food. After this first approach to the probiotic concept there have been several definition of these microorganisms up to the current definition given by the FAO/OMS (2001), who defines a probiotic as a live microorganism that when administered in adequate amounts has a beneficial effect for the health's host. This amount varies from one country to other; however, it is generally recognized that a probiotic product must contain between 10^6 and 10^8 CFU/g or between 10^8 and 10^{10} CFU/dose. In addition, probiotic microorganisms must have the GRAS (generally recognized as safe) status given by the Food and Drug Administration (FAD).

The probiotic strains most commonly used belong to the genders *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces boulardii*. Since probiotics are most frequently administered orally they must survive to passing through the gastrointestinal tract, tolerate bile salts, acid, and gastric enzymes, as well as being able to adhere to epithelial cells. Thus, it is important that probiotics are administered in food or pharmaceutical forms that confer protection to

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Table 16.1 Probiotics Used in Commercial Preparations (Mombelli and Gismondo, 2000; Tripathi and Giri, 2014)

Probiotic	Species
Lactobacillus sp	*L. acidophilus, L. casei, L. delbrueckii ssp, L. cellobiosus, L.curvatus, L. fermentum, L. lactis, L. plantarum, L. reuteri, Lactobaciulls brevis*
Bifidobacterium sp	*B. bifidum, B. adolescentis, B. animalis, B. infantis. B. thermophilum, B. longum*
Enterococcus sp	*Ent. Faecalis, ent. Faecium*
Streptococcus sp	*S. cremoris, S. salivarius, S. diacetylactis, S. intermedius*
Lactic acid bacteria not	*B. cereus, B. subtilis*
Saccaromyces	*S. bourlardii, S. cerevisiae*

the environmental and technological conditions. In addition, probiotic products must ensure a number of viable cells ($>10^6$ CFU/g) not only at the moment they are produced, but also at the end of the expiration date.

Commercial interest in probiotics is gradually increasing as it is reported that intestinal microbiota play an important role in different physiological and pathological conditions (Table 16.1). Probiotics are considered as a promising alternative in the prevention and treatment of different pathologies (Table 16.2). However, the main problem when considering the production of a probiotic product is their low resistance to environmental and technological conditions, such as pH, oxygen, light exposure, temperature, compression techniques, and so forth. Microencapsulation of probiotic bacteria is thus a good alternative in the production of probiotic food or nutraceuticals, since it could confer a high degree of microorganism protection and ensure an adequate dosage during all the shelf life, thus increasing the applications and the efficacy of these products.

2 Probiotics

Manipulation of the microbiota by probiotics in different locations (mainly the intestinal one but also that established in other tissues as the skin or the vagina) is gaining a lot of interest due to the

Table 16.2 Probiotics Used in Various Pathologies (Amara and Shibal, 2013)

Disease	Species	References
Eczema	*E. coli, B. bifidum* *B. lactis, Lacotococcus lactis*	Niers et al. (2009); Soh et al. (2009); Viljanen et al. (2005a,b)
Food allergies	*E. coli, Bacilus circulans PB7, L. plantarum DSMZ 12028*	Lodinova-Zadnikova et al. (2003) Bandyopadhyay and Das Mohapatra (2009); Cammarota et al. (2009)
Regeneration of the beneficial microbiota after treatment with antibiotics	*Enterococcus mundtii ST4 SA, L. plantarum 423, L. brevis KB290*	Botes et al. (2008); Zhou et al. (2005)
Gastroenteritis	*L. casei*	Yamada et al. (2009);
Intestinal hyperpermeability	*L. plantarum 299* *L. plantarum LP299*	Kennedy et al. (2000); Strowski and Wiedenmann (2009); White et al. (2006)
Vaginal candidiasis	*L. rhamnosus GR-1* *L. reuteri RC-14*	Martínez et al. (2009)
Urinary tract infections	*L. rhamnosus GR-1* *L. reuteri RC-13*	Anukam et al. (2009)
Lactose intolerance	*L. acidophilus*	Hawrelak (2003)
Intestinal dysbiosis	*L. johnsonii La1* *L. GG*	Hawrelak (2003); Silva et al. (1987); Bennett et al. (1996)
Inflammatory bowel disease	*B. infantis 35624* *E. coli DSM 17252* *B. infantis 35624*	Brenner and Chey (2009); Enck et al. (2009); Whorwell et al. (2006)
Traveler's diarrhea	*L. GG* *L. plantarum*	Hawrelak (2003); Michail and Abernathy (2002)
Radiation-induced diarrhea	*L. casei DN-144 001*	Giralt et al. (2008)
Crohn's disease	*Escherichiacoli strain Nissle 1917*	Boudeau et al. (2003)
Preventing colon cancer	*Enterococcus faecium M-74*	Mego et al. (2005); Thirabunyanon et al. (2009)
Ulcerative colitis	*L. acidophilus* *E. coli Nissle 1917* *Bifidobacterium*	Abdin and Saeid (2008); Adam et al. (2006); Imaoka et al. (2008)
Peptic ulcer	*L. acidophilus*	Iarovenko et al. (2007);
Atopy prevention	*L. rhamnosus GG*	Huurre et al. (2008); van der Aa et al. (2008)
Hipercolesteriolemia and cardiovascular disease	*E. faecium M-74* *L. plantarum* *Orpionibacterium Freudenreichii* *L. plantarum PH04*	Hlivak et al. (2005); Kiatpapan et al. (2001); Nguyen et al. (2007)

beneficial properties that these bacteria could exert for the host's health. It can be said that probiotic administration is one of the more promising therapies, both in nutrition and pharmaceuticals.

However, not all probiotics have the same properties and the effects are strain-dependant. Furthermore, it is important to note that the effect of one probiotic microorganism on a specific pathology is not applicable to another condition, and there is no universal microorganism that present all the different effects reported for probiotics. In fact, it has been reported that even different strains from the same species can have different effects on the host (Vasiljevic and Shah, 2008). Therefore, it can be concluded that when considering the administration of a probiotic product it has to be cautiously studied—not only the strain, but also the target tissue, way of administration, dosage, and duration. Generally it is considered that the recommended dose of probiotics to obtain beneficial effect is at least 5×10^9 CFU/day during at least 5 days.

Survival during passage through the gastrointestinal tract is key when probiotics are orally administered. Production of bile salts, acids, or pancreatic secretions has, among other roles, the ability to eliminate bacteria coming from food. Efficacy of probiotics depends on their capability to survive to these conditions to reach the intestine and to be able to multiply and adhere to epithelial cells.

Thus, to be considered as a probiotic, a microorganism has to meet a variety of criteria (Ramos-Cormenzana et al., 2005; Saulnier et al., 2009):

- To be able to survive to passage through the gastrointestinal tract.
- To have antimicrobial activity.
- To be able to colonize the final location (mucus, skin, etc.).
- To be alive at the moment of administration, although it has been reported that different strains can have positive effects even though they are inactivated (by heat for example).
- To be well characterized from the genotypic and phenotypic perspectives and to be deposited in a recognized culture collection.
- To have beneficial effect for the host demonstrated by generally recognized scientific proofs.
- To be safe for the host, with no detrimental effects demonstrated by in vitro and in vivo studies.

Finally, it is important to mention two other concepts related to probiotics (Guarner et al., 2011):

- Prebiotics: ingredients (mainly carbohydrates) that are selectively fermented by commensal microorganisms, thus enriching intestinal microbiota in those microorganisms that confer a beneficial effect for the host.
- Symbiotics: products that contain as many probiotics as products containing a mixture of probiotics and prebiotics.

2.1 Probiotic Cells

2.2.1 Lactic Acid Bacteria (LAB)

2.2.1.1 Gender Lactobacillus

Lactobacilli are essential for the dairy industry as they are used for the production of cheese, yogurt, and other fermented dairy products. They include a high number of gram-positive, catalase-negative, nonsporulating bacteria. These LAB are quimio-organotrophics, they ferment carbohydrates to produce lactic acid as the main final product of their metabolism. Some strains are present in the gastrointestinal tract both in humans and other animals and their administration can influence intestinal microbiota and impact human health and nutrition. In the following paragraphs, some examples of lactobacilli used in human nutrition are mentioned (Bernardeau et al., 2008).

Lactobacillus acidophilus LA-5 is frequently used in combination with *Bifidobacterium animalis* subsp. *Lactis* Bb-12 in infant formula, nutritional supplements, and fermented dairy products. It has been reported that regular consumption of this strain can influence the composition of intestinal microbiota, thus protecting the host from some diseases such as traveler's diarrhea and reinforcing the immune system (Möller and De Vrese, 2004). *Lactobacillus johnsonii* is also used in some probiotic products with similar effects.

Among the species *Lactobacillus casei* there are two frequently used strains, *L. casei* DN-114001 and *L. casei* Shirota, presented mainly in fermented dairy products. *Lactobacillus paracasei* F19 was isolated form the colon and has a good viability in dairy products as well as a high rate of survival through gastrointestinal tract (Crittenden et al., 2002; Desai et al., 2006).

The strain *Lactobacillus rhamnosus* GG (LGG) is probably the probiotic that is most widely spread all over the world. It was isolated from the intestine of a healthy human and its effects are well known as it has been the object of many reports. Its main applications are based on gastrointestinal protection and immune modulation. Many clinical trials have been performed with LGG in different conditions, thus reporting a positive role of this probiotic strain in acute diarrhea treatment, both in children and adults, as well as a possible role in the prevention of allergies and treatment of intestinal inflammation. More recently a group of lactobacilli strains (*Lactobacillus gasseri* CECT5714, *Lactobacillus fermentum* CECT5716, and *Lactobacillus salivarius* CECT5713) has been isolated from human breast milk, previously considered sterile, of healthy women. *L. fermentum* CECT5716 is currently used in infant formula for prevention of gastrointestinal infections and in pharmaceutical supplements for the prevention of mastitis.

Finally, *L. rhamnosus* GR-1 has different properties for its use as a urogenital probiotic, as adhesion to uroepithelial cells and the inhibition of growing and adhesion of pathogens. This strain was chosen for its production of different compounds with antibacterial effect, as biosurfactants (Reid et al., 2001).

2.2.1.2 Gender Bifidobacterium

This group of bacteria has an important impact on human health as it is one of the most common bacteria found in human microbiota, with *Bifidobacterium longum* subsp. *longum* as one of the ten more prevalent bacteria (Tap et al., 2009).

In addition, bifidobacteria is also the most common gender in feces from breastfed infants. They are all gram-positive bacteria, nonsporulating and strictly anaerobic, what make them very difficult to culture by traditional techniques. Their main conditions of growing are 31–41°C and pH of 6.5–7.0.

Bifidobacteria includes 30 characterized species with a high-diversity degree both in the phylogeny and in the genotype (Ventura et al., 2009). During the first years after birth, infants' intestinal microbiota is dominated by bifidobacteria. As the infant grows and the diet varies, the profile changes and these bacteria are substituted by other groups of microorganisms, and its presence considerably decreases in the elderly.

The main species of this gender presented in the human gut are *Bifidobacterium adolescentis*, *Bifidobacterium catenulatum*, *Bifidobacterium pseudocatenulatum*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *B. longum* subsp. *longum,* and *B. longum* subsp. *infantis*. It is widely accepted that its presence is beneficial for human health and well-being and for this reason some of them are used in food and nutraceuticals (Ward and Roy, 2005; Roy, 2001), specially *B. animalis* subsp. *lactis* (Bb12) as it is a strain more oxygen-tolerant and more resistant to pH and temperature conditions (Meile et al., 1997; Roy et al., 1996).

Bb12 is one of the most studied probiotic strains. It was originally isolated from human feces and it was chosen for its safety and efficacy as a probiotic, with a high capacity of adhesion and resistance to gastric acids and bile salts, what confers a high colonization capacity. It is one of the more clinically studied strains, with some reports demonstrating a positive role in the prevention of rotavirus infections. Its capacity to interact with immune cells has also been reported and it could have a role in immune modulation, especially in newborns and children (Möller and De Vrese, 2004).

Another strain from the same species, *B. animalis* subsp. *lactis* DN-173010, is also widely used in dairy products for digestive comfort, although its probiotic properties are less known.

2.1.2 Propionibacteria

Propionibacteria are mesophylic and nonsporulating with different degrees of oxygen tolerance, from anaerobic to aero tolerant. Since they are heterofermentative, they are able to metabolize carbohydrates, polyols, and pyruvate to form propionate, acetate (with some beneficial effects for human health) succinate, and carbon dioxide. They can be isolated from the soil, from plants and also from the digestive tract of ruminants. Lactic propionibacteria, as *Propionibacterium freudenreichii*, are essential during the cheese ripening process, especially Swiss cheese, and they have a history of use that helps to establish their safety for human (Meile et al., 2008).

Although the probiotic properties of this group of bacteria are less known, some strains have some probiotic properties, such as high resistance to gastric acids and bile salts, low nutritional requirements, survival through the gastrointestinal tract, as well as their metabolic colonic activity (Hervé et al., 2007).

2.1.3 Yeasts

Theoretically, any nonpathogen microorganism can have probiotic properties, provided that they meet the probiotic definition. It is the case of some yeast that apart from their nutritional value, they seem to have some beneficial properties in gastrointestinal disorders (Agarwal et al., 2000). Among them, *Saccharomyces boulardii* has been widely studied for its probiotic properties and is currently used in the treatment of acute diarrhea, especially in children, but with scientific evidences also showing benefits for adults (Czerucka and Rampal, 2002; Mitterdorfer et al., 2001).

Some strains of *Saccharomyces cerevisiae* have been widely studied for their probiotic properties, such as bacterial translocation and the preservation of the intestinal barrier integrity (Generoso et al., 2010; Klingberg et al., 2008; Martins et al., 2007). Other yeast species not belonging to *Sacharomyces* gender have also demonstrated probiotic properties, specially *Debaryomyces*, *Torulaspora*, *Kluyveromyces*, *Pichia* y *Candida* (Silva et al., 2011), as they have demonstrated the ability to survive the gastrointestinal passage in different animal models and to have some antimicrobial properties (Kumura et al., 2004; Psani and Kotzekidou, 2006; Tiago et al., 2009). However, *S. cerevisiae* var. *Boulardii*, is the only yeast strain with clinically proven effects and efficiency in double-blind human studies (Sazawal et al., 2006, Born et al., 1993; Buts et al., 2006; McFarland, 2007; Surawicz, 2003; Zanello et al., 2011).

2.2 Beneficial Effects of Probiotics on Human Health

As probiotics are normally consumed orally, it would be logical to think that their effects are limited to the gastrointestinal tract. However, the high quantity of studies carried out over the past two decades has demonstrated that probiotics can have an effect in other locations as the urogenital tract, the immune system or even the cardiovascular system. Whereas some of these effects have been widely studied in humans, other need further studies since they have been demonstrated just in vitro or in animal models. It has also be taken into account that the effect of probiotics are strain and dose dependent; thus the effects demonstrated for one strain in one particular condition cannot be extrapolated to other strains or conditions. For this reason, it is difficult to generalize when considering probiotics effects on the human body.

2.2.1 Lactose Intolerance

Lactose is digested in the first part of the intestine by the enzyme lactase. When there is a deficiency in the production of lactase, lactose can be accumulated in the intestine and cause digestive problems after the intake of dairy products, such as diarrhea or abdominal pain, partially due to gases and acids produced during lactose fermentation by the bacteria in the intestinal microbiota. Lactose intolerance can be of genetic cause, since a variable percentage of humans cannot produce lactase in enough amounts. Another type of lactose intolerance is secondary to microbiota alterations due to infections by bacteria or parasites.

One of the first effects proposed for probiotics was in the treatment of lactose intolerance. Probiotics can have a role by two different mechanisms. First, most of probiotics used are lactic acid bacteria that contain lactase and are able to hydrolyze lactose. This is the reason why intolerant people can normally consume yogurt, although in limited amounts (Jiang and Saviano, 1997). Another mechanism for probiotic effect on lactose intolerance is their ability to restore intestinal microbiota in the case of infections (Alm, 1982; Marteau et al., 1990).

It has been reported that the consumption of yogurt containing *L. bulgaricus* and *Streptococcus thermophilus,* reduces lactose intolerance symptoms, provided it contains at least 10^8 UFC/mL of yogurt (Salminen et al., 1996; Gorbach, 2000).

2.2.2 Diarrhea

One of the effects more studied for probiotics is in the prevention and treatment of different types of diarrhea, such as traveler's, antibiotic-associated, or rotavirus diarrhea.

L. acidophilus, L. rhamnosus, Lactobacillus GG, and *Lactobacillus reuteri*, have been reported to be effective in the prevention and treatment of diarrhea, decreasing the number of days needed for recovery (Lemberg et al., 2007; Sazawal et al., 2006). Other probiotics, as *L. fermentum* CECT5716 have demonstrated its role in the reduction of the incidence of gastrointestinal infections in infants (Maldonado et al., 2012).

2.2.3 *Inflammatory Bowel Disease*

The most accepted hypothesis for explaining the etiology of the inflammatory bowel disease (IBD) is the interaction of different environmental factors in genetically predisposed people, causing exacerbated immune responses against antigens in the intestinal lumen (Podolsky, 2002).

Probiotics modulate the immune response and this is one of the mechanism by which they can help in IBD treatment. But probiotics can also produce short chain fatty acids, such as propionic acetic and butyric acid, reducing gut pH and limiting the growth of potentially pathogenic bacteria and the damage they can cause. Finally, probiotics can also compete with pathogenic bacteria for the adhesion to epithelial cells, which also decreases the intestinal permeability and the passage of antigens to the lamina propria (Plevy, 2002).

Although many probiotics have been reported to have a positive role in IBD, those with the highest scientific evidence are the strain *Escherichia coli* Nissle 191, that is able to reduce the damage caused by *E.coli* enteroinvasive by 97% (Malchow, 1997; Rembacken et al., 1999) and a preparation of six different strains—*S. thermophilus, B.m breve, B. longum, B. infantis, L. acidophilus,* and *L. plantarum*—that have been reported to be effective in the treatment of IBD (Jonkers et al., 2012).

2.2.4 Helicobacter Pylori *Associated Gastritis*

In vitro and animal model studies have demonstrated that certain LAB can inhibit the growth of *Helicobacter pylori*, Gram-negative bacteria responsible for the chronic gastritis that increase the risk of gastric cancer. Some probiotics have demonstrated to be able to inhibit the urease activity of *H. pylori*, needed for the survival to gastric pH (Midolo et al., 1995). In vivo studies have reported that probiotics do not eradicate the microorganism but limit the amount and the inflammation it produces (Sgouras et al., 2005) and reduce the adverse effects caused by the antibiotic treatment used for the infection. Thus the use of probiotics before and after antibiotics is proposed to increase the efficacy of the therapy for eradication of *H. pylori* (Zhifa et al., 2015).

2.2.5 Irritable Bowel Syndrome (IBS)

IBS is a common disorder that affects the large intestine (colon) commonly causing cramping, abdominal pain, bloating, gas, diarrhea, and constipation. Although it is not totally known, it seems that an imbalance in the microbiota could be one of the causes of IBS and, thus, administration of probiotics could have beneficial effects.

It has been reported that *B. infantis* 35624 reduces abdominal pain and bloating in patients with IBS (O'Mahony et al., 2005). In a revision of studies, Moayyedi et al. (2010) concluded that probiotics have a potential in IBS treatment but further studies are needed with specific strains and dosages.

2.2.6 Colorectal Cancer

In developed countries colorectal cancer is one of the most prevalent both in man and women. 70% of the cases are associated with environmental factors, mainly nutrition, being the production of carcinogenic substances by the some bacteria of the gut microbiota one of the plausible causes of this pathology (Elmer, 2001).

Although the effect of probiotics on colorectal cancer it is not known, some strains have activities that could be of interest, as immunomodulation, inhibition of apoptosis, absorption of carcinogenic compounds, inhibition of the production of carcinogenic metabolites, and so forth (Hirayama and Rafter, 2000; Brandy et al., 2000; Goldin and Gorbach, 1984). Unfortunately, currently there are no conclusive studies about the role of probiotics, in spite of the fact that some reports related the consumption of fermented products with a reduced incidence (Malhotra, 1977).

2.2.7 Urinary Tract Disorders: Vaginitis

Vaginal mucosa is rich in nutrients such as glucose or amino acids, which allows for the colonization by bacteria that forms the vaginal microbiota. Lactobacilli are the predominant microorganisms with the main following strains: *Lactobacillus iners*, *Lactobacillus jensenii*, *Lactobacillus crispatus, L. gasseri, Lactobacillus vaginalis*, and *L. salivarius*. In addition, occasionally other species coming from the digestive tract are also found, such as *L. rhamnosus*, *L. casei,* and *Lactobacillus plantarum* (Martin et al., 2008; Zhou et al., 2010).

The presence of lactobacilli in the vaginal mucosa limits its colonization by potentially pathogen bacteria, since lactobacilli compete with those bacteria for nutrients, blocking their adhesion, and they also produce antimicrobial compounds and acids that decrease the pH, inhibiting the growth of pathogens (Reid, 2012).

All these protective mechanisms have made that lactobacilli are proposed for the maintenance of the vaginal mucosa integrity and prevention of disorders related to alteration of vaginal microbiota, such as vaginitis and vaginosis.

It has been suggested that the use of probiotics after antibiotic treatment allows a recolonization of the vaginal mucosa and causes a pH decrease, limiting the recovery of pathogens. For that reason probiotics could be useful in the prevention of recurrent urogenital infections.

In addition, lactobacilli are normally safe for its vaginal use, since there are few reports of infections by lactobacilli. However, more studies are needed to elucidate which strain could be more effective and the protocol of use (duration, dosage, etc.) (Beltrán and Guerra, 2012).

2.2.8 Immune System and Allergies

Almost 70% of the human immune system is located in the gut, where there is also the highest number and heterogeneity of bacteria. This is not by chance, since the interaction between bacteria and immune cells is key for the maturation of the immune system and the modulation of the immune response. It is well know that an imbalance of the intestinal microbiota is the basis of some immune disorders, such as allergies or autoimmune diseases (Takahashi, 2010; Shida and Nanno, 2008).

In fact, according to the hygiene hypothesis the current increase in autoimmune diseases is due to the low exposure to microorganisms, and probiotics could help to restore the balance between different immune response (Th1/Th2) (Borchers et al., 2009).

Therefore, the manipulation of microbiota by the use of probiotics could help in the prevention of allergies and autoimmune diseases, by specifically interacting with the immune cells, thus modulating the immune response and limiting those exacerbated reactions.

2.2.9 Hypercholesterolemia

Cholesterol is a molecule with a key role in the human body, but when its blood levels are above normal values, it can be a risk factor for cardiovascular disease (Fogli-Cawley et al., 2007) and metabolic syndrome, the disorder characterized by hyperglycemia, hypertension, and abdominal obesity (Isomaa et al., 2001).

Probiotics have also been studied for their effects on cholesterol levels both in animals and humans. It has been reported that *L. acidophilus* and *L. plantarum* can help to reduce total cholesterol and triglycerides levels in blood (Nguyen et al., 2007; Fukushima et al., 1999). Other probiotics, such as *B. longum* and *L. acidophilus* have also been reported to decrease total cholesterol levels

and in the case of *B. longum* even increase HDL (high-density lipoprotein)-cholesterol, beneficial to obtain a minor hypercholesterolemia (Anderson and Gilliland, 1999; Xiao et al., 2003).

In a recent metaanalysis the role of probiotics on cholesterolemia has been studied, concluding that a diet rich in probiotics decreases total cholesterol and LDL cholesterol concentration in plasma for participants with high, borderline high, and normal cholesterol levels (Guo et al., 2011). Different mechanisms of this probiotic effect have been proposed (Ooi and Liong, 2010):

- Probiotics produce short-chain fatty acids (SCFA) and among them propionate and acetate, which inhibit cholesterol synthesis and induce the synthesis of polyunsaturated fatty acids (Floch, 2010; De Preter et al., 2011).
- Bile salts.
- Induction of transformation of cholesterol in coprostanol, which is eliminated by feces (Lye et al., 2010).
- Absorption of cholesterol in their surface, as it has been reported for *L. gasseri* (Usman, 1999; Kimoto et al., 2002).

However, further studies are needed to elucidate those mechanisms, since most of them have been reported just in vitro (Ooi and Liong, 2010).

2.3 Probiotics as Nutraceuticals

While the original purpose of food fortification was to correct deficiencies of essential vitamins and minerals in food, there is a growing interest in supplementing foods with other bioactive ingredients such as antioxidants, phytochemicals, omega-3 fatty acids, and probiotic bacteria for added health benefits (Tallon et al., 2007). When used to prevent or cure diseases through food, these compounds are called nutraceuticals. Nutraceuticals are biologically active compounds present in some traditional or unusual food materials, and can be isolated, purified, and encapsulated to form ready-to-use food ingredients or pharmaceutical devices.

Nutraceuticals are categorized based on their chemical structures, such as vitamins, minerals, PUFAs, phytochemicals, probiotics/prebiotics, essential amino acids, specialty peptides and proteins, fibers, carotenoids, and polyols.

Currently, there are also numerous studies and reviews in the literature on the food engineering and processing perspective of delivery technologies/systems for various nutraceuticals. However, it seems hard to form a consensus on the types of delivery technologies or formulating systems that would be desirable for each individual nutraceutical. Nonetheless, due to the chemical

or biological complexity of many nutraceutical compounds in comparison with commonly used food processing agents, it is generally agreed in this field that the use of microencapsulation to protect, separate, modify, and/or mask these ingredients before incorporating them into formulated food products is an almost necessary practice (Poncelet et al., 1995).

To produce these beneficial effects in health, the probiotic should be metabolically stable and active in the product, surviving passage through the stomach to reach the intestine in higher amounts (Sanz, 2007). However, the main problem associated with probiotics is their low resistance to factors such as pH, postacidification (during storage) in fermented products, hydrogen peroxide production, oxygen toxicity (oxygen permeation through packaging), and storage temperatures (Kailasapathy, 2002). At the moment, probiotics in general are added to food matrices and they are also present in the supplement market in form of capsules, tablet, oral rehydration solutions, infant formulas, and urogenital formulas (Table 16.3). Indeed, several studies have revealed that the number of viable bacteria in some commercial products is below the desired level (Vinderola et al., 2000; Kailasapathy and Chin, 2000). In addition to production conditions, numerous

Table 16.3 Probiotics Pharmaceutical Forms in the Market

	Medicaments	
Name	**Dosage Form**	**CFU/Dosage Form**
Casenfilus	Oral rehydration solutions	*L. acidophilus*
Lactelol	Oral rehydration solutions	*L. acidophilus* *L. fermentum* *L. delbrueckii*
Lactofilus	Oral rehydration solutions	*L. acidophilus*
Salvecolon	Oral rehydration solutions	*Bacillus subtilis*
Infloran	Capsules	*L. acidophilus* *L. biphidus*
Lacteol	Capsules	*L. acidophilus* *L. fermentum* *L. delbrueckii*
Ultralevura	Capsules	*Saccharomices boulardii*

additional obstacles are encountered during gastrointestinal transit (pH, enzymes, bile salts, etc.), limiting the survival and functionality of probiotics and their health benefits after intake.

On the other hand, microencapsulation of cells has several benefits: protection from bacteriophages, freezing, and storage, and converting them into a powder form easier to application (Mortazavian et al., 2007). It also allows adequate release of encapsulated microorganism in the gut (Sultana et al., 2000). For this reason, in the following item we will review the different microencapsulation technologies with special focus on the ones used for probiotics.

3 Microencapsulation

Microencapsulation is not a new idea; it is a technology that has been in use for over 50 years in the pharmaceutical, biological, nutritional, and food science fields (Fig. 16.1). Briefly, it is a process whereby the functional ingredients are protected from their environment by entrapping them within a protective coating material and forming particles with diameters of a few nanometers to a few millimeters. The substance that is encapsulated may be called the active agent or core ingredient, while the substances that provide the protection are called the coating or shell materials. The latest advances in drug delivery have their roots in basic microencapsulation, while the technology has opened up a broad range of applications in other fields, for example, nutraceuticals or food products.

The microencapsulation systems can have various aims; a key objective is to protect the encapsulated substance from certain environmental conditions and thus avoid degradation (Gibbs et al., 1999; Schrooyen et al., 2001). This is mainly achieved through physical control exercised by the shell material (Champagne and

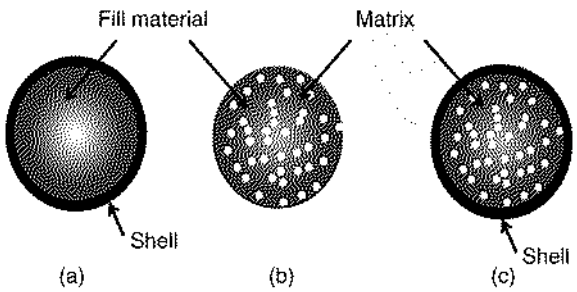

Figure 16.1. Schematic representation of encapsulation systems. (a) Reservoir type, (b) matrix type, and (c) coated matrix type.

Fustier, 2007; Desai and Park, 2005; Kailasapathy, 2002; López-Rubio et al., 2012). On the other hand, the coating material of encapsulates used in food products should be food grade and able to form a barrier protecting the active agent from adverse effects of moisture, heat, light, oxygen, and other reactive components present in the food matrix (Zuidam and Shimoni, 2010). In contrast to drugs, food products have to meet the additional criterion that the addition of the encapsulated bioactive ingredients should not adversely affect or alter the sensory properties of the food vehicle (Champagne and Fustier, 2007).

3.1 Techniques for Microencapsulation of Probiotics

The aim of probiotic microencapsulation is to protect cells from environmental and technological conditions, before addition to food matrices. In addition these microparticles must meet release requirements, given that its final application is colonization of the gastrointestinal tract to achieve the purposed probiotic effect (Naidu et al., 1999; Heidebach et al., 2012). Moreover, it is important that the size of the obtained microparticles is sufficiently small to avoid a negative sensorial impact on the food, they have been added to. Probably for these reasons, there are a few successful cases of probiotic microencapsulation, both in food and pharmaceutical applications, even though many approaches have been made.

3.1.1 Spray Drying

Spray drying is a method of producing a dry powder from a liquid or slurry by rapidly drying with a hot gas (Fig. 16.2). This method is utilized for foods and pharmaceuticals materials that are thermally sensitive. On this occasion, a solution containing the dissolved polymer matrix and the probiotic living cells is drying. The materials matrices used are generally gum arabic and starches because they tend to form spherical microparticles during the drying process (Chen and Chen, 2007; Kailasapathy, 2006; De Voss et al., 2010). The spray drying method is easily reproducible and has advantages such as the rapidity and the relatively low cost of the procedure.

The main problem for application of spray drying is the high temperature applied, which is not compatible with the survival of bacteria. In order to improve probiotic survival, protectors such as granular starch or trehalose are added to the media (Picot and Lacroix, 2003; Champagne et al., 2010) because they could improve culture viability during drying and storage (Semyonov et al., 2010).

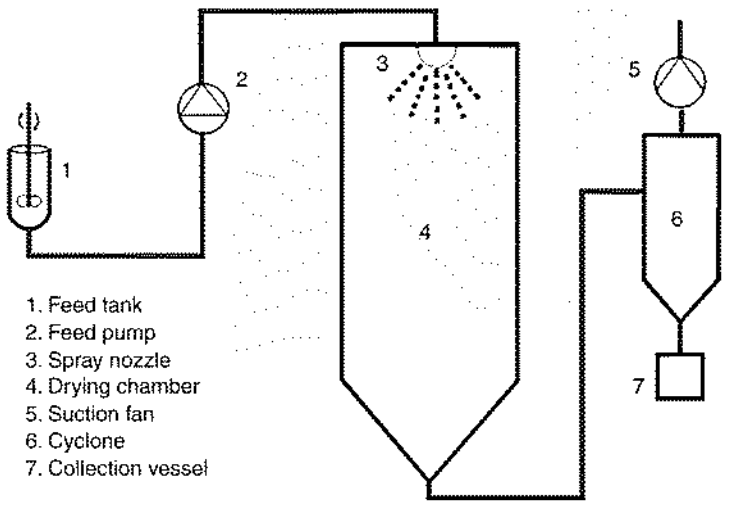

Figure 16.2. Microencapsulation by spray drying technology (Martin et al., 2013).

At present, there are several modifications of this method, such as spray freeze drying, spray chilling, ultrasonic vacuum spray dryer, and so forth.

3.1.1.1 Spray Freeze Drying

Spray freeze drying combines processing steps that are common to freeze drying and to spray drying. The probiotic cells in solution are atomized into a cryogenic liquid, as is the liquid nitrogen. This step generates a dispersion of frozen droplets that are then dried in a freeze dryer (Wang et al., 2004; Kailasapathy, 2006; De Voss et al., 2010; Semyonov et al., 2010).

Spray freeze drying has various advantages: it provides controlled size and results in a greater surface area than spray-dried capsules. The disadvantages of this technique are the use of high energy, and the long processing time, and the cost, this is 30–50 times more expensive than spray drying (Zuidam and Shimoni, 2010). They can protect against adverse environmental conditions with an additional cover (Semyonov et al., 2010).

3.1.1.2 Spray Chilling

Spray chilling is also called spray cooling and spray congealing. This process is similar to spray drying with respect to the production of small droplets. However, spray chilling is based on the injection of cold air, which enables the solidification of the particle. A molten matrix that contains the bioactive compound

is atomized so that it forms drops that quickly solidify when they contact with the cold air (Champagne and Fustier, 2007).

The spray chilling mainly uses fat matrices as carrier. The microparticles that are produced can present some disadvantages, which include a low encapsulation capacity and the expulsion of core material during storage, as a result of the crystalline structure and polymorphic arrangement characteristic of many lipid materials during the solidification and crystallization process. However, spray chilling is considered to be the cheapest encapsulation technology that has the possibility of industrial scale manufacture (Gouin, 2004) Moreover, this technology could be used to generate smaller beads, which may be desirable in food processing. Pedroso et al. (2012) used the spray chilling technology to microencapsulate *B. lactis* and *L. acidophilus,* using as wall materials inter esterifies fat with palm and palm kernel. The solid lipid microparticles developed were efficient in protecting the probiotics against the passage through gastric and intestinal fluids, and they could also be stored at low temperatures. In addition, the morphologies and sizes of the microparticles may facilitate the flow of material, while causing no harmful effects toward the food texture.

3.1.1.3 Ultrasonic Vacuum Spray Dryer

A technique based on spray drying which minimizes the thermal and oxidative stresses during the drying process has been developed. This system uses an ultrasonic nozzle, low temperatures, and vacuum atmosphere in the drier chamber. Semyonov et al. (2011) selected as wall material a mix of maltodextrin and trehalose because, as indicated before, these components can increase the survival by maintaining the membrane integrity of probiotic cells during the drying and storage, as well as promoting the stabilizing effect of the bacteria's proteins. The results showed that the combination of a protein and a carbohydrate contributed to retain a high viability after spray drying and to extend survival rates during storage.

3.1.1.4 Encapsulation by Coating and Agglomeration

Spray coating is the single most commonly used method to encapsulate probiotics intended for incorporation in nutraceutical products. Spray coating is particularly adapted to give multilayer coatings and has the advantage of being easy to scale up. However, it is important to highlight that spray coating is a technology with more problems for implementation (Champagne and Fustier, 2007; De Voss et al., 2010).

3.1.2 Ionic Gelification

3.1.2.1 Ionic Gelification and Emulsification

Emulsification is a chemical technique to encapsulate probiotic living cells and use alginate, carrageenan, and pectin, and so forth (hydrocolloids) as encapsulating materials (Fig. 16.3). The aim factor in this technique is the relationship between the discontinuous and the continuous phases. Moreover, an emulsifier and a surfactant are needed, because the encapsulation occurs in an emulsion. A calcium chloride (solidifying agent) is then added to the emulsion (Chen and Chen, 2007; Kailasapathy, 2009; De Voss et al., 2010). This technique is easy to scale-up and gives a high survival rate of the bacteria (Chen and Chen, 2007). They are obtained capsules have a small diameter but the main disadvantage is that it provides large size range and shape. However, the targeted microcapsules size can be charged by variation of the water/oil ratio and agitation speed (Kailasapathy, 2009). The gel beads can be placed in another polymer solution to create a coating layer that provides added protection to the cell or many other occasions improved organoleptic properties (Kailasapathy, 2009).

The results obtained for Martin et al. (2013) show that *L. fermentum* CECT5716 resists the process of microencapsulation in alginate beads with starch (Fig. 16.4), and the resultant microencapsulated probiotic preparation is stable at 4°C. Therefore, emulsification/ionic internal gelation technology with alginate and starch seems to be a suitable procedure for protecting *L. fermentum* and could allow the addition of this probiotic to food and pharmaceutical preparations. The emulsification/internal gelation technique to form alginate microparticles has been described previously by Poncelet et al. (1995). The procedure was modified as follows to encapsulate *L. fermentum*. A sodium alginate mixture was prepared by mixing with lyophilized culture and $CaCO_3$ and after homogenization, was added to 2 parts of soya oil (v/v) (continuous phase) containing 2.5% (w/v) Span 80. The mixture was stirred at 700 rpm for 10 min with a mechanical stirrer to form a uniform water-in-oil-emulsion. With continuous stirring, glacial acetic were added to the emulsion to initiate internal gelation. The supernatant was discarded and the microparticles were centrifuged, collected, and washed using sterile peptone water by vacuum filtration and stored at 4°C.

However, one problem with classical encapsulation technologies is the use of coatings which are not allowed in dairy products in some countries (Picot and Lacroix, 2004); the solution can be the use of milk proteins in which probiotics will be encapsulated

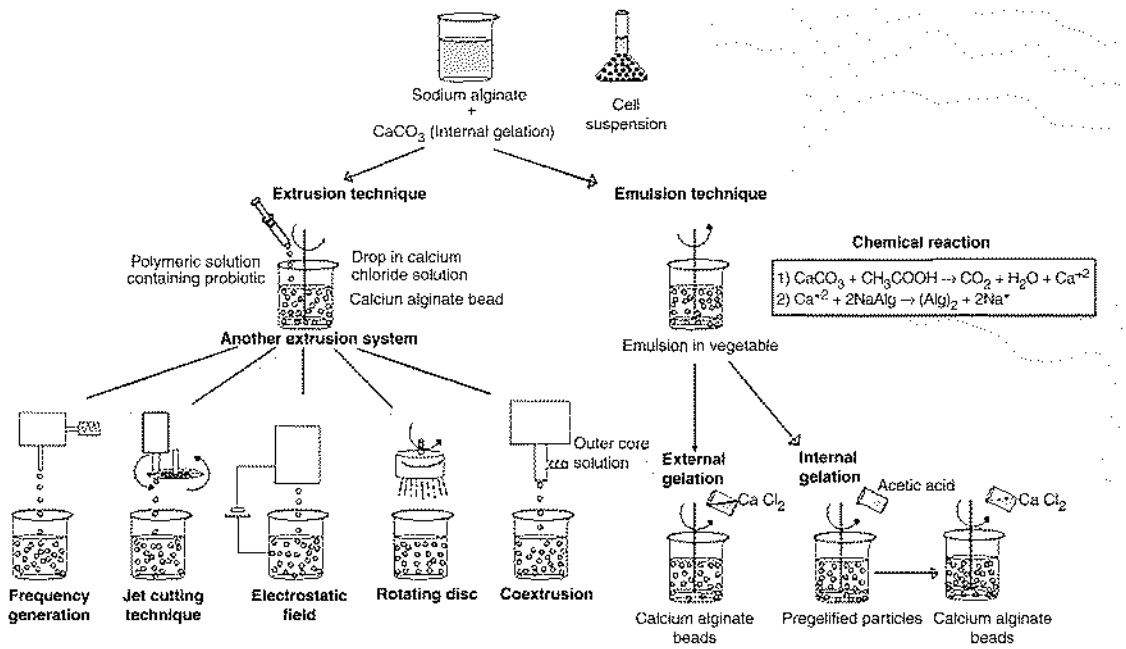

Figure 16.3. Microencapsulation by means of the emulsifying technique (Martín et al., 2013).

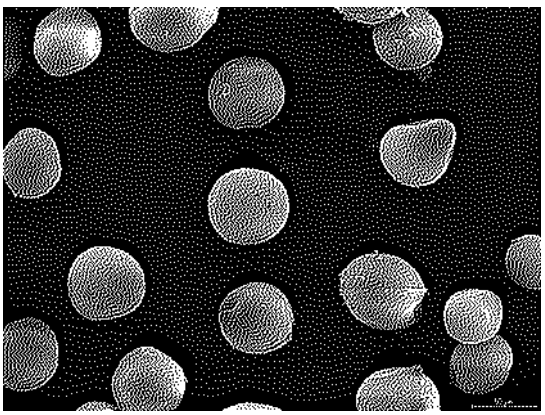

Figure 16.4. SEM images of alginate with *L. fermentum.*

by means of an enzymatic-induced gelation, being that the milk proteins are natural vehicles for probiotics, and they have excellent gelation properties (Livney, 2010). Heidebach et al. (2009) detailed an example of encapsulation by means of rennet gelation; this method gives water insoluble and spherical particles.

3.1.2.2 Ionic Gelification and Extrusion Method

In the technique of extrusion are employed hydrocolloids, as alginate and carrageenan to encapsulate probiotic living cells. The microencapsulation by extrusion of probiotic cells consists in projecting the solution containing the cells through a nozzle at high pressure. The use of coaxial flow or an electrostatic field is the other common technique to form droplets (Kailasapathy, 2002). The extrusion is an operation, which causes no damage to probiotic cells and gives high probiotic viability (Krasaekoopt et al., 2003; Chen and Chen, 2007; Kailasapathy, 2009; De Voss et al., 2010), also with this method does not involve deleterious solvents and can be done under anaerobic and aerobic conditions. The disadvantage of this method is scaling, since productivity is relatively low.

3.1.3 Interfacial Polymerization

Interfacial polymerization is an alternative technique which is performed in a single step. Interfacial polymerization is used to encapsulate microorganisms in order to improve their productivity in fermentation. It is performed by the formation of an emulsion. The continuous phase is an organic solvent and the discontinuous phase it is an aqueous suspension of the probiotic cells. During the polymerization process is added a biocompatible agent soluble in the continuous phase. The droplets obtained containing probiotic cells are enveloped in a thin and strong membrane (Kailasapathy, 2002).

3.1.4 Impinging Aerosol Technology

Impinging aerosol technology uses two separate aerosols, one with the microbial suspension in alginate solution and the other one with calcium chloride. The mixture of alginate is injected form the top of a cylinder meanwhile the calcium chloride is injected from the base. This technology produces alginate microparticles with an average diameter almost always lower to 40 μm (Sohail et al., 2011), addition is suitable for encapsulating heat labile and solvent sensitive materials, since they not used. Moreover, it has a large volume production capacity and microparticles could be spray or freeze dried. Sohail et al. (2011) demonstrated that microparticles obtained by impinging aerosol technology and extruded macroparticles (approximately 2 mm diameter) offered similar protection to *L. rhamnosus* GG in the acid and bile tolerance study. Moreover, Sohail et al. (2012) investigated the effect of microencapsulation on the survival of *L. rhamnosus* GG and *L. acidophilus* NCFM and their acidification in orange juice. They study the effect of temperature on two storage conditions (at 25°C for 9 days and at 4°C over 35 days). Encapsulation of these two bacteria did not

significantly enhance survivability but did reduce acidification at 25°C and 4°C. Unencapsulated *L. rhamnosus* GG was found to have excellent survivability in orange juice at both temperatures. However, unencapsulated *L. acidophilus* NCFM showed significant reduction in viability. In conclusion, the microencapsulation has potential in reducing acidification and possible negative sensory effects of probiotics in orange juice, specifically, but also in other fruit-based products; on the other hand *L. rhamnosus* GG showed excellent survival in orange juice.

3.1.5 Electrospinning

The combined use of two techniques, namely electrospray and spinning, results in a highly versatile technique called electrospinning (electro + spinning). In this technique, a high electric field is applied to a fluid which may be a melt or solution coming out from the tip of a die, which acts as one of the electrodes. This leads to the droplet deformation and finally to the ejection of a charged jet from the tip toward the counter electrode, leading to the formation of continuous fibers.

The advantages of electrospinning technique are the production of very thin fibers or capsules to the order of few nanometers with large surface areas. Moreover, the possibility of large-scale productions combined with the simplicity of the process makes this technique very attractive for many different applications (Agarwal et al., 2008). In that regard, probiotic encapsulation has been carried through electrospinning using a protein-based matrix (whey protein concentrate) and a carbohydrate-based matrix (pullulan). Whey protein concentrate microcapsules have proved a greater improvement in cell viability when compared to pullulan structures (López-Rubio et al., 2012).

3.2 Supporting Materials

3.2.1 Alginate

Alginic acid, also called algin or alginate, is an anionic polysaccharide widely in the cell walls of brown algae, where through binding with water it forms a viscous gum. Sodium alginate has a wide use across a wide variety of industries including food and pharmaceuticals. Alginate is both food- and skin-safe. As a flavorless gum, it is used by the foods industry to increase viscosity and as an emulsifier. It is also used in indigestion tablets. Alginate hydrogels are extensively used in cell encapsulation (Rowley et al., 1999) and calcium alginate is preferred for encapsulating probiotics because of its simplicity, biocompatibility, nontoxicity, and low cost (Krasaekoopt et al., 2003). However, some disadvantages are

attributed to the use of alginate is their sensitive to the acidic environment (Mortazavian et al., 2007) which is not compatible for the resistance of the microparticles in the stomach conditions. Other disadvantages are that the microparticles obtained are very porous, which is a drawback when the aim is to protect the cells from its environment (Gouin, 2004), and scaling-up of the process is very difficult. Nevertheless, this problem can be solved by mixing alginates with other polymer compounds, using different additives, coating the capsules by another compound or applying structural modification of the alginate (Krasaekoopt et al., 2003). For example, the mixing starch with alginate is used and it has shown suitable results in an improvement of probiotic encapsulation effectiveness (Sultana et al., 2000; Sun and Griffiths, 2000; Truelstrup-Hansen et al., 2002; Krasaekoopt et al., 2003).

3.2.2 Gellan Gum and Xanthan Gum

Gellan gum is a water-soluble anionic polysaccharide produced by *Pseudomonas elodea*, which is composed of a repeating unit of monomers, tetrasaccharide, that are two residues of D-glucose and one of each residues of D-glucuronic acid and L-rhamnose. Gellan gum is one of the most expensive hydrocolloids (Chen and Chen, 2007).

Xanthan gum is a polysaccharide secreted by the bacterium *Xanthomonas campestris* used as a stabilizer and food-thickening agent. It is composed of pentasaccharide repeat units, comprising mannose, glucose, and glucuronic acid. Also used in frozen foods and beverages, a mixture of xanthan–gellan gum has been used to encapsulate probiotic cells (Sultana et al., 2000; Sun and Griffiths, 2000) and the mixture presents high resistance toward acid conditions.

3.2.3 Carrageenan

Carrageenans or carrageenins are a family of linear sulfated polysaccharides that are extracted from red edible seaweeds. There are three main varieties of carrageenan, which differ in their degree of sulfation. Carrageenans are highly flexible molecules that have a helical structure. They are widely used in the food industry, for their gelling, stabilizing, and thickening properties. This gives them the ability to form a variety of different gels at room temperature. They are widely used in the food and other industries as thickening and stabilizing agents. All carrageenans are high-molecular-weight polysaccharides made up of repeating galactose units, sulfated and nonsulfated. The units are joined by alternating α-1,3 and β-1,4 glycosidic linkages. The technology

using the compound requires a temperature between 40°C and 50°C, at which the cells are added to the polymer solution. On produce gelation occurs by cooling the mixture to room temperature; by adding potassium ions, the microparticles are stabilized (Krasaekoopt et al., 2003). The encapsulation of probiotic cells in carrageenan beads keeps the bacteria in a viable state (Dinakar and Mistry, 1994) but the produced gels are not able to withstand stresses (Chen and Chen, 2007).

