# EMULSIONS

## Nanotechnology in the Agri-Food Industry, Volume 3

Edited By

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AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD
PARIS • SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1800, San Diego, CA 92101-4495, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK



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**British Library Cataloguing-in-Publication Data**
A catalogue record for this book is available from the British Library

**Library of Congress Cataloging-in-Publication Data**
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-804306-6



*Publisher:* Nikki Levy
*Acquisition Editor:* Patricia Osborn
*Editorial Project Manager:* Karen Miller
*Production Project Manager:* Caroline Johnson
*Designer:* Mark Rogers

Typeset by Thomson Digital

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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

With the increasing demand for healthier and safer food products, researchers are currently focusing their efforts on the utilization of nanotechnology to encapsulate, protect, and deliver functional compounds. Nanoemulsion technology is particularly suited for the development of suitable encapsulating systems for functional compounds, and they have many potential advantages compared with that of conventional emulsions for this purpose. These can be prepared by a variety of methods such as high-shear stirring, high-pressure homogenizers, self-emulsification, phase transitions, and phase inversion. In this book, the fundamentals of nanoemulsions, methods of preparation (both high-energy and low-energy techniques), and applications in the food industry are presented. This book brings together both basic and advanced knowledge about nanoemulsion, and presents an overview of the production methods, materials used (solvents, emulsifiers, and functional ingredients), and the current analytical techniques that can be used for the identification and characterization of nanoemulsions. Finally, the applications of nanoemulsion with special emphasis on systems suitable for utilization within the food industry are discussed.

Volume III contains 20 chapters prepared by outstanding international researchers from Brazil, Canada, China, Denmark, France, India, Iran, Iran, Israel, Jordan, Malaysia, Singapore, Tunisia, Turkey, and the United States.

Weiping Jin et al., in Chapter 1, *Nanoemulsions for Food: Properties, Production, Characterization, and Applications*, present an overview of nanoemulsions, covering the basic knowledge, production methods, characterization techniques, and the applications in the food industry.

In Chapter 2, prepared by Catherine Charcosset, *Preparation of Nanomaterials for Food Applications Using Membrane Emulsification and Membrane Mixing*, is highlighted the importance of membrane processes in the food processing industry, such as membrane emulsification and membrane mixing. For both processes, the principles and the possible applications in the food processing industry are described.

Chapter 3, prepared by Vanessa Mendonça Esquerdo et al., *Nanoemulsions Containing Unsaturated Fatty Acid Concentrates*, provides important information about nanoemulsions containing unsaturated fatty acid concentrates. This chapter introduces the delivery systems widely used for the unsaturated fatty acid

concentrates for incorporation in foods and beverages such as bulk oils, emulsions, and powders. The omega-3 digestion, bioavailability, and the ability to avoid oxidation of unsaturated fatty acids in nanoemulsions is also highlighted in this work.

Chapter 4, *Nanoformulations of Polyphenols for Prevention and Treatment of Cardiovascular and Metabolic Disorders*, prepared by Rakesh Pandeet Nankar et al., discusses the various nanotechnological applications to improve the functionalities of polyphenols and their mechanisms of action in preventing cardiovascular disorders and related metabolic diseases.

In Chapter 5, *Nanoemulsion: Preparation and its Application in Food Industry*, Priyakshree Borthakur et al. focus on the synthesis, characterization, and applications of nanoemulsion in the food industries. Nanoemulsions display enhanced activity in contrast to other conventional emulsions. Their preparation through various methods, such as: high-energy methods and low-energy methods and characterization through different methods, such as dynamic light scattering (DLS), zeta potential, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), small angle X-ray scattering (SAXS), transmission electron microscope (TEM), scanning electron microscope (SEM), small-angle neutron scattering (SANS), nuclear magnetic resonance (NMR), conductivity, viscosity, and atomic force microscopy (AFM) techniques are presented and their potential revealed.

G. Roshan Deen et al., in Chapter 6, *Formation and Properties of Nanoemulsions*, report the novel approaches on properties, formation, stability, and characteristics of nanoemulsions. Popular properties of nanoemulsions such as their stability, optical transparency, diffusion characteristics are discussed and also the impact of these properties in the food industry for flavor encapsulation, food preservation, food safety, and to maintain food quality.

In Chapter 7, *Application of Nanoemulsion Technology for Encapsulation and Release of Lipophilic Bioactive Compounds in Food*, Gabriel Abraham Cardoso-Ugarte et al. present recent literature concerning the different nanoemulsions methods of preparation, the emulsifiers used, and their impact on the release of the lipophilic bioactive compounds in some applications in food.

Vivek Erramreddy and Supratim Ghosh, in Chapter 8, *Gelation in Nanoemulsion: Structure Formation and Rheological Behavior*, critically reviews the fundamental research work on theory and modeling of nanocolloidal gelation with particular emphasis on the influence of nanodroplet size, charge, interactions, and effect of emulsifier types and concentrations. The nanostructure of the nanogels is discussed using small-angle neutron and X-ray scattering techniques while the bulk and interfacial rheological

behavior is reviewed for quantification of elasticity and gelation behavior of the nanogels. Finally, potential applications of the nanogels in food and related soft materials including fat reduction and controlled release applications are discussed.