3.2.4 Cellulose Acetate Phthalate

Cellulose acetate phthalate (CAP) is a polymer phthalate commonly used in the formulation of pharmaceuticals, such as the enteric coating of different pharmaceuticals forms (Mortazavian et al., 2007), the advantage of this component is that it is not soluble at acidic pH (less than 5) but it is soluble at pH higher than 6. It is a cellulose polymer where about half of the hydroxyls are esterified with acetyls. The encapsulation of probiotic bacteria using cellulose acetate phthalate provides good protection for microorganisms in simulated GI conditions (Fávaro-Tindale and Grosso, 2002), and it is widely used in release controlled of pharmaceutical formulations.

3.2.5 Chitosan

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit) can polymerase by means of a cross-link formation in the presence of anions and polyanions. It is made by treating shrimp and other crustacean shells with the alkali sodium hydroxide. Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans. Chitosan enhances the transport of polar drugs across epithelial surfaces, and is biocompatible and biodegradable. In acidic environments, protonation of the amino groups leads to an increase in solubility because chitosan is relatively insoluble in water, but can be dissolved by dilute acids. The implications of this are very important to biomedical applications which would make it a highly viscous dietary fiber; it may be useful as an antibacterial agent, and it can also be used to help deliver drugs through the skin. This component has not shown a good efficiency for increasing cell viability by encapsulation and it is preferably used as a coat but not as a capsule (Mortazavian et al., 2007). In fact, encapsulation of probiotic bacteria with chitosan and alginate coating provides protection in simulated GI conditions and it is a good way of delivery of viable

bacterial cells to the colon (Chávarri et al., 2010). However, chitosan has some disadvantages, increase the excretion of sterols and produce a reduction the digestibility of ileal fats; and it seems to have inhibitory effects on LAB (Groboillot et al., 1993).

3.2.6 Starch

Starch or amylum is a polysaccharide consisting of a large number of glucose units joined by glycosidic bonds. Starch consists mainly of amylose, a linear polymer of D-glucopyranose joined by a-1-4 glycosidic bond and amylopectin, a branched polymer of glucose joined by a-1-4 glycosidic bond and a-1-6 glycosidic bond for ramification (Sajilata et al., 2006). It consists of two types of molecules: the linear and helical amylose and the branched amylopectin. Pure starch is a white, tasteless, and odorless powder that is insoluble in cold water or alcohol. It is the most common carbohydrate in human diets and is contained in large amounts in such staple foods as potatoes, rice, and so forth. Raw starch will digest poorly in the duodenum and small intestine, while bacterial degradation will take place mainly in the colon, digestive enzymes have problems digesting crystalline structures. As an additive for food processing, food starches are typically used as thickeners and stabilizers in foods. In the pharmaceutical industry, starch is also used as an excipient, as tablet disintegrater or as binder.

Resistant starch can reach the colon where it will be fermented (Sajilata et al., 2006; Anal and Singh, 2007). This specificity means a better release of the bacterial cells in the large intestine; moreover, resistant starch can be used by probiotic bacteria in the large intestine by its prebiotic functionality (Mortazavian et al., 2007). Finally, resistant starch is an ideal surface for the adherence of the probiotic cells to the starch granules (Anal and Singh, 2007) and this can enhance probiotic delivery in a viable and a metabolically active state to the intestine (Crittenden et al., 2002).

3.2.7 Gelatin

Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen extracted from the skin, bones, and connective tissues of animals. During hydrolysis, the natural molecular bonds between individual collagen strands are broken down into a form that rearranges more easily. Gelatin readily dissolves in hot water, and sets to a gel on cooling, and is also soluble in most polar solvents. Gelatin solutions show viscoelastic flow, which makes a thermo reversible gel and was used for probiotic encapsulation, alone or in combination with other compounds. Due to its amphoteric nature, it is an excellent candidate for cooperation

with anionic polysaccharides. Moreover, gelatin is used as a carrier and coating (Krasaekoopt et al., 2003; Anal and Singh, 2007).

3.2.8 Milk Proteins

Milk proteins are natural vehicles for probiotics cells and, owing to their structural and physico-chemical properties, they can be used as a delivery system (Livney, 2010). There are 3 or 4 caseins in the milk of most species; the different caseins are distinct molecules but are similar in structure. All other proteins found in milk are grouped together under the name of whey proteins (Shapira et al., 2010). The major whey proteins in cow milk are beta-lactoglobulin and alpha-lactalbumin. For example, the proteins have excellent gelation properties and this specificity has been recently exploited by Heidebach et al. (2009) to encapsulate probiotic cells. The results of these studies are promising and using milk proteins is an interesting method because of their biocompatibility (Livney, 2010).

3.3 Encapsulation Efficiency

Besides bioavailability, another requirement for an effective microencapsulation system for micronutrients or nutraceuticals is acceptable encapsulation efficiency, which is a measure of how well the microparticles separate the core from the environment. It is generally defined as the ratio (in percentage) between the weight of the core ingredient actually encapsulated and its total weight used in the formulation. There are numerous studies in the literature that involve the use of specific methods to quantify the encapsulation efficiency for various delivery systems. Encapsulation yields (EY) of 100% were achieved for the encapsulation of various probiotic cells in large microcapsules (Krasaekoopt et al., 2004; 2006; Kushal et al., 2006; Leverrier et al., 2005; Sun and Griffiths, 2000; Talwalkar and Kailasapathy, 2003; Urbanska et al., 2007). The EY is usually calculated by comparing the probiotic colony forming units (CFU) per gram dry matter of the initial polymer-cell-solution versus the generated microparticles. The EY is therefore a combined parameter that describes the survival of viable cells and the efficacy of entrapment during the encapsulation procedure.

The main reason for an EY below 100% is mainly probiotic cell damage due to detrimental conditions caused by the encapsulation process itself, such as heating, shear stress, or the application of concentrated solutes. Furthermore, a physical loss of cells into the hardening solution during the encapsulation process can appear in significant numbers. It should also be noted that a disintegration process is required to measure the concentration of living cells in the microcapsules. In the case of alginate-based capsules,

dissolution of the capsules can be easily achieved by gently shaking them in a phosphate-buffer solution (Sheu et al., 1993). In contrast, for capsules based on irreversible gelation, mechanical disintegration methods are often required. An incomplete disintegration as well as detrimental influences of the disintegration process can shift the found EY toward lower levels (Annan et al., 2008).

3.4 Morphology and Size of Microparticles

Microencapsulation technologies can allow us to produce nutraceutical delivery systems for food matrices or dosage forms with different particle size ranges. An important goal is that the particles resemble the physical characteristics of the selected food vehicles in terms of shape, size, color, and appearance, which ensures the resulting fortified foods have desired physical, chemical, nutritional, and organoleptic properties, ultimately meeting the requirements of consumer acceptability and product shelf-life stability (Pedroza-Islas et al., 1999).

To date, research on probiotics encapsulation has focused on maintaining the viability by changing both pH and bile salts concentration. Among the most important challenges for cell encapsulation is the large size of microbial cells (typically 1–4 μm) or particles of freeze-dried culture (more than 100 μm), that limits cell loading for small capsules. In addition, when large capsules are produced, their size can negatively affect texture and sensorial profile of food products where they are added. Therefore, size and morphology of microparticles have been identified as one of the key factors for a successful application in foods or pharmaceutical products. The choice of wall materials and their core material loading were critical for obtaining desirable microparticles, that is, those that have smooth surface and are spherical in shape with a narrow size distribution (Figs. 16.5 and 16.6).

For evaluating the morphology and size of microparticles to be used in food fortification, several microscopy techniques (optical and scanning electron microscopy) and laser light scattering-based particle size distribution measurements can be used. However, scanning electron microscopy (SEM) is most widely used as it allows the analysis of the surface of the particle as well as its size and shape (Prasertmanakit et al., 2009) (Figs. 16.7 and 16.8).

4 Food and Pharmaceutical Applications

Food products containing encapsulated probiotic cells have been introduced on the market (probiotic ice cream, products like probiotic chocolate, nutrient bars, etc.), and product allows a

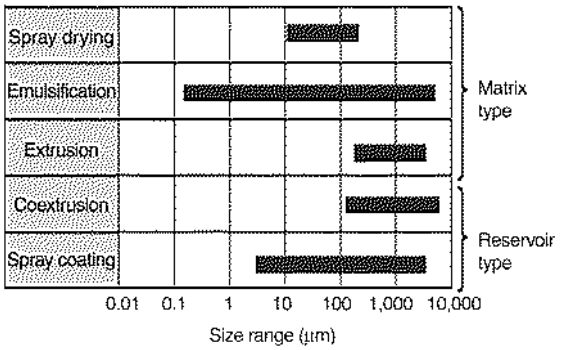

Figure 16.5. Probiotic encapsulation technologies: size range provided by each technique.

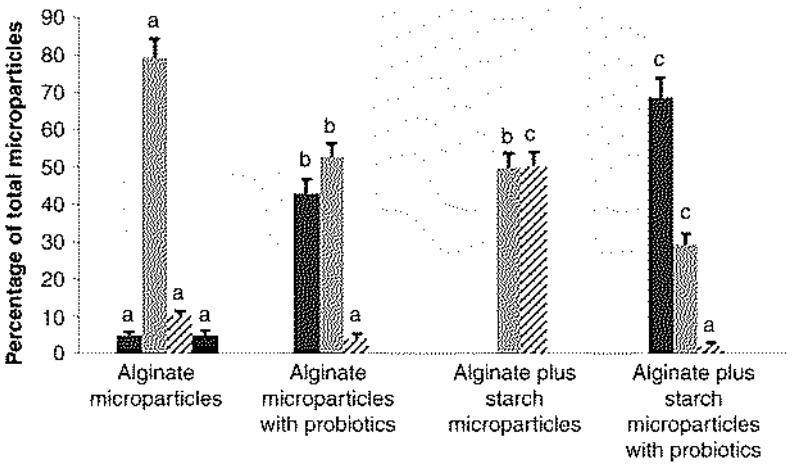

Figure 16.6. Particle size distribution expressed as percentage of total particles (Martin et al., 2013). For evaluating the morphology and size of microparticles to be used in food fortification, several microscopy techniques (optical and scanning electron microscopy) and laser light scattering-based particle size distribution measurements can be used. However, scanning electron microscopy (SEM) is most widely used as it allows the analysis of the surface of the particle as well as its size and shape (Prasertmanakit et al., 2009).

reduction of physical symptoms related to stress (Diop et al., 2008), particularly abdominal pain, nausea, and vomiting. However, the analysis of these products in several different countries has confirmed that probiotic strains exhibit poor survival in food such as fermented dairy products (Shah, 2000). In this respect, probiotic microorganisms present in food should survive in significant

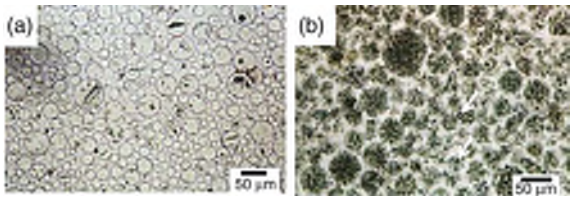

Figure 16.7. Optical microphotographs of calcium alginate blended with starch microparticles. (a) Microparticles without *L. fermentum*. (b) Microparticles with *L. fermentum*. Particles in (b) are more opaque than in (a). *White arrows* in (b) show residual oil drops (Martín et al., 2013).

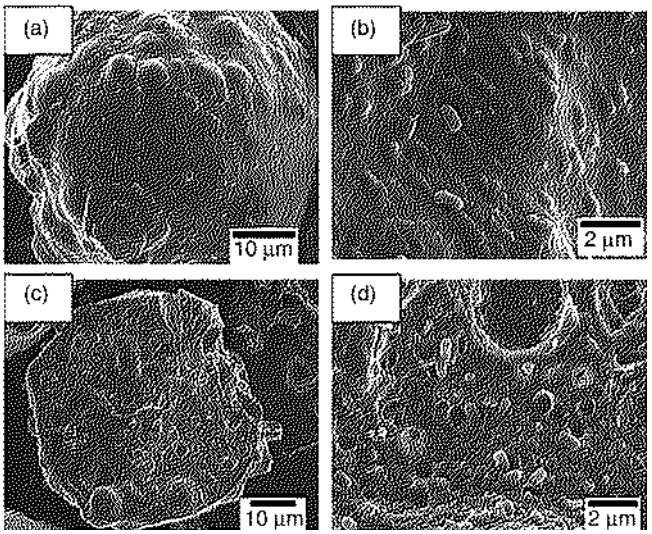

Figure 16.8. SEM images of alginate and starch beads with *L. fermentum*. (a) Surface of a microparticle. (b) Surface of a microparticle at high magnification. (c) Fractured alginate and starch microparticle loaded with *L. fermentum*. (d) Fractured alginate and starch microparticle loaded with *L. fermentum* at high magnification (Martín et al., 2013).

number (10^6–10^8 CFU/g), although the number varies from strain to strain. Growth, survival, and death of these microorganisms in food are largely governed by properties of the aliment (water availability, pH, and buffering capacity) in addition to the storage conditions (temperature, relative humidity and atmosphere). It has to be pointed out that food matrices should help probiotics to survive through the gastrointestinal tract and regulate the colonization of the gastrointestinal tract. Therefore, selection of suitable food systems to deliver probiotics is a vital factor that should be considered in developing functional probiotic foods.

Microencapsulation can also improve the viability of probiotic in some food matrices. In fact during the past few years, food products containing encapsulated probiotic cells have been introduced on the market (Burgain et al., 2011).

For example, Attune's innovative product line is found in the refrigerated yogurt section and advertising of this product highlights that it offers more calcium and fiber and less sugar than most yogurts. Kerry Group in Ireland has developed the first probiotic orange juice. The use of encapsulated probiotic cells can be better suited to survive harsh conditions in juices. Capsules containing cranberry present recognized effects on urinary disorders as cystitis. In this product, encapsulated probiotic cells have been incorporated and the chosen strain has positive effects on urinary microbiota.

On the other hand, as we mention in the second section, "Probiotics," in this chapter, probiotics can produce beneficial effect in some pathologies. However, to get this beneficial effect, they have to reach the gut in adequate amounts. As a result of the harsh condition associated to the gastrointestinal tract, using encapsulated probiotic could be an interesting option. However, only a few in vivo studies have been carried out to test the beneficial effect of encapsulated probiotic in various pathologies.

In this respect, probiotic have been used to regulate the glucose concentration. These microorganisms are known to have health effects in hypocholesterolemia (Bhatia et al., 2012) and immunomodulation (Kumar et al., 2011). In fact, there is a direct correlation between diabetes and immunomodulation. The result obtained by Bhatia et al. (2013) using encapsulated *Lactobacillus* (LB10) isolated from healthy buffalo milk and commercial probiotic from Lee Biotic Capsule (LCap) show that encapsulated probiotics have better efficacy as antidiabetic agents than the same probiotic in unencapsulated form. Microparticles were prepared using the extrusion technology. In the groups treated with unencapsulated bacteria (LB10) and (LCap), the decrease in glucose level observed was 37.85% and 36.50% respectively, whereas the group receiving encapsulated bacteria LB10 and encapsulated commercial showed a decrease of 41.84% and 40.97%, respectively. Moreover, the bacteria reduced the glucose level to normal within 14 days. Glibenclamide reduced the glucose level within 7 days. However, this drug created hypoglycemic conditions. This result suggests that encapsulation improves the survival of bacteria under gastrointestinal conditions and produces a significant reduction of total blood glucose level. Hence, for a sustained beneficial health effect of probiotics, encapsulation of bacteria could be a better alternative to decrease blood glucose levels.

Another factor which may be responsible for health benefits of probiotics is conversion of linoleic acid (LA) to conjugated linoleic acid (CLA). This acid has been shown to have anticholesterolemic action (Schlegel et al., 2012). Results obtained by Bhatia et al. (2012), show that encapsulated and unencapsulated *Lactobacillu*s (isolated from healthy buffalo milk) as well as drug (Atorvastatin) reduced the cholesterol level. Microparticles were developed using the extrusion process. The percentage of decrease in cholesterol level in encapsulated and unencapsulated bacteria is almost parallel to that of percentage decrease in cholesterol level of drug treated mice. The study also indicated that the effect of probiotics is independent from the encapsulation. According to these authors, this result could be because encapsulated bacteria could need a longer period of time to exert the effect since they are released in a slower but maintain way than unencapsulated bacteria.

Furthermore, it is known that the micromilieu of solid tumors provides an ideal environment for growth of facultative and strictly anaerobic bacteria (Cheng et al., 2008). It has been shown that some species such as *Lactobacillus* and *Clostridium* can colonize those environments, leading to regression of tumor growth (Cheng et al., 2008; Matsuzaki, 1998; Tuo et al., 2010; Zabala et al., 2001; Kim et al., 2010). Several studies have observed that live microorganisms might be used to colonize the tumor and exert a tumorolytic effect (concept of bacteriolytic therapy). However, these lytic properties of some bacteria could also be detrimental for nontumor cells; this is why, it would be advantageous to explore a relatively nonpathogenic strain and designate a specific injection site and avoiding the dissemination or minimize dispersion of the microorganism throughout the host. In testing the feasibility of such an approach, Dwivedi et al. (2012) prepared formulations of *L. casei* NCDO 161 microencapsulated by external gelation, and they confirmed that these formulations were toxic for tumor cells in vitro, and also investigated the effects on tumor growth in vivo following direct intratumoral injection. The study demonstrated the potential therapeutic benefit in the treatment of solid tumors.

In addition, Ruan et al. (2007) developed a gelatin microparticle to be tested in a hemorrhagic shock model, using *B. longun*, *B. bifidum*, and *B. adolescentis*. Authors demonstrated that rat pretreated with encapsulated and unencapsulated *Bifidobacteria*, showed decreases of total aerobes in cecum, magnitude of total aerobes to bacterial translocation levels of plasma endotoxin, and percentage of ileal villous damage when compared with rats treated with phosphate buffered saline. Encapsulated *Bifidobacteria* induced greater decreases than intact *Bifidobacteria* in this model, with the exception of a similar effect on ileal villous damage. In

addition, the incidence of bacterial translocation was decreased in hemorrhagic rats pretreated with *Bifidobacteria* compared with control. However, the magnitude of total anaerobes and *Bifidobacteria* were similar among hemorrhagic shocked rats receiving the different supplements.

5 Futures Trends and Conclusions

The microencapsulation technology has been investigated by many companies as a way to improve the resistance of probiotics cells in the acidic medium and especially to increase shelf life in food. In most cases, results are promising on a laboratory scale but the technologies used could be difficult for scaling up. For example, low production capacities and large particle sizes have been reported in the case of extrusion methods. Regarding the emulsion method, large-size dispersions have been obtained. Another important issue is the addition of some polysaccharides that are not allowed in dairy products in some European countries (Picot and Lacroix, 2003). As bacteria are sensitive and can be destroyed by heating, most of the foods containing probiotic microorganisms are found in the refrigerated section of supermarkets. Thus, the dairy sector, very well established in this section, has a major advantage when developing probiotic foods. Nevertheless, research is focused on expanding the food categories currently available.

Much current research focuses on extending these technologies to developing "engineered" foods and food ingredients containing both essential micronutrients and desirable nutraceuticals, consequently leading to novel functional food products with added nutritional value for health promotion and disease prevention. Clearly, to achieve this research goal, an integrated approach combining microencapsulation techniques appropriate to the selected food carriers is essential for meeting the principal fortification criteria: technical and economic feasibility, clinical effectiveness, and consumer acceptance (Iannitti and Palmieri, 2010).

On the other hand, consumer behavior toward novel foods should be taken into account. Microencapsulation can achieve a wide variety of functionalities according to the development of the technology and nowadays, encapsulated probiotic cells can be incorporated in large number of food products, such as cereals, ice cream, chocolate, and so forth. Many studies now that are doing research in probiotic encapsulation are focusing on reducing the particle size because a large size can negatively affect the sensorial properties and the textural of the product. The benefits provided by probiotics are now well documented; consumer requirements for food, beverage, and supplement products enriched with these ingredients will increase.

In conclusion, it is evident that the probiotic market has a strong future, given the great demand from consumers.

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17

NEW TRENDS IN FOOD SCIENCE: THE USE OF NUTRACEUTICALS AS AN ANTIINFLAMMATORY THERAPEUTIC TOOL IN EXERCISE

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1 Introduction

Exercise can have multiple effects on immune response, depending on the type of exercise and the intensity of effort relative to the individual's state of training. While acute or regular moderate exercise training might be beneficial, numerous studies have demonstrated that high-intensity exercise can temporarily weaken the immune system, rendering athletes more susceptible to illness. It is also well known that infrequent, strenuous, or prolonged exercise damages skeletal muscle through various ultra-structural changes and protein degradation and is associated with the development of an acute phase inflammatory response. Maintaining excessive training stress may lead to the development of a condition known as overtraining syndrome, characterized by muscle weakness and fatigue, delayed-onset muscle soreness, and an eventual decrease in performance. This situation may arise in both elite and recreational athletes (Paulsen et al., 2012; Hackney, 2013).

Nutraceuticals (a portmanteau of the words nutrition and pharmaceuticals) are compounds isolated or purified from foods which might be helpful in regulating immune function and improving the health and performance of athletes. Some of them, namely omega-3 polyunsaturated fatty acids (PUFAs), glutamine,

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and branched-chain amino acids (BCAAs) and various phytochemicals (quercetin, astaxanthin) have been shown to have immunomodulatory effects by decreasing the production of inflammatory eicosanoids, cytokines, and reactive oxygen species (ROS) (Aoi et al., 2003; Kargotich et al., 2005; Konrad et al., 2011; Howatson et al., 2012; Lembke et al., 2014). These effects make nutraceuticals attractive to athletes who seek to maintain good health and accelerate recovery during periods of intensive exercise training in order to ensure optimal performance in competition and avoid the consequences of overtraining.

Sports drinks may be an interesting vehicle to easily administer these compounds to athletes. These beverages are widely used as a way to stimulate rapid fluid absorption, to supply carbohydrates as substrate for use during exercise, and to promote overall recovery after exercise. Developing a product that could additionally mitigate muscle damage, soreness, and inflammation is also of great interest to companies, as it would allow them to gain the upper hand in such a competitive market (Maughan and Shirreffs, 2012).

However, the incorporation of these compounds in sports drinks raises some technological problems that nanotechnology could help solve. Some nutraceuticals are hard to dissolve in water due to their liposolubility. Others are only absorbed in limited quantities by the digestive tract or do not disseminate easily throughout the organism and consequently do not reach their sites of action in high enough concentrations to exert their desired effects.

The use of nanostructured delivery systems containing bioactive antiinflammatory ingredients would not only allow for a better dispersion of the bioactive compounds but would also improve their systemic bioavailability, optimizing their effect. This could pave the way for a next generation of sports drinks, which can be formulated for different target groups according to age, gender, occasion use, and physical requirement.

After a brief review of the effects of exercise on immunity and inflammatory mediators, this chapter will then focus on how nutraceuticals such as PUFAs, glutamine, BCAAs, or phytochemicals can influence those effects and, finally, on how nanotechnology constitutes a new trend in food industry that can be used to increase the availability and optimize the action of these substances.

2 Immune Function in Sports and Exercise

2.1 Overview of the Immune System

The immune system is responsible for defending the organism against pathogens. It is composed of a wide variety of elements and

complex biological processes. The immune system functions in an integrated fashion with the other systems of the organism, meaning that several factors such as age, gender, eating habits, medical status, and training and fitness level may affect its daily functioning.

The immune system can be divided into what are referred to as the Innate and Adaptive immune responses. In general, innate responses are the first line of immunological defense and act against pathogens in an indiscriminate fashion, while adaptive responses (which typically follow those of the innate immune system) tend to target specific pathogens and have an antigen-specific memory of such pathogens (Smith, 2004).

The major difference between innate immune responses and adaptive responses is that innate responses do not strengthen upon repeated exposure and therefore do not confer immunity (there is no memory function). In addition, innate responses are less specific in terms of pathogen recognition. So, whereas innate responses recognize classes of pathogens (eg, gram-negative bacteria) through toll-like receptors (TLRs), lymphocytes, which are part of the adaptive immune system, exhibit exquisite specificity for epitopes of individual pathogens (eg, influenza virus).

The innate branch of the immune system includes both soluble factors and cells. Soluble factors include complement proteins, which are activated through a proteolytic cascade and mediate phagocytosis, control inflammation, and interact with antibodies; interferons α/β, which limit viral infection; and antimicrobial peptides like defensins, which limit bacterial growth. Complement factors may also combine to form a membrane attack complex (MAC), which leads to lysis of pathogenic bacteria. Major cells of the innate immune system include neutrophils, which are first-line defenders against bacterial infection; dendritic cells (DCs), which serve to orchestrate immune responses; macrophages (Mφ's), that perform important phagocytic, regulatory, and antigen presentation functions, and natural killer (NK) cells, which recognize altered host cells (eg, virally infected or transformed). However, many body cells can initiate responses to pathogenic infection, even if they are not part of the innate immune system.

Based upon the literature, it appears that both acute and chronic exercise have the potential to alter both the number and function of cells of the innate immune system. Some animal studies have demonstrated the extent to which these changes alter susceptibility to herpes simplex (Murphy et al., 2004) and influenza virus (Lowder et al., 2006; Sim et al., 2009) infection. Unfortunately, not much is known regarding the way both acute and chronic exercise affect the innate immune cells, but also regarding

whether exercise-induced changes in immune function alter susceptibility to or the severity of infectious disease.

The adaptive immune system has two major components: the "humoral" and the "cell-mediated" responses. The humoral response is characterized by the production of antibodies by cells called bone marrow-derived cells, B lymphocytes, or B cells. These antibodies then bind to a specific surface antigen of the pathogen and lead to their elimination via phagocytosis or the activation of other effector cells of the immune system. "Cell-mediated" does not involve the production of antibodies and death of infected cells or cancer cells is induced directly by the cells of the immune system, such as cytotoxic T-lymphocytes.

The local response to infections or tissue injury involves the production of cytokines that are released at the site of inflammation. Cytokines are small polypeptides, which were originally discovered to have immunoregulatory roles (Majno and Joris, 2004). Some of these cytokines facilitate the influx of lymphocytes, neutrophils, monocytes, and other cells. In response to an acute infection or trauma, the cytokines and inhibitors may increase several-fold and decrease when the infection or trauma is healed. The local inflammatory response is accompanied by a systemic response known as the acute-phase response. This response includes the production of a large number of hepatocyte-derived acute phase proteins, such as C-reactive protein (CRP), and can be mimicked by the injection of the cytokines tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, or IL-6 into laboratory animals or humans (Majno and Joris, 2004; Pedersen, 2006; Edwards et al., 2007). The initial cytokines in the cytokine cascade are TNF-α, IL-1, IL-6, IL-1 receptor antagonist (IL-1ra), and soluble TNF-α receptors (sTNF-R). IL-1ra inhibits IL-1 signal transduction and sTNF-R represents the naturally occurring inhibitors of TNF-α (Majno and Joris, 2004; Luster et al., 2005). These acute phase reactants have considerable effects on the metabolism during acute illness, leading to hyperglycemia, insulin resistance, and increased glucogenesis (Falciglia, 2007). Elevation in these markers also increases proteolysis (Hasselgren, 1999), bone resorption (Smith et al., 2002), and dyslipidaemia (Marik, 2006), in addition to up regulating other members of the inflammatory cascade, each with their own downstream biologic effects.

2.2 Antiinflammatory Nature of Regular Moderate Exercise

Physical activity, inflammation, and immunity are tightly linked in a complex way (Febbraio, 2007). Regular and moderate

physical exercise reduces systemic inflammation but a conclusive explanation for this fact has yet to be found. However, several candidate mechanisms have been proposed.

It is known that exercise increases the release of epinephrine, cortisol, growth hormone, prolactin, and other factors that have immunomodulatory effects (Nieman, 2003). Furthermore, exercise results in decreased expression of Toll-like receptors on monocytes, suggesting an involvement in whole body inflammation. Pavlov and Tracey (2005) suggest in their "cholinergic anti-inflammatory pathway hypothesis," that exercise may alter the equilibrium between the sympathetic and parasympathetic nervous systems toward an antiinflammatory state.

The existence of an adipose tissue–innate immune system axis that plays a central role in the effects of physical activity in inflammation and immunity has also been suggested. According to this possibility, exercise is able to reduce body fat with a subsequent decrease in the production of proinflammatory adipokines. Exercise also reduces accumulation of macrophages in the adipose tissue and leads to a phenotype switch from M1 into M2 macrophages, which decrease inflammation (Woods et al., 2009).

These mechanisms, together, might underlie the antiinflammatory effect of exercise and are the basis of the adipocyte-macrophages-innate immune response hypothesis.

Excellent reviews of the evidence from epidemiological studies as well as clinical trials addressing the influence of physical activity and fitness on low-grade inflammation in the general adult population (Autenrieth et al., 2009), in athletes (Tomaszewski et al., 2003), and to a lesser extent in children and adolescents (Wärnberg et al., 2007; Nascimento et al., 2014) have been published. A wide range of inflammatory markers have been measured and assessed against physical activity; these include fibrinogen, cytokines, and leucocytes, although CRP is by far the most commonly used.

Our group found that lean nondiabetic ZDF rats submitted to a swimming protocol (1 h/day and 3 days/week) during 12 weeks did not present statistically significant modifications of cytokine levels IL-6 and TNF-α. However, the serum levels of CRP exhibit a significant decrease when compared to their age-matched sedentary group (Teixeira de Lemos et al., 2009).

The antiinflammatory nature of exercise training in chronic inflammation and its association with the macrophage/innate immune response was revised by Woods et al. (2009) in both cross-sectional and longitudinal studies. Almost all of the longitudinal studies analyzed reported an antiinflammatory effect of exercise training in subjects with chronic diseases, including heart disease

and metabolic syndrome. Furthermore, studies which featured shorter periods of exercise showed less antiinflammatory effect.

2.3 Exercise as an Immunosuppressant

The existence of a proinflammatory effect of exercise has been increasingly discussed. While moderate and regular exercise leads to a reduction of chronic inflammation, prolonged high-intensity training seems to result in increased systemic inflammation and elevated risk of infection. In fact, after this type of exercise, athletes exhibit a transient exercise-induced immunodepression (Gleeson et al., 2004).

The first step toward maladaptation may be overreaching. Functional overreaching is defined as a short-term decrement in performance as a result of increased training stress. It is a usual part of the training process of elite athletes and its recovery to regular performance occurs within a few days (Meeusen et al., 2006).

Overtraining is a persistent decrease in performance that takes several weeks or months to restore and may seriously harm the athlete's health (Halson and Jeukendrup, 2004; Meeusen et al., 2006). While alterations of several performance-related, endocrine, immunological, vegetative, and neuropsychological indicators as well as of physiological exercise responses have been associated with overreaching and overtraining (Urhausen and Kindermann, 2002), there is no evidence that diagnostic tools can discern acute functional from nonfunctional overreaching and overtraining (Halson and Jeukendrup, 2004; Meeusen et al., 2006).

According to the "cytokine tissue trauma hypothesis" of overtraining proposed by Smith (2004), muscle contraction and repetitive joint action cause microtrauma to tissues. If excessive, this tissue trauma results in the production of an abundance of proinflammatory cytokines, namely interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) (Smith, 2004; Walsh et al., 2011). This leads to the development of a sickness response or a chronic fatigue-like behavior in an athlete (Robson-Ansley et al. 2007), which is known as overtraining syndrome (OTS) (Fig. 17.1). Using 30-week-old lean ZDF rats forced to swim until exhaustion and then immediately sacrificed, our group has observed a statistically significant increase in both TNF-α (27,8%) and CRP (5%) when compared to nonexercised rats (Teixeira de Lemos et al., 2011).

It is now accepted that muscles release IL-6 and the plasma concentrations of CRP and of both pro- and antiinflammatory cytokines (TNF-α, IL-1, IL-1ra, IL-10, and sTNF-r) increase several times during acute exercise. The same can be observed regarding the various leukocyte subsets, such as neutrophils, lymphocytes

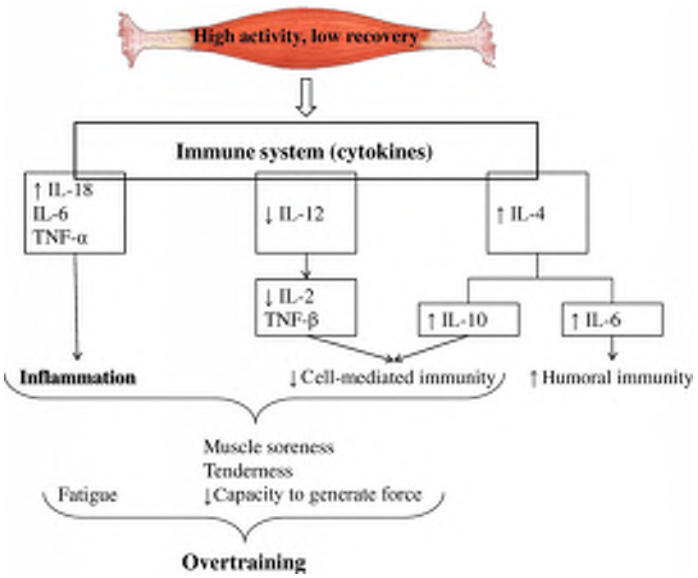

Figure 17.1. Scheme representing the etiology of overtraining by cytokine hypothesis. There is a time-dependant sensitization with amplification at each step to repeated intermittent stimuli over time.

(including their subsets T, B, and NK cells) and monocytes. Following the cessation of intense exercise, neutrophils and monocytes continue to increase into the recovery period, while other leukocytes decrease in number. Plasma concentrations of the above-mentioned cytokines remain elevated for some time.

Research by other investigators has produced findings that substantiate the role of proinflammatory cytokines, especially IL-6, as being key physiological mediators and modulators for development of many of the symptoms associated with OTS (Robson-Ansley et al., 2007; Walsh et al., 2011).

The intensity and duration of exercise are determining factors on the effects of physical activity. While it is commonly accepted that moderate exercise and training are key components of a healthy lifestyle and help to prevent or delay the onset of pathological conditions, strenuous exercise might exert deleterious effects on one's health.

2.4 Interactions of Exercise and Immune System and Decrease of Performance

2.4.1 Upper Respiratory Tract Illness

Athletes appear to experience more frequent episodes of upper respiratory symptoms and there is an established link between

training load and risk of respiratory illness (Walsh et al., 2011). In fact, strenuous long-term training has been associated with a chronic suppression of mucosal immunity lasting seven or more days (Bishop and Gleeson, 2009). Moreover, it has been found that several components of the immune system demonstrate a suppressed function for 3–12 h following a single bout of strenuous, intense, prolonged endurance exercise (Nieman, 2007). This period of increased vulnerability to URTI, known as the "open window" period, will in turn negatively affect training and performance (Costa et al., 2005).

Nieman (1997) and Peake et al. (2015) described the changes in cellular and soluble components of the immune system in the hours after intense physical exertion as well as in their biological regulators such as catecholamines, cortisol, blood flow, body temperature, and dehydration. Shortly after intense exercise, acute neutrophilia and lymphopenia occur, as well as a decrease in natural killer cell activity and T-cell function, a decrease in salivary IgA, and an increase in proinflammatory cytokines and chemokines.

Bacterial or viral agents or a reactivation of the Epstein-Barr virus are usually deemed responsible for recurrent URTI (Reid et al., 2004; Spence et al., 2007). However, it is hard to identify the cause for these symptoms, as inflammation is not always associated with infection. Other causes such as asthma, allergy, and unresolved nonrespiratory infections and autoimmune disease should also be considered (Spence et al., 2007).

As a consequence of the large and prolonged movements of air in endurance athletes, an increased risk of airway dysfunction was found, which might be due to substantial mechanical stress on the airways, exposure to agents capable of inducing airway injury (pollutants, irritants, allergens), and dehydration. Oxidative stress has been identified as a major factor in pollutant-induced bronchospasm but only a few studies have investigated the role of these agents in eliciting respiratory symptoms in athletes (Chimenti et al., 2009).

The relationship between exercise intensity/volume and susceptibility to URTI has been modeled in the form of a "J" curve (Nieman, 2007). This model suggests that moderate exercise may lower the risk for URTI when compared to sedentary individuals—it appears to be beneficial to a certain point (Walsh et al., 2011). However, as the intensity and length of exercise periods increase, so does the risk for URTI. Despite being based on epidemiological data from observing or self-reporting of symptoms of URTI, this model has been widely accepted by athletes, trainers, and scientists (Moreira et al., 2009).

2.4.2 Delayed-Onset Muscle Damage (DOMS)

Mechanical stimuli such as endurance exercise and eccentric muscle contractions can promote muscle fiber damage with functional impairment usually manifesting as pain, swelling, temporary strength decrease, and passive muscle tension increase (Leahy and Pintauro, 2013). Two different types of muscle soreness can be considered: acute soreness, which appears during and immediately after exercise; and delayed-onset muscle soreness (DOMS), felt 24–48 h after activity (Clarkson and Hubal, 2002; Connolly et al., 2003). Both types can have a big impact in athletes, modifying training schedules, bringing continual psychological discomfort and limiting performance.

Previous studies have shown that DOMS is mainly induced by mechanical stress, especially eccentric muscle contraction (Proske and Morgan, 2001) and disturbances of calcium homeostasis (Gissel and Clausen, 2001). The mechanical stress triggers an inflammatory response and the production of reactive oxygen species (ROS) that prolong inflammation and oxidative stress by promoting the activation of transcription factors such as nuclear factor-κB (NF-κB), a proinflammatory "master switch" which controls the production of inflammatory markers and mediators. The infiltration of phagocytes into the tissues expressing these mediators results in proteolysis and ultrastructural damage. Oxidative damage also occurs in other cellular components such as lipids, proteins, and DNA (Aoi et al., 2004). Julius (2013) presented an important and detailed review describing the mechanisms underlying this initial step of pain.

There are also metabolic changes concerning insulin-sensitive glucose transport into muscle, namely a decrease in glucose utilization caused by inhibition of insulin signal transduction in damaged muscles (Aoi et al., 2012). This effect is observed for several hours after exercise and often persists until the next day. This suggests that strenuous muscle-damaging exercise may be inappropriate for health promotion in patients with metabolic diseases. In addition, the transient reduction in glucose metabolism may represent a disadvantage during precompetition conditioning among athletes, as it may lead to a decrease in performance.

As a consequence of the repeated activation of the mechanisms which were previously described throughout this section, several biochemical changes may occur in the affected area and cause secondary muscle damage to arise. As described by Pedersen (2000), these changes include the local accumulation of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1b, IL-6, and IL-1 receptor antagonist (IL-1ra), similar to the sequential release of cytokines observed after trauma. This

also leads to the production of reactive oxygen species (ROS) that may further degrade muscle proteins and increase the local expression of cytokines (Pedersen, 2000; Powers and Jackson, 2008). This vicious cycle of muscle damage leads to persistent signs and symptoms of injury, thus decreasing performance.

3 Nutraceuticals for Maximizing Recovering and Performance in Athletes

Nutritional support of elite athletes has changed substantially in the past decades, based on emerging evidence from nutrition science. The emphasis on high daily carbohydrate and fluid intake, which used to be the norm, is now perceived as reductionist and was abandoned. Although athletes still need energy, macronutrients, and micronutrients, alternative nutrition strategies have been developed by sports nutrition specialists in order to modulate training-induced muscle adaptations in order to increase performance and prevent or treat several disorders. This change in paradigm has resulted from recent advancements in nutritional research, which have led to a better knowledge of the effects of chemical components found in foods (such as nutraceuticals), their bioavailability, and the interactions that may occur between themselves and with the human metabolism (Manach et al., 2009).

As shown earlier in this chapter, exercise may affect immune function. These effects may in part be influenced by the nutritional status of the athlete. The use of antioxidant and antiinflammatory nutraceuticals can, in some cases, attenuate oxidative stress (Goldfarb et al., 2005; Goldfarb et al., 2011), inflammation (Phillips et al., 2003), and muscle soreness (Bryer and Goldfarb, 2006), and improve strength recovery (Connolly et al., 2006; Trombold et al., 2010) after exercise.

The effects of several nutraceuticals such as omega-3 polyunsaturated fatty acids, glutamine, branched-chain amino acids, and phytochemicals in reducing inflammation resulting from intense exercise and accelerating recovery will be described in the following section.

3.1 Omega 3 Fatty Acids

Omega-3 fatty acids (n-3 FA) and omega-6 fatty acids (n-6 FA) are two distinct groups of polyunsaturated fatty acids (PUFAs). They are distinguished by the position of the first double bond in the carbon chain: while n-3 FA present the first double bond in

the third carbon atom counting from the methyl terminal (hence n-3), in n-6 FA the first double bond is six carbons away from the methyl terminal.

In humans, the two PUFA parent compounds alpha-linolenic acid (ALA; 18:3 n-3) and n-6 FA linoleic acid (LA; 18:2 n-6) suffer a series of elongation and desaturation steps in order to be converted into their more bioactive derivatives. The two predominant products of ALA metabolism are the long-chain n-3 docosahexaenoic acid (DHA; 22:6 n-3) and eicosapentaenoic acid (EPA; 20:5 n-3). However, in adults synthesis of these products is limited in occurrence and the levels of DHA and EPA are primarily determined by dietary sources. While EPA and DHA have antiinflammatory effects, arachidonic acid (AA), the downstream product of ALA, is typically proinflammatory (Deckelbaum and Torrejon, 2012; Mozaffarian and Wu, 2012). Food sources of omega-3 FA include fish oil, oily fish (small forage fish, such as sardines, herring and anchovies, and other larger pelagic fish, such as salmon, trout, tuna, and mackerel) and plants (seeds, nuts, and nut oils) (Fig. 17.2).

The ability of dietary omega-3 LC-PUFA to limit inflammation has been demonstrated in numerous studies of animals and humans under different conditions and using varied doses. Its intake is associated with reduced concentrations of CRP, proinflammatory eicosanoids, cytokines, chemokines, and other biomarkers of inflammation (Robinson et al., 2008).

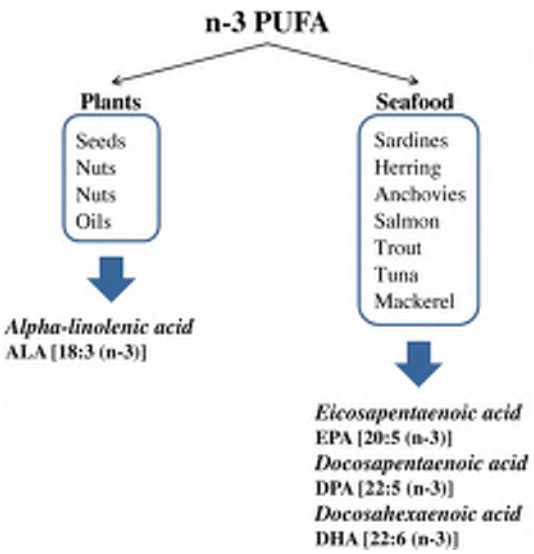

Figure 17.2. Origin and sources of (n-3) PUFA. Major (n-3) PUFA include ALA, EPA, DPA, and docosahexaenoic acid (DHA). ALA is the plant-derived (n-3) PUFA, found in certain seeds, nuts, and their oils.

In a systematic review, Rangel-Huerta et al. (2012) describe the effects of dietary omega-3 fatty acid supplementation on inflammatory biomarkers in healthy subjects and in patients with chronic and acute inflammatory diseases, including chronic renal disease, sepsis, and acute pancreatitis.

Therefore, considering the antiinflammatory properties of omega-3 fatty acids, their use as a dietary supplement might reduce exercise-induced muscle damage and DOMS, ameliorate the negative effects of intensive training in immune function, strengthen bones, and improve heart, lung, and cognitive functioning. Several studies have shown that an increase in omega-3 fatty acid intake offers a variety of performance-enhancing effects in both elite and children athletes (Heikkinen et al., 2011; Sanchez-Benito et al., 2007).

The works of Smith et al. (2011a,b), conducted in both healthy young and middle-aged adults, demonstrate that omega-3 fatty acids seem to have anabolic properties, increasing muscle cell size. This may occur by an insulin-sensitizing effect on protein metabolism (Gingras et al., 2007). Indeed, the improvement of muscle protein metabolism, lean body mass function and quality by omega-3 fatty acids combined with an anabolic stimulus was corroborated by the scientific literature (Macaluso et al., 2013; Di Girolamo et al., 2014; McDonald et al., 2013). This suggests that the ingestion of fish oil associated with strength or resistance training is a simple way of improving the muscle gains.

Omega-3 fatty acids may exert an important influence on muscle function, namely by helping to improve muscular strength, functional capacity, and physical performance as demonstrated by the works of Robinson et al. (2008), Hutchins-Wiese et al. (2013), and Rodacki et al. (2012).

Athletes are frequently prescribed nonsteroidal antiinflammatory drugs (NSAIDs; eg, aspirin and ibuprofen) postexercise to help alleviate the symptoms associated with muscle damage. NSAIDs are known to exert their local inflammation and pain-reducing effects through inhibition of the synthesis of prostaglandins from arachidonic acid by the cyclooxygenase iso-enzymes (COX), which are overexpressed in inflammation (Schoenfeld, 2012). However, inhibition of the initial inflammatory responses by NSAIDs alters the natural healing process of an injury and might therefore have a negative impact on the repair process. Omega-3 supplementation replaces the arachidonic acid molecules of cell membrane, reducing the production of proinflammatory molecules (Calder, 2013). It has been demonstrated that increased consumption of omega-3 fatty acids improves lipid profiles, reduces oxidative stress, and inhibits the production of proinflammatory mediators such

as leukotrienes, thromboxane, prostaglandins, and cytokines (Gopinath et al., 2011; Huang et al., 2011) as well as resulting in a decreased inflammatory response and a reduction in pain (Poudyal et al., 2011). Moreover, the increased concentration of omega-3 fatty acids in the muscle cell wall improves elasticity and flexibility and reduces the risk of muscle cell injury during heavy exercise. The antiinflammatory actions of supplementation with EPA and DHA, observed in several studies, suggest that it may represent an alternative strategy to NSAID use in preventing/alleviating the symptoms associated with exercise-induced muscle damage. Omega-3 fatty acids also positively influence muscle recovery, thus decreasing time between training sessions, improving performance and enhancing training adaptations (Jouris et al., 2011). Omega-3 supplementation may also be useful in the prevention and treatment of other inflammatory conditions (Fetterman and Zdanowicz, 2009).

The studies of Tartibian et al. (2011a,b), in untrained men and in healthy sedentary postmenopausal women, have shown that fish oil may be effective in ameliorating exercise-induced inflammation. In addition, others (Jouris et al., 2011; Tartibian et al., 2009) have demonstrated that daily supplementation with different doses of EPA and DHA reduce exercise-induced muscle soreness and muscle stiffness in healthy men and women.

Although regular exercise is beneficial to the immune system (Karacabey, 2005), intensive training coupled with inadequate diets can lead to the generation of free radicals as demonstrated by Venkatraman and Pendergast (2002) and Powers and Jackson (2008). Supplementation with omega-3 fatty acids may modify the antioxidant status of blood after exercise, increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (Poprzecki et al., 2009). Additionally, supplementation trials revealed that after a single bout of eccentric knee contractions, EPA and DHA supplementation reduced several markers of oxidative stress (Gray et al., 2013). All these results suggest that omega-3 fatty acids may help to improve the body's reaction to exercise-induced stress, with potential beneficial effects in immunity, since reactive oxygen species may suppress the activity of the immune system (Andrade et al., 2007; Santos et al., 2013).

Physical activity is widely known to exert positive effects on bone health (Pigozzi et al., 2009). The use of nutritional supplements such as calcium and magnesium for their benefits in bone health is also widespread. However, omega-3 fatty acid supplementation may exert an important action in bone health (through their antiinflammatory mechanisms) as well as by reducing the risk of hip fractures and by promoting higher bone mineral density

(Kelly et al., 2013; Järvinen et al., 2012; Orchard et al., 2013). Several supplementation trials confirmed that daily low doses of EPA and DHA may represent an important reinforce-bone formula. This action may be achieved through the synergy resulting from antiinflammatory activity and exercise (Tartibian et al., 2010b).

Studies involving healthy subjects (Walser et al., 2006; Buckley et al., 2009) have shown that supplementation with omega-3 fatty improved blood flow. Omega-3 fatty acid supplementation has also been proven effective at improving cardiovascular parameters in athletes, lowering heart rate, whole-body oxygen consumption, and the heart's oxygen consumption (Peoples et al., 2008; Buckley et al., 2009). Omega-3 impacts cardiovascular function (Rontoyanni et al., 2012) and can increase the positive effects of exercise in the heart's ability to pump blood and consequently deliver oxygen to working muscles.

Exercise strengthens the respiratory muscles and increases lung capacity, thus increasing the amount of oxygen that the lungs can handle. Preliminary research has shown that fish oil might also improve lung function during exercise (Tartibian et al., 2010a). Fish oil supplementation may reduce the use of asthma medication in athletes who suffer from coughing, wheezing, and dyspnea by improving pulmonary functioning (Mickleborough et al., 2003, 2006). These effects occur due to the reduction of various inflammatory mediators. While this research is still very incipient, it gives an indication that omega-3 supplementation may ameliorate lung functioning in athletes with and without exercise-induced airway constriction.

Omega-3 fatty acid supplementation has been shown to be associated with improvements in cognitive abilities such as attention, reaction times, and vigor in healthy subjects (Fontani et al., 2005, 2009). This is of great importance in sports that depend on such skills. The ability of EPA and DHA supplementation for increasing neuronal function by supporting synaptic membrane fluidity may serve to enhance decision making and reaction times in athletes (Gomez-Pinilla, 2011). Furthermore, exercise and omega-3 fatty acids may work synergistically to boost brain function, synaptic plasticity, and cognition, as reviewed by Gomez-Pinilla (2011). Based on the available research findings, omega-3 supplementation may constitute a strategy to enhance concentration and cognitive functioning, thus improving athletic performance.

3.2 Glutamine

Glutamine is an amino acid which plays an important role in many biological processes. It is synthesized from glutamic acid

(another amino acid) and ammonia by the enzyme glutamine synthetize. Despite the existence of this pathway, it can be considered a conditionally essential substrate for many immune cells, meaning that its synthesis may be insufficient to respond to the body's necessities in situations of high catabolic stress.

Glutamine has the potential to assist in the regeneration of glycogen levels with the advantage of not increasing insulin levels in the same way as glucose supplements do (Caballero, 2009). The conversion of glutamine to α-ketoglutarate is the preferential energy source for cells with high mitotic activity, such as leukocytes and enterocytes (Cruzat et al., 2014a).

Prolonged exercise is associated with a reduction in the plasma concentration of glutamine, which can suppress immune function, particularly during overtraining. This reduction may persist until 6 h after exercise. Therefore, it is very important to restore levels of this amino acid to optimize recovery and decrease the risk of overtraining (Cruzat et al., 2014a).

Cruzat et al. (2014a) have also described the effects of L-glutamine is the modulation of glutathione (antioxidant properties) and HSPs (with chaperone function and inflammatory control) synthesis. In vivo studies have shown that L-glutamine supplements (free along with L-alanine and glutamine containing dipeptides) are able to increase the hepatic and muscular concentration of L-glutamine, which in turn increase the tissue concentration of GSH (γ-glutamyl-cysteinyl-glycine), attenuating the oxidative stress induced by long duration physical exercise (Cruzat and Tirapegui, 2009). This antioxidant effect is attributed to the supply of L-glutamate from L-glutamine, especially from plasma to immune cells and skeletal muscles (Curi et al., 2007). When transported inside the cell, L-glutamine promotes the uptake of water, an increase in sodium ion Na^+ uptake and the release of potassium ions (K^+), thus increasing cellular volume and consequently resistance to injury (Usher-Smith et al., 2009). The augmented availability of L-glutamine increase neutrophil and lymphocyte activity and function (Pithon-Curi et al., 2002), for example, generating NADPH for the NADPH oxidase enzyme (Pithon-Curi et al., 2003), stimulating intermediary metabolism, and preventing apoptosis by maintaining mitochondrial function (Cury-Boaventura et al., 2008). These mechanisms suggest L-glutamine supplementation may attenuate muscle damage and inflammation induced by exhausting exercise (Cruzat et al., 2010).

Recent works by Petry et al. (2014) and Cruzat et al. (2014b) have reported glutamine-enhanced stimulation of the heat shock protein (HSP) response induced by acute or chronic inflammation. The heat shock protein 72 (HSP72) is an immune-regulatory

protein that is now receiving considerable attention. HSP72 can induce different inflammatory responses according to its location (intra vs extracellular), positioning this protein as a master regulator for the fine-tuned control of the immune system. Several studies have demonstrated that exercise is a physiological stimulus that promotes an increase in the eHSP70 concentration, depending on intensity and duration of exercise (Kotas et al., 2013; Cruzat et al., 2014b; Newsholme et al., 2014).

Based on several clinical nutritional studies that found benefits in attenuating the dramatic decrease in plasmatic and tissue L-glutamine levels (Wernerman, 2008), as well as immune cell function, including lymphocytes (Cruzat et al., 2014a) and neutrophils (Pithon-Curi et al., 2003), the use of L-glutamine as a nutritional supplement for sport and exercise had been raised. Other important publications have described the importance of L-glutamine in clinical nutrition (Wernerman, 2008; Roth, 2008).

Castell et al. (1996) showed that glutamine supplementation (5 g in 330 mL water) immediately after and 2 h after a marathon reduced the incidence of URTI (in the 7 days following the race). Furthermore, glutamine had a beneficial effect on digestive function, morbidity, and mortality. Glutamine also exerts effects on some aspects of immune cells in diseased or traumatized patients (Newsholme, 2001; Cavalcante et al., 2012). However, the same studies were not able to demonstrate that exercise-induced reductions in plasma glutamine levels cause impaired immunity or reduce host protection against viruses in athletes. This may be because L-glutamine reserves within the body are not completely depleted by exercise (Walsh et al., 2011).

Oral supplementation with L-glutamine in its free form poses potential problems, as it is highly metabolized by the gut, decreasing its absorption and lowering its effect in the body. Possible alternatives include exogenous administration of L-glutamine chemically attached to another amino acid usually as a dipeptide (eg, L-alanine, forming L-alanyl-L-glutamine) or using nanotechnology to increase bioavailability.

In humans (Castell and Newsholme, 1997) and animal models (Rogero et al., 2004), acute oral L-glutamine supplementation, in its free form or as a dipeptide, is able to increase the plasma L-glutamine concentration between 30 to 120 min after ingestion. Dipeptides containing L-glutamine are highly soluble and stable in solution and are often used in enteral nutrition and total parenteral nutrition (TPN) to achieve high L-glutamine and L-alanine blood concentrations. This effect has been attributed to the glycopeptide transport protein (PepT-1) in the intestinal cells (enterocytes), which is a very efficient transport mechanism for the

absorption of dipeptides and tripeptides (Nässl et al., 2011) allowing L-glutamine to bypass metabolism by enterocytes and proceed directly to the systemic circulation, thus increasing its availability to immune cells and other tissues (Rogero et al., 2006; Cruzat et al., 2014a). In the dipeptide or in its free form, L-alanine can also help L-glutamine avoid metabolism, allowing the latter to be used by high-demand tissues (Cruzat et al., 2014a).

In short, growing evidence in support of the immune mediating effects of L-glutamine has resulted in an increase in interest for use in supplementation. However, more studies in athletes are required to determine optimal supplementation strategies, including the use of dipeptides with and without free amino acids. The recent advances in nanotechnology make it a very promising way to develop other forms of administration and increase bioavailability of L-glutamine and nutraceuticals, something which will be explored later in this chapter.

3.3 Branched-Chain-Amino Acids (BCAAs)

Amino acids (AA) are quaternary compounds of carbon, hydrogen, oxygen and nitrogen and are the basic components of human proteins. They can be divided into natural (ie, endogenous synthesis) and essential amino acids (meaning they can only be obtained from the diet). L-valine, L-leucine, and L-isoleucine, usually referred as branched-chain-amino-acids (BCAAs) due to their chemical structure, are three of the nine AA identified as essential for protein synthesis in human body (Leahy and Pintauro, 2013). They represent 14–30% of the total AA used in muscle metabolism and 35–40% of the essential AA (Shimomura et al., 2012). Foods rich in protein, such as meat, poultry, fish, eggs, milk, and cheese can contain on average 15–20 g of BCAAs per 100 g of protein (Burke et al., 2009). After ingestion the concentration of BCAAs in plasma rapidly increases. They are then predominantly metabolized in the skeletal muscle, meaning they bypass liver metabolism. Although the liver cannot directly metabolize BCAAs, this tissue has an active system for the degradation of the α-branched-chain-keto acids (BCKA) derived from the corresponding BCAAs (Nicastro et al., 2012) by the branched-chain α-keto acid dehydrogenase (BCKD) enzyme, which contribute to gluconeogenesis (Newsholme et al., 2014).

BCAAs, especially leucine, are well-known nutritional supplements which may influence the adaptive responses of the skeletal muscle (Nicastro et al., 2012). BCAAs regulate protein metabolism, reduce protein oxidation, increase protein synthesis, stimulate mRNA translation, and suppress protein degradation in muscle

through different pathways, namely via mechanisms involving the mammalian target of rapamycin (mTOR) protein (Bolster et al., 2004; Williamson et al., 2006).

BCAAs supplementation, before and after exercise, is more and more considered as a selective anabolic strategy, giving muscle efficiency and plasticity, stimulating protein synthesis, muscle sarcomerogenesis and modulating glucose homeostasis following exercise (Spiering et al. 2008; da Luz et al., 2011; Layman, 2011).

Skeletal muscle oxidizes BCAAs more than any the other AA during physical exercise (PE) (Shimomura et al., 2006). Different studies suggest that oral supplementation can decrease the release of essential AA from exercising muscles with suppression of endogenous muscle-protein breakdown (MacLean et al., 1994; Shimomura et al., 2012), reducing the rise in serum creatine kinase level (Coombes and McNaughton, 2000; Shimomura et al., 2012) and increasing protein synthesis after exercise (Rasmussen et al., 2000; Shimomura et al., 2012; Pinheiro et al., 2015).

As previously stated in this chapter, during PE, mechanical stimuli like endurance exercise as well eccentric muscle contractions (da Luz et al., 2011; Leahy and Pintauro, 2013; Pinheiro et al., 2015) can promote muscle fiber damage with functional impairment usually manifested by pain, swelling, temporary strength decrease and increase passive muscle tension (Howatson et al., 2012; Pinheiro et al., 2015). There are two different types of muscle soreness related to physical exercise, acute soreness, which appears during and immediately after exercise; and delayed soreness (DOMS) felt 24–48 h after activity. Both can have a negative impact in athletes, modifying training schedules and limiting performance. DOMS is most prevalent at the beginning of sporting season, with the increase training intensity increases and most specifically in eccentric workload (Cheung et al., 2003; Nicastro et al., 2012; Ra et al., 2013).

In the study conducted by Bassit et al. (2002), acute and chronic BCAAs supplementation (about 6 g/d) given to *endurance* athletes resulted in attenuation of the fall in the plasma L-glutamine concentration and also modified the immune suppression promoted by the exercise. The results lead us to believe that the immune effects of BCAAs may be dependent on L-glutamine metabolism in the tissues, such as the skeletal muscle. In fact, in hyper-catabolic situations, such as burning, sepsis, and malnutrition, BCAAs administration can modulate inflammation through the L-glutamine pathway (D'Antona et al., 2010). However, considering the effects of exercise, this pathway deserves some considerations. When lymphocytes are maintained in vitro in a low level of L-glutamine, identical to the lowest plasma L-glutamine concentration

measured postexercise (300–400 μM), they perform equally well (Newsholme, 2001) as when L-glutamine is added at a higher concentrations than the resting plasma level (600 μM) (Hiscock and Pedersen, 2002). Consequently, BCAAs effects for sports and exercise with regard to immune function may occur independently of L-glutamine synthesis and stimulation.

Several studies suggest that the production of proinflammatory mediators, such as cytokines, prostaglandin E2 (PGE2) and nitric oxide by neutrophils, macrophages, and lymphocytes can be suppressed by the association of BCAAs with taurine (Gijón et al., 2000; Kudo and Murakami 2002). PGE2 sensitizes muscles' afferent nociceptors leading to muscle swelling and pain. Taurine may contribute to enhance the anabolic effects of BCAAs due to its capability of decreasing the levels of serum markers of inflammation and immune response to exercise.

More studies are designed to investigate the effects of BCAAs supplementation in exercise muscle damage in athletes (Nosaka et al., 2006; Shimomura et al., 2009; Sharp and Pearson, 2010; Jackman et al., 2010). All these studies include an exercise protocol (squatting, jumping, endurance, isometric, eccentric, concentric…) to induce muscle soreness in different muscle groups, a supplementation protocol (pre and/or postexercise during one or several days) containing isolated BCAAs or in association (carbohydrate, taurine, others, placebo), blood samples to quantify serum markers of muscle damage (creatine kinase, aldolase, lactate dehydrogenase, myoglobin, etc.), blood samples to quantify plasma AA concentrations (BCAAs, taurine, etc.), plasma elastase concentration (as an index of neutrophils activation) and several parameters of inflammation and immune response (prostaglandins, interleukins, and others). These study protocols also feature a subjective assessment of muscle soreness (VAS: visual analogic scale, numeric scale) and indirect markers of muscle damage via physical parameters (maximal voluntary contraction, vertical jump performance, limb circumference) and imaging (ultrasound measuring the cross-sectional area and volume of the muscle) (Yu et al., 2015).

In conclusion, according to the different data, isolated BCAAs supplementation or in association appears to be an interesting nutritional strategy to reduce/attenuate exercise-induced muscle damage and DOMS, while also contributing for a faster recovery process. The association of BCAAs and other supplements (arginine, taurine, carbohydrates, glutamine) can also increase the protective effect against muscle fatigue, inflammation, and loss of performance after different exercise protocols.

However, little is known regarding the objective effects of BCAAs supplementation in protecting muscle and controlling

inflammation, and future studies are needed to evaluate its impact in muscle physiology and function as well as in molecular pathways involved in muscle repair and potential regeneration.

3.4 Phytochemicals

Phytochemicals are chemical compounds derived from plants, many of which possess powerful antioxidant activity (Kähkönen et al., 1999; Dragland et al., 2003). Among nutraceutical compounds, phytochemicals have been widely studied due to their positive effects on human health (Fimognari et al., 2008; Davì et al., 2010; Asif and Acharya, 2013).

Several phytochemicals are useful in promoting the health effects of exercise, maintaining homeostasis, and preventing muscle aging. Human and animal studies have shown that caffeine, quercetin, and β-glucan are effective against URTI and changes in cytokine and hormone levels after exercise (Nieman et al., 2007; Fletcher and Bishop, 2012). Nieman et al. (2007) in a double-blind, placebo-controlled study with 40 cyclists showed that ingestion of quercetin (1000 mg) for 3 weeks significantly reduced URTI incidence during the 2-week period following 3 days of exhaustive exercise (Nieman et al., 2007). On the other hand, ingestion of a single quercetin did not suppress postexercise inflammation or immune changes when compared to treatment with a placebo (Konrad et al., 2011). Recently, β-glucan extracted from the *Pleurotus ostreatus* mushroom has also been shown to reduce the incidence of URTI symptoms, while also increasing the number of circulating NK cells during heavy physical training in athletes (Bergendiova et al., 2011). Astaxanthin, a carotenoid, can attenuate exercise-induced damage in mouse skeletal muscle and heart, including associated neutrophil infiltration that induces further damage (Aoi et al., 2003). In a study conducted in soccer players, the astaxanthin group exhibited significantly lower postexercise levels of creatine kinase and AST than the placebo group (Djordjevic et al., 2012).

Several nutritional interventions using phytochemicals such as allicin, panax ginseng, and curcumin have been proposed regarding the issue of delayed-onset muscle soreness (DOMS).

The antiinflammatory and antioxidant capacities of allicin, which is commonly found in garlic, are well known. Allicin is able to regulate cellular inflammatory response by inhibiting the expression of adhesion molecule-1 (Sela et al., 2004; Son et al., 2006). Furthermore, it also has antioxidant capabilities and demonstrated an ability to scavenge hydroxyl radicals and prevent lipid peroxidation (Xiao and Parkin, 2002). Su et al. (2008) reported

the reduction of exercise-induced muscle damage, IL-6 and an increase in antioxidant capacity in well-trained athletes supplemented with allicin.

Panax ginseng is an ancient and well-known herb of traditional Chinese medicine (Wei et al., 2014). Recent investigations have shown that ginsenosides, a group of saponins with triterpenoid dammarane structure which are present in ginseng, seem to be the main substances responsible for the antiinflammatory, antioxidant, antiapoptotic, and immunostimulant properties of ginseng (Wei et al., 2014). Due to these beneficial effects, ginseng supplementation has also been considered as a strategy in reducing DOMS. A study conducted by Pumpa et al. (2013) reported a reduction in DOMS at 96 h following downhill running among trained males ($n = 20$) after supplementation with 4 g of panax ginseng 1 h before and 4 days when compared to the placebo group. However, the difference was not as pronounced as in other nutritional interventions. Comparison of the biomarkers of inflammation has shown some conflicting results: the panax ginseng supplement group had higher IL-6 and TNF-α levels than the placebo group in the 24 h following exercise, and there was no difference in CRP levels between the groups. Other studies also reported that while ingestion of ginseng may reduce inflammatory response, that effect was not observed in the context of exercise (Jhun et al., 2014; Wei et al., 2014).

Curcumin, a powerful promoter of antioxidant response (Cuomo et al., 2011), is one of the best-investigated natural products (Steigerwalt et al., 2012).

Several mechanisms have been proposed to explain the beneficial effects of curcumin. Early experimental studies demonstrated that curcumin suppresses the activation of NF-κB (Singh and Aggarwal, 1995; Jobin et al., 1999) an effect of critical relevance in DOMS relief, since NF-κB is involved in the regulation of proteolysis and inflammation in muscle (Alamdari et al., 2009). Therefore, inhibition of NF-κB by curcumin may result in a muscle-protective effect. This is supported by reports that curcumin may prevent loss of muscle mass during sepsis and endotoxaemia and may stimulate muscle regeneration after traumatic injury (Thaloor et al., 1999). Other mechanisms besides NF-κB inhibition which may be responsible for the antiinflammatory and antioxidant properties of curcumin include induction of heat-shock response (Dunsmore et al., 2001), reduction in the expression of the proinflammatory enzyme cyclooxygenase-2 (COX-2) (Chun et al., 2003) and promotion of the antioxidant response by activation of the Nrf2 transcription factor (Shehzad and Lee, 2013). Experimental evidence indicates that curcumin

can lower inflammation and mitigate some of the negative effects resulting from eccentric exercise, including the release of proinflammatory cytokines and markers of muscle injury like creatine kinase (CK) (Davis et al., 2007).

3.5 Conclusions

The available literature seems to suggest that nutraceutical bioactive compounds, can also provide protection against exercise-induced muscle damage and overtraining thanks to their antioxidant and antiinflammatory properties. This topic has attracted a great research effort; however, scientific data do not allow a clear conclusion to be drawn. While in vitro and in vivo animal studies suggest that nutraceuticals could really play a role in improving endurance performance; most human trials have failed to demonstrate this hypothesis. Results can be affected by many factors including study design, dose, ingestion period, and the markers that were measured to verify the hypothesis. Nevertheless, the use of nutrients to modulate immune function (known as immunonutrition) represents an emerging area in health and sports nutrition. Omega-3 fatty acids, amino acids and phytochemicals all play a part in the regulation of key metabolic pathways in immune cells and the cellular response to oxidative stress, as depicted in Fig. 17.3. At the antiinflammatory molecular level, new findings have been reported such as enhancement of an immune-regulatory protein [heat shock protein (HSP)] levels, nitric oxide (NO) synthesis, and redox balance, all of which are essential for optimal immune function and recovery from intense periods of training.

Considering that athletes are at increased risk of URTI, overtraining syndrome, chronic inflammatory response, and oxidative stress, during and after periods of heavy exercise immunonutritional approaches may be considered for future recommendations in the sport science field (Fig. 17.3).

4 New Trends: The Use of Nanotechnology for Immune Modulation

Nanostructured delivery systems for health-promoting bioactive components (nutraceuticals) in functional food are an important and rapidly developing area in food nanotechnology. Most nutraceutical compounds cannot be added into foods or beverages in their pure form. Nanodelivery systems are being engineered to overcome this hurdle, aiming to enhance bioavailability with minimal adverse sensory effects.

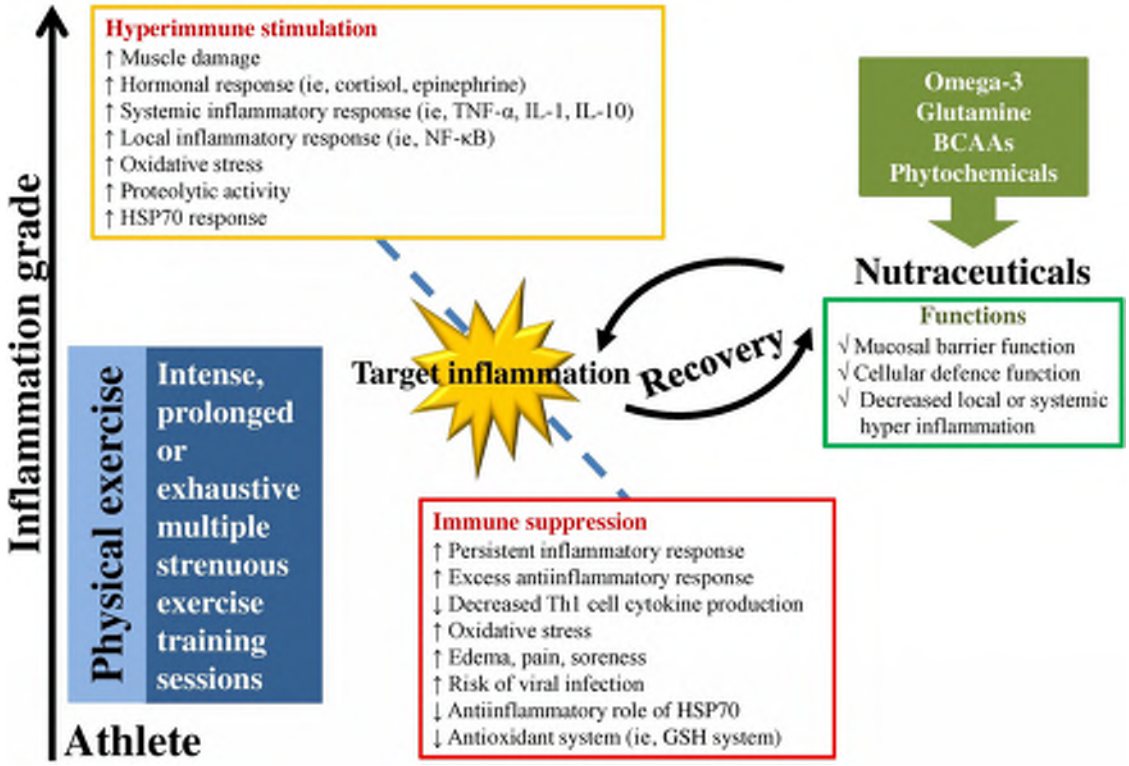

Figure 17.3. Biphasic immunoinflammatory response to severe exercise and the possible role of nutraceuticals. Immunoinflammatory response induced by severe exercise or heavy periods of training and the proposed role of specific nutrients (nutraceuticals) with immune benefits: tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-10 (IL-10), nuclear Factor-κB (NF-κB), glutathione (GSH), and heat shock protein 70 kda (HSP70).

The new possibilities brought by nanotechnology could allow athletes to maintain good health and optimize performance during periods of heavy exercise training and preparation for competition.

In the current section, some benefits of the use of nanotechnology for incorporate specifically nutraceutical compounds into foods or beverages will be described. The possibility of using sports beverages as a vehicle to deliver nutraceutical compounds to athletes allowing them to improve immune function and consequently optimize performance will then be discussed.

4.1 Nanotechnology and Functional Foods

In recent years, the food industry has been faced with an increasing demand for producing and introducing in the market a

whole different kind of foods. The use of nanotechnology in the food, beverage and allied sectors is still in its infancy, but there are clearly many opportunities to innovate and investigate new ways of working with these materials. The tools are becoming available to enable us to learn processes occurring in our everyday environment from a nanoscale point of view. The coming decades will bring further understanding of the surface chemistry of very small particles, foam and emulsion stability, the migration of particulates into food and water, the barrier properties of packaging, the development of thermochromic and photochromic materials as indicators of product shelf life and storage conditions and many more new applications for nanotechnology in the food sector (Maugghan, 2004). The use of nanotechnology may revolutionize food industry by providing stronger, high-barrier packaging materials, more potent antimicrobial agents, and a host of sensors, which can detect trace contaminants, gases or microbes in packaged foods (Duncan, 2010).

Nano is the scientific term meaning one billionth (1/1,000,000,000) and is derived from a Greek word meaning "dwarf." Nanotechnology is a multidisciplinary field that draws from the knowledge obtained from physical, chemical, biological, engineering, and electronic sciences, allowing for the manipulation of materials and structures at sizes which vary approximately between 1 and 100 nm (European Food Safety Authority, 2009). Nanomaterials naturally exist in many animal or plant products, including milk (such as casein, fat globules, lactose, and whey proteins), some fibrous structures present in fish and meat, or the molecular structure of cellulose fibrils of plant cells (Morris, 2011). Nanomaterials are being developed with enhanced mechanical and thermal properties to ensure better protection of foods from exterior mechanical, thermal, chemical, or microbiological effects. Nanocomposites, for instance, are nanoparticles bonded in polymers so that the materials have enhanced properties such a lighter weight and better recyclability, as well as spoilage and flavor issues (Dingman, 2008).

The food industry is particularly working on the development of new techniques, which include several areas such as new culinary textures, safety, healthier foods, sports foods and drinks, encapsulation, and packaging.

Most nutraceutical compounds cannot be added into foods or beverages in their pure form. The main reasons for this are either poor solubility, sensitivity to deterioration during processing, shelf life and digestion, undesired sensory attributes, or poor bioavailability, and often, several of these reasons combined, hampering the applicability and diminishing the efficacy of these compounds

in preventing diseases. Nanodelivery systems are engineered to overcome these hurdles. However, to be applicable and commercially viable, these delivery systems must be safe for food use, cost effective, label-friendly (particularly for certain consumer groups, like allergic consumers, vegetarians, religious, etc.) and they must not adversely affect the sensory properties of the enriched product. Enhanced bioavailability and minimal adverse sensory effects are the main advantages of nanodelivery systems.

4.1.1 Types of Nanostructured Delivery Systems for Functional Food

To respond to the new challenges of consumers such as producing new health food ingredients, adding specific nutrients or functional ingredients, modifying food compositions, masking undesirable flavors, reducing or removing undesirable food components or stabilizing ingredients, additional techniques were developed to assist traditional food technologies. Therefore, modern biotechnology, coupled with recent discoveries in gene science, revolutionizes the way foods are created and allows the possibility to manipulate their natural components (Hsieh and Ofori, 2007).

There is a large and increasing market for foodstuffs with enhanced vitamin and other supplements, where nanoencapsulation can have a major impact. Nanosized or nanoencapsulated food additives and supplements can improve dispersability of fat-soluble additives in food products, improve taste, enable hygienic food storage, reduce the use of fat, salt, sugar, and preservatives, and improve the uptake and bioavailability of nutrients and supplements (Chaudhry and Castle, 2011).

These encapsulation techniques enable the production of "healthy" foods, such as low-fat dairy and nondairy oils, low salt, and sugar products or foods that can contain certain substances by the incorporation of specific vitamins and minerals, and by making them more easily absorbable in nanoparticulate form. Other nanoscale phenomena, such as those included within the term "colloid science," are also exploited in nutraceutical and functional food formulations, manufacturing and processing. Certain companies have already created nanosized concentrates of vitamins A, D, E, K, and isoflavones.

Various types of nanodelivery systems for food applications have been reported and reviewed in the literature (Cerqueira et al., 2014; Yao et al., 2014, 2015; Walker et al., 2015; Norton et al., 2015). Norton et al. (2015) presented a very interesting review discussing the impact on health of both the macronutrients and micronutrients, and how rational design of food microstructure and nanostructure, and control of nutrient release and absorption

may be applied to improve human health. From a literature review we found the main types which have been studied and developed over the past 2 years. Nanoemulsions, nanostructured lipid carriers (NLC), Pickering emulsions, solid-lipid NPs (SLN), nanosuspensions, liposomes, and nanoliposomes, NPs and micelles made of proteins, polysaccharides, and their complexes or conjugates, and combinations with lipid or mineral components are some examples of recent developments in nanotechnology applied to food industry over the past 2 years (Norton et al., 2015; Yao et al., 2015). Nanostructured delivery systems are not the main focus of this section, which instead aims to emphasize how nanotechnology might play an important role in the modulation of immune system in athlete. Table 17.1 organizes the nutraceuticals with activity in immune-modulation of athletes (reported in Section 3) and the nanovehicle used in nanodelivery systems.

4.1.2 Sensory Aspects of Functional Food (Texture, Flavor, Odor)

Texture is a critical attribute that is essential to the overall quality and acceptability of most food products because, in addition to its relevance to mouth feel, it is also a property generally related to freshness (Isaksson et al., 2002).

One of the major advantages of nanodelivery systems in food is the fact that they usually evade our sensory perception, enabling enrichment of food and beverages with bioactives without adversely affecting the sensory attributes of the original product. Their nanometric size is certainly too small to be seen, but if kept below about 80 nm (and not at very high concentrations, or with a particle refractive index not very different from that of the solution), they hardly scatter any visible light, hence maintaining transparency. Such "stealthy" vehicles are very useful, for example, for enriching clear beverages with water-insoluble nutraceuticals, or ones which have undesirable flavors or odors. Solid lipid nanoparticules (SLNs), and protein complexes were reported to mask bitterness (Shpigelman et al., 2012; Fathi et al., 2013). Markman and Livney (2012) have considered that the nanovehicles useful for clear beverage enrichment include, for example, nanoemulsions, protein nanoparticles and protein-polysaccharide conjugates Also mouth-feel attributes of nanoparticles are nonexistent, as our tongue ceases to sense particulates below about 10 μm in size. Niosomes containing resveratrol did not cause any changes in the textural properties of regular yoghurt (Pando et al., 2015). Nanocomplexes of high amylase corn starch (HACS) encapsulating omega-3 fatty acids and silymarin were incorporated into bread formulation, and up to 2.5% had no adverse effect on bread quality parameters and sensory properties (Mogo et al., 2013).

Table 17.1 Nutraceuticals with Activity in Immune-Modulation of Athletes (previously referred) and Nanovehicle Used for Nanodelivery Systems

Bioactive Compounds		Nanovehicle	References
Fatty acids	Omega 3	Nanoemulsions	Salminen et al. (2013); Cho et al. (2014); Lane et al. (2014); Walker et al. (2015)
		SLNs	Salminen et al. (2013)
		NLCs	Salminen et al. (2013)
		Zein nanoparticles	Soltani and Madadlou (2015)
		Casein micelles	Ghasemi and Abbasi (2014)
		Electro spun zein fibers	Moomand and Lim (2014)
		Amylose inclusion complexes	Mogol et al. (2013)
Peptides	Glutamine BCAAs	Nanoemulsions	Adjonu et al. (2014)
Phytochemicals	Curcumin	Casein NPs	Pan et al. (2013)
		Phosphocasein micelles	Benzaria et al. (2013)
	Astaxanthin	NLCs	Tamjidi et al. (2014)
	Quercetin	Lecithin/chitosan NPs	Souza et al. (2014)
		Nanoemulsion	Pool et al. (2013)

Solid-lipid, SLNs; nanostructured lipid carriers, NLCs; branched-chain amino acids, BCAAs; nanoparticles, NPs.

4.1.3 Improvement of Bioavailability of Bioactive Compounds

Compounds must be able to reach the tissue of action to exert their positive effect in health. This depends on the substance's bioavailability: compounds with higher bioavailability are more likely to reach systemic circulation and consequently their tissues of action, while substances with lower bioavailability are less likely to exert their effects. Therefore, knowing their bioavailability is fundamental when studying functional foods and health claims attributed to food components. However, due to it being a pharmacokinetic property, bioavailability is dependent on several different phases: liberation, absorption, distribution, metabolism,

and elimination. A huge advantage of NPs is their improvement of micronutrient bioavailability, thanks to their nanometric size (Oehlke et al., 2014; Ting et al., 2014; Yao et al., 2015). The improved oral bioavailability is obtained by several mechanisms: increasing bioactive stability during digestion; enhancing nutraceutical solubility in intestinal fluids; enhancing nutraceutical transport and absorption via the intestinal epithelia; and decreasing first-pass metabolism in the gut and liver (Oehlke et al., 2014; Ting et al., 2014; Yao et al., 2015). The materials and their physical state, the nanostructure of the vehicles and surrounding food matrix affect bioavailability. Penalva et al. (2015), using casein nanoparticles for the oral delivery of folic acid observed, in vitro, that casein nanoparticles acted as gastro-resistant devices and, as a result, folic acid was only released under simulated intestinal conditions. In vivo animals treated with folic acid-loaded casein nanoparticles displayed significantly higher serum levels than those observed in animals receiving an aqueous solution of the vitamin. As a consequence, the oral bioavailability of folic acid when administered as casein nanoparticles was calculated to be around 52%, a 50% higher than the traditional aqueous solution.

A study found that calcium absorption of a whey protein hydrolysate-calcium chelate in Caco-2 cells was significantly higher than those of calcium gluconate or calcium chloride ($CaCl_2$) (Xixi et al., 2015).

A clinical study has shown that the bioavailability of vitamin D_3 encapsulated in reformed-casein micelles in 1% fat milk was at least as high as the one obtained using an aqueous Tween-80-emulsified vitamin D_3 supplement (Haham et al., 2012).

For example, Fernandez-Garcia et al. (2008) used several in vitro digestion models in order to assess whether emulsifier system enhanced the bioaccessibility of carotenoids. Various studies were also undertaken comparing the bioavailability of PUFAs delivered through new systems such as microcapsules, liposomes, gel emulsions, and plant spore exines to that of fish oil. The results demonstrated that the new delivery systems improved bioavailability of PUFAs (Matalanis and McClements, 2012; Israeli-Lev and Livney, 2014; David et al., 2014). A clinical trial demonstrated that ω-3 fatty acid absorption from a nanoemulsion was significantly higher than from bulk oil (Lane et al., 2014).

4.1.4 Health Aspects of Nano Enrichment of Food: Safety and Efficacy

The increased bioavailability of bioactives in NPs is also an important safety concern, particularly when the added nutraceutical or the nanovehicle material may have toxic effects above a certain

dose. This requires a prudent safety evaluation and regulatory approval, particularly when introducing new materials, which have no record of safe human consumption. Increased bioavailability in nanovehicles may require reevaluation of the Recommended Daily Allowance (RDA) and the Tolerable Upper Intake Level (UL) of bioactive compounds.

Health-promoting attributes of nanoencapsulated bioactives are being studied extensively; however, more in vivo and clinical studies of their safety and health promotion are still needed. Casein-encapsulated curcumin showed higher antioxidant activity and higher anticancer activity than unencapsulated curcumin, as evaluated by in vitro cell proliferation assay using human colon cancer cells (Pan et al., 2013). Epigallocatechin-3-gallate (EGCG) encapsulated in casein micelles caused a decrease in proliferation of cancer cells, while in healthy cells, neither free nor encapsulated EGCG affected cell proliferation at low concentrations, and its bioefficacy was not diminished regardless of digestion extent (Haratifar et al., 2014). EGCG-loaded chitosan-NPs orally administered to mice implanted with subcutaneous tumor xenograft, resulted in significant tumor growth inhibition, compared with free EGCG and control groups (Khan et al., 2014). Calcium content, bone mineral density and biomechanical properties were significantly higher in rats following egg-phosphopeptide supplementation compared to control (Huang et al., 2014). Conjugated linolenic acid-rich oil nanocapsules significantly reduced the blood lipids, tissue lipids and plasma viscosity in high-fat diet induced hypercholesterolemia in rats (Sengupta et al., 2015).

The potential applications of nanotechnologies in the food sector has not come unnoticed to food technologists and engineers, as they attempt to identify new processes to reinvent food products that would cater to consumers on a global scale. However, concerns over consumer health and the safety of nanoparticles in food products need to be properly addressed (Sonkaria et al., 2012).

4.2 Sports Drinks to Modulate the Immune System and Increase Performance

The development of innovative functional foods for athletes requires an adequate study of the nutritional, physiological, and biochemical changes that occur due to training and the practice of different sports. Suitable functional foods must also be designed to be suitable before, during and after training. Follow-up studies must be carried out to assess the efficiency of a proposed food formula in athletes. Screening of phytochemicals and nutrients

constituents of the prepared food products is very important in order to establish the suitable quantities of food that each athlete should ingest.

In the case of sports athletes, the creation of special drinks that decrease in infection and increase performance may be useful.

Sports drinks have three main objectives: intake of enough carbohydrates to maintain an adequate concentration of glucose in the blood and delay the use of the glycogen reserves; intake of electrolytes, namely sodium; and supplying water, avoiding dehydration (Gil, 2010).

The active ingredients in sports drinks need to be absorbed quickly to assist performance and maintaining the health of the athlete. As previously stated, these benefits can be achieved by including nutraceuticals in nanoparticulate form.

The delivery of hydrophobic nutraceuticals can be achieved, for example, through the use of nanosized carriers. Their nanoscopic size allows for greater bioavailability with a minimal impact on sensorial characteristics, including, for example, the transparency of clear food systems like certain beverages. Entrapment and encapsulation by these nanocarriers may protect nutraceuticals against degradation by oxidation and other enzymatic or chemical reactions during production and shelf-life, further preventing the development of undesired sensorial properties and the loss of metabolic value (Zimet and Livney, 2009). The growing knowledge on digestive disintegration (Mackie, 2012) and absorption mechanisms facilitates a reversed-engineering approach to construct these vehicles so that they will only be degraded in an adequate time and location in the digestive tract to and optimize the survival of the bioactive through production, shelf life, and digestion, and maximize its bioavailability. When considering nanoencapsulation versus microencapsulation, only some of the technologies used for microencapsulation may be scaled down and applied for nanoencapsulation. Nanodelivery systems may enable enrichment of clear beverages with water-insoluble nutraceuticals, minimize sensory effects and facilitate better bioavailability than microdelivery systems, while the latter may offer higher protection and barrier for sustained release.

4.3 Future Trends Evolving Sports Beverages

Many nanoenabled products are currently under research and development, and may enter the market in the near future. As for any other regulated product, applicants seeking market approval have to demonstrate that use of such new products does not pose safety risks to the consumer and the environment. Several

countries all over the world have been active in examining the appropriateness of their regulatory frameworks for dealing with nanotechnologies (Amenta et al., 2015).

Despite of the opinion of the American College of Sports Medicine in 1984 stating that cool water was the best drink to ingest during endurance exercise, recent evidences disapprove this theory. The sports nutrition industry is shaped by a variety of factors. New beverages that make us run faster, lift more, and perform better increase knowledge considerably around this area (Greenwood et al., 2008).

Spano (2010) characterized three distinct trends in sports drink formulation. The first and most notable involved the categorization of beverages into segments that cater to the needs of athletes, by type. The second trend feeds off the concept of "nutrient timing," that is, strategically consuming certain nutrients within proximity of training times, to facilitate greater training adaptations and/or shorten recovery time. The third trend involves a crossover between sports drinks and functional beverages. As technology advances, a fourth trend can be proposes: using nanotechnology as way to include bioactive compounds in sports beverages and also to control their time of release. The possibility of including substances such as omega-3 fatty acids, glutamine, branched-chain amino acids and phytonutrients, whose benefits were already described in previous sections of this chapter, in these products is definitely interesting and nanotechnology can make this achievable.

"Smart" packaging is also another possibility. Nanotechnology can allow the maintenance of an internal ambient temperature for longer, thus increasing the lifespan of contents. It may also confer antibacterial and sun-blocking properties. Nanodevices placed in packaging would enable easy tracking of large quantities of product.

The accumulated knowledge and improved technological capabilities bring about an exciting plethora of innovative nanostructured delivery systems for nutraceuticals (Fig. 17.4). These delivery systems may act by controlling the structure matrix, the release time and locale of release, and even the dosage of bioactive contents. This knowledge allows delivery of nutraceuticals based on individual needs. Personalized sports drinks considering individual needs of the athlete based on type of sport, time of duration, and age and sex of the athlete will be able to prevent and/or decrease main problems associated with heavy exercise (Figure 17.4).

In conclusion, the future materialization of nanotechnology used in functional food for athletes will allow for personalized nutrition conferring higher immune protection and better mental capabilities, thus increasing performance.

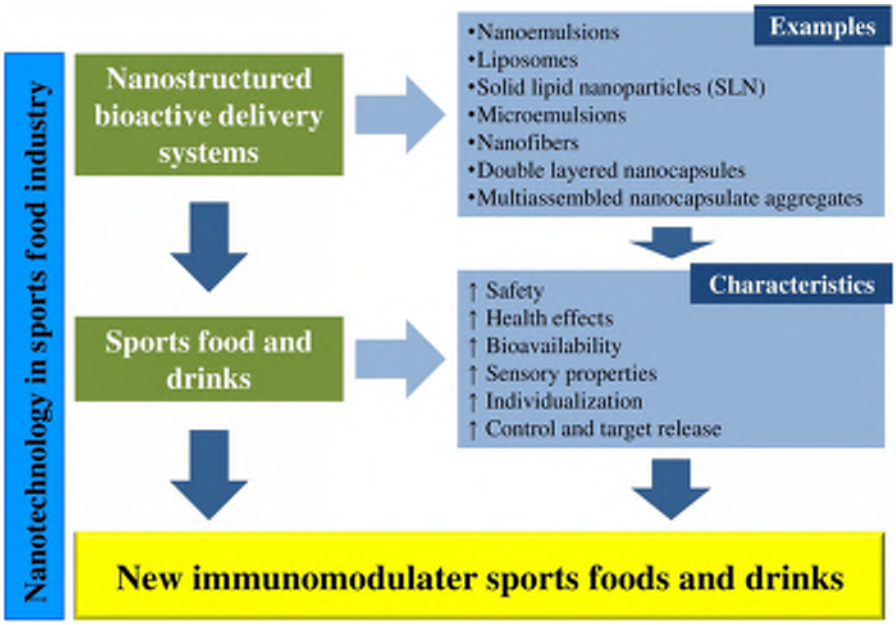

Figure 17.4. New trends in sports food and drinks: nanotechnology allowing the design of immunomodulating foods and drinks for athletes. The nanodelivery systems solubilize and protect nutraceuticals during processing, shelf life, and digestion. These nanodelivery systems allow the design of foods and drinks with improved sensory impact, stability, ability to control release of nutraceuticals, bioavailability, and beneficial health effects, thus representing a new trend in food science. In addition they allow personalizing the dose of nutraceuticals according to age, sex of the athlete, and duration and type of sport. So, nanodelivery systems can be engineered to introduce immunomodulatory substances in sports foods and drinks in order to increase performance and decrease fatigue, lesions (DOMS), overtraining syndrome, and upper respiratory tract infections (URTI).

5 Concluding Remarks

Physical exercise has been proven to have numerous health benefits when practiced regularly and with a moderate intensity. However, athletes often engage in high-intensity exercise as part of their training regimes. This may temporarily weaken their immune systems, rendering them more susceptible to illness. Intense exercise may also activate several proinflammatory pathways in the body, leading to muscle soreness, weakness, and fatigue, eventually leading to a decrease in performance.

Bioactive compounds, also known as nutraceuticals, present anti-inflammatory and antioxidant proprieties. Nutritional supplements such as omega-3, glutamine, BCAAs, and several phytochemicals

are known to exert a positive impact on the cellular oxidative stress response and in the regulation of the immune system.

Supplying athletes with these substances may be helpful in preventing or optimizing the action of therapeutic agents against exercise-induced muscle damage, overtraining, and upper respiratory tract infections (URTI), thus improving performance.

Production of innovative functional foods useful for athletes requires proper knowledge of the nutritional, physiological, and biochemical changes that occur due to exercise. Suitable functional foods must be designed to be effective before, during, and after training. Follow-up studies must be carried out for assessment of the efficiency of a proposed food formula in athletes. Quantifying the phytochemicals and nutrients present in food products is very important in order to establish the suitable quantities of food that each athlete should ingest.

However, most nutraceutical compounds cannot be added into foods or beverages in their pure form. This is where the recent developments in nanotechnology comes into play, as the use of nanostructured delivery systems for nutraceuticals in sports drinks will allow a better control over the structure matrix, the release time and local of release and even the dosage of bioactive contents, without negative modifications in aspect or flavor of the beverage. This may even allow for creation of personalized sports drinks which take individual needs of the athlete based in type of sport, time of duration, and age and sex of the athlete into account, which could be helpful in preventing and/or decreasing the main problems associated with specific types of exercise.

There are many challenges that science has not dealt with regarding a nutritional approach to immune modulation in athletes, and hence there is need for further investigations. Future materialization of nanotechnology used in functional food and beverages for athletes will allow for personalized nutrition conferring higher immune protection and ultimately increasing performance.

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FUNCTIONAL FOOD INGREDIENTS AND NUTRACEUTICALS, MILK PROTEINS AS NUTRACEUTICALS NANOSCIENCE AND FOOD INDUSTRY

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1 Introduction

Nutraceutical foods have shown a sustainable and upward trend gaining popularity with health-conscious consumers. Nutraceutical foods typically contain a small amount of nutraceutical ingredient(s) with specific and scientifically documented health benefits in humans such as improved cardiovascular status. Although nutraceutical ingredients such as omega-3 oil, vitamins, antioxidants, and fiber are typically added at low concentrations, these concentrations are physiologically meaningful and trigger beneficial health effects in humans (Matak et al., 2015). The European Nutraceutical Association (www.enaonline.org) defines nutraceutical as nutritional products that have effects that are relevant to health. In contrast to pharmaceuticals, however, these are not synthetic substances or chemical compounds formulated for specific indications. These are products that contain nutrients (partly in concentrated form) and are assigned to the category of food (Gonzalez-Sarrias et al., 2013). The effectiveness of nutraceutical products in preventing diseases depends on preserving the bioavailability of the active ingredients (Chen et al., 2006).

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Reducing the particle size may improve the bioavailability, delivery properties, and solubility of the nutraceuticals due to more surface area per unit volume and thus their biological activity. The bioavailabilities of these nutraceuticals are supposed to increase as a nanocarrier allows them to enter the bloodstream from the gut more easily (Lee, 2015).

Many milk-derived peptides reveal multifunctional bioactivities and can be produced on an industrial scale, and have already been considered for application both as dietary supplements in functional food and as drugs. Hence, these peptides are claimed to be health-enhancing nutraceuticals for food and pharmaceutical preparations (Meisel, 1997).

Phytochemicals, which are food supplements with health benefits, are commonly used as part of the daily diet. Because of their low solubility, many phytochemicals are poorly absorbed by the human body; thus one of the most important and interesting applications for encapsulation of phytochemicals is to enhance the bioavailability of phytochemicals by changing the pharmacokinetics (PK) and biodistribution (BD). To enhance nutritional quality and stability of the functional foods, one option is to encapsulate the functional ingredients using food grade or generally recognized as safe (GRAS) materials that can exhibit controlled-release behavior (Huang et al., 2010).

Nanotechnologies may provide new ways and tools for controlling properties and structuring foods, introducing new features, and creating added value. The phenomena that take place at the nanometer scale offer lots of opportunities for innovation that have the potential to impact substantially the food industry worldwide, as nanotechnologies can be applied to the whole food chain, from production to processing, safety, packaging, transportation, storage, and delivery (Rossi et al., 2014). Nanotechnology is the ability to understand, control, and manipulate matter at the level of individual atoms and molecules, as well as at the supramolecular level, involving clusters of molecules (in the range of about 0.1–100 nm), in order to create materials, devices, and systems with new properties and functions because of their small structure (Mura et al., 2014). Some of the nanoencapsulated hydrophilic nutraceuticals are ascorbic acid, polyphenols, and nanoencapsulated lipophilic nutraceuticals include lycopene, β-carotene, lutein, phytosterols, and DHA (docosahexaenoic acid). Hydrophilic compounds show faster release rates and their release kinetics is determined by the appropriate combination of diffusion and erosion mechanisms. Lipophilic compounds often resulted in incomplete release due to poor solubility and low dissolution rates by an erosion mechanism.

Although lipophilic compounds are highly permeable through the intestine via active transport and facilitated diffusion, hydrophilic compounds have low permeability that are absorbed only by active transport mechanism (Lee, 2015). The main lipid-based nanoencapsulation systems that can potentially be used in food and food supplements are nanoliposomes, archaeosomes and nanocochleates. Natural polymers that are used are albumin (protein), gelatin (protein), alginate (saccharide), collagen (protein), chitosan (saccharide), and the milk protein α-lactalbumin (Garcia et al., 2010). A starch-like nanoparticle can also help to stop lipids from oxidizing and therefore improve the stability of oil-in-water emulsions. The health benefits of anticancer compound curcumin, the natural pigment that gives the spice turmeric its yellow color, could be enhanced by encapsulation in nanoemulsions (Lee, 2015). This review will focus on: (1) nanodelivery systems, (2) milk proteins with nanotechnologic applications, and (3) nanoemulsion-encapsulated phytochemicals.

2 Nanodelivery Systems

Bioactive peptides have been identified in various food proteins such as caseins, soy, canola, and are finding great applications in the development of nutrition tailored functional foods (Phelan et al., 2009; Wang and de Mejia, 2005). However, such applications are limited due to the lack of appropriate delivery systems capable of protecting bioactive peptides from degradation (eg, conformational changes and denaturation) during processing as well as during administration because the structure and functionality of food proteins/peptide are positively correlated. In addition, the majority of bioactive peptides are not absorbed from the gastrointestinal tract into the blood, possibly due to poor delivery systems, although their effects have been proposed to be mediated directly in the gut through receptors on the intestinal walls (Korhonen and Pihlanto, 2006; Lee et al., 2007; Phelan et al., 2009).

A suitable delivery system should protect bioactive peptides from interactions with other food components, stabilize the peptides during processing and administration, and should also enhance their absorption and transport across the intestinal mucosa to target sites (Balcao et al., 2013; Prego et al., 2006; Yang and McClements, 2013). Nanodelivery systems [eg, nanoencapsulation, nanoemulsion, nanovessicles (liposomes)] present a mechanism for the structural and functional stabilisation of bioactive proteins/peptides against denaturation by enzymatic digestion and a way to increase their biopharmaceutical and food applications (Balcao et al., 2013; Prego et al., 2006) (Fig. 18.1.). These

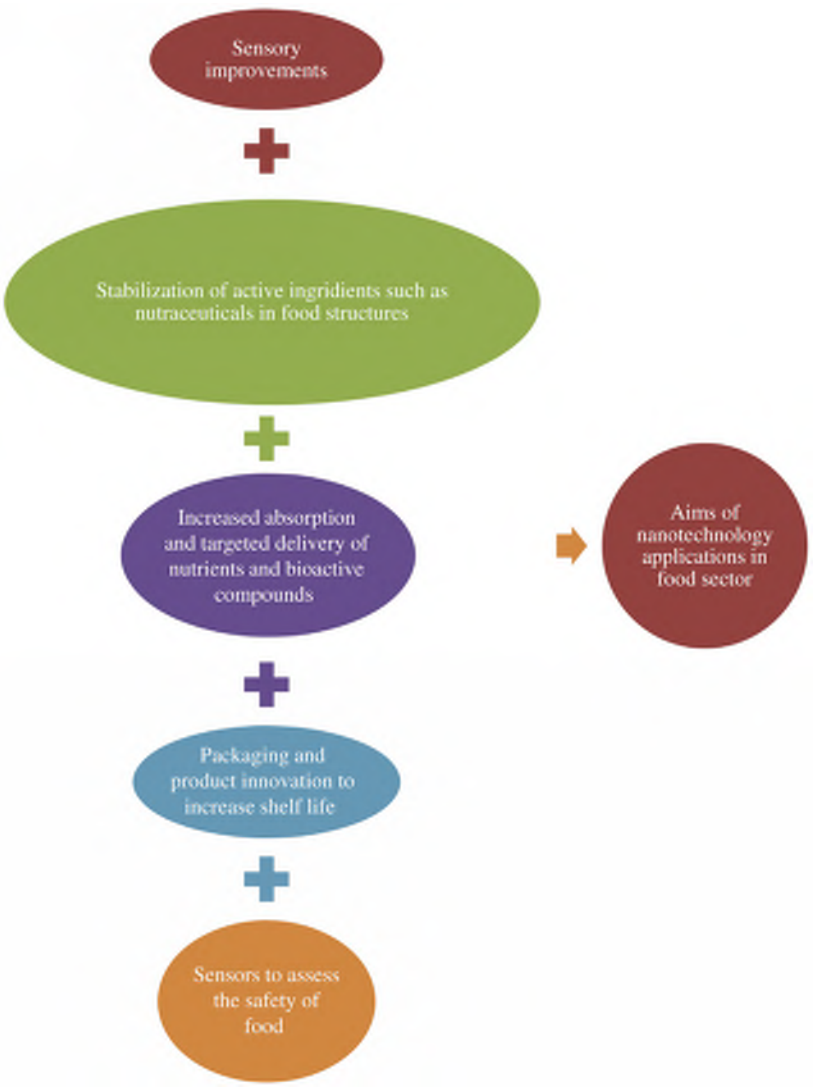

Figure 18.1. Aims of nanotechnology applications in food sector.

delivery systems typically consist of micronutrients trapped within nanoparticles ($r < 500$ nm) that may be fabricated from surfactants, lipids, proteins, and/or carbohydrates. The small size of the particles in these systems has a number of advantages over conventional delivery systems: higher stability to aggregation and gravitational separation; higher optical clarity; and improved bioavailability (Joye et al., 2014). In principle, protein nanoparticles are biodegradable, nonantigenic, metabolizable and easily

modifiable for surface alterations and the covalent attachment of other molecules. All of these characteristics make these protein-derived materials suitable for use as carriers for bioactive compounds found in foods, such as peptides, vitamins, and antioxidants. Furthermore, nanoparticles may improve the water solubility, thermal stability and oral availability of a number of compounds (Arroyo-Maya et al., 2014).

2.1 Nanoencapsulation

The application of nanotechnology to the food industry involves several benefits, such as products with new properties (less sugars and fats, new textures, colors, nutrients, flavors, and tastes), improved food security, processing, and traceability, new packaging and products having extended shelf life and improved microbial food safety (Garcia et al., 2010; Mura et al., 2014). In general, the physicochemical properties suchas particle size, size distribution, surface area, shape, solubility, encapsulation efficiency,and releasing mechanisms were reported to be altered by the encapsulation technique anddelivery system (Lee, 2015). An important class of nanoparticles for application within food are nanodelivery systems. When used as a food additive or supplement, the delivery systems are commonly builtfrom natural biopolymers of nanosize scale such as peptides, polysaccharides, or lipid monomers (Garcia et al., 2010).

Two building strategies are currently used in nanotechnology: (1) the top-down approach, in which nanolevel structures are generated by breaking up bulk materials, using milling, nanolithography, or precision engineering; and (2) the newer bottom-up approach, which allows nanostructures to be built from individual atoms or molecules that are capable of self-assembling (Garcia et al., 2010). Mechanical approaches (top down) are capable of producing nanoparticles, typically in the 100–1000 nm range, whereas chemical methods (bottom up) tend to produce 10–100 nm particles (Acosta, 2009). A top-down approach such as emulsification and emulsification-solvent evaporation involves the application of precise tools that allow size reduction and structure shaping for desired application of the nanomaterials being developed. In the bottom-up approach such as the supercritical fluid technique, inclusion complexation, coacervation and nanoprecipitation, materials are constructed by self-assembly and self-organization of molecules (Lee, 2015).

2.1.1 Top-Down Approaches

Top-down approaches are produced through different size reduction (mechanical) processes. The potential advantage of

mechanical processes over chemical processes is that mechanical processes require minimal use of chemical additives, which mitigates the concerns regarding the regulations imposed on such formulation ingredients. There are two types of mechanical process-mills for the nanonization (asopposed to micronization) of solid particles, and microfluidic processes for the nanonization of liquids or melts. There are challenges in the production of nanoparticle systems with mechanical processes. These challenges include creating high energy density bursts to break down the particle, preventing the reaggregation of the particles, and segregating large and small particles. In the case of mill processes, the first challenge is typically met by shearing or colliding either the particles onto themselves (jet mill) or using other small and hard particles as grinding media (bead and ball mills) (Acosta, 2009).

Ball mill processes have been used for the production of iron nanoparticles in aqueous suspension. The smallest particle size attainable with ball mill technology is close to 20 nm, but this is only achievable if the particle is produced by the precipitation of the solid from a supersaturated solution (Peukert et al., 2005; Todaka et al., 2003). The bead mill process, on the other hand, is capable of producing nanoparticles as small as 20 nm from micrometer size crystalline drugs. Due to the relative simplicity and the ability to process a wide range of materials, this process is considered to be one of the most promising milling methods for the production of solid nanoparticles (Inkyo et al., 2006).

Microfluidization is an established technology in food processing, specially for dairy products (Tunick et al., 2002; Olson et al., 2003) and it has been used in the production of submicron liposomes for the delivery of ferrous sulfate, ascorbic acid and for the delivery of other poorly absorbed hydrophilic compounds (Vuillemard, 1991; Kosaraju et al., 2006). Microfluidization (colloid mills) processes and related liquid-based technologies use the flow-induced shear of liquids, hot melts and other soft aggregates to produce or maintain nanosized dispersion of the processed material. Such flow-induced shear is typically obtained by inducing large pressure drops across small nozzles. Colloid mills also face the challenge of stabilizing the product against coalescence (Tunick et al., 2002; Olson et al., 2003). Microfluidization is also an important technique for encapsulating probiotic cultures (Feijoo et al., 1997). Furthermore, microfluidization constitutes the basis for the production of solid lipid nanoparticles (Liedtke et al., 2000). Chen and Wagner (2004) used microfluidization to produce100 nm vitamin E nanoparticles stabilized by a starch coating, suitable for fortified beverages. Tan and Nakajima (2005) prepared 60–140 nm β-carotene nanodispersions by

microfluidization–emulsification followed by solvent evaporation. The methods of inducing the precipitation/solidificationinto a disperse state with mechanical shear include: the spray freezing into a cryogenic liquid, atmospheric freeze drying, and the spinning disk processing (SDP) method (Anantachoke et al., 2006). SDP, which has a jet, is impinged onto a heated rotating disk. The centrifugal force breaks down the jet into small particles and the heat transferred from the rotating disk into the liquid induces a fast evaporation of the solvent, leaving behind a fine mist of particles. SDP has been used to generate 40–100 nm β-carotene particles stabilized by polyglycerol esters of fatty acids (Chen et al., 2006; Sanguansri and Augustin, 2006).

According to Gil-Ch́avez et al. (2013), ultrasound technology is one of the other encapsulation techniques. Ultrasound-assisted extraction (UAE) has been credited with more high recovery of targeted compounds with lower solvent consumption and/or faster analysis and bioactivity properties than conventional solvent extraction. Its better extraction efficiency is related to the phenomenon called acoustic cavitation. Some researchers stated that adequacy of ultrasound intensity is provided which the expansion cycle can create cavities or microbubbles in the liquid. Once formed, bubbles will absorb the energy from the sound waves and grow during the expansion cycles and recompress during the compression cycle. Further, bubbles may start another rarefaction cycle or collapse leading shock waves of extreme conditions of pressure and temperature (several hundred atmospheres and around 5000 K of temperature). Nowadays, UAE is extensively used for the extraction of proteins, sugars, polysaccharides-protein complex, oil, as valuable molecules (Gil-Ch́avez et al., 2013). However some researchers showed that extraction variables, extraction time, temperature, and frequency influenced strongly antioxidant capacity and profiles (Chukwumah et al., 2009; Ghafoor et al., 2009; Hossain et al., 2011, Gil-Ch́avez et al., 2013). Therefore UAE should be carefullyused in the extraction of unstable compounds as carotenoidssince a major degradation has been observed compared with other technologies (Gil-Ch́avez et al., 2013).

2.1.2 Bottom-Up Approaches

The term *chemical processes* refers to those methods of nanoparticle preparation where either chemical reactions and/or the self-assembly of surfactant and polymers are the primary drivers of the process. There are typically five components involved in chemical methods: the solute of interest, an internal (dispersed) solvent, an external solvent (typically water), a surfactant that is dispersed in the external solvent(s), and in some cases a polymer

that is soluble in the internal solvent, but insoluble in the external one (Acosta, 2009).

In all these chemical processes there are two important objectives that are undermentioned: (1) to find a quick way to produce a solid network that will make up the body of the nanoparticle, and (2) the objective is to protect that the nanoparticle from agglomeration using surfactants or other emulsifiers.

In most of the cases reviewed earlier, the solid network is produced by a polymer such as polylactic acid, poly-(lactic-*co*-glycolic) acid or a similar polymer, and the hydrophobic drug (eg, β-carotene) is deposited as a small nanosized crystal aggregate in the particle. In such cases, the solubility of the crystal-forming active ingredients could be further improved if the active ingredient is dissolved as a solid solution in a solid lipid matrix (Kipp, 2004).

The chemical methods of producing organic nanoparticles classified according to the nature of the internal solvent. There are three types of internal solvents: a lipophilic solvent, an amphiphilic solvent and a hydrophilic solvent. The lipophilic solvent method is basically a process of emulsification/homogenization where the presence of a surfactant and/or polymers reduces the energy required for emulsification (by reducing the interfacial tension) and protects the nanodroplets against coalescence (Freitas and Müller, 1998; Harivardhan and Murthy, 2005). The amphiphilic solvent method consists of dissolving the solute in a polar organic (internal) solvent such as acetone or methylene chloride (containing a predissolved lipophilicpolymer), and mixing this system with an aqueous solution containing a surfactant or hydrocolloids. This method has been recently used to produce 20–80 nm β-carotene nanoparticles using acetone as the amphiphilic solvent, poly-(lacticco-glycolic) acid as stabilizing polymer, and either Tween 20 or gelatin as emulsifier (Ribeiro et al., 2008). The hydrophilic (internal) solvent method involves the use of water-soluble alcohols as the internal solvent. In this case, the organic solute and a stabilizing polymer are dissolved in alcohol, and upon mixing with an aqueous solution containing the emulsifier, nanodroplets are spontaneously formed. In recent years, there is growing interest in using these reactive methods to transform globulin proteins and casein micelles in nanocapsules for the delivery of hydrophobic nutraceuticals and hydrophilic minerals (Chen et al., 2006; Semo et al., 2007). The external solvent is later evaporated through spray drying or lyophilization technologies. Solid lipid nanoparticles (SLN) are prepared using this lipophilic solvent method (Freitas and Müller, 1998; Harivardhan and Murthy, 2005), which has received more attention due to their ease of preparation and for being amenable to a wide range of active ingredients. There are various ways to produce

SLN; however, the simplest method involves melting a lipid matrix (eg, saturated fatty acids), and dissolving the hydrophobic active ingredient in this hot melt. This hot melt is then emulsified in an oil-in-water nanoemulsion produced by either microfluidization (Üner, 2006; Müller et al., 2000). Recently, microemulsion-based solid nanoparticles have been formulated for the controlled release of tea polyphenols (Ma et al., 2007; Kristl et al., 2003).

2.2 Nanoemulsions

Nanoemulsions have small droplet size that allows for efficient delivery, accelerated release, and rapid absorption of hydrophobic bioactive drug and food agents such as vitamin E, omega 3 fatty acids, flavonoids, and various phyto-polyphenolic compounds. Nanoemulsions are a technology that has food and pharmaceutical applications, and their evolution has paralleled the development of efficient emulsification technologies. An efficient emulsification process is able to form emulsions with small droplet sizes and narrow size distributions. These characteristics are, however, a function of the two opposing forces: droplet breakup and droplet–droplet coalescence. These properties have been identified in several works as being dependent upon several processes including: homogenizing mechanism, type, concentration, and interfacial properties of surfactant/emulsifier, dispersed phase volume/mass fraction and viscosity, time scale of surfactant adsorption onto the surfaces of newly created droplet, frequency and timescale of inter droplet–droplet collision (Adjonu et al., 2014a).

2.3 Liposomes

Liposomes are spherical bilayer membrane structures with aqueous cores, so unlike lipophilic-containing micro emulsions, they can be used to contain and deliver hydrophilic, or water-soluble, ingredients. Moreover, their internal pH is adjustable, so they can contain ingredients that otherwise would not be stable under certain circumstances. As with microemulsions, there is a lot of engineering that can be done and different materials that can be used, leading to a range of differently shaped and sized final products. For example, depending on how the phospho lipids base materials are put together, one could form either multiple vesicular structures or single onion-shaped vesicles. Antioxidant peptides have been extensively examined as potential biopreservatives in food recent technology. However, stability issues like proteolytic degradation and the potential interactionof peptide with food components might result in a decrease in their activity (Da Silva Malheiros et al., 2010). The great advantage of nanoliposomes

over other encapsulation technologies (spray-drying, extrusion, fluidized beds) is their stability (Desai and Park, 2005). Liposome entrapment has been shown to stabilize the encapsulated materials against a range of environmental and chemical changes, including enzymatic and chemical modification, as well as buffering against extreme pH and temperature (Taylor et al., 2005). The ability of nanoliposomes to trap water-soluble substances has been employed in various pharmaceutical and cosmetic applications to protect and control the release of active compounds. Thus, the entrapment of antioxidants into nanoliposomes might represent an alternative to overcome some problems related to the direct application of bacteriocins in food, such as proteolytic degradation or interaction with food components (Mozafari et al., 2008). Another significant advantage of liposome is that it can incorporate and simultaneously release two materials with different solubilities. One example of this is the incorporation of two antioxidant agents namely alpha-tocopherol (a lipid-soluble molecule) and glutathione (a water-soluble molecule) in the same lipid vesicle (Mozafari and Mortazavi, 2005). Encapsulation of antioxidants into nanoliposomes is mainly reported to be achieved by thin film hydration method, and phosphatidyl choline is the most common phospholipid employed in liposome manufacture (Da Silva Malheiros et al., 2010). Liposome encapsulated curcumin has shown enhanced bioavailability and inhibition to pancreatic and colorectal cancer in vitro and in vivo (Li et al., 2007).

Maherani et al. (2012) have successfully encapsulated these natural antioxidant peptides by nanoliposomes to overcome the limitations related to the direct application in food. Bio Delivery Sciences International has developed Bioral nanocochleate nutrient delivery system, which is a phosphatidyl serine carrier (–50 nm), derived from soya bean (GRAS status). This system has been used for protecting micronutrients and antioxidants from degradation during manufacture and storage (Chaudhry and Groves, 2010). The undesirable tastes of functional ingredients can be minimized in the finished food product if they are carried in nanostructured delivery systems. For example, the application is the entrapment of proteolytic enzymes in liposomes for cheese production (Mozafari et al., 2006; Walde and Ichikawa, 2001), thus reducing the production time to half without losing flavor and texture properties.

3 Milk Proteins

Milk proteins with natural bioactive vehicles have structural and physicochemical properties. These properties include binding of ions and small molecules, excellent surface and self-assembly

properties; superb gelation properties; pH-responsive gel swelling behavior, useful for programmable release; interactions with other macromolecules to form complexes and conjugates with synergistic combinations of properties; various shielding capabilities, essential for protecting sensitive payload; biocompatibility and biodegradability, enabling to control the bioaccessibility of the bioactive, and promote its bioavailability (Livney, 2010). Milk proteins are currently the main source of a range of biologically active peptides (Table 18.1). Concentrates of these peptides are potential health-enhancing nutraceuticals for food and pharmaceutical applications. Several bioactive peptides may be used as nutraceuticals, for example, in the treatment of diarrhea, hypertension, thrombosis, dental diseases, as well as mineral malabsorption, and immunodeficiency (Severin and Wenshui, 2005). Several milk proteins possess important functional properties, including their ability to bind hydrophobic molecules, interact with other biopolymers, stabilize emulsions, form gels, and to some extent retard oxidation, allowing them to serve as excellent materials for the encapsulation and delivery of bioactive compounds (Table 18.2). Particularly, whey proteins are widely used in formulated foods because they have high nutritional value and excellent functional properties (Kontopidis et al., 2004). Dairy proteins have been extensively used as emulsifiers in foods as they adsorb to the oil droplet interface, forming a strong and cohesive protective film that helps prevent droplet aggregation (Lee and McClements, 2010). Proteins hold promise as building blocks for self-assembled systems because of their exquisite three-dimensional structures and evolutionarily fine-tuned functions (Rajagopal and Schneider, 2004). Recently, water-insoluble hydrogels based on proteins were successfully applied as matrix material for the microencapsulation of probiotic cells, as a promising alternative to polysaccharide hydrogels such as alginate. Protective effects against adverse conditions, especially under low pH-gastric conditions were explained by the buffering capacity of the dense protein matrix (Heidebach et al., 2010). The self-assembled protein and peptide nanostructures can be further organized to form various nanowiresi, nanotubes, and nanoparticles via their molecular-recognition functions (Esmaeilzadeh et al., 2013).

3.1 Casein and Casein Micelles

Casein, which accounts for about 80% of milk protein, is organized in micelles. Proteins such as caseins, whey proteins, and soy proteins are large complex amphipathic molecules containing combinations of ionic, polar, and nonpolar regions; thus, they are

Table 18.1 Nanotechnology Applications of Milk Proteins

Milk Proteins	Nanospheres/ Particles 	Core-Shell Nanostructures/ Emulsions/Multiple Emulsions 	Nanotubes 	Nanocomposite Films
Casein micelles	Ma et al. (2013); Nakagawa and Kagemoto (2013)	Semo et al. (2007); Roach et al. (2009)		
Caseinate	Day et al. (2007); Patten et al. (2009); Nielsen and Jacobsen (2009); Bonnet et al. (2009); Ai and Wei (2008); Burgar et al. (2009)	Sugiarto et al. (2009); Chu et al. (2007)		
β-Casein		Shapira et al. (2010); Esmaili et al. (2011)		
Whey proteins	Sugiarto et al. (2009); Rodríguez et al. (2015)	Benichou et al. (2007); Lee and McClements (2010); Shah et al., 2012; Ach et.al. (2015)		Zolfi et al. (2014)
β-Lactoglobulin	Hong and McClements (2007); Sandra et al. (2008)	Zimet and Livney (2009); Gunasekaran et al. (2007); Pérez et al. (2014); Ach et.al. (2015)		
α-Lactalbumin		Eren et al. (2015); Ach et.al. (2015)	Graveland-Bikker and De Kruif (2006); Ipsen and Otte (2003)	
Bovine serum albumin		Rodrigues et al. (2009); Li et al. (2008); Maghsoudi et al. (2008); Crisante et al. (2009); Jahanshahi et al. (2008); Yang et al. (2007); Yang et al. (2008)		

Table 18.2 Different Application of Nanotechnology in Milk Proteins with Bioactive Properties

Properties	Milk Proteins	Application	References
Chitosan	β-Lg[a]	Complex coacervation	Guzey and McClements (2006)
Green tea, epigallocatechin, gallate	β-Lg	Nanoemulsions	Shpigelman et al. (2010)
Green tea, polyphenols	β-Lg	Nanoemulsions	Von Staszewski et al. (2014)
pectin	β-Lg	Complex coacervation	Girard et al. (2003a,b, 2004)
Acacia gum	β-Lg	Complex coacervation	Schmitt et al. (2001); Mekhloufi et al. (2005); Sanchez et al. (2006)
Sodium alginate	β-Lg	Complex Coacervation	Harnsilawata et al. (2006)
Folic acid	β-Lg	Nanoemulsions	Pérez et al. (2014)
Lysozyme	β-Lg	complex Coacervation	Howell et al. (1995)
Lf	β-Lg	Nanocomposite films	Tokle et al., 2012
Naringin	β-Lg	Nanoemulsions	Shpigelman et al. (2014)
Tangeretin	β-Lg	Nanoparticles	Chen et al. (2014)
Resveratrol	β-Lg	Nanoemulsion	Liang et al. (2008)
Kefiran	WPI	Nanocomposite films	Zolfi et al. (2014)
Acacia gum	Whey protein	Complex coacervation	Weinbreck et al. (2003a,b); Weinbreck et al. (2004a,b); Weinbreck and Wientjes (2004)
B-Carotene	WPI hydrolysates	Nanoemulsions	Chu et al. (2007)
Carrageenan	Whey protein	Complex coacervation	Weinbreck et al. (2004c)
Fish oil	WPI	Nanoemulsion	Serfert et al. (2014)
β-Carotene	WPC	Nanoemulsions	Chu et al. (2007)
Curcumin	β Casein	Nanoemulsion	Esmaili et al. (2011)
β-carotene	Casein-grafted dextran	Nanoemulsion	Pan et al. (2007)
DHA	Caseinat complex	Nanoemulsion	Semo et al. (2007)
Vitamins	α-lactalbumin	Nanotubes, nanoencapsulation	Graveland-Bikker and De Kruif (2006), Srinivas et al. (2010)

[a] β-Lg, β-Lactoglobulin; WPI, whey protein isolate; WPC: whey protein concentrate

surface-active and strongly interact with other food compounds (Dickinson, 1999). The interfacial membranes formed by proteins are electrically charged; hence, the major mechanism preventing particle aggregation in protein-stabilized dispersions is electrostatic repulsion (McClements, 2005). Casein micelles (CM) are designed by nature to concentrate, stabilize, and transport essential nutrients, mainly calcium and protein, for the neonate (De Kruif and Holt, 2003). A CM is, in effect, a natural nanodelivery system. Casein micelles contain ~3.4 g of H_2O per gram dry matter (Morris et al., 2000) and the dry matter consists of ~93% protein and ~7% inorganic material, collectively referred to as micellar calcium phosphate (MCP) (De Kruif and Holt, 2003). According to some researchs, MCP is present in the form of socalled nanoclusters (De Kruif and Holt, 2003; De Kruif (Kees) and Huppertz, 2012).

Due to supersaturation of calciumphosphate in the mammary gland (Neville, 2005), nanometer-sized clusters of amorphous calcium phosphate are formed, the growth of which is stopped by absorption of the centers of phosphorylation of αs1-, αs2-, and β-casein onto the surface of the calciumphosphate nanoclusters. These thermodynamically stable nanoclusters have a radius of ~9 nm and consist of a core (~2 nm) of amorphous calcium phosphate and a shell of caseins (Smyth et al., 2004; Little and Holt, 2004). The more hydrophobic parts of the casein protrude from the surface of the nanoclusters and self-associate into larger structures (De Kruif and Holt, 2003). The association process is terminated when the surface of the particle becomes hydrophilic, through the adsorption of κ-casein and β-casein, which have hydrophilic C-termini that protrude into the surrounding solvent, stabilizing casein micelles as a polyelectrolyte brush (De Kruif and Zhulina, 1996).

Casein micelles in milk are the delivery vehicle to, the neonate for high levels of calcium and phosphate, required for its bone growth. Approximately 70% of bone and ~90% of tooth enamel of the rapidly growing neonate consists of calcium phosphate, which is in the hydroxyapatite form ($Ca_5(PO_4)_3OH$). For bone and tooth mineralization, the neonate depends solely on calcium and phosphate from milk; for bovine milk the levels are~30 mmol L^{-1} calcium and ~20 mmol L^{-1} inorganic phosphate (De Kruif (Kees) and Huppertz, 2012). Like most other calcium phosphates, hydroxyapatiteis only sparingly soluble in water, having a solubility product of 2.34×10^{-59}, which corresponds to a solubility of~0.4 mmol L^{-1}. Even though the solubility of calciumphosphates is slightly higher in milk serum than in water (Chaplin, 1984), this concentration is still far too low to sustain the demands of the neonate. To solve this problem, and prevent pathological calcification of

the mammary gland, the transport of calciumphosphate in milk is in an encapsulated yet bioavailable form, that is, casein micelles (Neville, 2005).

Nanovehicles may improve the bioavailability of oil-soluble nutraceuticals, thanks to their nanoscopic size and immense numbers per unit mass. Another important potential benefit is the minimization of adverse effects on sensorial characteristics such as transparency of clear food systems like certain beverages. Lastly, the entrapment and encapsulation may provide protection against degradation of the nutraceuticals by oxidation and other chemical and enzymatic reactions during production and shelf life, thus preventing development of undesired flavors and odors, and loss of metabolic value. In vitro, live animal and human trials suggest that ω-3 fatty acids have beneficial effects on human health, including the prevention and sometimes curing of cognitive disorders, cardiac and circulatory disorders, immune dysfunction, and inflammatory disorders (Ruxton et al., 2004; Ruxton et al., 2005; Shahidi and Miraliakbari, 2005). However, the enrichment of nonfat foods or beverages with these fatty acids encounters great difficulties. Because, they are very hydrophobic, and practically insoluble in water, even in the free fatty acid form. The apparent pKa of docosahexaenoicacid (DHA) is 8.5, and in dilute basic aqueous systems at room temperature, it self-assembles into micelles forming transparent systems. However, a decrease of the pH causes DHA to aggregate in the forms of vesicles and oil droplets (turbid systems), which finally leads to macroscopic phase separation (Namani et al., 2007). Moreover, and more challenging difficulty is the high sensitivity of these highly unsaturated fatty acids to oxidative degradation, resulting in off-flavors and odors, and negating its health benefits. Factors affecting the oxidation rates include fatty acid composition, storage conditions, presence of oxygen, prooxidants, and antioxidants, and the physical state of the lipid (Yoshii et al., 2002). A recent study by Zimet et al. (2011) reported about two systems based on casein (casein nanostructures and reformed casein micelles) that showed markable protective effect against docosahexaenoic acidoxidation. These systems proved to have a good colloidal stability and to preserve the effect of their functional ingredient up to 37 days at 4°C.

3.2 Whey Proteins

Whey proteins (α-lactalbumin, β-lactoglobulin, bovine serum albumin, lactoferrins, and immunoglobulins) constitute about 20% of the total protein in milk (~80% caseins) and have a high nutritional value owing to their high essential amino acid content.

They are valued as important emulsifiers in food due to their amphiphilic properties (possessing both hydrophobic and hydrophilic residues) (Adjonu et al., 2014b).

Whey proteins possess globular/rigid structures with buried hydrophobic residues which tend to negatively affect their functionality (Gauthier and Pouliot, 2003). Controlled enzymatic hydrolysis of whey proteins produces peptides that are smaller, possess fewer secondary and tertiary structures, and have a partially exposed hydrophobic core (Christiansen et al., 2004; Gauthier and Pouliot, 2003; Tirok et al., 2001). These characteristics account for their higher rate of diffusion to the oil/water interface and their ability to cover a larger area of the interface than the intact protein (Davis et al., 2005; O'Regan and Mulvihill, 2010). Their amphiphilic nature allows them to adsorb onto the surfaces of oil droplets (Tirok et al., 2001), and stabilize the newly created emulsion droplets against destabilisation (Van der Ven et al., 2001). The most important functional properties of whey proteins are solubility, viscosity, gel formation, emulsification, foaming, and like many other albumins, the capacity to form nanoparticles and could obtain nanoparticles of bovine α-lactalbumin. α-lactalbumin products range from 100 to 200 nm in size, so they could be used as carriers of bioactive compounds (Esmaeilzadeh et al., 2013).

Whey protein as a by-product of cheese making is a very well known material to form biodegradable films. Whey proteins have already widespread uses in food products because of their high-nutritional value and suitable ability to form gels, emulsions and foams. This functionality enables whey proteins to be good candidates for biodegradable films (Zhou et al., 2009). Although whey-protein films can act as an excellent oxygen barrier at low or intermediate relative humidity, these films without any modification have high water vapor permeability due to the high proportion of hydrophilic amino acids in their structures and relatively low mechanical properties (Osés et al., 2009). Unfortunately, so far the use of films based on proteins or polysaccharides for food packaging has been strongly limited because of the poor barrier and weak mechanical properties. Different physical and chemical modifications including biopolymers blending (Kristo et al., 2007), addition of plasticizing agents (Ghanbarzadeh and Almasi, 2011), and nanofillers addition have been applied to improve the properties of biodegradable films. Zolfi et al. (2014) developed biodegradable kefiran-whey protein isolate-titanium dioxide (TiO_2) blend films. They showed that the water vapor permeability, moisture content, moisture absorption, and water solubility decreased by increasing the nano-TiO_2 content. Moreover embedding lycopene into whey protein matrices enhanced its bioavailability to that equal

to tomato paste, the most valuable food source of bioavailable lycopene for humans (Parada and Aguilera, 2007). Sugiarto et al. (2009) researched that determining systematicallythe binding of iron to sodium caseinate and whey protein isolate (WPI), and on determining how this binding is affected by changes in pH and iron concentration. They found that the amount of iron bound to sodium caseinate is independent of pH in the range 5.5–7.0, but similar changes in pH decreases the ability of whey proteins to bind iron.

3.3 β-Lactoglobulin

Beta-lactoglobulin (β-Lg) (18.4 kDa, 162 amino acid residues), the major whey protein, auilera belongs to the lipocalin protein-family, many of whose members are known to bind small hydrophobic molecules (Kontopidis et al., 2004). It has recently been shown to present some antioxidant activity, apparently thanks to its free thiol group (Liu et al., 2007). β-Lg folds up into an eight-stranded, antiparallel β-barrel with a three-turn α-helix on the outer surface and a ninth β-strand flanking the first strand (Brownlow et al., 1997). The secondary structure of bovine β-Lg consists of an eight-stranded, flattened β-barrel, which folds into a calyx, and a flanking three-turn α-helix. In its native state two intramolecular disulphide bridges are formed between four of the five Cysteine (Cys/C) residues, whereby Cys at the position 121 (Cys121) represents a free sulfhydryl group (Hoffmann and van Mil, 1997). The tertiary structure of the β-Lg monomer is sensitive to unfolding at temperatures above 60°C, leading to the exposure of interior hydrophobic residues and the free sulfhydryl group of Cys121 (Manderson et al., 1998). Shpigelman et al. (2010) have previously studied the binding of the green tea epigallocatechin gallate (EGCG) and they found that the binding of EGCG to partially heat denatured β-Lg was significantly stronger than the binding to native β-Lg. Physiological pH β-Lg is mostly found as dimers, and at pH values below 3.5 and above 7.5 the protein tends to be monomeric. The solvent-accessible conical β-barrel, or calyx, forms the main ligand binding site, although there are indications for the existence of a second ligand binding site in a crevice near the α-helix on the external surface of the β-barrel (Kontopidis et al., 2004). A possible third binding site was suggested to be located at the dimer interface (Harvey et al., 2007). Since the finding of the retinol-β-Lg complex, many other hydrophobic ligands have been observed to bind to β-Lg, such as retinoic acid (Lange et al., 1998; Lynen et al., 2003; Narayan and Berliner, 1997), cholesterol (Wang et al., 1997), vitamin D (Wang et al., 1997; Wang et al., 1999), and

fatty acids (Narayan and Berliner, 1997; Perez and Calvo, 1995; Spector and Fletcher, 1970). Among the fatty acids found to bind to β-Lg were palmitate (Spector and Fletcher, 1970; Wang et al., 1999), stearate, oleate, laurate (Spector and Fletcher, 1970), myristic acid, and conjugated linoleic acid (Considine et al., 2007). Watanabe and Klostermeyer (1976) found that heating β-Lg leads to the formation of soluble high molecular weight polymers. Sulfhydryl-initiated disulfide exchange reactions play an important role in the polymerization process. It is known that heating whey proteins, under careful control of the initial solution conditions (pH, mineral salt content, and protein concentration) and heating conditions (temperature and holding time), can produce aggregates or polymers that do not gel.

Zimet and Livney (2009) researched that complex formation with the polysaccharide may be utilized for providing additional protection and stability for the sensitive nutraceutical bound to β-Lg. Electrostatic interactions between proteins and polysaccharides may lead to complex coacervation and sedimentation, or to formation of soluble complexes, depending on various factors including the type of polysaccharide used (anionic/cationic), pH, ionic strength, and the ratio of polysaccharide toprotein (Grinberg and Tolstoguzov, 1997; De Kruif et al., 2004; Schmitt et al., 1998). Depending on the pH of the target food system, different polysaccharides can be used to create these complexes. Several previous studies have focused on β-Lg-pectin interactions and complex formation and it has been found that interactions between β-Lg and pectin mainly occur through ionic and hydrogen bonds (Girard et al., 2002; Girard et al., 2003a). In addition, it has been shown that the maximum amounts of protein were complexed with low molecular weight pectin at 4.5pH, value that falls between the pI of β-Lg and the pKa of pectin (Girard et al., 2002).

Complex coacervation has been widely used industrially as a means of encapsulating functional components, including oils, flavors, drugs, cosmetics, pesticides, live cells, and vaccines (Cooper et al., 2005; De Kruif et al., 2004; Renard et al., 2002; Madene et al., 2006) there have been studies of complex coacervation between β-Lg and acacia gum (Schmitt et al., 2001; Mekhloufi et al., 2005; Sanchez et al., 2006), between β-Lg and pectin (Girard et al., 2003a,b, 2004), between β-Lg and sodium alginate (Harnsilawata et al., 2006), between whey proteins and acacia gum (Weinbreck et al., 2003a,b; Weinbreck et al., 2004a,b; Weinbreck and Wientjes, 2004), and between whey proteins and carrageenan (Weinbreck et al., 2004c). Coacervates have also been formed between proteins and cationic polysaccharides. For example, there have been studies of complex coacervation between

bovine serum albumin and chitosan (Chen and Tsaih, 1997), between ovalbumin and chitosan (Delben and Stefancich, 1998; Van der Lubben et al., 2001; Yu et al., 2006), and between β-Lg and chitosan (Guzey and McClements, 2006). The use of whey proteins and specifically β-Lg as carrier for bioactive compounds is based mainly on the entrapment of these components in whey protein hydrogels. Hydrogels are a water-swollen network that can hold a large amount of water while maintaining a network structure (Qui and Park 2001).

Complex coacervation provides food scientists with a relatively simple method of creating novel hydrogel particles that can be utilized for encapsulation purposes. A major advantage entirely from food-grade ingredients (proteins and polysaccharides) using simple processing operations (eg, pH adjustment and mixing). Nevertheless, there are several factors that currently limit the more widespread application of complex coacervation in the food industry. First, the coacervate phase is stable over only a relatively narrow range of pH values, and a protein polysaccharide coacervate will tend to either disintegrate (when the pH is adjusted so that the molecules have strong similar charges) or form precipitates (when the pH is adjusted so that the molecules have strong opposite charges). Second, the coacervate phase is held together by relatively weak electrostatic interactions that may be disrupted when the ionic strength is increased, which may limit their application in some foods. Third, the particles in a coacervate suspension will tend to coalesce over time, leading to a gradual increase in the mean particle size and eventually to macroscopic phase separation. Fourth, the current methods used to form more stable hydrogel particles by cross-linking the coacervate phase, such as gluteraldehyde treatment, are approved for only a limited range of food applications, for example, gelatin and gum arabic. There is, therefore a need to find alternative methods of forming stable hydrogel particles that are more suitable for utilization within foods (Hong and McClements, 2007). Howell et al. (1995) suggested that it was food-derived protein–protein interactions between β-Lg and egg protein lysozyme. It was observed that β-Lg and lysozyme (Lyso) could form insoluble but reversible complexes, depending on the pH, ionic force, and concentration of the individual protein. β-Lg/Lyso microspheres can serve as a potential food-grade vehicle for bioactives in the formulation of food products and pharmaceuticals.

The interactions of β-Lg with various ligands may modulate its physicochemical properties and colloidal properties, which in turn can have implications to the biological and digestive fate of the β-Lg/ligand complexes. For example, β-Lg interactions with

physiological surfactants, namely phosphatidylcholine, has been reported to affect its susceptibility to gastrointestinal proteolysis (Mandalari et al., 2009). This is part of a spur of interest in milk proteins as vehicles for the controlled and targeted delivery of nutraceuticals (Benshitrit et al., 2012; Livney, 2010; Pérez et al. 2014) characterizes the interactions between β-Lg and folic acid (FA) at different load ratio and their functional implications, in terms of colloidal behavior and digestibility. They were found to be accompanied by a shift in zeta-potential values at pH = 5 for pure β-lg (0.95 ± 0.09 mV) versus β-lg/FA nanocomplexes (–20.13 ± 1.29 mV).

The surface properties of the encapsulant, such as charge and hydrophobicity, influenced delivery mostly. The success of delivery is highly dependent on cationic polymers (such as chitosan, polylysine, and Lf), have demonstrated significantly higher mucoadhesive capacity and cellular internalization compared with anionic macromolecules. This phenomenon was largely attributed to two factors. The first factor is the affinity of the polycations to the negatively charged glycoproteins, the latter of which are abundant on the membrane of epithelia cells or tissues. The second advantage for cationic encapsulants is their ability to acquire a negatively charged corona consisting mostly of serum proteins, which bind strongly to specific receptors on the cell membrane and thus promote the cellular internalization (Teng et al., 2014).

Fibrils are isolated by β-Lg, bovine serum albumin, egg albumin, and lysozyme, as well as soy glycinin and soy protein and they can be obtained by heating the protein solution at high temperatures (>80°C) for several hours under acidic conditions (pH∼2) and low ionic strength (Bolder et al., 2006). During fibrillation, not all protein monomers are converted to fibrils (ie, less than 40%), and therefore aggregated spherulites coexist with nonaggregated peptides in fibril solutions (Akkermans et al., 2008). There are two major fibril assembly pathways: one possible pathway involves partial denaturation of the protein and subsequent β-sheet alignment. The second theory assumes that peptides resulting from denaturation and hydrolysis at high temperatures assemble into amyloid structures (Jones and Mezzenga, 2012). Serfert et al. (2014) reported on microencapsulation of fish oil by spray-drying as well as oxidative stability of the oil in emulsions and microcapsules in dependence of WPI conformation and they argued that WPI fibrils exerted a significantly higher elasticity at the oil–water (O/W) interface and a better emulsifying activity at a fixed oil content compared to native WPI (Fig. 18.2). The β-Lg protein was also discovered to be an efficient and selective dispersant for carbon nanotubes (CTNs) with certain diameters (Karchemsky et al., 2013).

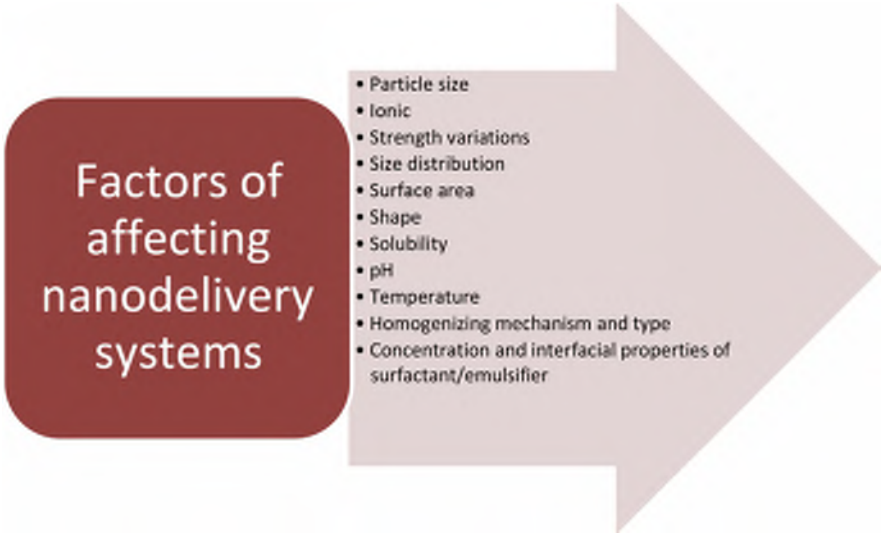

Figure 18.2. Factors of affecting nanodelivery systems.

3.4 α-Lactalbumin

α-lactalbumin, a major whey protein, has been shown to form nanotubular gels when subjected to limited hydrolysis by a protease specific to glutamic and aspartic acid bonds. The gel stiffness of the resulting gels is extremely pH sensitive with an optimum stiffness at pH 7, and the gels are also very sensitive to mechanical strain, but are able to partly regain structure under quiescent conditions (Ipsen and Otte, 2003). Partial hydrolysis of α-lactalbumin by a serine protease from *Bacillus licheniformis* (BLP) under appropriate conditions leads to the formation of nanotubular structures. Ipsen et al. (2001) proposed that the mechanism behind the self-assembly of the partially hydrolyzed α-lactalbumin into long tubes is a spatially restricted creation of ionic bonds between Ca^{2+} and carboxyl acid groups on peptide fragments resulting from the action of BLP on α-lactalbumin. Proteolysis of α-lactalbumin with BLP in the presence of Ca^{2+} thus results in formation of a strong gel with a microstructure not previously observed in food protein systems.

Bovine α-lactalbumin (BLA) is a small acidic protein with structure and functionality that are significant from several points of view (Bomhoff et al., 2006). It has been reported that this small protein (14.2 kDa) has diverse biological and pharmaceutical functions, such as its important role in lactose biosynthesis in the mammary gland and the induction of apoptosis in tumor cells by some of its forms (Permyakov and Berliner, 2000). BLA is genetically and structurally homologous to lysozyme (Goers et al., 2002).

BLA is a two-domain protein that consists of a single polypeptide chain of 123 amino acid residues. The α-domain of BLA is comprised of the two short 310 and three major α-helices. A one short 310 helix and three-stranded antiparallel β-sheet are the components of the smaller β-domain. The α and β domains are separated by a single strong calcium binding site called calcium binding loop. Two disulfide bridges make connection between the α and β domains and the loop is formed by one of the two bridges. The structure of BLA is stabilized by four disulfide bridges. One of the most important characteristics of bovine α-lactalbumin is its active ability to bind to metal cations (Permyakov and Berliner, 1994). The binding of the Ca^{2+} to BLA caused pronounced stability in the native structure of BLA (Bomhoff et al., 2006).

Particularly relevant to the food sector is the possibility to obtain nanotubes from milk protein α-lactalbumin by partial hydrolysis. The resulting α-lactalbumin nanotubes are able to increase viscosity owing to their high aspect ratio (ie, large surface area) and stiffness, which requires less protein. In addition, these high protein-density nanotubes could also be used as thickener alternatives (http://www.counteragingwise.com/pdf/CY12938a.pdf). The most special features of α-lactalbumin protein nanotubes are its cavities and hollow cores. Due to the cavities, they could well serve as vehicles for drug or other encapculated molecules such as vitamins and enzyme and also for doping of diverse ligand ions like Fe, Mn, Ca, and Zn melts encapsulates in diet nutrition (Esmaeilzadeh et al., 2013). Moreover, α-lactalbumin nanotubes have cavities of 8 nm in diameter, which might enable the binding of food components, such as vitamins or enzymes. These cavaties could also be used to encapsulate and protect nutraceuticals or to mask undesirable flavor or aroma compounds. Because these nanotubes consist of milk protein, they are considered food-grade materials, which should make their introduction into the marketplace relatively easy and might facilitate widespread applications in nanoencapsulating of nutrients, supplements, and pharmaceuticals (http://www.counteragingwise.com/pdf/CY12938a.pdf). In past years, different nanoparticle systems were developed to control drug release, to protect degradable drugs (peptides or proteins), to limit the toxicity of anticancers, or to enhance the transport of drugs across mucosal barriers or the brain. Systems consisted of bovine serum albumin or polyallylamine nanoparticles alone or entrapped in a polyurethane and then loaded with cefamandole nafate, chosen as a drug model by Crisante et al. (2009). Results showed that nanoparticles alone were able to adsorb high antibiotic amounts due to their high surface/volume ratio. For these systems the drug delivery was at least 50% with respect to nanoparticles alone with a prolonged antimicrobial activity of up to 9 days.

A recent pharmacological research showed that As_2O_3 (arsenic trioxide) can inhibit growth of many kinds of solid tumors. Nevertheless, the usage of As_2O_3 as an antitumor drug in clinic has been limited by the toxicity and poor retention time in the tumor cells, that is, As_2O_3 can inhibit tumor cells while showing severe toxicity effect on normal tissue. Fortunately, since the first reports on the preparation of uniformly sized albumin microspheres or nanoparticles, these biodegradable, biocompatible particles have been utilized as drug carrier systems to improve the efficacy and reduce the toxicity of some anticancer agents. Yang et al. (2008) studied on bovine serum albumin (BSA) nanoparticles containing arsenic trioxide. The results indicated that the anticancer efficacy of the As_2O_3-loaded BSA nanoparticles was very obvious.

Albumin nanoparticles have been already proposed in biomedical application as quantum dot carriers or in formulations for drug sustained release, like anticancer molecules, monoclonal antibodies and antiviral. The antibiotic-loaded system consisted of a carboxylated polyurethane (PEUA) containing bovine serum albumin nanoparticles (BSAnp), which act as a drug reservoir. In order to better understand the PEUA/BSAnp composite drug release properties and the mechanical behavior, a detailed physical investigation on the material and on its interaction with absorbed water was carried out (Martinelli et al., 2011).

The gel made of α-lactalbumin nanotubes are strong gels, compared to other protein gels at equal concentrations. Therefore, the nanotubes could serve as a gelation agent and it has some additional properties. Firstly, the gel formation is reversible, which can be a desirable characteristic for a particular application. Secondly, the gel is transparent, which can be a desired characteristic as well. Last, because α-lactalbumin nanotubes can be disassemled in a controllable way, for example, by changing the pH to acidic values, the gel structure can easily be broken down by the same means. All these properties of the gel provide a novel gelation agent with novel functional properties (Graveland-Bikker and De Kruif, 2006). Zhanga et al. (2011) determined that the optimum balance between antimicrobial efficacy and toxicity was achieved by treating freshly prepared silver and reduced α-lactalbumin nanocomposite with ultraviolet radiation, which resulted in a dramatic reduction in toxicity and maintenance of antimicrobial activity.

3.5 Lactoferrin

Lactoferrin (Lf), a member of the transferrin protein family, is one of the most abundant glycoproteins in human and ruminant milks (Froehlich et al., 2010). Lf are single-chain polypeptides of about 80,000 Da, containing 1–4 glycans, depending on

the species, and makes an important contribution to the host defense system. It eliminates pathogens such as bacteria, viruses, and fungi; stimulates and protects cells involved in the host defense mechanisms; and controls cytokine response (Steijns, 2001). Human milk is rich in lactoferrin, with a concentration around 1–2 mg/mL (Lonnerdal, 2003; Smilowitz et al., 2013), whereas lactoferrin concentration in ruminant milk is 10–100 times lower than in human milk (in the range of 0.02–0.2 mg/mL) (Hiss et al., 2008). Lf exhibits an array of biological activities, including antioxidant, antibacterial, antiviral activities, iron (and other metals) binding and immunomodulation (Lonnerdal, 2003; Legrand et al., 2005; Seganti et al., 2004). Lf's function is modulated by both the polypeptide chain and its glycosylation (Barboza et al., 2012). The discovery of lactoferrin's functional properties has resulted in increased supplementation of bovine milk-based infant formula with bovine milk Lf as a means to enable health claims (Boland et al., 2001). Bezault et al. (1994) found that Lf decreased solid tumor growth and strongly inhibited experimental metastasis in mice. In addition, Campbell et al. (1992) had demonstrated that Lf may be downregulated in some cancers, such as human breast carcinoma and showed that it may regulate cell proliferation.

Lactoferrin (Lf) has been found to have two thermal denaturation temperatures (61 and 93°C), and the formation and size of Lf nanoparticles depended on the temperature and duration of the thermal treatment. The obtained nanoparticles were resistant to subsequent change in pH (3–11) and ionic strength (0–200 mM NaCl). Lf nanoparticles can be used to encapsulate and deliver bioactive compounds or to deliver Lf nanoparticle rather than individual molecules when Lf is used as a functional component. Recently, the formation of lactoferrin nanoparticles by controlled thermal treatment has been described (Bengoechea et al., 2011). Lf has been found to be a source of bioactive peptides, for example, lactoferricin that can be released during gastric digestion (Conesa et al., 2010; Elbarbary et al., 2010; Flores-Villasenor et al., 2010; Nagpal et al., 2011). Lf digestion and the digestion of Lf-based nanoparticles in the alimentary tract is of importance as digestion is likely to be affected by various colloidal and physiological aspects, as previously shown for other proteins (Dupont et al., 2010; Macierzanka et al., 2009; Mackie and Macierzanka, 2010). Tokle et al. (2012) studied the "premix" approach that involved mixing two globular proteins with different isoelectric points prior to emulsion formation: β-Lg and Lf and droplets coated by β-Lg were unstable to aggregation near their isoelectric point (pH∼5), whereas those coated by Lf were stable across the whole pH range.

4 Nanoemulsion-Encapsulated Phytochemicals

4.1 Carotenoids

Carotenoids are a diverse group of lipophilic compounds that contribute to the yellow to red colors of many foods. They are polyenes consisting of 3–13 conjugated double bonds and in some cases 6-carbon ring structures at one or both ends of the molecule. Carotenoids containing oxygen are known as xanthophylls (eg, lutein and zeaxanthin) while those without oxygen are known as carotenes (eg, lycopene and β-carotene). Endogenous carotenoids in foods are generally stable. However, as food additives, carotenoids are relatively unstable in food systems because they are susceptible to light, oxygen, and autooxidation. Consequently, dispersion of carotenoids into ingredient systems can result in their rapid degradation. Carotenoids can be degraded by reactions that cause the loss of double bonds or scission of the molecule. In addition, the double bonds in carotenoids can undergo isomerization to the cis configuration. Isomerization reactions might actually be beneficial since cis isomers of carotenoids such as lycopene are thought to be more bioavailable and bioactive (McClements et al., 2007). β-carotene nanoemulsions formed by two WPI hydrolysates (with degree of hydrolysis (DH) 8.1% and 18.1%) had smaller droplet sizes (110.3 and 30.4 nm, respectively) than whey protein concentrate (WPC) and soy protein isolate (SPI) stabilized nanoemulsions (145.3 and 196.3 nm, respectively). Zeta potential measurement showed the WPI hydrolysates had droplet surface charge comparable to unhydrolysed SC and SPI but significantly higher than unhydrolyzed WPI and WPC (Chu et al., 2007).

4.2 Zein

Zein is considered as a generally recognized as safe (GRAS) and food-grade ingredient by the Food and Drug Administration (FDA). It consists of three parts of lipophilic and one part of hydrophilicamino acid residues. Because of its high hydrophobicity, zein has been successfully applied as a promising carrier for encapsulation and controlled release of fat-soluble compounds (eg, gitoxin, fish oil, etc.) in the pharmacuetical and food areas (Muthuselvi and Dhathathreyan, 2006). Zein, a prolamin and the major component of cornprotein, has been an important material in science and industry owing to its unique properties and molecular structure. By dissolving zein, either in ethanol or in acetone, biodegradable zein films with good tensile and water-barrier properties can be

obtained (Lai et al., 1999; Shukla and Cheryan, 2001; Lawton, 2002; Yoshino et al., 2002; Lee et al., 2005). Nanotechnology approaches are expected to yield new applications for zein in specialty foods and in the biodegradable plastics industry. Zein can form a meshwork consisting of tubular structures, which can be microbiologically resistant and inert, for example, after treatment with formaldehyde (Lawton, 2002; Guo et al., 2005; Torres-Giner et al., 2007). However, zein nanobeads or nanoparticles can be used as edible carriers for flavor compounds or for encapsulation of nutraceuticals, as well as to improve the strength of plastic and bioactive food packages (Lawton, 2002;Guo et al., 2005; Torres-Giner et al., 2007). Controlling the uniformity and organization of zein films at the nano level are crucial in terms of mechanical and tensile properties (Lai et al., 1999). Wu et al. (2012) have demonstrated that the entrapment of essential oils within zein nanostructure allows their dispersion in water, enhancing their potential for use as antioxidant and antimicrobial in food preservation and control of human pathogenic bacterium, *Escherichia coli*.

4.3 V-Amylose

Starch is a major plant food material and ingredient that is made up of two glucose polymers; a linear amylose and branched amylopectin (Buléon et al., 1998). Amylose consists of mainly α-(1→4)-glucan linkages with a few occasional α-(1→6)-glucan linkages (Obiro et al., 2012). The amylose molecules are about 15–30% of starch depending on the type of starch; starches having >40% amylose are referred to as high-amylose starches (Obiro et al., 2012). Amylose can form single helical inclusion complexes, generically known as V-amylose (Morrison et al., 1993; Rappenecker and Zugenmaier, 1981; Godet et al., 1993). The V-amylose complexes have been shown to form with a diverse range of compounds such as alcohols, (Hinkle and Zobel, 1968; Buléon et al., 1990; Helbert and Chanzy, 1994) fatty acids (Godet et al., 1996), potassium hydroxide (Sarko and Biloski, 1980) iodine (Immel and Lichtenthaler, 2000), flavor compounds (Conde-Petit et al., 2006; Nuessli et al., 1997; Nuessli et al., 2003; Biais et al., 2006) and hydrophobic organic polymers (Kadokawa et al., 2001; Kadokawa et al., 2002; Kadokawa et al., 2003; Kaneko and Kadokawa, 2005; Kaneko et al., 2008; Liquin et al., 2008). V-amylose complexes have shown potential in various food-related applications such as nanoencapsulation of sensitive bioactive (Lalush et al., 2005; Lesmes et al., 2009) or flavor compounds (Conde-Petit et al., 2006; Nuessli et al., 1997), formation of amylose nanotubes (Kim et al., 2003) and modification of starch rheological functionality

(Nuessli et al., 1995; D'Silva et al., 2011). Since amylose is an inherent food component and natural ingredient, the application of V-amylose as an ingredient in food products would raise limited legal or regulatory issues if the included ligands are food grade (Obiro et al., 2012).

4.4 Chitosan

Chitosan is a modified natural carbohydrate polymer derived from chitin that is found in a wide range of natural sources such as crustaceans, fungi, insects, and some algae (Peniston and Johnson, 1980). It is a linear polysaccharide consisting of randomly distributed N acetyl-*d*-glucosamine and -(1,4)-linked d-glucosamine units. Chitosan has been widely considered as a versatile polymer used in pharmaceutical and nutraceutical areas as wall materials for development of delivery systems, due to its favorable biological properties such as biodegradability, biocompatibility, and low toxicity (Sahoo et al., 2009). Removal of most of the acetyl groups of chitin by treatment with strong alkali yields chitosan (Li et al., 1997), that is, 2-amino-2-deoxy-β-D-glucose. A sharp nomenclature with respect to the degree of *N*-deacetylation has not been defined between chitin and chitosan. In general, chitin with a degree of deacetylation of above 70% is considered to be chitosan (No and Meyers, 1992).

Compared with other delivery systems, nano- or microparticles prepared by chitosan have a special feature of being able to adhere to mucosal surface and transiently open tight junctions between epithelial cells, due to its positive surface charge of chitosan molecules (Liu and Park, 2009). According to Luo et al. (2011), α-tocopherol/zein–chitosan complex was successfully prepared under mild conditions. Physicochemical analyses suggested that electrostatic interactions, hydrogen bonds, and hydrophobic interactions are the main forces in α-tocopherol/zein–chitosan complex. By coating α-tocopherol zein nanoparticles with chitosan, particle size was dramatically reduced and zeta potential was increased to be highly positive, depending on different formulations.

Polysaccharide-based nanostructures are also interesting for encapsulation of food ingredients, like chitosan, due to both biocompatibility and biotolerability properties. Polysaccharidesare polymers of monosaccharides (carbohydrates) that are linked together by glycosidic bonds. In the organism, these polymers are broken down to saccharides by the colonie microflora and are able to protect functional food ingredients from hostile conditions, such as those found in biological systems (eg, stomach

acidic pH). The glycosidic linkages are hydrolyzed on arrival in the colon, which triggers the release of the entrapped bioactive compounds (Luykx et al., 2008). Recently, Tang et al. (2013) developed self-assembled nanostructures composed of chitosan and an edible polypeptide, poly (γ-glutamic acid), for oral delivery of tea catechins, which can be used as food additives for drinks, foods, and dietary supplements.

4.5 Phenolic Compounds

Flavonoids are important antioxidants due to their high redox potential, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. The structure consists of two benzene rings, A and B on either side of a three-carbon ring, which forms heterocyclic ring, C. The aromatic ring A is derived from the acetate/malonate pathway, whereas ring B is derived from phenylalanine through the shikimate pathway. Flavanones are characterized by the presence of a saturated three-carbon chain and an oxygen atom in the C4 position. Flavanones are present in high concentrations in citrus fruits. Apart from citrus fruits, flavanones are also present in tomatoes and certain aromatic plants, such as mint. Isoflavones are phytochemicals found in many plants and plant-derived foods, which impart health benefits. Some physiological effects are attributed to their structural similarities to β-estradiols, and thus, they are occasionally referred to as "phytoestrogens" (Babbar et al., 2015).

Naringin (naringenin-7-rhamnoglucoside) is more soluble and very bitter glycosylated polyphenol of the flavanones class, a group of flavonoids that occurs almost exclusively in citrus plants, and they show promise for supplementation of staple foods and beverages, as a kind of preventive medicine (Erlund, 2004). Shpigelman et al. (2014) have used native and preheated β-Lg based nanovehicles. Using UV spectrophotometry and intrinsic fluorescence methods, they have found that naringenin forms complexes with preheated and nonpreheated β-Lg, with K_a in the order of 104 M^{-1}.

Tangeretin (5, 6, 7, 8, 40-pentamethoxyflavone) is a flavonoid (polymethoxyflavone or PMF) found in citrus fruits. There has been considerable interest in the use of PMFs as functional ingredients in foods and pharmaceuticals because of the potential of their beneficial activities, such as anticarcinogenic activities and antiinflammatory activities (Li et al., 2009; Mak et al., 1996; Manthey and Guthrie, 2002). However, the extensive application of PMFs is currently limited because they have low water-solubility, which makes them difficult to be incorporated into liquid food

products (Li et al., 2009). Chen et al. (2014) produced tangeretin-loaded protein nanoparticles with mixing an organic phase containing zein and tangeretin by an aqueous phase containing β-Lg and then converted into powder by freeze-drying.

Phenolic acids largely consist of two subgroups: the hydroxybenzoic acid and the hydroxycinnamic acid. Previous studies have shown that hydroxycinnamic acids are generally more abundant than hydroxybenzoic acids in fruits and that they differ in the pattern of their aromatic rings. Tannins can be subdivided into hydrolyzable and condensed tannins. The most widely studied condensed tannins are (−) epicatechin and (+) catechin. Epigallocatechin formed by addition of gallic acid to epicatechin showed higher antioxidant ability than epicatechin (Babbar et al., 2015).

Green tea catechins are unstable in neutral and alkaline solutions while they remain relatively stable in acidic conditions. In a sodium phosphate buffer (pH 7.4) 80% of Epigallocatechin-3-gallate (EGCG) were lost after 3 h and even in acidic commercial soft drinks the green tea catechins degraded by at least 50% during the first month of storage (Su et al., 2003). EGCG degradation is temperature, oxygen, concentration, and pH dependent (Zimeri and Tong, 1999), and it was demonstrated that it followed a pseudo first-order kinetics (Wang et al., 2008). The EGCG degradation products turn the solution from colorless to yellowish brown (Mizooku et al., 2003) through oxidation and dimer formation (Hong et al., 2002). Obviously, this browning effect is undesired in bottled green tea beverages (Wang et al., 2003) and the oxidative dimerization and polymerization lower the antioxidative potential, as it was shown that black tea, which has many polymerized catechin molecules, has a lower antioxidant capacity than green tea (Lee et al., 2002). Catechins cause an unpleasant flavor due to their bitterness and astringency (Rossetti et al., 2009), therefore palatability decreases with increasing catechins concentrations (Narukawa et al., 2010). Taste time-intensity experiments showed that the onset of bitterness occurs earlier than that of astringency (Peleg et al., 1999; Rossetti et al., 2009). The leading theory regarding the astringency states that the precipitation of saliva proteins by polyphenols leads to loss of lubricity in the oral cavity. The major proteins in saliva are a group of proteins containing a large amount of proline, commonly referred to as proline-rich proteins. Recently this theory was questioned by the fact that while epicatechin did not alter the lubricating properties of a salivary film, it was still perceived to be astringent. Additionally, milk mitigated the astringency of EGCG solutions while considerably reducing saliva lubricity (Rossetti et al., 2009). Shpigelman et al. (2012) found that although particle size increased with rising EGCG concentration,

zeta potential stayed around 40 mV upto 8:1 M EGCG:β-Lg ratio, suggesting particles are very stable in solution. Maltodextrin and skim milk proteins were shown to reduce the astringency of catechins (Rossetti et al., 2009).

5 Nanoemulsions and Lipids

5.1 Omega-3 Fatty Acids

Omega-3 (ω-3) fatty acids are unsaturated fatty acids that have a double bond that is 3 carbon atoms from the methyl end of the molecule. The most common ω-3 fatty acids are α-linolenic acid (ALA, 18:3), eicosapentaenoicacid (EPA, 20:5), and docosahexaenoicacid (DHA, 22:6). Of these three, the long chain ω-3 fatty acids EPAand DHA are the most bioactive. Omega-3 fatty acids can have a major impact on health because they have numerous physiological roles such as impacting cell membrane fluidity, cellular signaling, gene expression, and eicosanoid metabolism. Because of these, ω-3 fatty acids has been attributed with the ability to decrease the risks of cardiovascular disease, diseases affected by immune response disorders (eg, type 2 diabetes, inflammatory bowel diseases, and rheumatoid arthritis), and mental disorders, as well as benefit infant development. Dietary ω-3fatty acids strongly suggests that large portions of the population would benefit from increased consumption of ω-3 fatty acids, making them an excellent candidate for incorporation into functional foods. However, numerous challenges exist in the production, transportation, and storage of ω-3 fatty-acid-fortified functional foods, since these lipids are extremely susceptible to oxidative deterioration. Oxidation of ω-3 fatty acids is a complex chemical reaction that often requires multiple antioxidant hurdle technologies for adequate stabilization Encapsulation of omega-3 fatty acids has been found to be an excellent method for stabilization (McClements et al., 2007). Von Staszewski et al. (2014) formulated (O/W) emulsions containing liver fish oil rich in ω-3 fatty acids using green tea polyphenols and β-Lg at pH 6, but showed that surface pressure and dilatational properties decreased.

6 Conclusions

Nanotechnology is becoming increasingly important for the food sector. Edible nanolaminates could find applications in fresh fruits and vegetables, bakery products, and confectionery, where they might protect the food from moisture, lipids, gases, off-flavors, and odors. Natural biopolymers of nanosize scale,

such as polysaccharides, can be used for the encapsulation of vitamins, prebiotics, and probiotics and for delivery systems of drugs or nutraceuticals. Milk and milk proteins are used as nanospheres, nanoparticles, nanoemulsions, nanotubes, and nanocomposite films. In this context, milk protein with structural and physicochemical properties is an excellent tool for production of nanolevel bioactive compounds. However, studies need to be made on humans and animals to determine the efficacy and bioavailability of milk protein on their systems. The important issues are the continuation of nanotechnological applications, the regulation of commercialization by governments, thus making consumers aware of the presence of nanofoods in their products. Furthermore, all these possible applications should be evaluated economically and acceptance by consumers.

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19

PROTEIN-BASED DIETARY SUPPLEMENTS AS NUTRACEUTICALS

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1 Introduction

We begin with a discussion of proteins to understand the important points of this topic. Before defining proteins, it is suitable to start with the definition of amino acids. Amino acids have basically simple structure and contain amino, NH_2, and carboxyl group, COOH. There are hundreds of amino acids found in nature, while only 20 amino acids can be found in protein structures. Thus, proteins can basically be defined as macromolecules that are mainly amino acids. They have important functions for living organisms such as DNA replication, catalyzing metabolic reactions, and molecule's transportation from one location to another.

Most microorganisms and plants can biosynthesize 20 amino acids, while animals should acquire some of the amino acids through diet (Voet and Voet, 2004). The amino acids that an organism cannot synthesize on its own are identified as essential amino acids (valine, leucine, isoleucine, lysine, methionine, threonine, phenylalanine, and tryptophan). Amino acids are also important dietary sources of nitrogen. These types of amino acids are the main components of proteins. People should add these amino acids to their diet to meet nutritional requirements. There are many food alternatives in natural forms which can be used to meet these requirements. Thus, proteins can be divided into groups according to their origins such as animal (fishery and terrestrial animals), plants (oilseed, aquatic, and grain legumes), milk proteins, and single cell proteins (a group of microorganisms including unicellular algae, fungi, bacteria, cyanobacteria, and yeast) (Abdel-Fattah and El-Sayed, 1999; Göğüş and Fadıloğlu, 2006).

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From a nutrient perspective, protein has many important functions, such as necessity for the repair and growth of body tissues and providing a source of energy. The main sources of protein are animal sources such as meat, fish, eggs, and dairy foods, and cereal products such as nuts and so forth (Göğüş and Fadıloğlu, 2006). Generally humans can easily intake necessary protein from these sources by following a balanced diet.

Additionally, a dietary supplement has another definition, which is a product intended for ingestion that contains a "dietary ingredient" intended to add further nutritional value to supplement the diet. A "dietary ingredient" may be one, or any combination, of the following substances, such as a vitamin, a mineral, an herb or other botanical, an amino acid, a dietary substance for use by people to supplement their diet by increasing the total dietary intake, a concentrate, metabolite, constituent, or extract (http://www.fda.gov/AboutFDA/Transparency/Basics/ucm195635.htm).

After these definitions, we should focus on why people need supplements and especially why protein-based dietary supplements are preferred.

We can start from the beginning: the human need to maintain dietary requirements through food. For generations, people have used food from nature to meet basic requirements. This perspective is changed day by day and will be changed further in the future. Lifestyle, requirements, and habits have changed over the years. Recently people have become conscious about industrial food products. They want to know what they eat and which reactions and biochemical changes occur when foods are taken into their system. On the other hand, there are some differences between people and dietary behaviors which may be denoted as dietary approach of consumers. There are some consumers and scientists (mostly doctors) who prefer food from nature and do not agree with the use of dietary supplements, while others are not as strictly against dietary supplements. The latter kind of consumers and scientists can also be divided into two groups. Some of them prefer to use these supplements only if they consist of natural ingredients and do not include chemical compounds, while others discriminate among types of supplements based on the trustworthiness of the company that offers the product. Because of these kinds of variable consumer attitudes, it is hard to say which dietary supplements should or should not be used. That is why there should be positive and/or negative views of these supplements presented by scientific studies.

Last, we can summarize these perspectives as variable food habits: there are many consumers, doctors, and scientists who have lots and diverse ideas about what constitutes a balanced

diet. Some suggest a Paleolithic diet, which mainly is concerned with the benefits of unprocessed and raw foods. There are also some people who practice yoga and believe in the philosophy of yoga, which includes vegetarianism. These people also prefer raw and vegetarian food. The third group can be defined as consumers who avoid sugar and sugar acids users, meaning they avoid the use of carbohydrates and processed foods, which they believe are the main causes of health problems. The last group, generally food scientists and dieticians, are mainly concerned about maintaining a balanced diet in which all types of food and nutrients should be taken as needed. The most important point on which all people can agree is the importance of proteins in a diet. Generally the protein needs of people can be supplied with a balanced diet, but there are some exceptions, such as elderly people who have health problem, athletes who need extra protein to support muscles, vegetarians, who cannot acquire enough protein through vegan diets, and so forth. For those kinds of exceptions and some specific period of life, protein-based dietary supplements can be a good way to ensure a balanced diet.

Nanotechnology, which can be defined briefly as a field of science and technology, which is generally interested in particles that are smaller than 1–100 nm (a billionth of a meter), has grown rapidly in recent years (Senturk et al., 2013). Due to being a new technology and according to a food industrial perspective, this technology needs some detailed studies and researches about whether there is any toxic effect on humans or accumulation problems on human organs. These questions do not mean that this technology is not safe, but only that there are still some points that should be defined, clarified, and proved by scientific studies. This kind of research is ongoing and still needs time to be clarified.

2 Nanotechnology

Nanotechnology is a rapidly developing area of technology and science, which is generally interested in particles between 1 and 100 nm. This technology shows great potential to provide new biological, physical, and chemical features to materials at a molecular or atomic scale. By comparison with macro-sized materials, nanoscale structures have been shown to possess advanced and novel features; therefor they can be included in many kinds of fields of interest, especially electronic, computer, textile, and pharmaceutical industries. Applications of nanotechnology in the food industry have been developing more slowly than other areas because of their complex structures and sensitivities. Some applications in the food industry are improving the

uptake, absorption, and bioavailability of nutrients and supplements in the body, producing new or improved tastes and textures of foods, improving food packaging materials, developing nanosensors that can give information about the freshness or spoilage of products during transportation, and storage of food products (Otles and Yalcin, 2013).

These emerging technologies have shown great potential in nutraceuticals and functional foods for delivering bioactive compounds in functional foods to improve human health (Chen et al., 2006a; Chau et al., 2007). Nanocapsules may be incorporated in dairy products without modifying their sensory properties. This kind of study introduces new possibilities for encapsulation and delivery of sensitive health-promoting substances using natural GRAS (generally regarded as safe) ingredients (Semo et al., 2007). With this point of view, we try to deeply understand nanobased supplements construction and mechanism by looking into the type of nanosubstance and trying to figure out the mechanisms. Furthermore, it is good to have an idea about the synthesis approaches of nanoparticles, which are generally categorized into top–down and bottom-up approaches. The top–down approach can be briefly defined as production of the small size by grinding and cutting methods. The bottom-up method is also known as self-assembly and can be described as the building of materials from individual atoms and molecules (Sürengil and Kılınç, 2011; Devi et al., 2011; Hernández-Cázares et al., 2010). When we are concerned about these definitions from a food perspective, naturally nanoscale food components production could be also be used for these types of production approach or there could be an alternative way to work on food at nanoscale without adding nanoparticles and/or materials by using the top–down approach. Thus we can use nanotechnology on food in the safest way possible. While we are looking from the nanotechnology side to protein-based dietary supplements, nanogrinding can be a good alternative to make these supplements more effective, efficient, and suitable.

When we are looking from perspective of supplements, nanotechnology has uses as a functional food, such as the samples that can be briefly categorized as colloidal metal nanoparticles, delivery systems like nanoclusters and nanoclustered foods and/or drinks as nutrients. Their functions also can be briefly defined as claims of enhanced desirable uptake of metals, protected and targeted delivery of content, and enhanced uptake (Senturk et al., 2013), while there are some naturally nanobased supplements that can be found in nature. Furthermore, there are lots of products produced by using nanotechnology for the food industry. These products were further classified as: supplements,

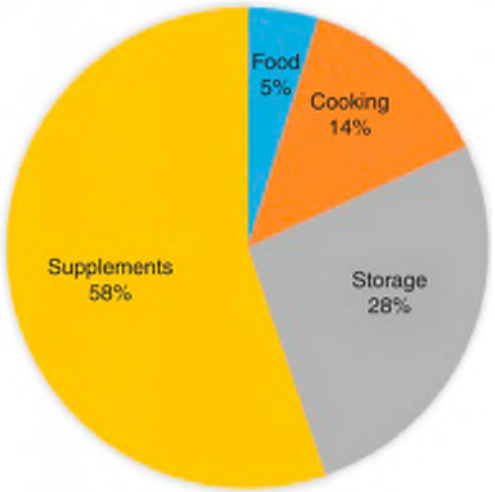

Figure 19.1. Distribution of nanotechnology products classified as food and beverage. From Senturk et al. (2013), WWICS (2007).

storage, cooking, and food (Fig. 19.1). One nanofood—slim shake chocolate—is indicated as being "low in fat and calories," "no artificial sweeteners," "tastes delicious," and as containing "nanoclusters," while nanoclusters are defined as: "tiny particles, 100,000th the size of a single grain of sand, … designed to carry nutrition into your cells" (Senturk et al., 2013; WWICS, 2007).

Thus, application of nanotechnology to dietary supplements has not only benefits because of the effectiveness of the particles but also because of nutraceutical and functional effects such as decreased fat and calories and/or increased functional effects.

Even though nanotechnology and nanoscience applications are rapidly emerging areas, while we are looking from the health and toxicity point of view about food intake, there are some open issues on people's minds. From this point, we should be aware of regulations and rules, which are formed to control the production of nanofoods. If there are still open questions, regulations should be reviewed and revised to address needs. These open issues can only be overcome by using or selecting certificated supplements. Furthermore, there should be more scientific studies, which support the positive issues of these supplements, associated with the indications of any negative effects occurred or proved. Other researchers can also focus attention on the literatures that suggest many dilemmas regarding nanomaterials and examine potential health risks. While there are some proposed applications about nanotechnologies development, there are still restrictions

because of potential risks (Cushen et al., 2012). Additionally, special attention should also be given to consumer attitudes toward food nanotechnology. Taking lessons from the GM arguments across European countries, it is crucial to discuss the benefits and risks of this highly promising technology. Governments should consider appropriate labeling and should also establish regulations that will help to increase consumer acceptability (Sozer and Kokini, 2008).

After overcoming these problems, there would be an upsurge of nanotechnology-based food products and supplements. Some proved toxicological studies and researches would overcome the doubts and worries of consumers.

2.1 Nanodelivery Systems

After giving the preceding information about protein, supplements, and nanotechnology, examples can be given of nanodelivery systems. We can also talk about nanonutrient delivery systems, which is a part of dietary supplements according to new technological developments. The attributes can be defined as; containing low to moderate sugar, trans-fat and sodium content; significantly reduced energy density; an increasing amount of whole dietary and grain fiber, and a high quantity of vegetable and milk proteins, or bioactive ingredients, such as nutrients which have health-sustaining properties (Senturk et al., 2013).

Organic constituents that are naturally present in foods such as proteins, carbohydrates, and fats can vary in size from large polymers to simpler molecules in the nanorange. Organic nanomaterials can be synthesized for specific purposes, such as the encapsulation of nutrients to increase bioavailability, enhance taste, texture, and consistency of foodstuffs, or mask an undesirable taste or odor. Functionalities of such nanomaterials such as particle size, size distribution, potential agglomeration, and surface charge, can be affected by the biological matrix in which they are held, such as the composition of a food (Powers et al., 2006). The science of the production of nanoderived food ingredients is still in its infancy; nevertheless, it shows much promise with the prospect of improving product functionality without compromising product quality or safety (Cushen et al., 2012). Additionally, milk proteins are natural bioactive compounds, which can also be used as delivery systems. We can give a description of their delivery system as the binding of small molecules with self-assembly properties. Especially casein micelle (CM) are used in a nanodelivery system as encapsulating agents, while other materials are also used, such as pectin-based nanomaterials for hydrophobic

nutraceuticals or bioactive carrying (Livney, 2010). For example, one of the nanonutrient delivery systems is a CM, which can be defined as a natural nanodelivery system and originates from cow milk that contains 30–35 g of protein per liter, and thus casein, which accounts for about 80% of milk protein, is organized in micelles. CM are designed by nature to concentrate, stabilize, and transport essential nutrients, mainly calcium and protein, for the neonate (Semo et al., 2007). On the other hand, caseinophosphopeptides can form soluble organophosphate salts and may function as carriers for different minerals, especially calcium. In relation to their mode of action, bioactive peptides may reach target sites (eg, receptors and enzymes) at the luminal side of the intestinal tract or, after absorption, in peripheral organs. Milk-derived peptides can be produced on an industrialscale, and as a consequence, these peptides have already been considered for application both as dietary supplements in "functional foods" and as drugs (Meisel, 1997). According to a research, casein films are known for their high tensile strength properties and favored as acceptable coatings for tablets. Naturally occurring genipin and a natural tissue enzyme, transglutaminase, were used as cross-linkers to prepare novel casein-based hydrogels for the controlled release of bioactive substances. Casein floating beads were developed to increase the residence time of drugs in the stomach based on their emulsifying and bubble-forming properties. Casein-based micro particles entrapping bioactive molecules were prepared via emulsification-chemical cross-linking with glutaraldehyde, enzymatic cross-linking by transglutaminase, simple coacervation, and electrostatic complexion. Casein nanoformulations were also prepared to deliver nutraceuticals and synthetic drugs via enzymatic crosslinking, graft copolymerization, heat-gelation and polyelectrolyte ionic complexion (Elzoghby et al., 2011).

As a result, there are lots of natural nanodelivery systems which can be formed by spontaneous self-assembly or produced at the nanolevel. There are some positive effects of these dimensions, which could be briefly summarized as high potential effect, better bioactive properties, protection of nutrients, and improving product functionality, and so forth.

3 Nutraceuticals

After a brief introduction, we first define nutraceuticals, which leads to our discussion of protein dietary supplements. The main idea of the use of these supplements begins with their nutraceutical attributes. According to another definition, the term

nutraceutical comes from "nutrition" and "pharmaceutical" (Brower, 1998; Kalra, 2003). By the way, the nutraceuticals definition comes from the concept of "personalized medicine." Additionally, we can also define nutraceuticals as phytochemicals or functional foods, which are natural bioactive, chemical compounds that have health promoting, disease preventing, or medicinal properties. Nutraceuticals are found in a mosaic of products emerging from the food industry, the herbal and dietary supplement market, the pharmaceutical industry, and the newly merged pharmaceutical/agribusiness/nutrition conglomerates (Dureja et al., 2003). The consumption of nutraceutical molecules, whether in foods or as dietary supplements, cannot provide the expected benefits if the molecules of interest lose their bioactivity before they are absorbed in the small intestine. It is therefore crucial to protect them against conditions encountered in food processing (temperature, oxygen, and light) and in the gastrointestinal tract (pH, presence of enzymes, and other nutrients) (Maltais et al., 2010).

Dietary supplements should have correlations with nutraceuticals, which cannot dissociate from each other. Nanotechnology usage within these systems provides lots of beneficial effects, such as to improve nutraceutical compound bioavailability (Goldstein et al., 1980; Chen et al., 2006b). As a result, there are lots of benefits to using nutraceuticals in a diet. Additionally, nutraceuticals with nanotechnology applications have effects like health promoting, disease preventing, or medicinal properties.

4 Protein-Based Dietary Supplements

While there are many choices of products including protein-based dietary supplements, the first one that comes to mind is protein powders, which come in various forms. The three common ones are whey, soy, and casein protein. "Whey is the most commonly used, because it's a water-soluble milk protein," says Peter Horvath, PhD, associate professor in the Department of Exercise and Nutrition Sciences at the State University of New York at Buffalo. "It's also a complete protein, so it's got all those advantages." (Complete proteins contain all nine of the amino acids necessary for human dietary needs). People who are vegan may prefer soy protein, although Horvath notes that its taste is sometimes considered to be more unpleasant, and it doesn't dissolve as well in water (http://www.webmd.com/vitamins-and-supplements/lifestyle-guide-11/protein-powder).

Cow's milk is considered to be a basic food in many diets, and is rich in a variety of essential nutrients. Achievements in

separation techniques in the dairy industry and enzyme technology offer opportunities to isolate, concentrate, or modify these compounds, so that their application in functional foods, dietary supplements, nutraceuticals, and medical foods has become possible. One may conclude that lactoferrin makes an important contribution to the host defense system. It eliminates pathogens such as bacteria, viruses, and fungi, stimulates and protects cells involved in the host defense mechanisms, and controls the cytokine response. Current commercial applications of bovine lactoferrin include infant formulas, nutritional iron supplements and drinks, fermented milks, chewing gums, immune-enhancing nutraceuticals, cosmetic formulas, and feed and pet-care supplements (Steijns, 2001).

Whey is a coproduct of cheese-making and casein manufacture in the dairy industry. After the casein curd separates from the milk, following coagulation of the casein proteins through the action of chymosin (rennet) or organic acid/mineral, the remaining watery and thin liquid is called whey. Whey protein-based ingredients provide the industry with an excellent choice, and these ingredients start from a firm traditional foundation (Zadow, 1994; Smithers, 2008).

CM are in effect nanocapsules created by nature to deliver nutrients, such as calcium, phosphate, and protein, to the neonate. A novel approach is herein presented, to harness CM for nanoencapsulation and stabilization of hydrophobic nutraceutical substances for enrichment of nonfat or low-fat food products. Such nanocapsules may be incorporated in dairy products without modifying their sensory properties (Semo et al., 2007). Along with increased market demands on nutritionally fortified foods, edible coatings and films containing high concentrations of nutraceuticals would provide alternative ways to fortify foods that otherwise cannot be, such as fresh fruits, vegetables, and other unprocessed food items. Products could be either coated or wrapped with nutritionally fortified coatings or films. Calcium caseinate (CC) and whey protein isolate (WPI) films were prepared to contain 5 or 10% gluconal cal (GC), a mixture of calcium lactate and gluconate, or 0.1 or 0.2% R-tocopheryl acetate(VE), respectively. These types of films may be used for wrapping or coating to enhance the nutritional value of foods. The concentration of GC and VE added to the films must be carefully selected to meet required water barrier and mechanical properties of the films depending on their specific applications (Mei and Zhao, 2003).

Botanical dietary supplements—also called botanical herbs and/or nutraceuticals—could be defined as plant-derived materials with medical benefits aimed at disease prevention or treatment

that go beyond satisfying basic nutritional requirements (Raskin et al., 2002). The need for plant-derived nutrients is expected to grow due to economic and environmental factors as well as to support the development of new, safe, and healthy foods which may respond to consumers' increasing awareness of the impact of dietary habits on human well-being.

Legume seeds are an abundant source of proteins and, among them, lupin is one of the richest. Indeed, lupin seed deserves greater interest as a result of its chemical composition and augmented availability in many countries in recent years. Lupin is a nonstarch leguminous seed with a high protein content, almost as high as that of soybean (about 35% of the dry weight), and a relatively low oil content (Duranti et al., 2008). The swelling of soy protein filamentous hydrogels and tablets thereof and the release of riboflavin from these drug delivery devices were investigated under simulated gastrointestinal conditions in the presence or absence of digestive proteases. Considering their nonsynthetic nature, they should be of great interest for the development of innovative functional foods (Maltais et al., 2010). Soy protein has been shown to be adequate for proper growth and development of animals and humans. Soy-based formulas (usually supplemented with methionine and various minerals and vitamins) are commonly consumed by infants, especially by those allergic to cow-milk-based formulas. Other than for long-term feeding of premature infants, soy protein is of such high quality that it can serve as the sole protein source in the human diet. Defatted soy flour, for example, is low in total fat, saturated fatty acids, sodium, and sugar and is devoid of cholesterol. This product is rich in fiber and low in calories compared with animal foods when evaluated on a dry weight or on an equal-protein basis (Erdman and Fordyce, 1989). Another plant-derived material, grain legumes are a valuable source of food proteins. Their exploitation is expected to grow in relation to the food needs of a growing world (Duranti, 2006). Legumes are the cheapest sources of supplementary proteins in Indian diets. They are also good sources of vitamins and minerals. It has been noted that germinated legumes are rich in vitamin C and in some there is an increase in the riboflavin as well as niacin contents upon germination. The activity of many enzymes like amylase, protease, phytase, and lipase will increase during germination. Processed legumes such as puffed Bengal gram contains proteins of fairly high biological value and is a good supplement to the diets of children. There are many varieties of legumes such as red gram (pigeon pea, *Cajanus cajan*), black gram (*Vigna mungo* L.),

broadbean (*Vicia faba* L.), Bengal gram (chickpea, *Cicer arietinum* L.), cowpea (*Vigna unguiculata* L.), field bean (*Dolichos lablab*), green gram (*Phaseolus aureus* Roxb), horse gram (*Dolichos biflorus*), and so forth, which are commonly used in India, especially by the poorer classes of the population. Additionally, a variety of dietary fiber fractions are available commercially as supplements for use in dietetic and other functional foods such as bakery products, high-fiber biscuits, and bread. The fiber incorporation in such foods might cause undesirable changes in the food, particularly their flavor, texture, and mouth feel, which have been partially overcome by modifying the polysaccharides by physical, chemical, and enzymatic treatments (Swaminathan, 1974, 1988; Thompson, 2000; Tharanathan and Mahadevamma, 2003).

Spirulina (a good source of protein, which includes main amino acids), a type of blue-green algae that has been consumed for thousands of years as a primary food source for the Aztecs and Mayans, contains high levels of antioxidants, for example, carotenoids, especially h-carotene, and phycocyanin and phycocyanobilin. In vivo studies also indicate that chronic treatment with spirulina for 6 weeks increased retinoid levels in liver (Mitchell et al., 1990; Annapurna et al., 1991; Reddy et al., 2000; Careri et al., 2001; Wang et al., 2005).

Consequently, there are lots of protein-based dietary supplements that have beneficial effects for human well-being, such as disease prevention or treatment, and reducing risk of obesity, diabetes, and gastrointestinal disease with fortification of food items. Nanodelivery systems and nanocoating applications provided better water barrier and mechanical properties to supplements by their usage.

4.1 Why Humans Need Protein-Based Dietary Supplements

A deficiency of protein supplies relative to needs can lead to loss of lean body mass, particularly muscle loss. As a result, older people are at considerably higher risk for conditions such as sarcopenia and osteoporosis than are young people. In turn, sarcopenia and osteoporosis can take a high personal toll on older people: falls and fractures, disabilities, loss of independence, and death (Cruz-Jentoft et al., 2010; De Souza Genaro and Martini, 2010; Clegg et al., 2013; Landi et al., 2013). These conditions also increase financial costs to the health-care system because of the extra care that is needed (Janssen et al., 2004).

PROT-AGE Study Group reviewed evidence in the following five areas (Bauer et al., 2013):

1. Protein needs for older people in good health.
2. Protein needs for older people with specific acute or chronic diseases.
3. Role of exercise along with dietary protein for recovering and maintaining muscle strength and function in older people.
4. Practical aspects of providing dietary protein (such as source and quality of dietary proteins, timing of protein intake, and intake of protein-sparing energy).
5. Use of functional outcomes to assess the impact of age- and disease-related muscle loss and the effects of interventions.

Currently there is no consensus on whether dietary protein needs change with advancing age. The current recommendation for protein intake for all men and women aged 19 years and older is 0.8 g/kg per day, established by the Institute of Medicine and based primarily on data from short-term nitrogen balance studies in young adults. A concern about the broad scope of this recommendation is that the original data set used for the estimate was derived from nitrogen balance studies performed on young men (Paddon-Jones et al., 2008; Rand et al., 2003; Trumbo et al., 2002). Additionally, older adults who have acute or chronic diseases need even more dietary protein. Indeed, new evidence suggests that higher dietary protein ingestion is beneficial to support good health, promote recovery from illness, and maintain functionality in older adults (defined as age >65 years) (Kurpad and Vaz, 2000; Morse et al., 2001; Chernoff, 2004; Morley et al., 2010; Walrand et al., 2011; Bauer et al., 2013).

On the other hand, when we review very-high-protein diets (>45% energy), we find them to be associated with a host of adverse events, including nausea, diarrhea, increased calcium excretion from diets high in sulfur-containing amino acids, and increased morbidity. However, diets containing a moderate amount of protein (20–35% energy) do not appear to be associated with negative health outcomes. Furthermore, in human clinical trials, apart from isolated cases of intolerance or hypersensitivity, there have been no reports of toxicity associated with amino acid administration (Paddon-Jones et al., 2008; Allen et al., 1979; Lemon, 1996; Hayashi, 2003). Consequently, there are no toxicity reports about high protein usage, although some negative health effects of high protein usage have been found. A balanced protein intake with diet, which means a proper dosage of protein intake via food and/or supplements can be achieved and avoid these difficulties by considering people's lifestyles and individual needs.

4.2 Beneficial Effect of Protein-Based Dietary Supplements

Food proteins are not only a source of constructive and energetic compounds in the form of amino acids, but they also may play a bioactive roles by themselves and/or can be the precursors of biologically active peptides with various physiological functions. From this point of view, the best-known examples are casein-derived peptides, which have been proved to possess immuno-modulating, antihypertensive, antithrombotic, and opioid activities (Kostyra, 1996; Duranti, 2006).

Soy protein with isoflavones intact was associated with significant decreases in serum total cholesterol (by 0.22 mmol/L, or 3.77%), LDL cholesterol (by 0.21 mmol/L, or 5.25%), and triacylglycerols (by 0.10 mmol/L, or 7.27%), and significant increases in serum HDL cholesterol (by 0.04 mmol/L, or 3.03%). The reductions in total and LDL cholesterol were larger in men than in women. Initial total cholesterol concentrations had a powerful effect on changes in total and HDL cholesterol, especially in subjects with hypercholesterolemia. Studies with intakes >80 mg showed better effects on the lipid profile (Zhan and Ho, 2005). Consumption of soy protein has recently been shown to improve the blood lipid levels in nondiabetic subjects. The purpose of this study was to evaluate if a dietary supplement of soy protein, isoflavones, and cotyledon fiber (Abalon) affects cardiovascular risk markers, blood glucose, and insulin levels in type 2 diabetic subjects. Twenty type 2 diabetic subjects participated in a crossover trial. They were randomized to double-blind supplementation for 6 weeks with Abalon [soy protein (50 g/day) with high levels of isoflavones (minimum 165 mg/day) and cotyledon fiber (20 g/day)] or placebo [casein (50 g/day) and cellulose (20 g/day)], separated by a 3-week wash-out period. Consequently, these results indicate beneficial effects of dietary supplementation with Abalon on cardiovascular risk markers in type 2 diabetic subjects. This improvement is seen even in individuals with near normal lipid values. Ingestion of soy products has been shown to further improve the effectiveness of low-fat diets in nondiabetic subjects. Thus, a dietary supplementation with Abalon in type 2 diabetic patients may provide an acceptable and effective option for blood lipid control, thereby postponing the requirement for drug therapy for these patients (Hermansen et al., 2001).

Besides, hypercholesterolemia is a major modifiable risk factor for cardiovascular disease. Some, but not all, studies have shown that soy protein intake decreases total and low-density lipoprotein cholesterol and triglycerides and increases

high-density lipoprotein cholesterol. Soy protein supplementation was associated with a significant reduction in mean serum total cholesterol, low-density lipoprotein cholesterol, and triglycerides, and a significant increase in high-density lipoprotein cholesterol. Meta regression analyses indicated a dose-response relation between soy protein and isoflavone supplementation and net changes in serum lipids. These results show that soy protein supplementation reduces serum lipids among adults with or without hypercholesterolemia. After all, replacing foods high in saturated fat, trans-saturated fat, and cholesterol with soy protein might have a beneficial effect on coronary risk factors (Reynolds et al., 2006).

A research was designed to compare the acute response of mixed muscle protein synthesis (MPS) to rapidly (such as, whey hydrolysate, and soy) and slowly (such as micellar casein) digested proteins both at rest and after resistance exercise. Three groups of healthy young men performed unilateral leg resistance exercise followed by the consumption of a drink containing an equivalent content of essential amino acids (10 g) as either whey hydrolysate, micellar casein, or soy protein isolate. Mixed MPS was determined by a primed constant infusion of L-phenylalanine. Ingestion of whey protein resulted in a larger increase in blood essential amino acid, branched-chain amino acid, and leucine concentrations than either casein or soy. A similar result was observed after exercise (whey > soy > casein); MPS following whey consumption was 122% greater than casein and 31% greater than soy. MPS was also greater with soy consumption at rest (64%) and following resistance exercise (69%) compared with casein. We conclude that the feeding-induced simulation of MPS in young men is greater after whey hydrolysate or soy protein consumption than casein, both at rest and after resistance exercise; moreover, despite both being fast proteins, whey hydrolysate stimulated MPS to a greater degree than soy after resistance exercise. These differences might be related to how quickly the proteins are digested (such as fast vs slow) or possibly to small differences in leucine content of each protein (Tang et al., 2009).

According to researches about soy protein and isoflavones, there are some contrasts while some of them indicate these molecules potentially improve risk factors for cardiovascular disease, while other researches indicate their beneficial health effect—especially for cardiovascular disease—because of their high content of polyunsaturated fats, fibers, minerals, and vitamins (Sacks et al., 2006). Additionally, another study shows that "high soy protein diet could inhibit breast cell proliferation by the ability of soy act as antagonists" (Hargreaves et al., 1999).

By the way, there are plant-derived proteins. One of them is legumes, which are second only to grasses (cereals) in providing a quantity of food crops for world populations. In comparison to cereal grains, the seeds of legumes are rich in high-quality protein, providing man with a highly nutritious food resource. The major staple foods, such as beans, soybean, lentils, peas, and chickpeas, are all legumes. There are a great number of food uses for grain legumes, such as the leguminous seeds that are grown for seed production and consumption. More recently, other scientists' boards have claimed that "grain legumes effectively contribute to a balanced diet and can prevent widely diffused diseases, including type II diabetes and cardiovascular diseases" (Leterme, 2002; Duranti, 2006).

Recently, research has evaluated the potential for nutritional supplementation and resistance training to positively influence body composition and muscle strength, size, and function in apparently healthy older adults and frail elderly people. It was reported that a group of men who consumed 280 kcal of mixed macronutrient liquid supplements twice daily separate from their meals increased their total energy, protein, and fat intakes, and gained 2.2 kg body weight over a 12-week period of lower-body resistance training, while these parameters were unchanged in the group of men who did not consume the supplements. Training-induced hypertrophy of the mid-thigh muscle groups was greater in the supplemented (13%) than the nonsupplemented (5%) men. Correlation analyses using data from both groups combined revealed that the change in mid-thigh muscle area was positively related to energy, protein, and fat intakes. The conclusion from this study, that nutrition can influence muscle hypertrophy in older men who train using high-intensity resistance exercises was partially supported. This research group reported that older men who ingested a protein-containing(10 g protein, 100 kcal energy) liquid supplement immediately before exercising experienced increases in fat-free mass and muscle hypertrophy over a 12-week training period. However, men who consumed the same supplement 2 h after each exercise session experienced no change or modest reductions in these parameters after training (Campbell, 2007). In recent years, seed dietary proteins have also started to assume a key position in the interests among nutritionists, not solely for their nutrient role, but also for a number of both adverse and beneficial effects that they may exert on the human body, including food intolerance, allergies, inhibition of endogenous hydrolytic enzymes, and hypolipidemic, hypoglycemic, hypotensive, anticarcinogenic, and antiobesity activities, respectively (Duranti et al., 2008).

Furthermore, free radicals are involved in neurodegenerative disorders, such as ischemia and aging. It was demonstrated that treatment with diets enriched with blueberry, spinach, or spirulina have been indicated to reduce neurodegenerative changes in aged animals. To indicate this effect a study was done with rats that were fed with equal amounts of diet (blueberry, spinach, and spirulina) or with control diet. It was found that animals that received blueberry, spinach, or spirulina-enriched diets had a significant reduction in the volume of infarction in the cerebral cortex and an increase in post-stroke locomotors activity. There was no difference in blood biochemistry, blood CO_2, and electrolyte levels among all groups, suggesting that the protection was not indirectly mediated through the changes in physiological functions. Animals treated with blueberry, spinach, or spirulina had significantly lower caspase-3 activity in the ischemic hemisphere. As a result, these data referred that chronic treatment with blueberry, spinach, or spirulina reduces ischemia/reperfusion-induced apoptosis and cerebral infarction (Wang et al., 2005).

According to another research, spirulina represents a blue-green alga that is produced as a dietary supplement for modulating immune functions, as well as ameliorating a variety of diseases. This double-blind, placebo-controlled study evaluated the effectiveness and tolerability of spirulina for treating patients with allergic rhinitis. Spirulina consumption significantly improved the symptoms and physical findings compared with placebo including nasal discharge, sneezing, nasal congestion, and itching. Spirulina is clinically effective on allergic rhinitis when compared with placebo (Cingi et al., 2008). Another study also supports the health effect of spirulina. They briefly defined spirulina along with its related species (Spirulina maxima), is well known as a protein-rich food for dietary supplement (Vonshak, 1997). The effects of spirulina supplementation on preventing skeletal muscle damage on untrained human beings were examined by these studies. Sixteen students volunteered to take Spirulina platens in addition to their normal diet for 3 weeks. Blood samples were taken after finishing the Bruce incremental treadmill exercise before and after treatment. The results showed that plasma concentrations of malondialdehyde (MDA, is the organic compound and this reactive species occurs naturally and is a marker for oxidative stress) were significantly decreased after supplementation with spirulina (Lu et al., 2006). One more research about spirulina is concern about the effect of lead on malondialdehyde, conjugated dienes, and hydroperoxides with vitamin C, E, or spirulina in rats. It indicated that vitamin C, vitamin E, and spirulina had significant

antioxidant activity thereby protecting the animals from lead-induced toxicity (Upasina et al., 2001).

According to a research, female athletes are more likely to use supplements for general health benefits, whereas male athletes mostly use supplements to improve performance and increase strength. Previous studies have found carbohydrate and vitamin/mineral supplements to be the most popular among university athletes, followed closely by creatine and protein supplements (Kristiansen et al., 2005; Burns et al., 2004; Krumbach et al., 1999; Froiland et al., 2004; Green et al., 2001).

On the other hand, there is some evidence which indicates high protein intakes to increase some health risks such as kidney function, especially for elderly people. This point should be taken into consideration before giving protein-based dietary supplements to patients (Paddon-Jones et al., 2008).

Consequently, there are various protein-based dietary supplements which are used for human well-being against a diversity of health problems. These supplements have lots of beneficial effects with and/or without nanotechnology applications.

5 Conclusions

Food proteins are the main macromolecule and nutritional ingredient, which cannot be excluded from a balanced diet. Additionally, food proteins are not only a source of constructive and energetic compounds as the amino acids, but also may play a bioactive role by themselves, and/or can be the precursors of biologically active peptides with various physiological functions. Generally protein requirements of people could be supplied with a balanced diet, although there are some exceptions such as elderly people who have health problems, athletes who need extra protein to maintain muscles, vegetarians, who cannot have enough protein by following vegan diets, and so forth. For those kinds of exceptions and some specific periods of life, protein-based dietary supplements can be a good way to have a balanced diet. Even if there are lots of beneficial effects of protein based dietary supplements usage, a high prevalence usage, which means high dosage intake of supplements can cause negative effects (although safe supplements usage) and health problems to users. Very-high-protein diets (>45% energy), which have been associated with a host of adverse events, including nausea, diarrhea, increased calcium excretion from diets high in sulfur-containing amino acids, and increased morbidity. However, diets containing a moderate amount of protein (20–35% energy) do not appear to be associated

with negative health outcomes. There are no toxicity reports about high protein usage, while there are some negative health effects of high protein usage have been found. A balanced protein intake in the diet, which means a proper dosage of protein intake via food and/or supplements, can avoid these difficulties by considering people's lifestyles and occasion. As a result, if the dosage can be controlled, there are lots of beneficial effects for health, especially for exceptions.

Consequently, we can indicate that protein-based dietary supplements, which have beneficial effects for human well-being such as disease prevention or treatment, can defend humans against obesity, diabetes, and gastrointestinal disease, through fortification of food items. Nanodelivery systems and nanocoating applications provided better water barrier and mechanical properties to supplements.

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20

NUTRACEUTICALS-LOADED CHITOSAN NANOPARTICLES FOR CHEMOPREVENTION AND CANCER FATIGUE

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1 Nutraceuticals

Active components in dietary ingredients are called *nutraceuticals*, a term that combines "nutrition" and "pharmaceutical" (Kalra, 2003). Nutraceuticals in a form of vegetable or in a form of food ingredients have a great importance in our daily life to maintain a human health and their well-being. However, the usage of the nutraceutical rich foods is dramatically decreased in recent days, it is mainly due to time management at workplace and easy availability of the fast foods. Consumption of diets rich in highly saturated fats, sugars, and salts has shown to accumulate reactive oxygen species (ROS), which further has shown to damage or alter the normal cellular metabolism (Lee et al., 2004). Disrupted cellular pathways have shown to associate with serious health-related issues such as cancer, diabetes, obesity, and cardiovascular diseases (McGinnis and Foege, 1993, 2004; Guenther et al., 2007). Therefore, it is essential to maintain the balanced diet for human health and their well-being. Many nutraceutical plants have been traditionally used in the Indian health care system (Ayurveda) for thousands of years due to their interesting antioxidant activities. Antioxidant substances are of particular interest, because they reduce the action of ROS, thereby protecting the body from various diseases. Ayurveda (Ayus meaning life, and veda, meaning knowledge) is one of the oldest forms of traditional medicine used to create harmony between the body, mind, and spirit with the universe. Every individual in this universe is made up of five basic

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elements: space or ether, air, fire, water, and earth. These elements combine in the human body to form three life forces or energies, called doshas. Doshas are of three types: Vata (space and air), Pitta (fire and water), and Kapha (water and earth). Each dosha controls a different body function and every individual inherits distinct mix of the three doshas. Our health and well-being is linked to the balance of the three doshas (tridoshas). Disturbances in any of the three major doshas lead to poor health and sickness. According to the University of Minnesota's Center for Spirituality & Healing, more than 90% of Indians use some form of Ayurvedic medicine to balance the harmony between doshas. Ayurvedic treatments include mainly herbal remedies, dietary restrictions, yoga, massage, meditation, and breathing exercises called pranayama. Herbal medicine is one of the widely used remedial systems of medicine. Herbal products are exclusively derived from plant sources such as seeds, roots, leaves, bark, or flowers. It is estimated that 40% of the world's populations depend directly on plant-based medicine for their health care (Mathi et al., 2015). According to the WHO more than one million people rely on herbal medicines to some extent and also listed 21,000 plants for medicinal uses around the world (Pimentel et al., 2005). Medicinal plants used in traditional system are found to contain steroids, alkaloids, and terpenoids in herbal preparations (Ngarivhume and Van't Klooster, 2015). So far, 12,000 chemical compounds with various biological activities have been isolated from medicinal plants (Tapsell and Hemphill, 2006). This number is estimated to be less than 10% of the total available medicinal compounds. From the beginning of human civilization, medicinal plants have been used for the treatment of different ailments including cancer, diabetes, and other cardiovascular diseases under traditional healing systems. In fact, in recent times researchers from different countries have shown great interest in exploring the use of phytotherapy in the form of extracts, decoctions, and juices (Ramalingum and Mahomoodally, 2014). Interest is mainly due to the fact that the effect of herbal formulations does not differ greatly from conventional drugs, but instead protect the body by restoring the immunological surveillance (Tavakoli and Miar, 2012). Herbal extracts such as curcumin, tea polyphenols, and micronutrients such as vitamins and minerals are naturally acts as a strong antioxidant and have a scavenging effect on active oxygen radical. They have important applications in food industry due to health benefits against cancer and cancer-associated illness. The effectiveness of polyphenols and vitamins in preventing diseases depends on preserving the bioavailability of the active ingredients. Unfortunately, the bioavailability of nutraceuticals is lower because of insufficient gastric residence time, low

permeability and solubility within the gut, as well as instability under conditions encountered in food processing and storage (temperature, oxygen, light) (Bell, 2001). One possible way of improving intestinal uptake is to nanoencapsulate the bioactive nutraceutical substances. Nanoencapsulation-based delivery systems can improve the bioavailability of the nutraceutical components due to the small particle size and more surface area per unit volume (Acosta, 2009). Nanoencapsulation has been shown to enhance the gastrointestinal absorption of polyphenols, vitamins, and also protection against degradation (Eccleston, 2002). Nanoencapsulation is a rapidly expanding technology with a lot of potential applications in food industries. It is a process by which small particles of core materials are packed within a wall material to form capsules. Thus, the production of nutraceuticals in a nanoencapsulated form improves their bioavailability to a large extent. Nanotechnology is one of the rapidly emerging active research fields with potential applications in many industries. The global nano-enabled packaging market for food and beverages was estimated to be $6.5 billion in 2013 and will grow at a compound annual growth rate of 12.7% to reach about $15.0 billion in 2020. On the other hand the use of nanomaterial in the food-packaging market is expected to reach $20 billion by 2020. Nanoencapsulation is one of the advancement of nanotechnology in food sector mainly for protection, controlled delivery, and enhanced bioavailabilty of nutrients and bioactive substances (Bumbudsanpharoke and Ko, 2015).

1.1 Importance of Nutraceuticals

Nutraceuticals refer to products that range from dietary supplements such as vitamins to herbal substances with high nutritional value. They have been extensively used to treat several diseases due to their strong antiinflammatory and antioxidant activity (Al-Okbi, 2014). Nutraceuticals are often taken to maintain health and prevent disease to improve the quality of life (Brower, 2005). Such products include dietary supplements. A dietary supplement is a product that is intended to supplement the diet that bears or contains one or more of the following dietary ingredients: a vitamin, a mineral, an herb, or other botanicals, amino acids, or a dietary substance used to supplement the diet by increasing the total dietary intake, or a concentrate, metabolite, constituent extract, or combinations of these ingredients (Halsted, 2003). Cancer patients routinely take multiple dietary supplements to prevent recurrence of cancer, reduce the adverse effects of cancer therapy, and to improve quality of life by reducing oxidative stress (Nicolson and Settineri, 2011). Oxidative stress is defined as an

imbalance between the production of oxidants or ROS or reactive nitrogen species (ROS or RNS), also called free radicals, and their elimination by protective mechanisms or antioxidants (Halliwell, 2006; Brown et al., 1997). The major site of ROS generation inside the cells is the mitochondria. During respiration, oxygen is reduced by the mitochondria, then forms superoxide (O^{-2}) or the dismutation product H_2O_2 (Murphy, 2009). Superoxide and peroxide reacts with transition metals like Fe^{2+}, Cu^{+}, and break down the hydrogen peroxide to the reactive hydroxyl radical (*OH) (Asad et al., 2004). The hydroxyl radical damages the important components of the cell, such as proteins, lipids, and DNA, results genomic instability, which ultimately lead to pathogenesis of cancer (Gill and Narendra Tuteja, 2010; Wiseman and Halliwell, 1996). Regular dietary components include green tea polyphenols (eg, EGCG), curcumin (Di-feruloyl-methane), and other dietary supplements such as vitamins that are shown to reduce the oxidative damage and maintain cellular homeostasis.

1.2 Polyphenols

Polyphenols are one of the most numerous and ubiquitous groups of plant metabolites, being an integral part of human diets. It is the major antioxidant in our diet. Application of polyphenols has been areas of great interest in the functional foods, nutraceutical, and pharmaceutical industries (Manach et al., 2004). Polyphenols possess a high spectrum of biological activities, including antioxidant, antiinflammatory, antibacterial, and antiviral functions (Bennick, 2002). The majority of preclinical research and epidemiological data suggests that plant polyphenols can slow the progression of certain cancers, reduce the risks of cardiovascular disease, neurodegenerative diseases, diabetes, and osteoporosis, and act as potential chemopreventive and anticancer agents in humans (Arts and Hollman, 2005). Epigallocatechin-3-gallate (EGCG) is one of the major polyphenols in green tea, which is a widely consumed beverage worldwide. Green tea is characterized by the dihydroxyl or trihydroxyl substitutions on the B ring and the *m*-5,7-dihydroxyl substitutions on the A ring. The B ring appears to be the principal site of antioxidant reactions, and the antioxidant activity is further increased by the trihydroxyl structure in the D ring (gallate) in EGCG (Sang et al., 2011). The polyphenolic structure allows electron delocalization, conferring the ability to quench free radicals. A pubmed search in Aug. 2015 under the title "Tea and Cancer" yielded 3,916 citations, while "EGCG and cancer signaling" yielded 333 publications. The EGCG is shown to reduce oxidative DNA damage in smokers by reducing the formation of

8-hydroxydeoxyguanosisne (8-OXO-DG) positive cells (Schwartz et al., 2005) Tea polyphenols enter cells through passive diffusion; however, a majority of them predominantly effluxed from intestinal lumen or liver and excreted in the feces (Jemnitz et al., 2010). It suggests, the bioavailability of tea polyphenols is poor. To increase the bioavailability, Sosa et al. (2011) encapsulated green tea by coprecipitation with biodegradable polymer using high-pressure antisolvent coprecipitation technique. Complexes were formed between green tea polyphenols and α-lactoglobulin to evaluate the impact of complexation on protein gelation and polyphenol antiproliferative activity against tumor cell lines. Particle size and charge of protein–polyphenol complexes depend on protein nature and pH. At pH 6 they had the smallest size and were soluble. The presence of polyphenols accelerated the gelation of both α-lactoglobulin and mainly affected viscoelasticity of α-lactoglobulin gels (von Staszewski et al., 2012). Polyphenol complexation by proteins did not inhibit its antiproliferative activity. The hypocholesterolemic effects of green tea extract were compared with green tea extract in C57BL/6 mice. Antioxidant and hypolipidemic effects of nanoemulsified green tea extract were also investigated. In composition analysis, both contained similar total catechin concentrations. The antioxidative effect of green tea extract was comparable with that of nanoemulsified green tea extract. Their results suggested that nanoemulsification significantly increased hypocholesterolemic effects of green tea extract in vivo due to increased bioavailability (Kim et al., 2012).

1.3 Curcumin

Curcumin is a major component of turmeric and is commonly used as a spice and food-coloring agent. According to the Food and Agriculture Organization and the World Health Organization the optimal daily intake of curcumin is 0–1 mg/kg and doses up to 12 g/day in nontoxic (Lao et al., 2006). The molecular formula of curcumin is $C_{21}H_{20}O_6$ and is not as soluble in water as it dissolves in organic solvents such as dimethyl sulfoxide, ethanol, methanol, and acetone. Chemically, curcumin is 1,7-bis(4-hydroxy-3-methoxyphenol)-1,6-heptadiene-3,5-dione and it comprises of two phenolic rings; each ring is replaced with methoxy ether and attached to each other by an aliphatic unsaturated heptene linker with an α, β diketonic functionality on carbon-3 and -5 (Scapagnini et al., 2002). Research studies suggested that Diketone group undergo reversible tautomerization between enolic and ketonic-forms (Fig. 20.1). The diketo form prevails in acidic and neutral aqueous solutions while the enol form prevails in

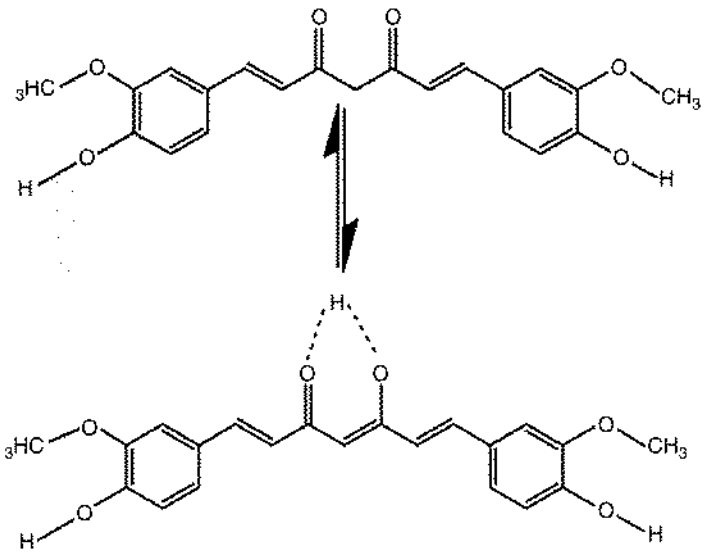


Figure 20.1. The structure of curcumin is shown, illustrating the keto-enol tautomers.

alkaline solutions, and is more stable than the diketo form (Vyas et al., 2013). Based on its tautomerism, curcumin was shown to interact with a wide range of enzymes involved in the initiation and progression of various cancers. Several clinical trials of oral curcumin have been carried out to further investigate the therapeutic activity of this compound (Cheng et al., 2001; Sharma et al., 2004; Goel et al., 2008; Carroll et al., 2011). Curcumin treatment decreased the levels of pyrimidopurinone-deoxyguanosine M1G adducts in malignant colorectal tissue (Sharma et al., 2001). Oral administration of the 8 gm of curcumin decreased the expression of cyclooxygenase gene (COX-2) in pancreatic cancer patients (Shishodia et al., 2007; Anand et al., 2008a). A promising advantage of oral curcumin is that it displays minimal side effects in clinical applications as the drug (Cheng et al., 2001; Sharma et al., 2004). Along the lines curcumin also decreased malondialdehyde and 8-hydroxydeoxyguanosine and increased vitamin C and E in the serum and saliva of patients with precancerous lesions (Rai et al., 2010). However, the practical availability of curcumin may be limited by its low bioavailability due to efficient first pass metabolism, poor gastrointestinal absorption, rapid elimination and poor aqueous solubility (Goel et al., 2008; Carroll et al., 2011). Therefore, an efficient drug delivery based on the nanoparticulate system is anticipated for the successful medical application of curcumin.

A database search of curcumin in combination with the term "*nano,*" which includes nanoparticle, nanoparticles, nanotechnology, nanoencapsulation, and so on, reveals patent applications prior to publication in the literature. The number of patent applications on "curcumin and nano" were 61 in 2007, which represented 34% of the total number of patent applications on "curcumin" in that year (179 patent applications). Many of these patents are related to improved drug delivery of curcumin with nanoparticles. Thus the use of nanotechnology to efficiently deliver curcumin for therapeutic use seems to be an active area in terms of patent application. However, the number of published literature reports on "curcumin and nano" was only16 in 2007, which accounts for only 2.5% of total literature reports on "curcumin" (638 papers). Nonetheless, the number of the literature reports on "curcumin and nano" markedly increased from 2007 onward with 157 publications in 2011–2012 alone. This trend suggests that a strategic decision was made to protect intellectual property for advances in nanotechnology related to pharmaceutical applications of curcumin prior to publication.

1.4 Vitamin C

Ascorbic acid or vitamin C is water-soluble and is present in many vegetables and fruits (Li and Schellhorn, 2007). Ascorbic acid is essential for collagen synthesis in living organism, which protects tissue and cells from oxidation reactions by free radicals and other reactive oxygen-derived species (Carr and Frei, 1999; Frei et al., 1989; Jacob and Sotoudeh, 2002). Besides that it can enhance the efficacy of other fat-soluble antioxidants such as α-tocopherol (vitamin E) and β-carotene (Buettner and Jurkiewicz, 1996). Vitamin C is one of the most important antioxidants to maintain the physiological process of human and it shown to reduce the risk of cancer (Esposito and Cervellati, 2002; Jacobs et al., 2001; Shiau and Hsu, 1999). Vitamin C is not synthesized in digestive tract of humans due to the absence of L-gulonolactone oxidase enzyme, which is responsible for the synthesis of vitamin C (Iqbal et al., 2004). The role for vitamin C (ascorbic acid) in cancer therapy emerged 30 years ago and in recent years its usage has increased due to potential cytotoxic effect to many neoplastic cell lines (Cameron and Campbell, 1974). Administration of ascorbic acid doses is crucial for the proper design of clinical trials; high doses can trigger hemolysis in glucose-6-phosphate dehydrogenase deficiency variants, especially in the presence of infection and fever (Levine et al., 1999). Acute tumor hemorrhage and necrosis have been reported within days after starting IV ascorbic acid in

patients with advanced cancer. There is increasing evidence that vitamin C (ascorbate) is selectively toxic to some types of tumor cells, functioning as a prooxidant (Park, 2013). Vitamin C induced growth arrest in lung, breast, glioblastoma cancer cell lines of murine, rat or human origin in millimolar concentrations, which is also evident from in vitro colony formation assay (De Laurenzi et al., 1995; Park et al., 1971; Chen et al., 2005). Its cytotoxicity is mediated by the generation of H_2O_2 the presence of transition metals such as iron (Frei and Lawson, 2008). "Free" or "loosely bound" iron is highly cytotoxic to normal cells and is stored in the body usually bound to a protein called as ferritin (Blatt et al., 1990; Selig et al., 1993). The proposed mechanism as follows, In the presence of O_2, Fe^{2+} is oxidized to Fe^{3+} and $O^{2\bullet-}$ is formed and it can further be disproportionate to H_2O_2. The resulting Fe^{3+} can be re-reduced to Fe^{2+} by ascorbate [via ascorbyl radical ($AA^{\bullet}$) formation]. H_2O_2 is a stable molecule and easily penetrates cell membranes by diffusion. Within the cell, it causes oxidative damage such as DNA strand breaks, results in depletion of cellular NAD^+ and ATP pools, and finally causes cell death. On the other hand, the formation of highly insoluble (ie, inactive) $Fe(OH)_3$ is prevented by aerobic glycolysis in the tumor microenvironment (release of lactate) (Deubzer et al., 2010; Bruchelt et al., 1991) (Fig. 20.2). On the other hand, recently it was found that the total intracellular concentration of iron remains unaffected in the presence of extracellular Asc, suggesting that Asc-induced cancer cell death is not related to iron deficiency using two cancer cell lines (LNCap and PC3) with different metastatic potentials (Mojić et al., 2014). Rather it was found that H_2O_2 undergo decomposition and produces hydroxyl free radical (*OH) via Fenton reaction at the physiological concentration of iron. However, extracellularly produced (*OH) are unable to cross the cellular membrane, therefore, they are buffered by extracellular proteins and some by Asc and membrane lipids. Under such settings ROS remain in the extracellular compartment leaving cancer cells unharmed. On the other hand, Asc shown to upregulate the expression of hypoxia inducible factor-1 (HIF-1) levels, a transcription factor that is involved in the regulation of different aspects of cancer cell biology (Sinnberg et al., 2014).

Vitamin C is sensitive to high temperature, oxygen and light, and most of its functionality is lost during processing and storage of food and feeds because of the exposure to high temperature, oxygen and light (Soliman et al., 1987). It was found that approximately 75% of the initial amount of supplemented vitamin C in shrimp feeds was lost during processing at ambient temperature (Shiau and Hsu, 1993). The utilization of more stable forms of

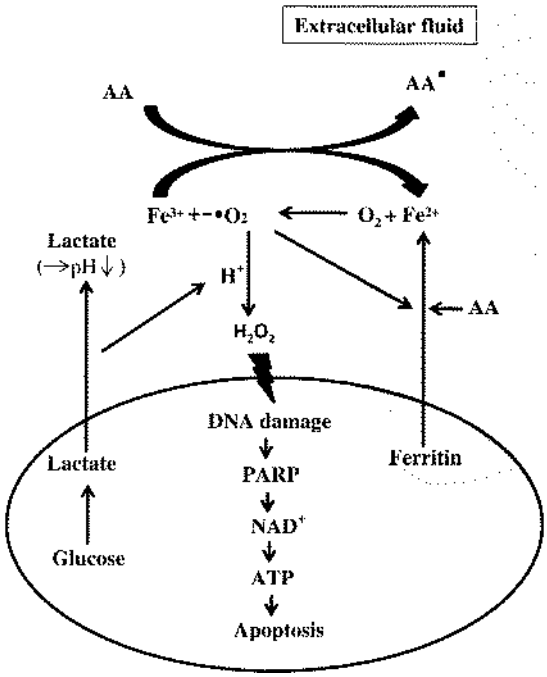


Figure 20.2. Redox activity of vitamin C/Fe system. Model for the generation of H_2O_2 in the tumor microenvironment.

vitamin C is therefore a crucial requirement for human and animal nutrition. The enhanced stability of vitamin C is a suitable option to realize its sustainability. The permeability of the unmodified vitamin C (an active form) through skin is obstructed by a major barrier, stratum corneum (Zhang et al., 1999). Therefore, a carefully designed topical delivery system for ascorbic acid is of importance for effective delivery of this compound. From the benefits of nanotechnology which are of interest in medical, pharmaceutical, and cosmetic fields, preparation of ascorbic acid loaded nanoparticles could enhance ascorbic acid utility by protecting the entrapped ascorbic acid from environment, control releasing rate at releasing area or absorbing area and could be designed to be a drug delivery system to the preferred target sites such as hair follicles.

1.5 Vitamin D

Vitamin D is a fat-soluble vitamin and it exists in two forms such as ergocalciferol (D_2) or cholecalciferol (D_3). It is synthesized in the sebaceous glands of the skin by the action of ultraviolet light in the UVB range (290–310 nm) on 7-dehydrocholesterol in

the skin (Norman, 1998). The epidermal pigment melanin (Orazio et al., 2013) efficiently absorbs UVB, results causing to open the 5,7-diene of the B ring of the sterol nucleus (Wacker and Holick, 2013) and isomerize to form the energetically more stable s-*trans*, s-*cis*-previtamin D_3. 7-Dehydrocholesterol content of skin decline with age, such that the vitamin D biogenic response to solar irradiation is also diminished in older people. Evidence suggests that vitamin D_3 is approximately three times more effective at maintaining serum concentrations because the binding protein has a higher affinity to vitamin D_3 than vitamin D_2. Vitamin D_3 is converted to 25OH-D_3 by an enzyme 25α hydroxylase in the liver and 25OH-D_3 is converted to 1,25 diOH-D_3 by an enzyme 1 α hydroxylase in the kidney (Bringhurst et al., 2005). The chief food sources of vitamin D in Western diets are fortified milk, juices, and cereals; and fatty fish (Holick, 2004). In the presence of bile salts vitamin D is absorbed by small intestine as similar to other hydrophobic substances, enters the lymphatic circulation, predominantly (about 90% of the total amount absorbed) in association with chylomicra. Chylomicrons are lipoprotein particles and they enable to transport vitamin D into liver, within a few hours after being absorbed across the small intestine or synthesized in the skin. In the liver, lipoproteins is converted by hydroxylation of side chain carbon C-25 of vitamin D_3 or previtamin D_3 to yield 25OH-D_3, also called calcidiol, the major circulating form of the vitamin D (Haddad et al., 1993; Jones et al., 1998; DeLuca, 2004). Low levels of calcium (Ca^{2+}) and phosphate (PO^{4-}) in the serum stimulates the parathyroid gland to increase the production of the parathyroid hormone and these further increases the production of 1-hydroxylase enzymes by the kidney. This enzyme hydroxylates at the C-1 position of the A ring of vitamin D to yield 1,25-$(OH)_2$-D_3 (calcitriol) in the kidney (Suda et al., 2002; Yamamoto et al., 1984) (Fig. 20.3).

Unlike other vitamins, calcitriol is a hormone that acts as a messenger to tell other cells what to do. Calcitriol works by binding to the vitamin D receptor (VDR), which is highly expressed in virtually every cell type including epithelial cells (including skin, breast, prostate, and colon, which are at risk for carcinogenesis) and immune cells (such as macrophages and dendritic cells) (Haussler et al., 2011). The best characterized mechanism of action for the calcitriol–VDR complex involves heterodimerization with retinoid X receptor (RXR) and binding to vitamin D response elements (VDREs) in target genes to regulate transcription. An excessive intake of vitamin D can also cause a raised calcium level. Malignant hyercalcemia is a frequent complication of metastatic bone disease. Hypercalcemia of malignancy is caused by

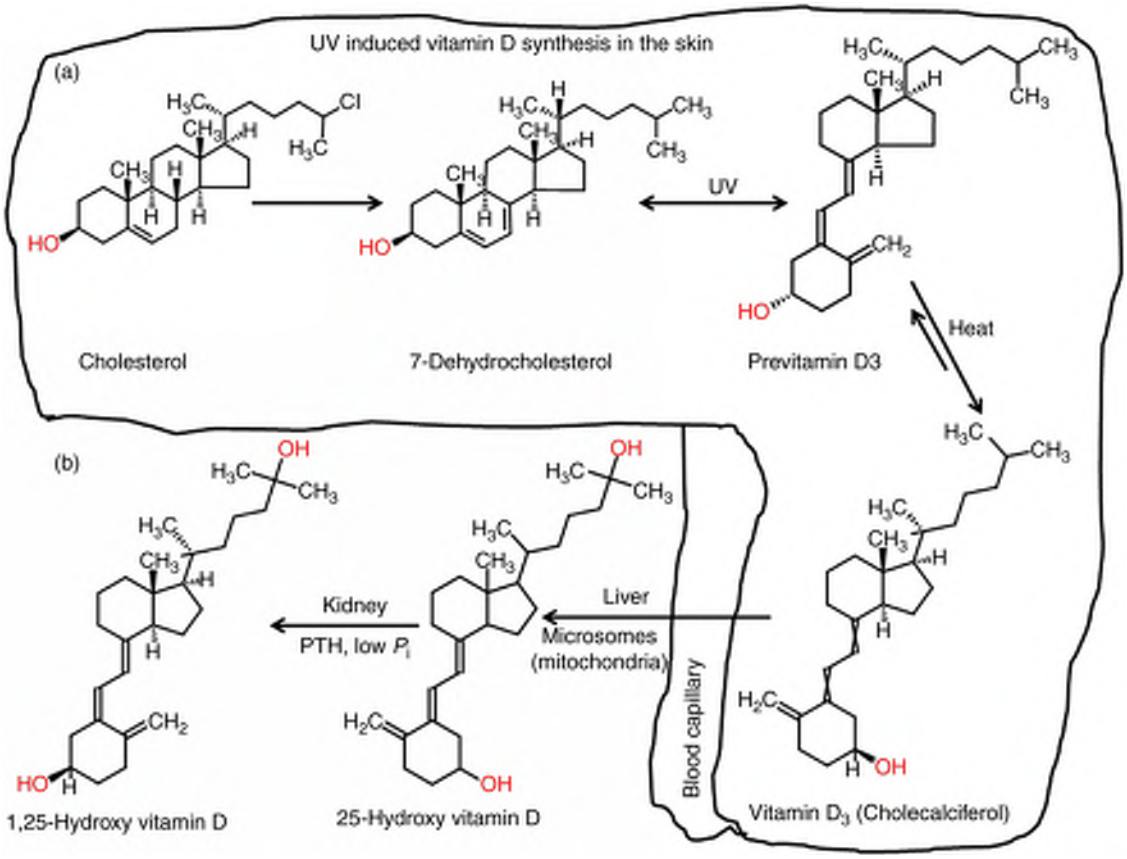


Figure 20.3. Synthesis of vitamin D_3. (a) The conversion of 7-dehydrocholesterol to previtamin D_3 by 290–310 nm UV light and the temperature dependent equilibrium between previtamin D_3 and vitamin D_3; (b) The functional metabolism of vitamin D_3. A CYP2R1 enzyme in the liver converts vitamin D_3 to 25OH-D_3, the circulating form of vitamin D_3, and a CYP27B1 enzyme converts it to the 1,25-$(OH)_2D_3$ in the proximal convoluted tubule of the kidney to the final hormone, 1α,25-$(OH)_2D_3$.

tumor production of PTH-related peptide (PTRrP) in 80% of cases (Dusso, 2003). Symptoms of a raised calcium level include thirst and increased urination and leads to fatigue (Milanesi et al., 2013).

Vitamin D protects against inflammation and reduces the incidence and severity of many types of cancer (Woloszynska-Read et al., 2011). Epidemiological studies indicate that maintenance of serum 25 D levels above 40 ng/mL (100 nmol/L) correlates with reduced risk of breast, colon, and rectal cancer (Keum and Giovannucci, 2014). The vitamin D receptor (VDR) is highly expressed in epithelial cells at risk for carcinogenesis (including those resident in the skin, breast, prostate, and colon). In epithelial cells, activation of VDR by its ligand, 1,25-dihydroxyvitamin D

(1,25D), triggers genomic changes that contribute to maintenance of differentiation via regulation of the cell cycle and apoptosis. Vitamin D also regulates inflammatory pathways and modulates cross talk between epithelial cells and immune cells in the context of cancer. Both epithelial and immune cells express the vitamin D metabolizing enzyme cytochrome P450 27B1 (CYP27B1), which enables autocrine generation of 1,25D from the circulating vitamin D metabolite 25-hydroxyvitamin D (25D), critically linking overall vitamin D status with antitumor and antiinflammatory actions (Kong, 2013). On the other hand, vitamin D deficiency is related to Obesity (Wortsman et al., 2000), because the more of the vitamin is required based on the weight of the body. In addition, vitamin D supplements shown to interact with several types of medications. For example, weight loss drugs including orlistat (Xenical, and Alli) and the cholesterol-lowering drug cholestyramine (Questran, LoCholest, Prevalite) shown to reduce your body's absorption of vitamin D and other fat-soluble vitamins. Drugs to control epileptic seizures phenobarbital and phenytoin (Dilantin) can increase the metabolism of vitamin D and reduce calcium absorption. Statins and diuretics can increase vitamin D levels (Robien et al., 2013; Verrotti et al., 2010; Yavuz and Ertugrul, 2012). Overall it suggests that vitamin D is one of the important nutraceutical agents with therapeutical value and encapsulation of bioactives in lipid-based carrier systems like nanoliposomes preserves their native properties against oxidation over time along with providing its stable aqueous dispersion.

1.6 Vitamin B_{12}

Vitamin B_{12} (B_{12}) or cyanocobalamin is soluble in water. It is stable at acidic environment (pH 4–7) and at temperature (until 120°C) without significant loss (Watanabe and Miyamoto, 2003). Cobalamins are organometallic compounds, consisting of a chiral corrin ring coordinating with a central positively charged cobalt ion through four nitrogen atoms and possessing seven amide side chains. A 5,6-demethylbenimidazole ribonucleotide moiety is linked to side chain of coordinates cobalt as the lower axial (alpha-side) lignd. The upper axial (beta-side) ligand can be exchangeable with cyano (CN), hydroxyl (OH), methyl (Me), or 5′-deoxyadenosyl (Ado) group (Sterling et al., 2013). Vitamin B_{12} uptake by the cells depends on the three transport proteins. The three transport proteins are haptocorrin (HC), intrinsic factor (IF), and transcobalamin (TC) (Furger et al., 2013). B_{12} from food is first captured by salivary haptocorrin (salivary B_{12}-binding protein) in the stomach. In the stomach haptocorrin–B_{12} complex

undergo proteolysis by pancreatic proteases in the duodenum. Next, released B_{12} preferentially binds to intrinsic factor (IF; gastric B_{12}-binding protein) in the proximal ileum. This complex protects the vitamin from side chain modification of the corrin ring by intestinal bacteria, while also protecting IF from hydrolytic attack by pepsin and chymotrypsin. The IF–B_{12} complex can enter the enterocyte involves cubam receptor-mediated endocytosis, in which IF being degraded within the enterocyte of lysosomes and the vitamin being transferred to a specific carrier protein, transcobalmin (TC). The essential role of TC is to transport absorbed B_{12} in the bloodstream to cells of the body. The membrane-bound receptor proteins (CD320) for TC is available in all cells, called TC receptors. Cellular uptake of vitamin B_{12} involves TC receptor mediated pinocytosis (Fig. 20.4). After the cellular uptake, the TC-receptor complex is degraded in the lysosome to yield the free vitamin, which further converted into methylcobalamin (MeB_{12}) in the cytosol by the cytoplasmic methione synthase and Adenosylcobalamin ($AdoB_{12}$) by mitochondrial methylmalonyl-CoA mutase. The predominant form in human plasma is methylcobalamin (Nielsen et al., 2012; Moestrup, 2006; Allen et al., 1978; Gueant et al., 1986; Quadros et al., 2009; Del Corral and Carmel, 1990; Froese and Gravel, 2010; Banerjee et al., 2009). Vitamin B_{12} maintains proper nerve function, is involved in production of DNA, blood cell formation, and production of amino acids. Deficiency of vitamin B_{12} can result in blood conditions, such as anemia, neurological disorders, and increased risk for cardiovascular disease (Mahmood, 2014). A number of research studies showed that low levels of vitamin B_{12} is associated with colon, stomach and breast cancers (Plant and Tisman, 2006; Herbert, 2001, 2002; Wu et al., 1999). A study of breast cancer shows that the higher dietary intakes of vitamin B_{12} and folate—another B-complex vitamin—were associated with lower breast cancer risk (Zhang et al., 2008; Rohan et al., 2000). This specific of vitamin B_{12} uptake system has been explored in the last years to enhance the oral delivery of poorly available therapeutic molecules including, granulocyte colony stimulating factor, erythropoietin, and luteinizing hormone-releasing hormone. However, in spite of the numerous advances for the oral delivery of small peptides and proteins, some disadvantages have limited the efficiency of this strategy (Russell-Jones, 2004; Lim and Shen, 2005; Bai et al., 2005). The directly linked macromolecular drugs to vitamin B_{12} are susceptible to the degradation within the gastrointestinal tract. In addition, according to the limited uptake capacity of vitamin B_{12} (2.4 μg/day for adult), the amount of the therapeutic molecule that can be delivered as conjugate is limited (Bor et al., 2006). An attractive

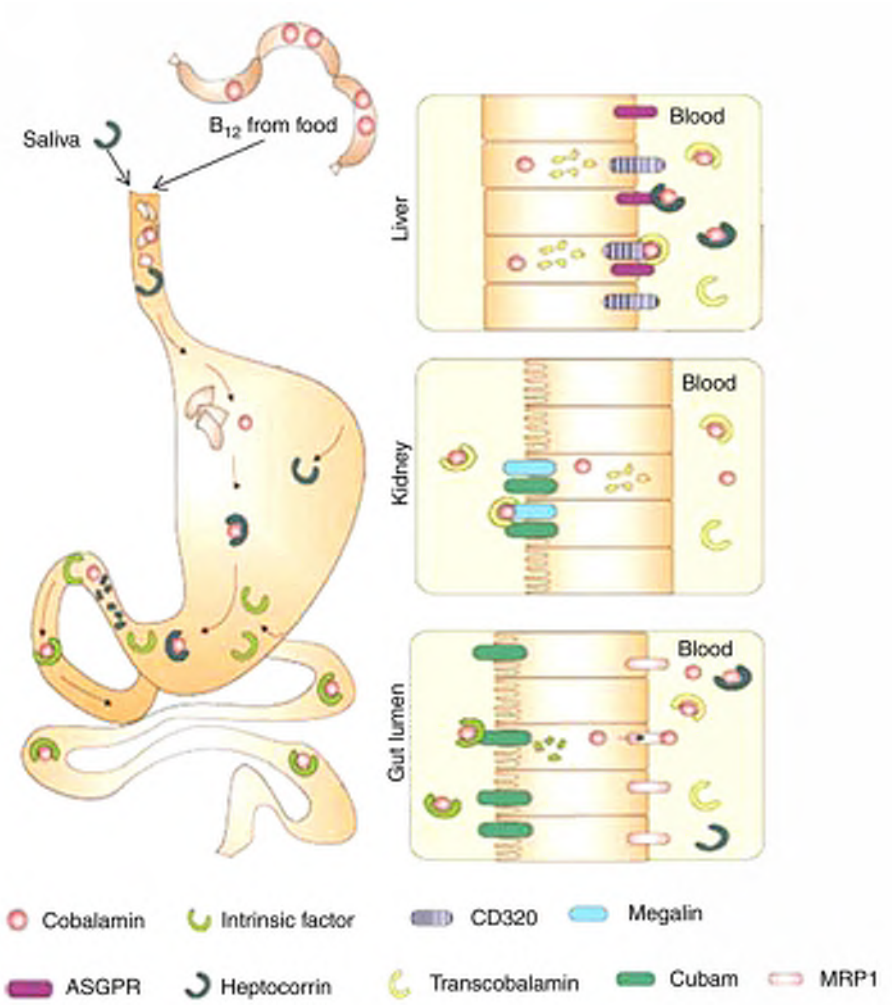


Figure 20.4. Absorption and transport of vitamin B_{12} (Cobalamin) in humans. Cobalamin (Cbl) from food is first bound to HC present in the saliva and moved to gastric environment. In the duodenum HC is degraded by pancreatic enzymes and Cbl is transferred to IF. The Cbl-IF complex is then endocytosed by the receptor complex cubam. In the intestinal cells, IF is degraded and Cbl is released into blood plasma from the basolateral side of the cell by a multidrug resistance protein (MRP1). In the blood plasma Cbl is bound either to HC or TC, where only TC is responsible for the Cbl uptake into cells. This uptake is mediated by the TC receptor CD320. In the kidney, megalin instead of CD320 is the receptor responsible for uptake of Cbl. Modified from Nielsen et al. (2012).

solution to overcome these problems would be the incorporation of drug in nanoparticles coated with vitamin B_{12}. This can offer both high loading capacity of the therapeutic molecules and the protection against the aggressive gut conditions (pH and enzymatic degradation).

1.7 Connection between Iron and Vitamins

Iron plays a key role in the transport of oxygen and its deficiency result the lack of hemoglobin, which deliver the oxygen throughout the body. Iron can exist both in the ferrous (Fe^{+2}) and ferric (Fe^{+3}) states and hence in biological redox reactions can serve as an electron donor or acceptor. Iron in the ferrous state is highly capable of binding with oxygen. In the ferric state iron binding capacity of hemoglobin is markedly reduced (Pittman, 2011; Wollenberg and Rummel, 1987). Vitamin C and other antioxidants in various foodstuffs are capable of reversibly binding with ferric ions and convert them to ferrous ions. Vitamin A deficiency limits the body's ability to use iron, although it does not cause actual iron deficiency (Kroner, 2011). There may also be effects on the body that are not due to the low hemoglobin but are rather due to an interruption of other iron-related reactions (Beard, 2001). For example, iron exhibits a catalytic activity for the formation of biologically active form of vitamin D in liver and kidney, using intermediates of cholesterol metabolism. People with vitamin deficiency kidney failure on dialysis often develop iron-deficiency anemia because of iron losses, as well as a decrease in erythropoietin, a hormone secreted by the kidney that stimulates red blood cell production (Haug et al., 1998; Aukrust et al., 1999; Masuhara and Migicovsky, 1963). On the other hand, vitamins had been shown to antagonize the absorption of Iron absorption. For example vitamin D, by enhancing the absorption of calcium, can lead to poor iron absorption. Cobalt, which is an integral part of vitamin B_{12}, competes with iron and vice-versa on an absorptive level. Similar antagonistic effects were found between iron and vitamin E at cellular level, either through increased metabolic demands of iron produced by vitamin E or by free radical production caused by excess tissue iron accumulation. Excess tissue iron levels contribute to lipid peroxidation, thereby increasing the antioxidant requirement for vitamin (Watts, 1998) (Fig. 20.5).

2 Cancer

Cell is a building block of disease and organ construction. Composition of membrane (protein–lipid complexes), nucleus (cell brain or command center), cytoplasm (space between nucleus and membrane), mitochondria (powerhouse of the cells), endoplasmic reticulum (transportation of proteins), golgi body (storage and transportation), and lysosomes (detoxification organelles). Cells vary in size, composition depending on which organic matter they are designed to maintain (Khoshmanesh et al., 2008). Cell

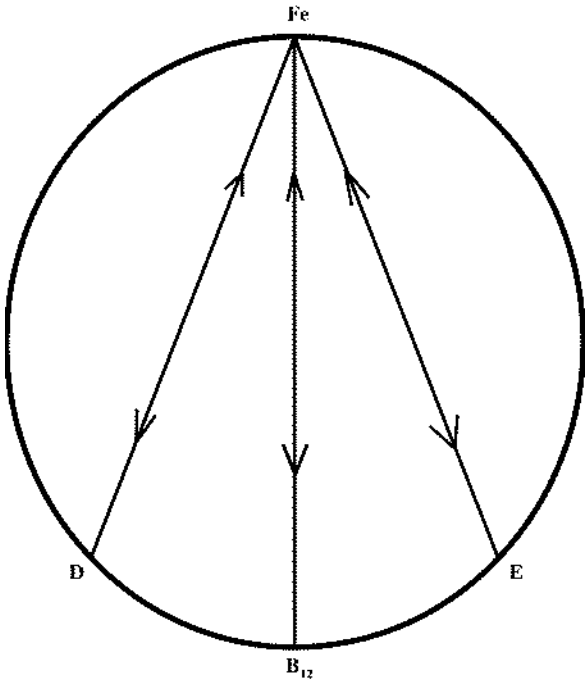


Figure 20.5. Illustration depicting the antagonistic effects of iron and vitamins. Modified from Watts (1998).

growth is controlled by equilibrium between proliferation, differentiation, and cell death. Proliferation is defined as the expansion of cells by the continuous division of single cells into daughter cells. Differentiation is the process whereby an undifferentiated stem cell (unspecialized cell) acquires the features of a differentiated cell (specialized cell). Division of one cell to daughter cell is a dynamic process that results in the duplication and transmission of genetic information from one cell generation to the next (Evan et al., 1994). The cell cycle can be divided into mitosis (cell division or cytokinesis) (M-phase) and interphase. Interphase can be subdivided into the DNA synthesis period (S-phase), and the pre and post-DNA synthesis gap phases (G_1 and G_2 phases, respectively). Cells in the G_1 phase are responsive to growth factors and other environmental signals such as nutrient availability. Following cell division (mitosis and cytokinesis), the daughter cells confront two possible fates. A daughter cell may decide to enter immediately into another round of growth and division, thereby remaining in the active growth cycle. This leads to repeated rounds of cell division and results, in turn, in an exponentially increasing cell population. As an alternative, a daughter cell may decide to cease

active growth and exit the active cell cycle to enter a state of quiescence termed G_0. A cell in the G_0 phase may perform normal cellular functions, but is not preparing for division (Cooper, 2000). In normal cells, cell cycle progression is controlled by the sequential activation and inactivation of cell cycle dependent proteins such as p53 and pRb (retinoblastoma protein). Loss of p53 function often seen in cancer cells, resulting uncontrolled cell proliferation results cancer, which further results in the formation of lesion or lump in our body is called as tumor (Pucci et al., 2000). Tumors are broadly classified into benign and malignant. Benign tumors grew locally and remain confined to the local environment of the tissue, they do not spread to other parts of the body, and they can be surgically removed depending on the site. On the other hand malignant tumors grow rapidly and spread from the site of the origin through systematic or lymphatic circulation. This property of cancer cells is called metastasis (Jawad and Scully, 2010). The etiology of cancer is linked to several natural substances in the environment and life style choices in the modern society. These lifestyle choices include excessive alcohol consumption, cigarette smoking, and lack of exercise, poor diet, and hormones. All these factors influence the regulation of cell growth and differentiation in our body (Anand et al., 2008b). Cancer cells in the tissue can be identified by their characteristic features such as: (1) large nucleus, (2) abnormal chromosomal numbers, and structures, (3) altered cell cycle, and (4) enhanced energy metabolism (Baba and Catoi, 2007).

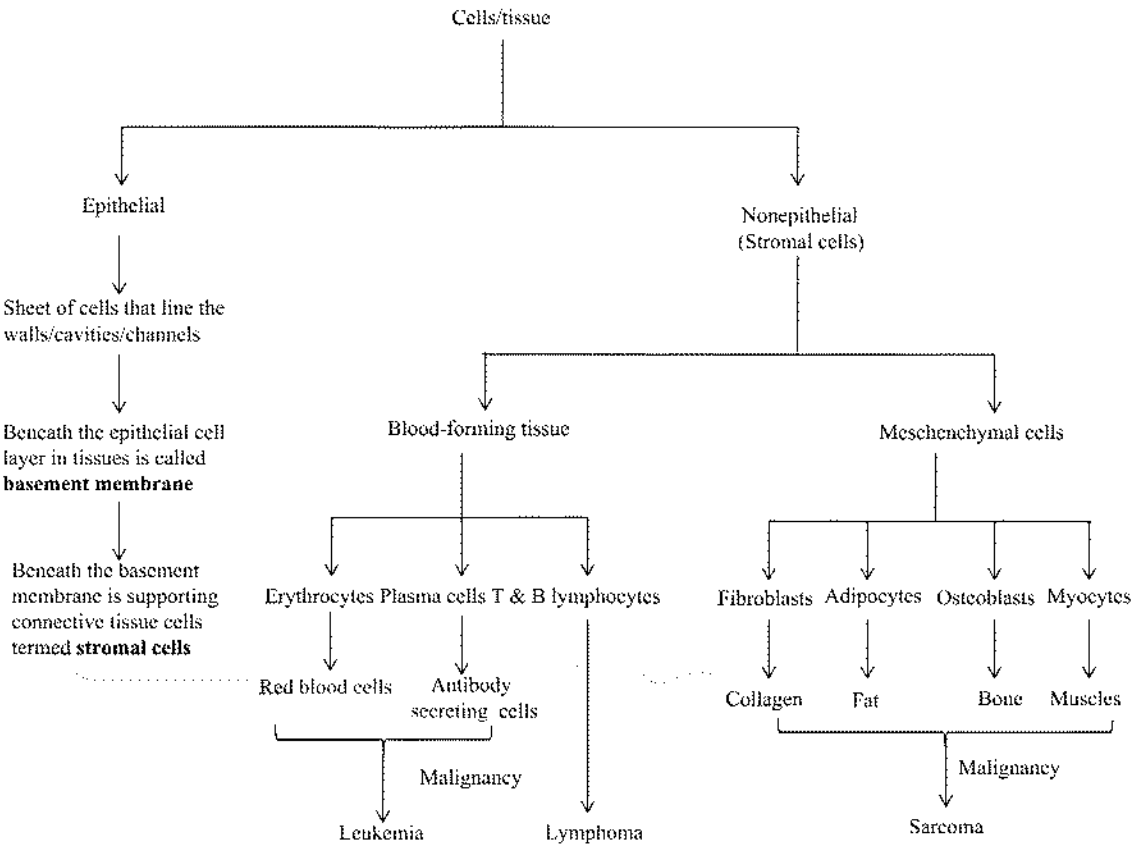
2.1 Types of Cancer

There are two major types of cells they are germ cells and somatic cells. Germ cells also called embryonic cells or stem cells or unspecialized cells, because they can differentiate into multiple cell types depending on the necessity. Somatic cells are called specialized cells because they are final differentiated cells. Examples are skin cells, lung cells, neuronal cells. Cells can be of two different origins, epithelial and nonepithelial. Epithelial cells are formed sheets of cells that line the walls/cavities and channels. Nonepithelial cells are also called stromal cells; they are located beneath the epithelial cell layer in tissue. Stromal cells consist of hematopoietic and meschenchymal stem cells. Hematopoietic stem cells are responsible for blood forming cells such as erythrocytes, plasma cells, T and B lymphocytes. Erythrocytes are responsible for formation of red blood cells and plasma cells are responsible for generation of antibodies, T and B lymphocytes are responsible for immunity. Tumors can be generated either from

epithelial or nonepithelial origin. Origin of cancer from epithelial cells is called carcinoma and from nonepithelial cells is called as sarcoma. Carcinoma is of two types, squamous cell carcinoma and adeno-carcinomas. Squamous-cell carcinoma is carcinoma of the epithelial sheets covering the cavity or channel, for example, skin cancer. Adeno-carcinoma is a cancer of an epithelium that originates in glandular tissue, for example, stomach cancer. Sarcomas are the malignant tumors of connective tissue or organs that originate from mesoderm, for example, osteosarcoma of bone, chondrosarcoma of cartilage, angiosarcoma of blood vessels, and so forth. Cancers of muscles are also sarcomas. Sarcomas constitute about 2% of the tumors diagnosed. Cancers of nonepithelial sources are fibro sarcoma (fibroblasts), liposarcoma (adipocytes), and osteosarcoma (osteoblasts). Leukemia is the malignant tumors of stem cells of hemopoietic tissues. They are liquid tumors affecting blood cells, particularly the white blood cells growing in the bone marrow, for example, chronic myelocytic leukemia, acute T cell leukemia, and so forth. Lymphoma is a malignant tumor of secondary lymphoid organs like spleen and lymph nodes, for example, Burkitt lymphoma (Skarin, 2003; Young and Heath, 2003). Cell types and their associated cancers are given below (Flow Diagram 20.1).

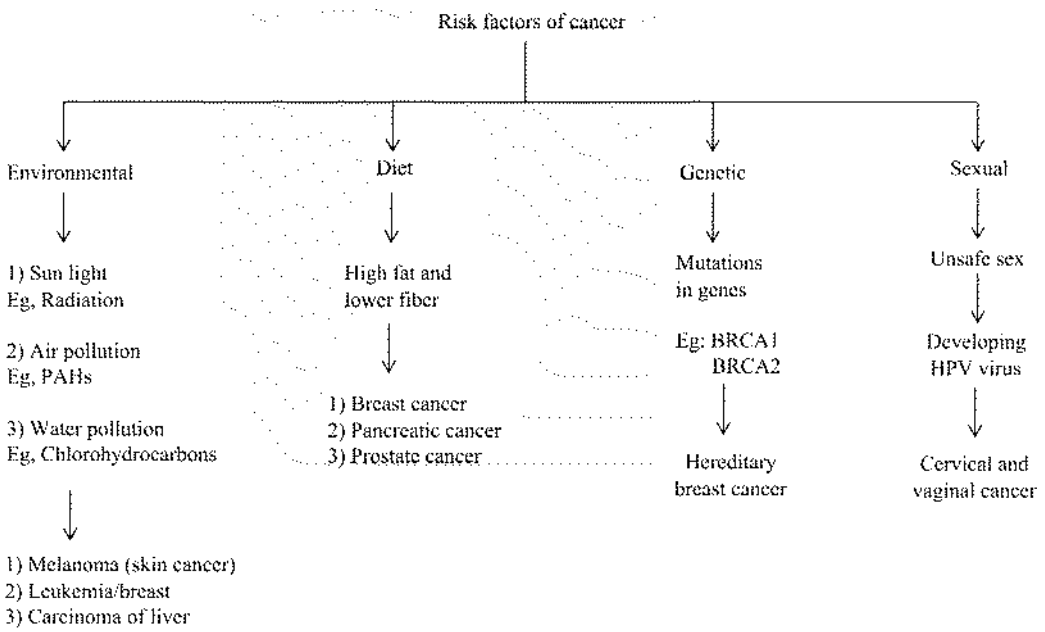
2.2 Causes of Cancer

Cancer-causing agents are called carcinogens, and processes are called carcinogenesis. Carcinogens are broadly classified into three types, physical, chemical, and biological agents; physical carcinogens produce cancer by some physical mechanisms. Examples in this category include radiation. Ionizing radiations like x-rays and gamma rays and nonionizing radiations like UV (200–400 nm wavelength) cause irreparable DNA damage leading to skin cancer. One of the best-studied chemical carcinogens is polycyclic aromatic hydrocarbons (PAHs). The risk factors and their associated cancers are given in Flow Diagram 20.2. Main targets of chemical carcinogens are proto-oncogenes, oncogenes, and tumor suppressors. Proto oncogenes are present in normal cells and they are activated upon interaction with carcinogens. An oncogene is a gene capable of transforming a normal cell into a tumor cells. Tumor suppressor genes protect cells from DNA damage and mutations. Inactivation of tumor suppressor cause unrestricted cell proliferation, for example, Polycyclic aromatic hydrocarbons (PAHs) cause mutations in the normal cells and activate the action of proto-oncogene c-Myc. c-Myc belongs to the Myc family of transcription factor, and it dimerizes with basic helix loop helix



Flow Diagram 1. Depicts the origin of cancer.

transcription factor Max. Myc-Max dimeric complex translocates to the nucleus and down-regulates the expression of cell-cell adhesion molecule, E-cadherin upon binding to the consensus E-box sequence (CACGTG). Loss of E-cadherin by deletion or gene silencing is by far the most common adhesion molecule alteration in several cancer types that includes cancers of bladder, stomach, breast, colon, kidneys, and prostate. Moreover, loss of E-cadherin function is associate in epithelial mesenchymal-like transition and the acquisition of a number of phenotypic and genotypic alterations leading to increased angiogenesis, radio-resistance, genomic instability, invasiveness and antiapoptotic functions (Fig. 20.6) (Botlagunta et al., 2008, 2010, 2011; Hanahan and Weinberg, 2011).



Flow Diagram 2. Depicts the factors associated in the development.

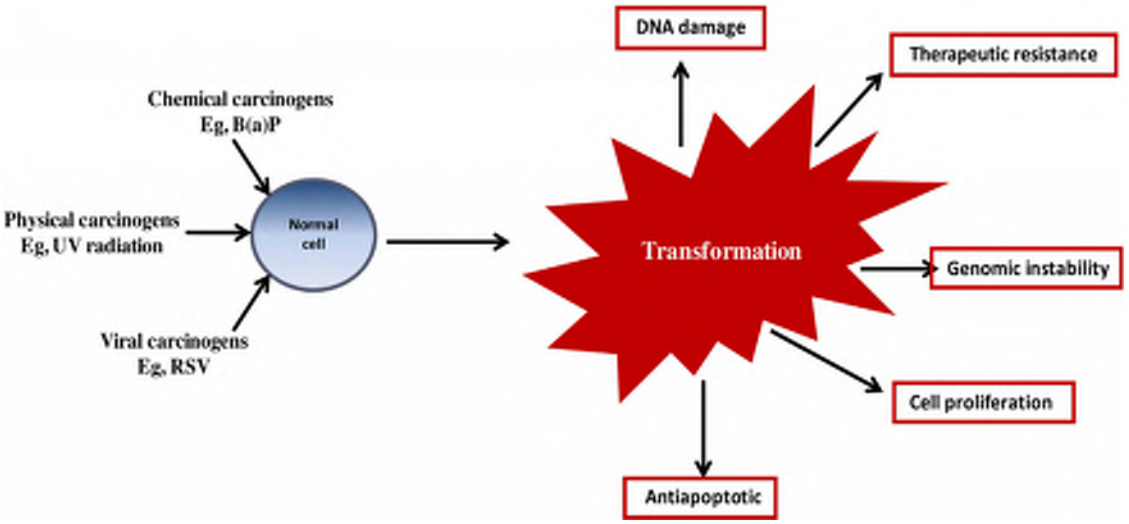


Figure 20.6. Effect of carcinogens in the transformation of normal cell into cancerous cell, which possesses increased angiogenesis, radio-resistance, genomic instability, invasiveness, and antiapoptotic functions.

2.3 Cancer Detection and Diagnosis

Early detection and diagnosis prevents cancer dissemination from its origin. It is well understood that the majority of cancer deaths is due to the dissemination of tumor cells from the primary tumor and their growth at distal organs, that is, treatment is unlikely to succeed once a local benign lesion metastasizes. Thus, cancer detection and diagnosis is of extreme importance with respect to the possibility of increasing survivability. Cancer detection is based on biopsy and histopathological evaluation of the tissue and blood and bone marrow tests. In biopsy, a piece of the suspected tissue is cut into thin sections and stained with hematoxylin and eosin (H&E) and examined under microscope (histopathological studies) to see rapidly dividing cancer cells by a pathologist (Brown et al., 2012; Soini et al., 1998). In addition, antibodies against cancer specific antigens such as Her-2/neu, EGFR, BRCA1, and BRACA2 generally used for detection of cancers of different types (Ong et al., 2012; Banin Hirata et al., 2014). Noninvasive in vivo magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT) is very useful for the detection of cancers in internal organs (Ehling et al., 2013; Fass, 2008).

2.4 Treatment of Cancer

Depending upon the location and the grade of the tumor cancer can be treated by various methods such as surgery, radiation therapy, chemotherapy, and immunotherapy. Benign tumor can be completely removed by surgery. Removal of the breast cancer surgery is called mastectomy, removal of cancer in the prostate is called as prostatectomy, and lung cancer surgery for nonsmall cell lung cancer. Preliminary goal of the surgery isremoval of either the tumor or the entire organ. In case this is not possible, when the tumor cells disseminate to other sites of the body from the site of the origin, a process is called metastasis. Radiotherapy is also called radiation therapy; in this method ionizing radiation can be administered externally via external beam radiotherapy (EBRT) or internally via brachytherapy to kill cancer cells and shrink the tumors. Chemotherapy is the treatment of cancer with drugs; these drugs are called anticancer drugs. Immunotherapy is designed to induce the patient's own immune system to fight the tumor (Urruticoechea et al., 2010). Among all therapaeutic approaches, chemotherapy is one of the highly recognized cancer therapies that prevents cancer recurrence following surgery. Chemotherapy can cause burning sensation at the place where it is injected,

tingling and shivering in the hands and feet, and pain in the tips of the fingers, and in the radiotherapy treatment can give irritation, stomachache, or vomiting sensation (Ortiz-Tudela et al., 2014; Aslam et al., 2014). A number of chemotherapeutic agents have been used for chemoprevention and they often have shown to contribute chemotherapy related cancer fatigue (CRF).

2.5 Cancer Fatigue

Fatigue is a common symptom of the cancer patients. Cancer fatigue results when lack of energy leads to tiredness. Fatigue in the normal person is short term and improves by taking rest. But in the cancer patient, fatigue is different, is not relieved by rest, and can go on for day, weeks, even months. We can also classify the fatigue in the cancer patients as due to acute fatigue and chronic fatigue. But in cancer patients probably we can see chronic fatigue; it may not end even when the treatment is complete. Fatigue in the cancer patients is due chemotherapy, radiotherapy, and so forth (Cruz et al., 2015; Bower, 2014; Escalante et al., 2010; Koornstra et al., 2014). CRF is caused by anemia, in which the body does not have enough healthy red blood cells and cannot properly transport oxygen and nutrients (Dicato et al., 2010; Dicato, 2003). Decreased concentration of hemoglobin in blood (14.5–15.5 g/100 mL of blood) leads to depletion of iron, which is an essential factor in the formation of active vitamin D. Vitamin D metabolism is essential in terms of formation of calcitriol, a key compound associated with intestinal absorption, transport of calcium, mineralization, and calcification of bones. The consequence of lack of iron supply to cells could lead to defective bone desorption, finally leading to both osteomalacia and osteoporosis (Toxqui and Vaquero, 2015). Apart from red blood cells, patients receiving cancer chemotherapy lack white blood cell count; therefore they are susceptible to infection from the disease, which is further shown to elevate inflammatory mediators such as interleukins (Gafter-Gvili et al., 2012; Karthikeyan and Lip, 2006). From the published reports, it is clear that cancer drugs induce cell death in rapidly growing cancer cells, but they are also shown to harm healthy cells by straining the immune system, causing cancer-related fatigue. For example, pemetrexed, treated nonsmall-cell lung cancer (NSCLC) patients showed serious life-threatening infections caused by combination of several side effects such as myelosuppression, mucositis, and diarrhea (Wang et al., 2004; Pierard-Franchimont et al., 2011). Several different approaches, including dietary changes and nutritional supplements, can be effective in increasing energy levels in cancer patients. Supplementation of multiple micronutrients is

recommended because many different types of free radicals are produced in different organs/cells and each antioxidant has a different affinity for each of these free radicals, depending upon the cellular environment (Ravindran et al., 2009). For example, Vitamin E is more effective as a quencher of free radicals in reduced oxygen pressure (hypoxia), whereas vitamins C and A were more effective in higher oxygen pressures (Vile and Winterbourn, 1988). Similarly, vitamin C is necessary to protect cellular components in aqueous environments, whereas carotenoids, and vitamins A and E protect cellular components in nonaqueous environments. Vitamin C also plays an important role in maintaining cellular levels of vitamin E by recycling the vitamin E radical (oxidized) to the reduced (antioxidant) form (Guo and Packer, 2000; Niki, 1987; Stoyanovsky et al., 1995). Along the lines, micronutrients vitamin C, vitamin A (including retinoids), and polar carotenoids including beta-carotene have shown enhanced growth-inhibitory effects of several chemotherapeutic agents (5-FU, vincristine, Adriamycin, bleomycin, 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboximide (DTIC), cisplatin, tamoxifen, cyclophosphamide, mutamycin, chlorozotocin, and carmustine) on some cancer cells in culture (Prasad, 2010). Overall it suggests the neutraceutical substances possess anticancer activity and reduce cancer fatigue. However, they should be taken with caution, because ingestion of higher doses of antioxidants may induce harmful effects. For example, vitamin A at doses of 10,000 IU or more can cause birth defects in pregnant women, and beta-carotene can produce bronzing of the skin at doses of 50 mg or more. The discoloration is reversible on discontinuation. Vitamin C as ascorbic acid at high doses, 10 g or more, can cause diarrhea in some individuals Vitamin E at doses of 2,000 IU or more can induce clotting defects after long-term consumption and vitamin B_6 at high doses may produce peripheral neuropathy (Prasad et al., 2000). It may be due to either direct/indirect interaction between antioxidants and cancer therapeutic agents. This can be easily overcome by developing multiple neutraceutical (herbal composition and vitamins)—drug composite nanoparticle formulations with the help of different nanomaterials such as nanocapsules, nanogels, herbal nanoparticles, nanotablets, nanopaste, nanopowder, and nanoemulsions.

3 Nanotechnology

The nanotechnology basis of drug delivery is revolutionizing medicine in many fields of disease treatment (Farokhzad and Langer, 2009). Many types of nanoparticles such as phospholipid

vesicles (liposomes) (Began et al., 1999; Li et al., 2005), micelles (Kunwar et al., 2006), solid lipid nanoparticles (Li and Schellhorn 2007), nanoemulsion (Sou et al., 2008), polymeric nanoparticles, proteins, cyclodextrin (Thangapazham et al., 2008) have been tested as drug delivery carriers. It is witnessing that lipid based formulations such as saquinavir (Fortovase, Roche Pharmaceuticals, NJ, United States), ritonavir (Norvir, Abbott Laboratories, IL, USA), cyclosporin (Neoral, Novartis Pharmaceutical Ltd., Surrey, UK) were approved by regulatory agencies and given impetus for development of lipid based formulations for the improved oral delivery of poorly water soluble drugs. Nanoemulsion drug delivery have been reported to improve the bioavailability of hydrophobic drugs very effectively (McClements and Rao, 2011; Velikov and Pelan, 2008), increased in vivo performance of anticancer drugs, for example, dacarbazine (Tagne et al., 2008) and camptothecin (Han et al., 2009). Nanoemulsions are heterogeneous dispersions consists of oil and water, stabilized by surface active agents, having globule size range of 20–200 nm (Gutiérrez et al., 2008). They are suitable to improve dissolution and bioavailability of poorly water-soluble compounds. Their preparation does not require various complicated equipments as in the case of nano tablets or nano capsules. Most of the emulsions are generally oil-in-water systems for therapeutic applications in which maximum quantity of drug is loaded in oil phase and further emulsification is performed using various techniques. These are nonequilibrium systems and cannot be prepared spontaneously to achieve required consistency. Hence, energy input is required either mechanically or chemically to make a homogenized dispersion of droplets. High-pressure homogenizers, high-shear stirring, ultrasound generators, and Microfluidizer are the common instruments that can be used to produce homogenous dispersions with smallest droplet sizes (Solans et al., 2005).

3.1 High-Pressure Homogenization (HPH)

Homogenization is a fluid mechanical process, which is used to reduce the relative heterogeneity of a system and to create a stable dispersions or nanoemulsions. This can be achieved under high pressure using high pressure homogenization equipments (HPH). HPH pumps are able to deliver hydrostatic pressure between 1,500 psi (10 MPa) and 60,000 psi (410 MPa) to a liquid regardless of the flow rate. High-pressure homogenizers are further classified into laboratory scale high-pressure homogenizers, pilot scale high-pressure homogenizers and production scale high-pressure homogenizers. The laboratory high-pressure homogenizer (Fig. 20.7)

Figure 20.7. Photograph showing Stansted laboratory high-pressure homogenizer.

works in the pressure range between 1,500 psi (10 MPa) and 60,000 psi (410 MPa) with flow rate from drop level to 200 mL/min with precooling or prewarming temperature control. The Ultra High Pressure Pilot Scale Homogenizer (Fig. 20.8) has the capability to produce flow rates up to 16 L/h and pressures up to 400 MPa (58,000 psi) with temperature controlling option to avoid degradation of drugs. The ultra-high-pressure production homogenizer

Figure 20.8. Photograph showing ultra high-pressure pilot scale homogenizer.

(Fig. 20.1) produces flow rates up to 240 L/h and pressures up to 400 MPa (58,000 psi) and having outstanding ease of use and of cleaning and requires only simple maintenance (http://www.homogenizersystems.com/production_high_pressure_homogenizers.html).

3.2 Ultrasonication

Nanoparticles are also prepared by ultrasonication method, which utilizes a lead zirconate titanate piezoelectric quartz crystal, under the influence of an alternating current the lead expands and contracts; by this way energy is transformed to mechanical vibrations. These vibrations reach the horn tip and vibrate at peak-to-peak amplitude. The emulsification is performed by placing the horn tip with suitable amplitude. The monitoring and controlling

of temperature is essential to prevent degradation of thermolabile components (Jafari et al., 2006).

3.3 Phase Inversion Temperature

Phase inversion temperature is the temperature at which the surfactant has equal hydrophobic and hydrophilic tendencies (Ee et al., 2008). This phenomenon is associated with the solubility property of nonionic surfactants like polyoxyethylenes change with temperature. At low temperatures surfactant is hydrophilic in nature and forms O/W dispersion, further changes to lipophilic and turns to W/O dispersion with raise in temperature due to dehydration of the polyoxyethylene chains. As phase inversion temperature increases emulsion stability also increases and the maximum HLB temperature is approximately 55°C for oil-in-water and 0°C for water-in-oil systems. Kinetically stable emulsions of oil-in-water or water-in-oil with very small droplet size and narrow size distribution can be prepared by rapidly cooling or heating. If the heating or cooling process is slow, coalescence predominates and polydisperse emulsions are formed (Ozawa et al., 1997).

3.4 Microfluidizer

Microfluidizer consists of a filter, pneumatic pump, and an interaction chamber. The coarse emulsion is introduced into microfluidizer works in the range of 20–124 MPa. The pneumatic pump produces a pressure up to 124 MPa using compressed air. The interaction chamber can be cooled with water to decrease the temperature during operation and monitored with a thermometer. The initial temperature rises up to 27°C at 35 MPa, further shoot up to 41°C at 105 MPa after three cycles. The sample required for each run is 500 mL and at least three cycles should be run for the preparation of nanoemulsions. An interim sample to be taken after each cycle until target size range is achieved (Jafari et al., 2006). The samples are powdered using lyophilizer.

3.5 Spray Dryer

In spray drying, an atomized spray is contacted with hot gas which is used as the drying medium. Evaporation takes place to yield dried particles, which are subsequently separated from the gas stream by a variety of methods (Anandharamakrishnan et al., 2007, 2008, 2010). Normally, it comes at the endpoint of the processing line, as it is an important step to control the final product quality. It has some advantages such as, rapid drying rates, a wide range of operating temperatures and short residence times.

Spray drying is widely used to produce food products such as whey, instant coffee, milk, tea, and soups, as well as health-care and pharmaceutical products, such as vitamins, enzymes, and bacteria (Anandharamakrishnan et al., 2007, 2008, 2010; Raghavendra et al., 2009; Rastogi et al., 2003).

3.6 Parameters for Evaluation of Formulation

Validation of bioactive content in the nanoparticles is very important to aid in the process of formulation studies, monitor the stability of nutraceutical substances and their release studies. The quality of analytical data is essential for scrutinizing the integrity, purity, quality, strength, and potency of the manufactured nutraceuticals.

These analytical procedures include specificity, linearity, accuracy, precision, range, detection limit, quantitation limit, and robustness which ensure accuracy, reproducibility, and reliability for the intended purpose of use. The most widely used equipment for the validation is High Performance Liquid Chromatography with UV detector (Chandran and Singh, 2007).

3.6.1 Specificity

It is the ability to estimate unequivocally the drug in the presence of components which may be expected to be present. Typically these might include formulation excipients like oils, surfactants, cosurfactants, various cosolvents, impurities, degradants, matrix, and so forth.

3.6.2 Accuracy

It is the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

3.6.3 Precision

It is the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. This parameter may be considered at three levels: repeatability, intermediate precision, and reproducibility. Statistical parameters like variance, standard deviation, or coefficient of variation of a series of measurements are used to determine the precision of studies.

3.6.3.1 Repeatability

It expresses the precision under the same operating conditions over a short interval of time. It is also termed intra-assay precision.

3.6.3.2 Intermediate Precision

It expresses within-laboratories variations: different days, different analysts, different equipment, and so forth.

3.6.3.3 Reproducibility

It expresses the precision between laboratories (collaborative studies).

3.6.4 Detection Limit

It is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

3.6.5 Quantitation Limit

It is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. It is used particularly for the determination of impurities and/or degradation products.

3.6.6 Linearity

It is the ability of a method to obtain test results which are directly proportional to the concentration of nutraceutical in the sample.

3.6.7 Range

It is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which the analytical procedure has a suitable level of precision, accuracy, and linearity.

3.6.8 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

3.7 Key Benefits of Nanotechnology-Based Drug Delivery

- Provides multifunctionality: targeting, delivery, reporting
- Provides improved therapeutic index
- Provides lowered toxic side effects
- Delivers multiple drugs directly to tumor site
- Enables nucleic acid delivery
- Enables nondrug therapies

3.8 Chitosan-Biodegradable Polymers

Chitosan is a natural biopolymer consisting of β-1 → 4 linked 2-amino-2-deoxy-glucopyranose (GlcN) and 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) residues. Chitosan is produced by deacetylation of chitin, which is isolated from the exoskeleton of crustaceans, such as crabs and shrimps and cell walls of fungi.

3.9 Preparation of Nanoparticles by Various Methods

The large scale productions of nanoparticles are by total encapsulation method or layer by layer coating method. In total encapsulation method the hydrophilic and hydrophobic, drugs and vitamins totally encapsulated inside the chitosan matrix. But in layer-by-layer coating the drugs and vitamins form layersone above the other. This coating depends on the pH of the medium, stirring speed, stirring time, and encapsulation efficiency. These methods are again classified into water/oil or oil/water methods. For the encapsulation of vitamins and drugs we employed both water/oil (W/O) or oil/water (O/W) mixing methods for layer-by-layer coating of vitamins and drugs (Fig. 20.9).

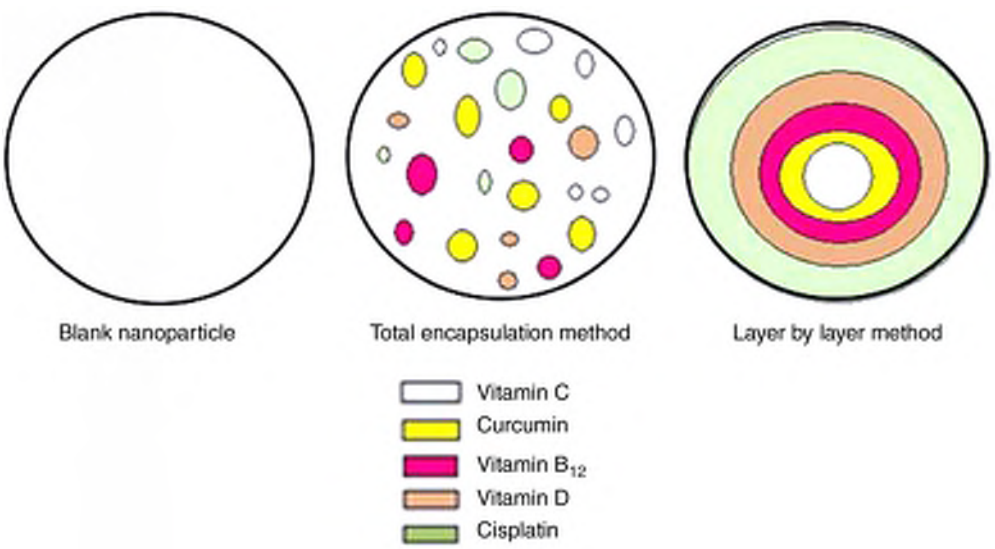


Figure 20.9. Schematic representation depicting the encapsulation of drugs and vitamins by layer by layer method.

4 Preparation of Blank Chitosan Nanoparticles (W/W)

Blank chitosan nanoparticles are prepared by ionic gelation method (Calvo et al., 1997). The chitosan solution was obtained by dissolving low molecular weight chitosan (400 mg) in 1% (v/v) acetic acid solution. Chitosan nanoparticles were prepared spontaneously upon addition of 40 mL of TPP solution (200 mg/100 mL) into chitosan solution under gentle magnetic stirring at room temperature for 1 h. Upon stirring, molecular linkages were formed between TPP phosphates and chitosan amino groups. The volume ratio of chitosan solution 0.1% (w/v):TPP solution 0.2% (w/v) was 2:1 and the opaque suspension was assigned as nanoparticles with overall positive surface charge.

4.1 Preparation of Vitamin and Drug-Loaded Chitosan Nanoparticles

For the preparation of vitamin C, B_{12}, and curcumin, vitamin D chitosan nanoparticles were prepared by W/W/W and W/O/W emulsion method. In brief, 1 gram of vitamin C and 1 mg of vitamin B_{12} were dissolved in 4 and 1 mL of ultra pure water, 30 mg of curcumin in 200 μL acetone, and 1 mg of vitamin D in 1 mL of ethanol solutions were added drop-wise to 0.4 gram of chitosan in 1% acetic acid solution and homogenized at 500 rpm for 5–10 h using a magnetic stirrer. Then 40 mL of TPP solution (200 mg/100 mL) were added dropwise under constant stirring at 500 rpm for 10 h. For the preparation of different combination of chitosan nanoparticles, 1 g of vitamin C in 4 mL of ultra pure water is mixed with 0.2% (w/v) TPP solution, likewise remaining vitamins and drugs for various combinations. For example, for the preparation of vitamin C–curcumin–vitamin B_{12} nanoparticles (W/W/O/W/W), the vitamin C solution was added to aqueous chitosan solution and homogenized at 500 rpm for 4 h using magnetic stirrer. The 25 mg/200 μL curcumin in acetone was slowly added to this solution at 500 rpm for 3 h. The 1 mg of vitamin B_{12} in 1 mL of high pure water was added to 0.2% (w/v) TPP solution and the nanoparticles were formed by adding this solution in dropwise under constant magnetic stirring at 500 rpm for 8 h. For the preparation of vitamin C–curcumin–vitamin B_{12}–vitamin D nanoparticles (W/W/O/W/O/W), the vitamin C solution was added to aqueous chitosan solution and homogenized at 500 rpm for 3 h using magnetic stirrer. The 25 mg/200 μL curcumin in acetone was slowly added to this solution at 500 rpm for 3 h. The 1 mg of vitamin B_{12} in 1 mL of high

pure water was added to this solution at 500 rpm for 3 h. The 1 mg of vitamin D in 1 mL of ethanol was added to 0.2% (w/v) TPP solution and the nanoparticles are formed by adding this solution in dropwise under constant magnetic stirring at 500 rpm for 6 h. The layered nanoparticles are formed after 6–12 h. Following time period, nanoparticles were collected by centrifugation at 15,000 rpm in a 10 μL glycerol bed for 30 min at 15°C. The supernatants were discarded and resultant nanoparticles were resuspended in 5% sucrose solution and lyophilized. The ratio of various vitamins and drug combination used for the preparation of nanoparticles are given in (Table 20.1). Following preparation of the nanoparticles, the samples were coated with gold using a Cressington gold sputter coater. Then the samples were observed under at 10.00 KX magnifications. SEM images of blank chitosan nanoparticles and chitosan nanoparticles with various combinations of vitamins and drugs show that all particles are spherical in shape. The size of nanoparticles increases in different combinations with various concentrations. The nanoparticles loaded with multiple drugs and vitamins showed maximum size than single drug or vitamin. The nanoparticles loaded with cisplatin and multiple vitamins showed maximum size than single drug or vitamin (Fig. 20.10). Next, the stability of nanoparticles is measured with the zeta potential value is shown in (Table 20.2). The mean diameter of the various nanoparticles ranged from 98 ± 5.27 to 238 ± 3.43 nm. The B_{12} + C + D_3 + curcumin + cisplatin-loaded chitosan nanoparticles

Table 20.1 Concentration of Vitamins and Drugs Used for the Preparation of Nanoparticles

S. No.	Combinations	Ratio (mg)
1	Chitosan : Vitamin B_{12}	100: 1
2	Chitosan : Vitamin C	100: 75
3	Chitosan : Vitamin D_3	100: 1
4	Chitosan : Curcumin	100: 6
5	Chitosan : Cisplatin	100: 2
6	Chitosan : Vitamin B_{12}: Vitamin C: Vitamin D_3	100: 1: 75: 1
7	Chitosan : Vitamin B_{12}: Vitamin C: Vitamin D_3: Curcumin	100: 1: 75: 1: 6
8	Chitosan : Vitamin B_{12}: Vitamin C: Vitamin D_3: Cisplatin	100: 1: 75: 1: 2
9	Chitosan : Vitamin B_{12}: Vitamin C: Vitamin D_3: Curcumin: Cisplatin	100: 1: 75: 1: 6: 2

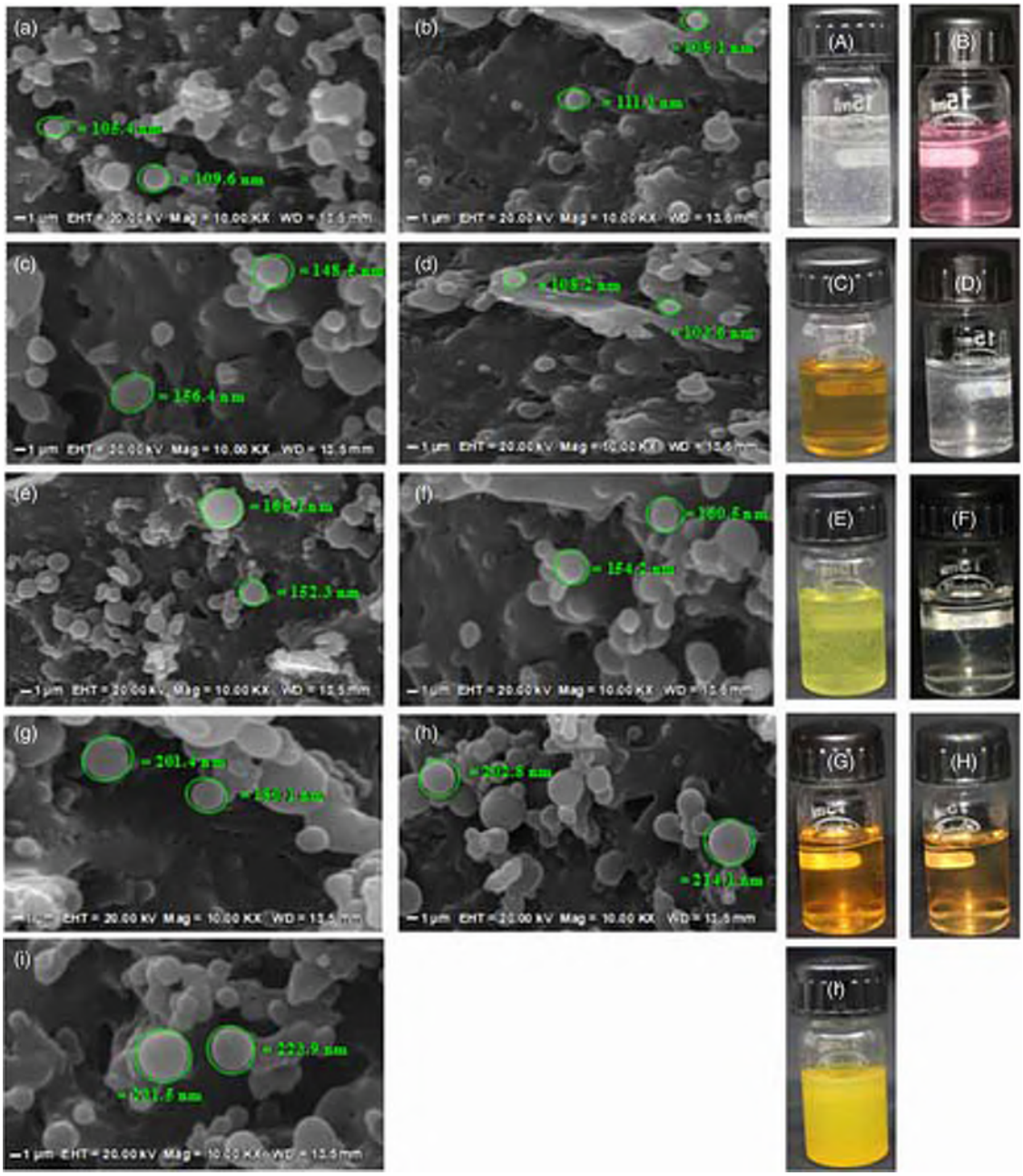


Figure 20.10. SEM images of vitamin and drug loaded chitosan nanoparticles. (a) (A) Blank chitosan NPs; (b) (B) Vitamin B12-loaded NPs; (c) (C) Vitamin C-loaded NPs; (d) (D) Vitamin D3-loaded NPs; (e) (E) Curcumin-loaded NPs; (f) (F) Cisplatin-loaded NPs; (g) (G) B12 + C + D3 + Curcumin-loaded NPs; (h) (H) B12 + C + D3 + Cisplatin-loaded NPs; (i) (I) B12 + C + D3 + Curcumin + Cisplatin-loaded NPs.

Table 20.2 Mean Diameter, Polydispersity Index, and Zeta Potential of Drug- and Vitamin-Encapsulated Chitosan Nanoparticles

S. No.	Nanoparticles	Mean Diameter (nm)	Polydispersity Index	Zeta Potential (mv)
1	Blank nanoparticles	98 ± 5.27	0.173 ± 0.044	24.12 ± 0.52
2	Vitamin B_{12} loaded nanoparticles	107 ± 2.16	0.187 ± 0.012	26.23 ± 1.24
3	Vitamin C loaded nanoparticles	192 ± 3.61	0.184 ± 0.036	24.37 ± 0.47
4	Vitamin D_3 loaded nanoparticles	103 ± 4.04	0.213 ± 0.021	22.44 ± 0.65
5	Curcumin loaded nanoparticles	166 ± 3.66	0.217 ± 0.059	27.15 ± 1.18
6	Cisplatin loaded nanoparticles	148 ± 2.05	0.221 ± 0.046	25.18 ± 0.27
7	B_{12}+C+D_3 loaded nanoparticles	200 ± 2.58	0.273 ± 0.025	26.34 ± 0.36
8	B_{12}+C+D_3+Curcumin loaded nanoparticles	212 ± 2.15	0.227 ± 0.028	24.63 ± 0.64
9	B_{12}+C+D_3+Cisplatin loaded nanoparticles	208 ± 4.22	0.296 ± 0.043	25.26 ± 0.39
10	B_{12}+C+D_3+Curcumin+Cisplatin loaded nanoparticles	238 ± 3.43	0.303 ± 0.066	24.12 ± 0.43

Note: Chitosan = 0.1% w/v, TPP = 0.04% w/v.

were greater than 200 nm in diameter. Zeta potential values were recorded to determine the stability of the B_{12} + C + D_3 + curcumin + cisplatin-loaded chitosan nanoparticles. The value ranged from 24.12 ± 0.43 mV.

4.2 Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

Fourier transform infrared (FT-IR) spectra were analyzed using an Alpha FT-IR spectrometer (Bruker, France) at 4 cm^{-1} resolution. The lyophilized nanoparticles were mixed with potassium bromide and pressed to a pellet form to investigate the chemical reactions between the drug and nanoparticles. Initially, we scanned chitosan, TPP and chitosan nanoparticles absorption peaks using FT-IR spectra. In the spectra of chitosan in (Fig. 20.11a), the 1649.13 cm^{-1} represents the primary amino group present in the chitosan, while the stretching bands at 1026.99 cm^{-1}, 1062.52 cm^{-1},

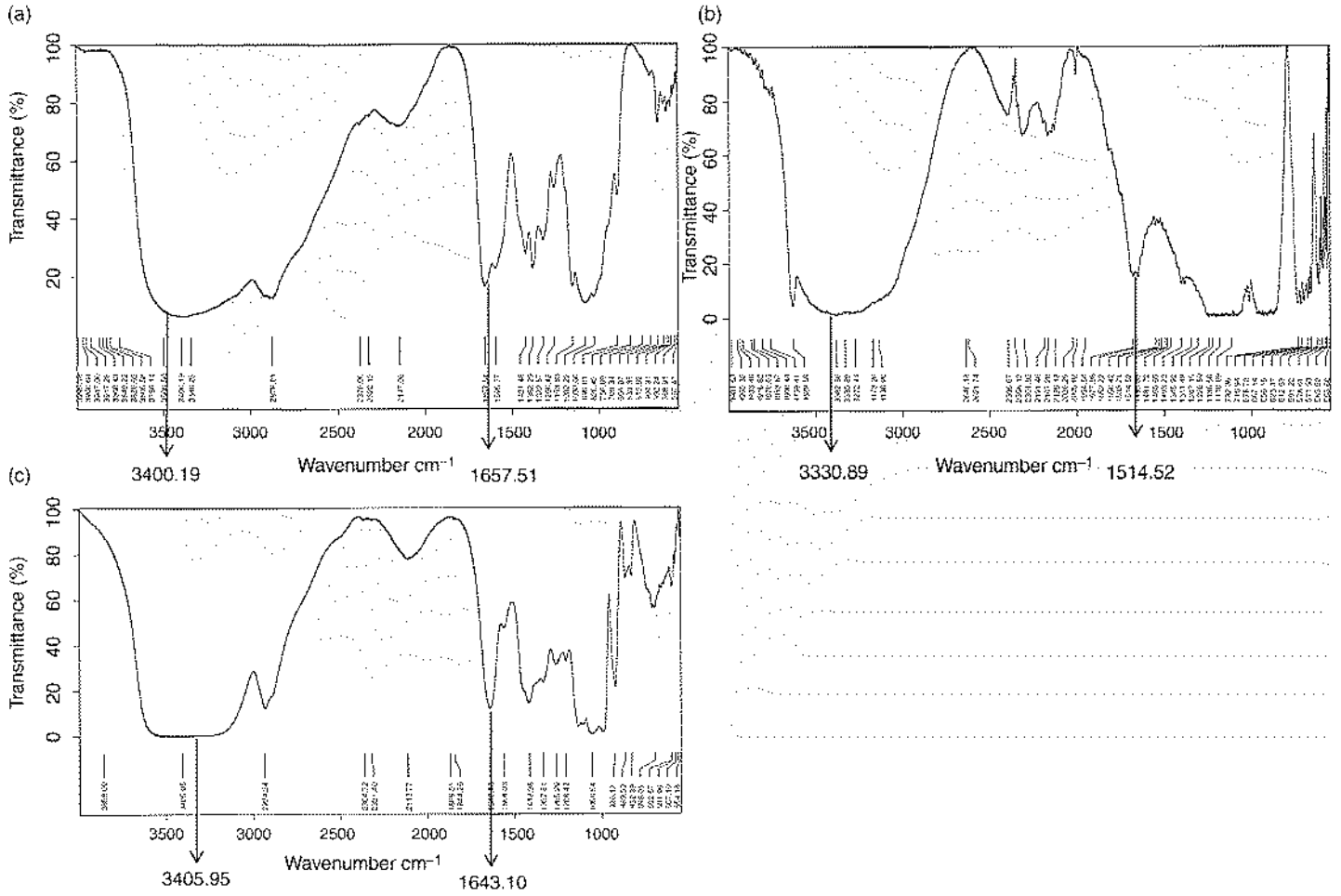


Figure 20.11. FT-IR characterization of chitosan nanoparticles. FT-IR spectrum of (a) Chitosan; (b) TPP (c) Chitosan NPs.

and 3329.03 cm^{-1} are due to the C-O, C-H, and hydroxyl group present in the chitosan, respectively. The peak at 2874.88 cm^{-1} represents the stretching band of methylene in chitosan structure. For blank chitosan nanoparticles (Fig. 20.11b), the amino band is shifted to 1636.11cm^{-1} which indicates the ionic interaction between TPP and NH_2 of chitosan. The broad band is maximum at 3229.48 cm^{-1} represents hydrogenic bonds between the hydroxyl groups in chitosan with TPP (Fig. 20.13C). It suggests that nanoparticles are formed in the medium, leads to positive charge for the chitosan due to electrostatic interaction between the primary amino group in the chitosan and TPP. These interaction leads to a decrease in chitosan solubility and nanoparticles formation.

The vitamin B12 (Cobalamin) show possible interaction with the chitosan nanoparticles. The stretched peak of vitamin B_{12}, at 3304.14 cm^{-1} is shifted to 3314.93 cm^{-1} in vitamin B_{12} nanoparticles due to the interaction between the vitamin B_{12} and chitosan nanoparticles (Fig. 20.12). Vitamin C (ascorbic acid) shares the characteristic of having a positively charged cation and a negatively charged anion in the dissolved form. These charges show the affinity toward the chitosan. The electrostatic and chemical reaction occurs between the chitosan and the vitamin C. In the spectra of vitamin C nanoparticles the negative charge of ascorbate ion is interacts with the positive charge of the chitosan nanoparticles and the spectra is shifted from 1496.44 to 1641.14 cm^{-1}. And the broad band is shifted from 3212.60 to 3342.17 cm^{-1} in the vitamin C nanoparticles (Fig. 20.13). The vitamin D_3 (cholecalciferol) spectra show good interaction with the chitosan nanoparticles. Broad band of vitamin D_3 at 3313.45 cm^{-1} is shifted to 3458.56 cm^{-1} in vitamin D_3 loaded nanoparticles due the interaction of vitamin D_3 with chitosan nanoparticles (Fig. 20.14). Curcumin spectra also show good interaction with the chitosan nanoparticles. Broad band of curcumin at 3354.10 cm^{-1} is shifted to 3492.75 cm^{-1}, and characteristic peak of curcumin 1512.57 cm^{-1} is shifted to 1644.02 cm^{-1} in curcumin loaded nanoparticles due to the interaction of curcumin with chitosan nanoparticles (Fig. 20.15).

Cisplatin is a neutral complex because the two positive charges of platinum (II) ion are neutralized by the two negative charges supplied by the chloride ions. This neutral nature helps while loading in to the chitosan nanoparticles. Interaction with the chitosan nanoparticles changes the characteristic peak of cisplatin from 3458.18 to 3418.82 cm^{-1}. But the narrow band was almost remaining same from 1641.29 to 1640.41 cm^{-1} due to the complex ions present in the cisplatin. And this indicates the encapsulation of cisplatin in the nanoparticle (Fig. 20.16a).

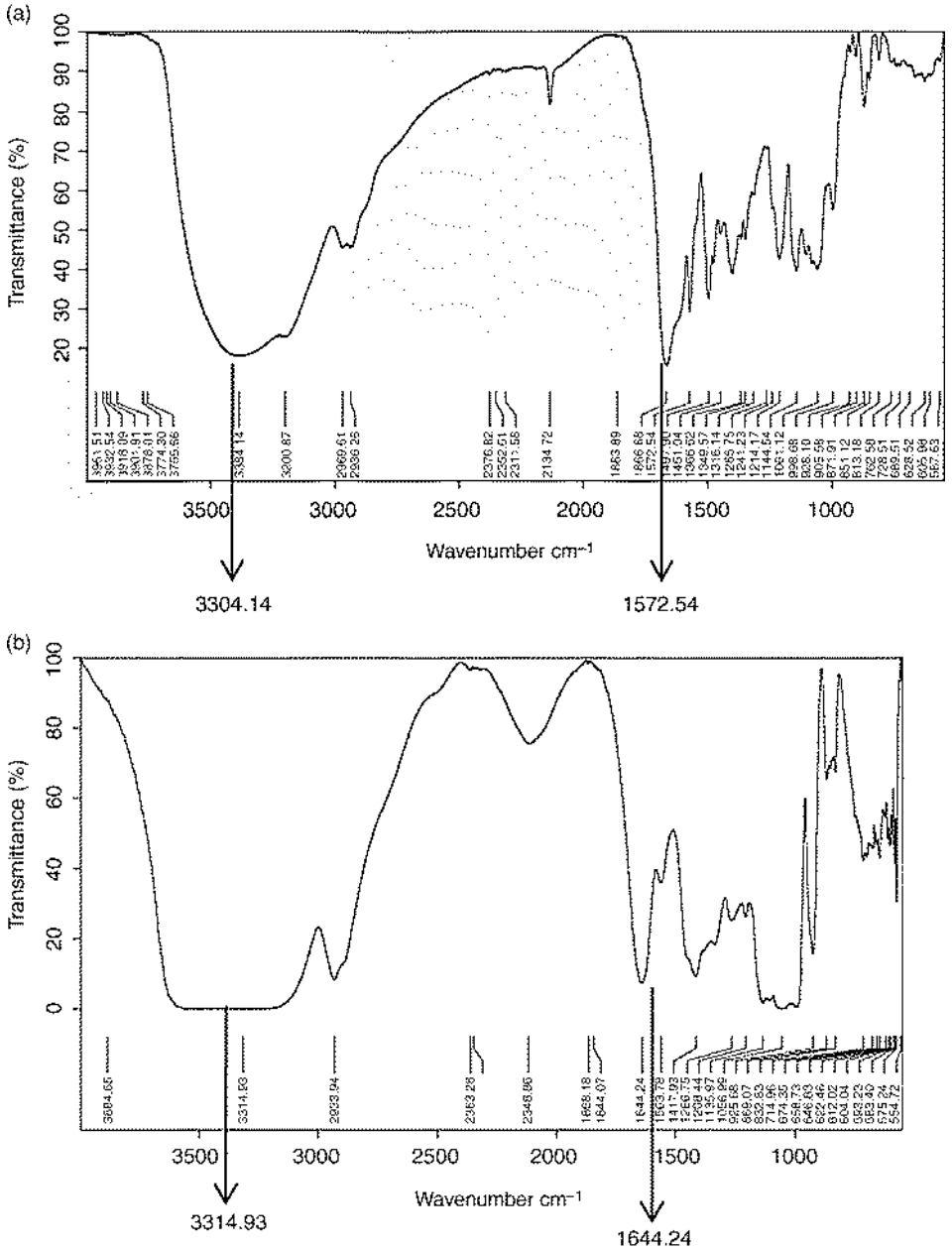


Figure 20.12. FT-IR characterization of chitosan nanoparticles. FT-IR spectrum of (a) vitamin B12; (b) vitamin B12-encapsulated chitosan nanoparticles.

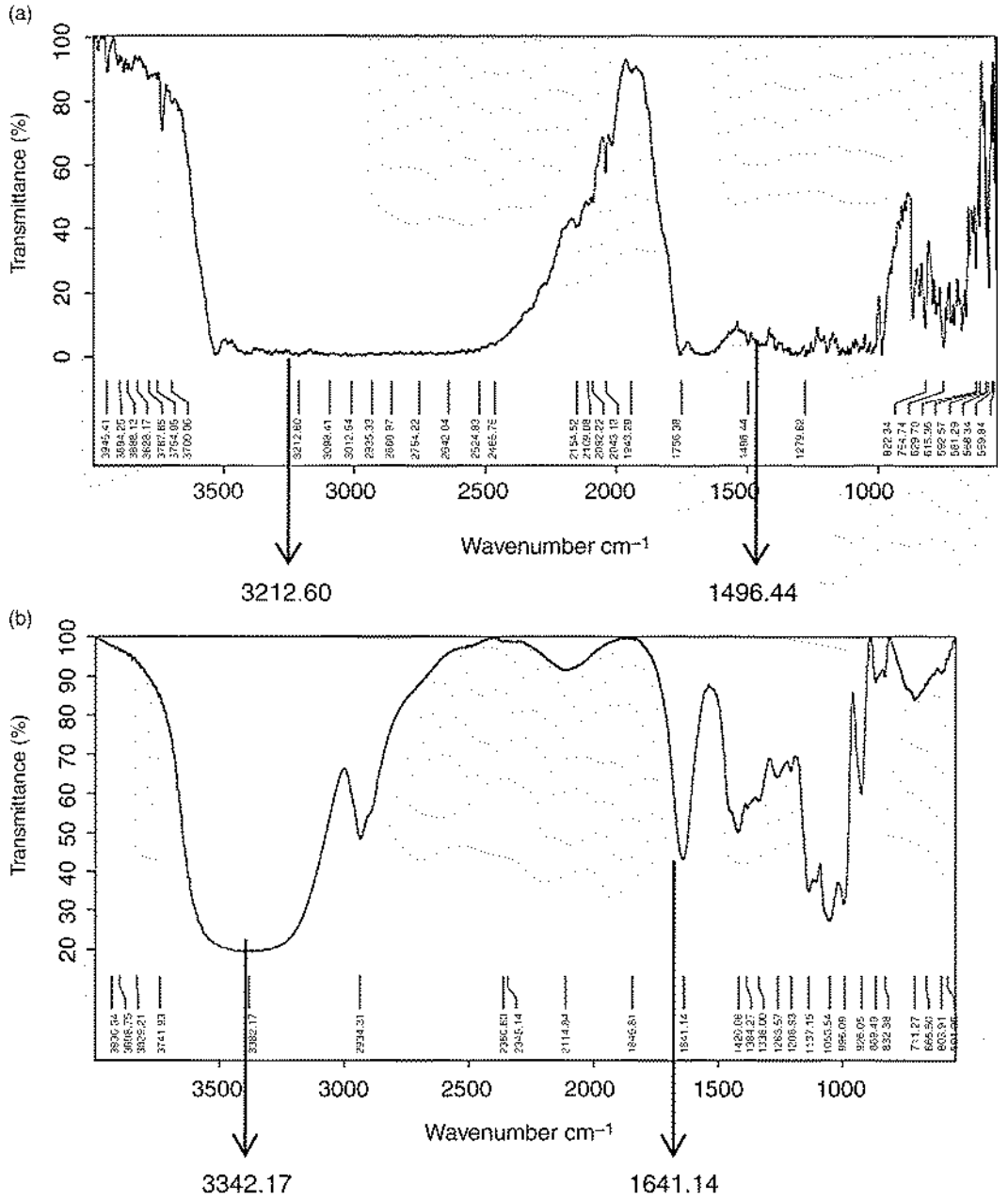


Figure 20.13. FT-IR characterization of chitosan nanoparticles. FT-IR spectrum of (a) vitamin C; (b) vitamin C-encapsulated chitosan nanoparticles.

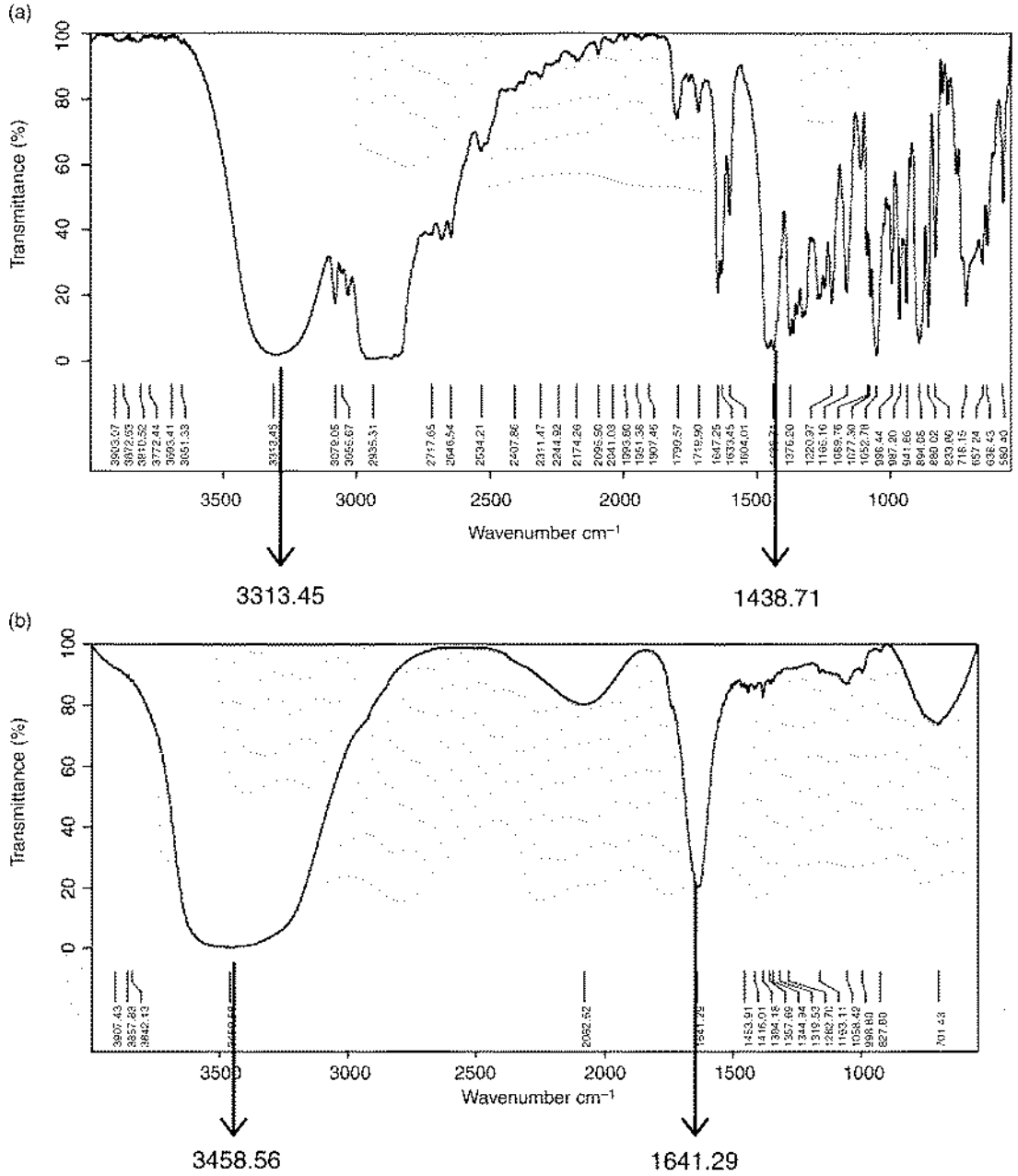


Figure 20.14. FT-IR characterization of chitosan nanoparticles. FT-IR spectrum of (a) vitamin D3; (b) vitamin D3-encapsulated chitosan nanoparticles.

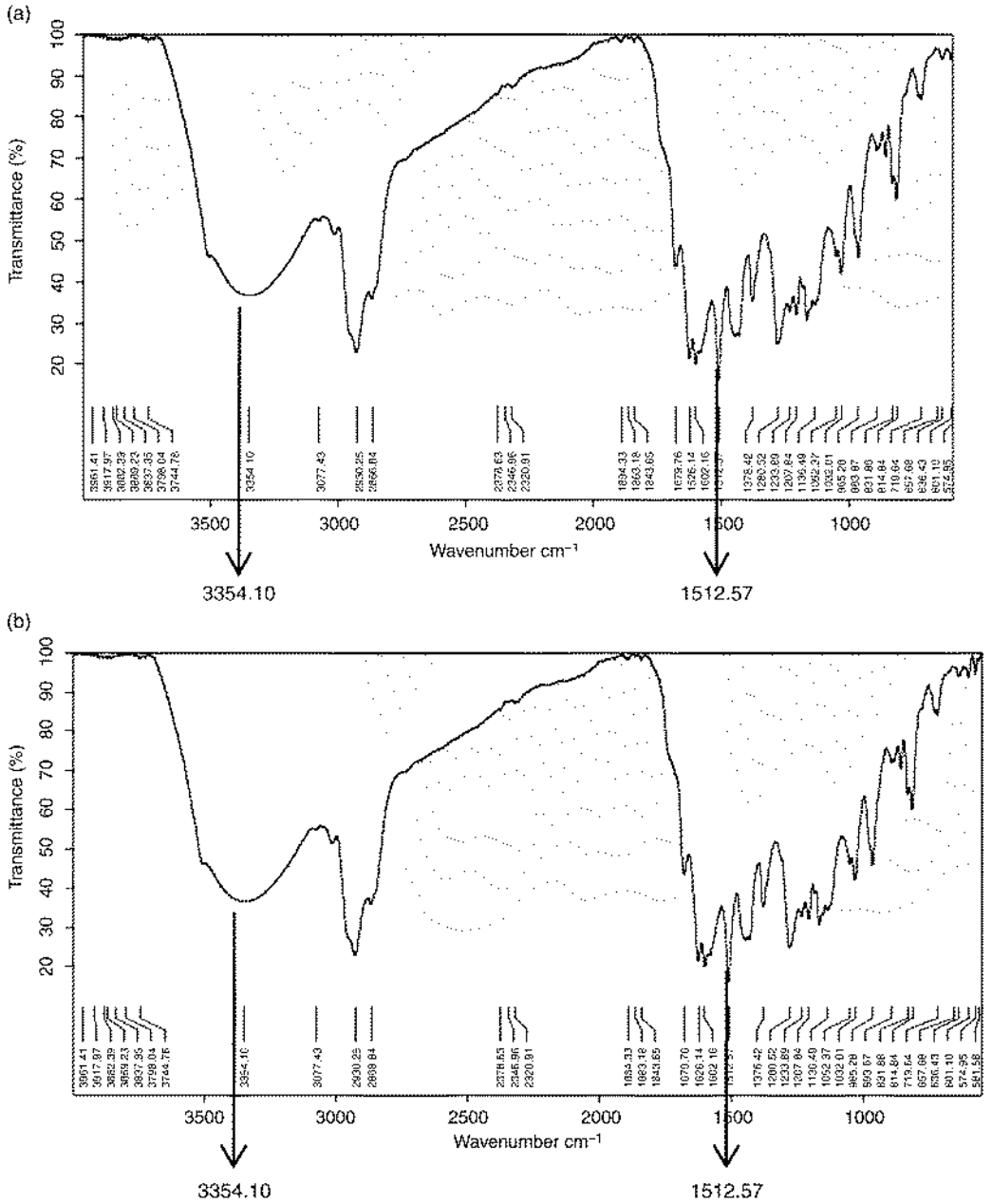


Figure 20.15. FT-IR characterization of chitosan nanoparticles. FT-IR spectrum of (a) curcumin; (b) curcumin-encapsulated chitosan nanoparticles.

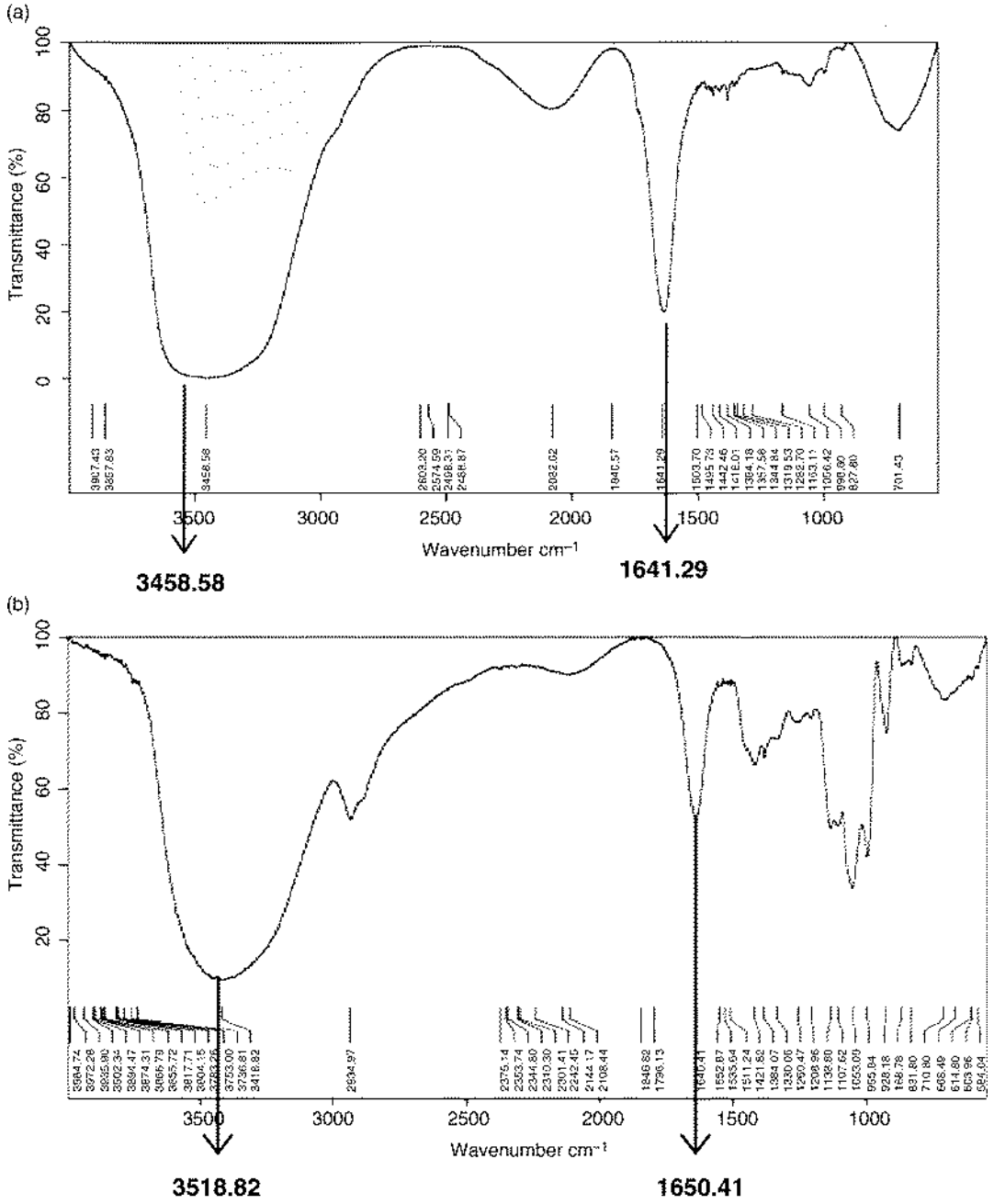


Figure 20.16. FT-IR characterization of chitosan nanoparticles. FT-IR spectrum of (a) cisplatin; (b) cisplatin-encapsulated chitosan nanoparticles.

The combination of vitamins in nanoparticles changes the narrow band at 1636.22 cm^{-1} and the broadband at 3428.55 cm^{-1}. But when the cisplatin is added to this combination again the peak slightly changes to 1638.93 and 3422.47 cm^{-1}. This indicates the effect of cisplatin was very less due to its neutral behavior. But these synergistic combinations showed better interaction with the chitosan nanoparticles. Absence of minor peaks of vitamins in synergistic combinations indicates that no free vitamin was available on the nanoparticle surface. These changes are due to the molecular distribution of the vitamins in the molecular scaffold of the polymeric nanoparticles. The small vibrational bands at combinatorial nanoparticles indicate the strong interaction of vitamins with the nanoparticles (Fig. 20.17).

4.3 Evaluation of Encapsulation Efficiency (EE)

To determine the encapsulation efficiency, initially we identified specific wavelength of each and every vitamin and drugs using UV-Vis spectrophotometer (THERMO Scientific and USA). All vitamins displayed different wavelength spectra and we have selected a maximum absorbance wavelength for each vitamin. Vitamin B_{12} showed a maximum absorbance at 230 nm, vitamin C at 265 nm, vitamin D_3 at 260 nm, curcumin at 420 nm, and cisplatin at 300 nm, respectively. No cross-reactivity was detected across the vitamins and drugs, which is evident from different absorbance values. For the preparation of standard curve, samples were serially diluted from 2 μg to 10 mg and the absorbance values were recorded and they were transported into Excel sheet for the preparation of standard curve (Fig. 20.18). To determine the encapsulation efficiency 4 mg of various combination of nanoparticles are dispersed in phosphate-buffered saline solution (PBS; pH = 7.4) as a release medium in a dialysis membrane sac (12 kDa; Sigma Aldrich). The dialysis sac is placed in a beaker containing PBS (50 mL) and incubated at 37°C in continuous shaking incubator under mild agitation (80–100 rpm). The release of drug-loaded chitosan nanoparticles from the dialysis bag for a period of 60 h are determined spectrophotometrically (Biospectrophotometer, Eppendorf) by reading absorbance at 230, 265, 260, 420, and 310 nm for vitamin B_{12}, vitamin C, vitamin D_3, and cisplatin. The EE of the nanoparticles were calculated by the following equation:

$$\text{EE}(\%) = [(\text{Total drug} - \text{Free drug}) / (\text{Total drug})] \times 100$$

The effect of different combinations of cisplatin and vitamins (B_{12}, C, and D_3) on the encapsulation efficiency (EE) of chitosan nanoparticles was determined (Table 20.3). The maximum

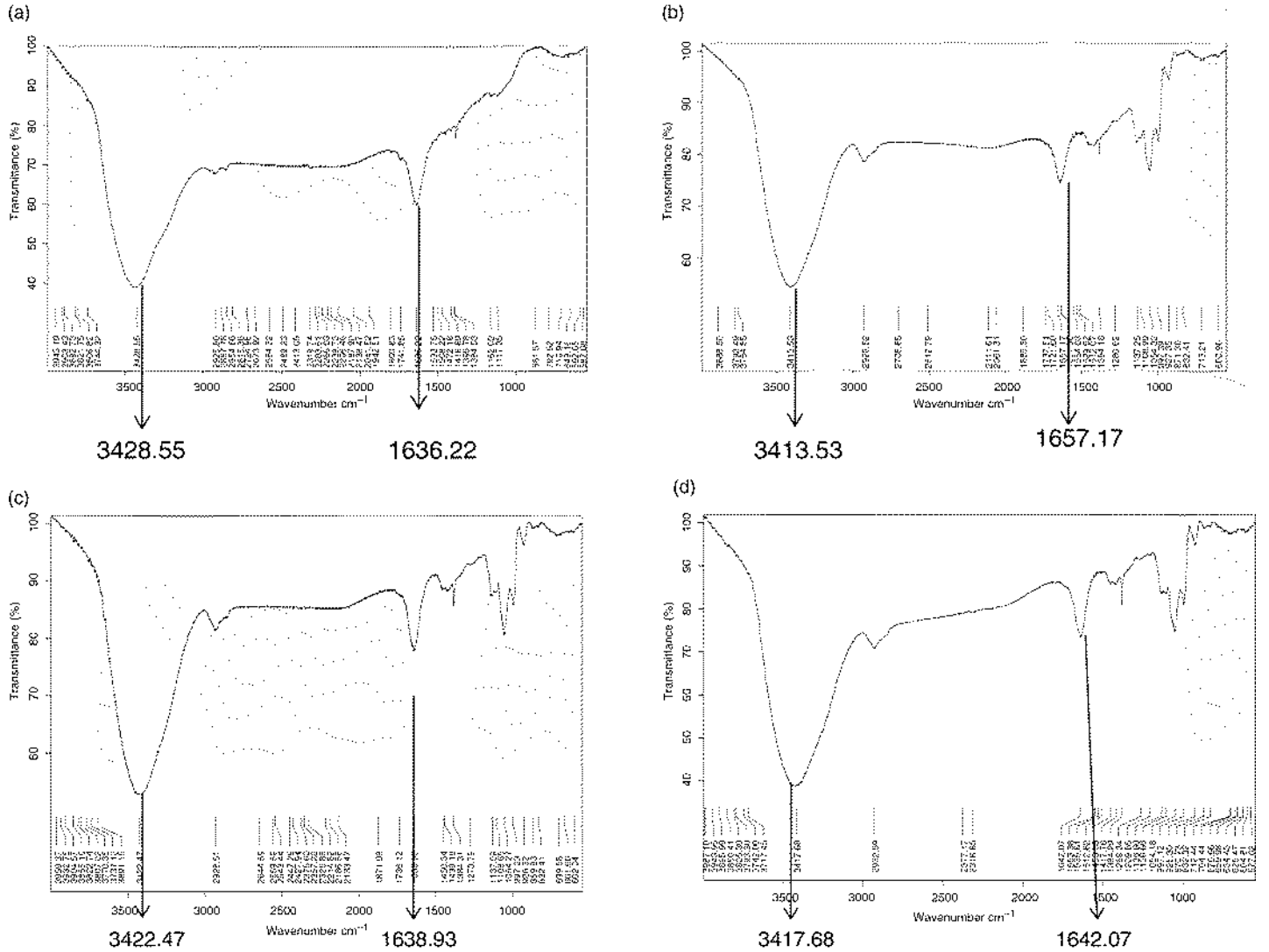


Figure 20.17. FT-IR characterization of drug- and vitamin-encapsulated chitosan nanoparticles. FT-IR spectrum of (a) B12 + C + D3 NP; (b) B12 + C + D3 + CMN NP; (c) B12 + C + D3 + CIS NP; and (d) B12 + C + D3 + CMN + CIS NP.

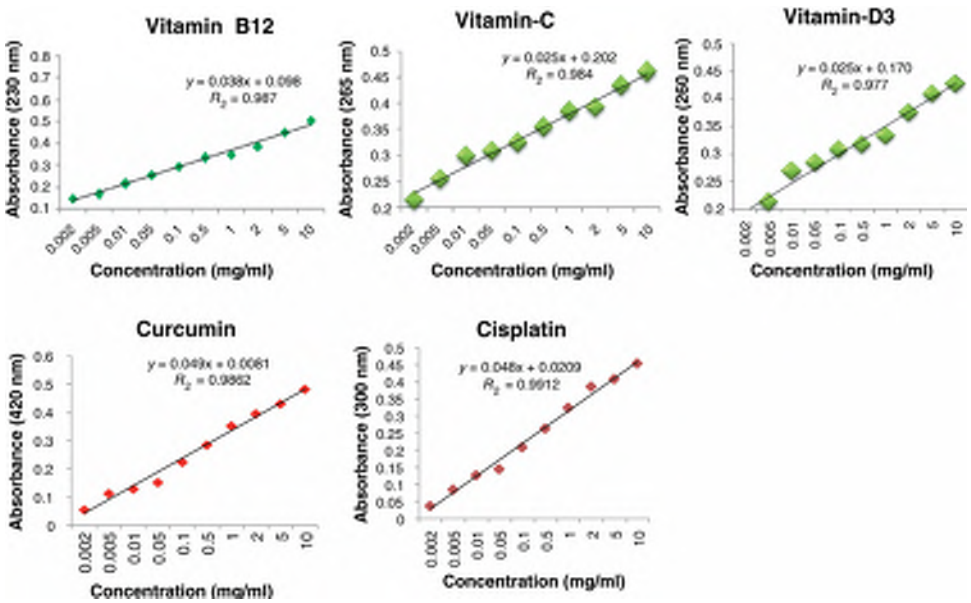


Figure 20.18. Linear graph representing the standard concentration of vitamins (B12, C, and D3), curcumin, and cisplatin.

Table 20.3 Encapsulation Efficiency in Vitamins and Drugs

S. No.	Nanoparticles	EE (%)
1	Vitamin B_{12}–loaded nanoparticles	65.05 ± 0.44
2	Vitamin C–loaded nanoparticles	56.9 ± 1.12
3	Vitamin D_3–loaded nanoparticles	64.6 ± 0.56
4	Curcumin-loaded nanoparticles	66.3 ± 0.38
5	Cisplatin-loaded nanoparticles	62.7 ± 1.25
6	B_{12}+C+D_3–loaded nanoparticles	61.6 ± 1.64
7	B_{12}+C+D_3+curcumin–loaded nanoparticles	60.04 ± 0.58
8	B_{12}+C+D_3+cisplatin–loaded nanoparticles	61.2 ± 1.43
9	B_{12}+C+D_3+curcumin+cisplatin–loaded nanoparticles	58.8 ± 1.54

Note: Chitosan = 0.1% w/v, TPP = 0.04% w/v.

encapsulation efficiency was achieved for vitamin B_{12}- and vitamin D_3-loaded nanoparticles. The vitamin C and its combinations show less encapsulation efficiency due to high vitamin C concentration.

4.4 Antiproliferative Assay

Uncontrolled growth of cells is called proliferation, which is one of the main characteristic features of cancer cells. The antiproliferative activity of vitamins and drug-loaded nanoparticles were evaluated using yeast strain (BY4741) as a cancer model system (Simon, 2001). The single healthy colony from the strain was inoculated into sterilized yeast, peptone, and dextrose broth and incubated at 37°C overnight, referred to as seed broth. The seeded broth was diluted with the blank culture media until the absorbance (OD) reaches 0.1 at 600 nm. To validate the action of vitamin and drug loaded nanoparticle, the 1 mL of overnight yeast culture was transferred into respective tubes. The first tube was kept as a control without any nanoparticles. The other tubes were distributed with vitamins (C, B_{12}, and D), curcumin- and cisplatin-loaded nanoparticles. The standard vitamins (C, B_{12}, and D), curcumin and cisplatin were also taken into respective control tubes. All tubes were incubated at 37°C and absorbance was observed at 600 nm for predetermined time intervals (2, 4, 6, 12, 24, 48, and 72 h) using UV-Vis spectrophotometer (DYNAMICA Halo DB20). The results showed that vitamin C–loaded nanoparticles showed good growth arrest with respect to vitamin B_{12} and vitamin D_3. The cisplatin-loaded nanoparticles showed better growth arrest than the pure drug. The cells exposed to the synergistic drugs and vitamin-loaded nanoparticles showed delayed and better growth arrest than single vitamin or drug (Fig. 20.19). Overall, it suggests that the synergistic combinations have good impact over the single drug or vitamin.

4.5 Blood Compatibility

The blood compatibility study was conducted with the help of hemolysis assay to investigate the action of nanoparticles with the human red blood cells. The 5 mL of blood samples were obtained from two healthy individuals (25–30 years old) and added with EDTA. The contents mixed properly by centrifugation at 1,500 rpm for 10 min and serum discarded. Finally red blood cells (RBCs) were washed 3 times with the phosphate buffer solution (PBS) for 7 min at 1000 rpm. The washed Red blood cells were resuspended in PBS and diluted to prepare erythrocyte stock solution.

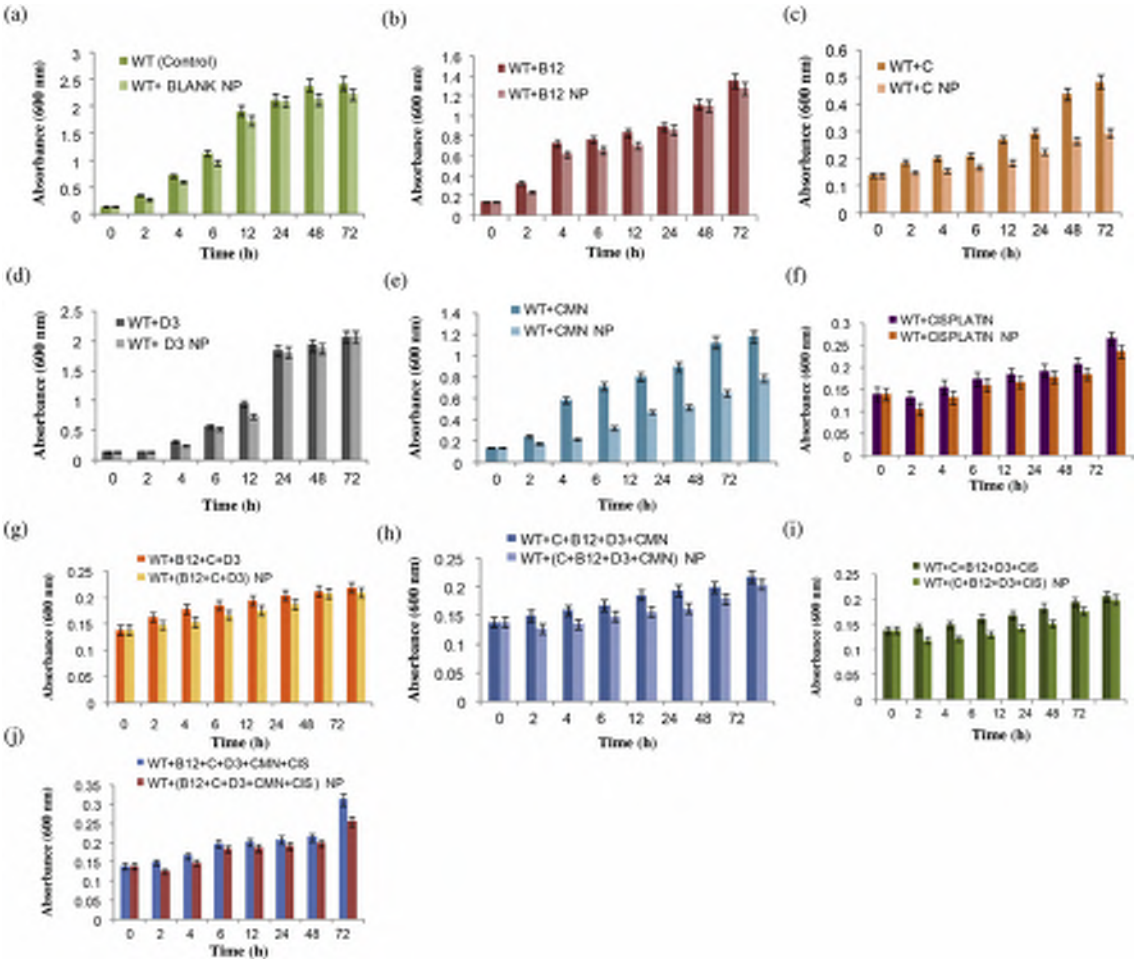


Figure 20.19. Antiproliferative activity of (a) Blank chitosan nanoparticles; (b) Vitamin B12–loaded nanoparticles; (c) Vitamin C–loaded nanoparticles; (d) Vitamin D3–loaded nanoparticles; (e) Curcumin-loaded nanoparticles; (f) Cisplatin-loaded nanoparticles; (g) B12 + C + D3–loaded nanoparticles; (h) B12 + C + D3 + curcumin–loaded nanoparticles; (i) B12 + C + D3 + cisplatin–loaded nanoparticles; (j) B12 + C + D3 + curcumin + cisplatin–loaded nanoparticles.

The freeze-dried samples were redispersed and sonicated in PBS to give 0.2 % suspensions. The drug containing nanoparticles suspensions was added to erythrocyte stock solution. Then the mixtures were incubated at 37°C in continuous shaking water bath for 1 h. The mixtures were then centrifuged at 1,000 rpm for 5 min and the supernatant read in a spectrophotometer at 540 nm. The saline solution used as negative control (0% lysis) and 0.1% triton in PBS used as positive control (100% lysis). The amount of release of hemoglobin was monitored in spectrophotometer (Biospectrophotometer, Dynamica) at 540 nm. The percentage of hemolysis was calculated using the formula:

$$\text{Hemolysis}(\%) = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the positive control}} \times 100$$

The different samples were taken in four different concentrations 100, 75, 50, and 25 µg, respectively, for the analysis. The percent of lysis was calculated with this assay. The nanoparticles

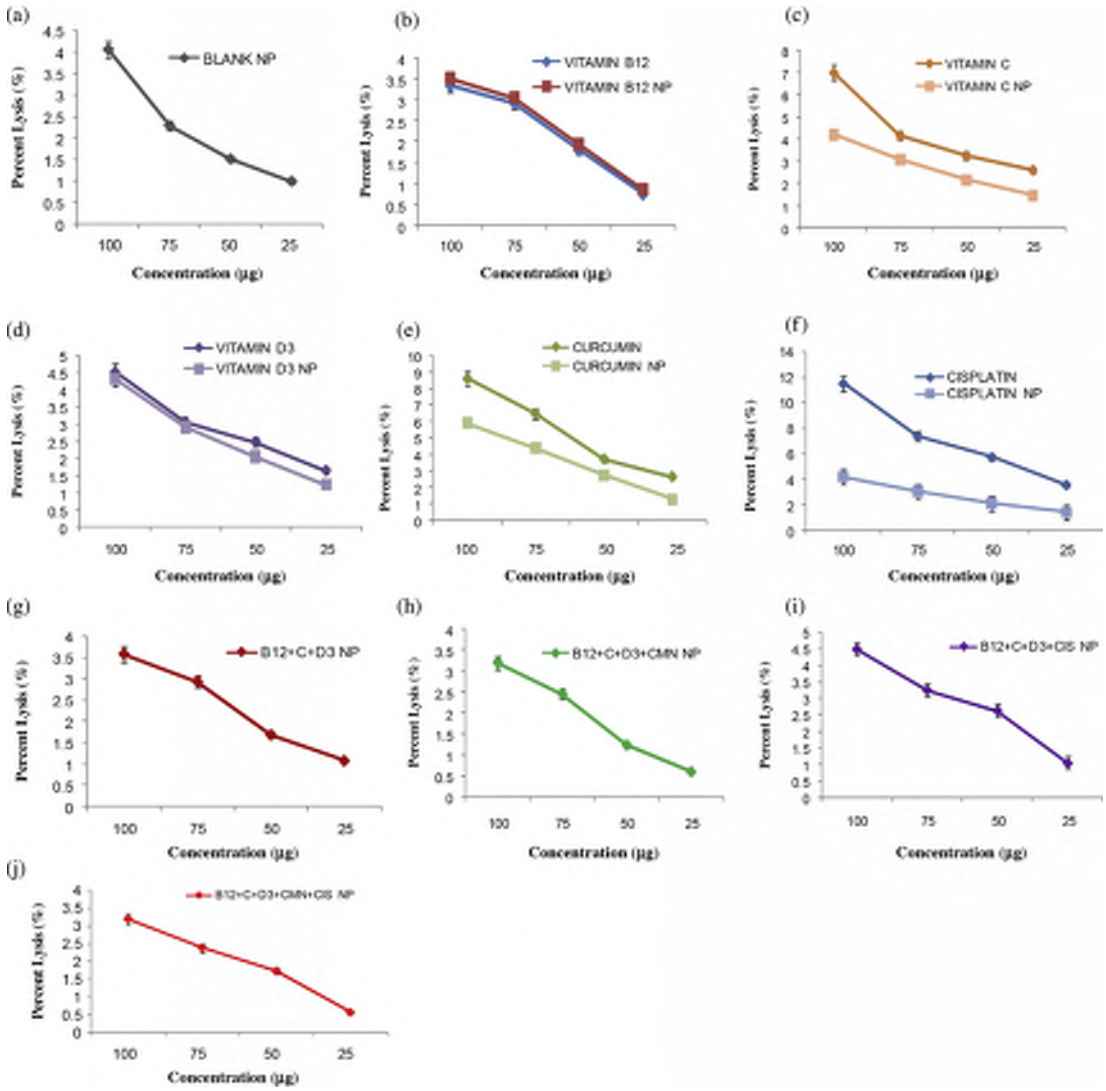


Figure 20.20. Blood compatibility of (a) Blank chitosan nanoparticles; (b) Vitamin B12–loaded nanoparticles; (c) Vitamin C–loaded nanoparticles; (d) Vitamin D3–loaded nanoparticles; (e) Curcumin-loaded nanoparticles; (f) Cisplatin-loaded nanoparticles; (g) B12 + C + D3 loaded–nanoparticles (h) B12 + C + D3 + curcumin–loaded nanoparticles (i) B12 + C + D3 + Cisplatin loaded nanoparticles (j) B12 + C + D3 + curcumin + cisplatin–loaded nanoparticles.

combinations showed less lysis than the individual drugs and vitamins. The vitamin B_{12} encapsulated nanoparticles showed lysis when compared to vitamin C and D_3. But the hemolysis was still higher for the drug cisplatin (Fig. 20.20). From these readings, it can be concluded that the drug- and vitamin-loaded nanoparticles shows less hemolysis than single drugs or vitamins. So these combinations can be used for drug delivery purposes.

5 Conclusions

Ayurveda or phytomedicine is one of the oldest forms of health care known to mankind, which uses various polyphenols such as alkaloids, steroids, tannins, and flavonoids from medicinal plants to treat not only communicable diseases but also noncommunicable diseases. On the other hand, vitamins and minerals from food sources play a critical role for the maintenance of body and mind balance. The formulation of the nanobased packed food should be supported by validated scientific data. In spite of the importance, taking any nutraceutical substances in excess is dangerous and they should be taken with caution. Therefore, it is essential to monitor the food safety regulations such as manufacture and expiration of validity period, foods for special dietary uses and for special medical purposes.

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