Chapter 9, *Nanoemulsion-Based Delivery Systems: Preparation and Application in the Food Industry*, by Solmaz Maleki Dizaj et al., discusses fundamental principles of emulsification process, the role of components, the types of nanoemulsions, their production methods, as well as the application of nanoemulsions in the food industry.

Chapter 10, *Biopolymers-Embedded Nanoemulsions and other Nanotechnological Approaches for Safety, Quality, and Storability Enhancement of Food Products: Active Edible Coatings and Films*, prepared by Hadar Arnon-Rips and Elena Poverenov, provides a brief general description of active edible films and coatings, which includes a discussion of the raw materials that are used to form them, their physical and mechanical properties and applications on various food products. The utilization of different nanotechnology methods such as nanoemulsions, nanoparticles, nanocellulose crystals, and fibers, and layer-by-layer techniques for the formation of active edible films and coatings are reviewed with referred literature examples.

Nesrine Mahfoudhi et al., in Chapter 11, *Nanoemulsions as Potential Delivery Systems for Bioactive Compounds in Food Systems: Preparation, Characterization, and Applications in Food Industry*, focus on the nanoemulsion technology and its applications for the nanoencapsulation of food bioactive compounds. Recently, nanoemulsions become one of the most interesting fields of application in the food industry, once they act as delivery systems for bioactive compounds. However, the main limitation for their application is their limited long-term stability as well as their nonstraightforward preparation methods.

Md. Saifullah et al., in Chapter 12, *Production, Stability and Application of Micro- and Nanoemulsion in Food Production and the Food Processing Industry*, describes the emulsification techniques for micro- and nanoemulsion, emulsion droplet properties, physicochemical properties of nanoemulsions, stability, approaches used for observing the properties of micro- and nanoemulsion, possible risk, and potential application in food production and food processing industry.

In Chapter 13, *Nanostructural Characterization of Food Grade Microemulsions: Ultrasonic Resonator Technology*, Soleiman Abbasi and Martin G. Scanlon describe microemulsions (definition, formulation, thermodynamic aspects, and applications); the use of ultrasound measurement principles (velocity and

attenuation and their relation to compressibility and particle sizing); and finally, the characterization of nonfood and food grade emulsions, nano- and microemulsions using various ultrasonic resonator technology (URT) devices.

Varun Garg et al., in Chapter 14, *Application of Self-Emulsifying Delivery Systems for Effective Delivery of Nutraceuticals*, compile the available data of various Self-Emulsifying Delivery Systems reported for improving the delivery of nutraceuticals and describe their future potential after critically viewing their advantages and limitations.

Dorota Bartusik et al., in Chapter 15, *The Synthesis and Application of Vitamins in Nanoemulsion Delivery Systems*, discuss the properties of nanoemulsions in the area of vitamin delivery and analyze recently reported vitamin nanoemulsion delivery systems. The authors present an overview of the efficient delivery of active ingredients and possibilities for controlled release and targeting, and discuss the synthesis and fabrication of vitamin nanoemulsion delivery systems and the factors that optimize particle size and distribution in vivo.

In Chapter 16, *Emulsified Protein Filaments: Types, Preparation, Nutritional, Functional, and Biological Properties of Mayonnaise*, Muhammad Hussein Alu'datt et al. cover the various types of mayonnaise and their chemical, physicochemical, and nutritional properties, and the nutritional advantages of the inclusion of emulsion protein filaments from legumes in food products.

Chapter 17, *Trends and Methods for Nanobased Delivery for Nutraceuticals*, by Anupama R. et al., addresses various aspects of nanotechnology applicable to nutraceuticals. Furthermore, challenges ahead, including regulatory issues of using nanotechnology-based delivery in food science, are also presented, which may trigger not only enhanced markets for these materials but also motivate further research in this fertile field for the pharmaceutical industry.

Chapter 18, *Nanoemulsions as Delivery Vehicles for Food and Pharmaceuticals*, prepared by Khushwinder Kaur, presents an up-to-date review related to the versatility of nanosystems that can dissolve large quantities of hydrophobics (drug/nutraceuticals/vitamins, etc.) along with their mutual compatibility and has the ability to protect them from hydrolysis and enzymatic degradation. As an ideal vehicle for the delivery of food and pharmaceutical components, these nanosystems prevent the food oxidation/degradation by protecting them in surfactant-coated droplets and ensure frequent and controlled release of therapeutic agents.

Shishu et al., in Chapter 19, *Nanoemulsions: An Emerging Technology in the Food Industry*, provide insights into nanoemulsion composition, processing, properties, and potential applications for utilization within food industry.

Ozcan Konur, in Chapter 20, *Scientometric Overview Regarding Nanoemulsions Used in the Food Industry*, highlights important papers that influenced the development of this research field as well as determines the key research areas in this field. The research in this field has strong public policy implications providing strong incentives for the key stakeholders involved in this area.

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# 1

# NANOEMULSIONS FOR FOOD: PROPERTIES, PRODUCTION, CHARACTERIZATION, AND APPLICATIONS

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## 1 Introduction

An online literature survey, using database provided by "Web of Science" (Thomson Reuters), reveals that the terminology of "nanoemulsion" first appeared in 1996 (McClements, 2012). In the past 20 years, nanoemulsions have aroused great interest among researchers. More than 5000 items related to nanoemulsions have been published up to now. After 2008, a tremendous increase of published research is observed (Fig. 1.1a). The number of publications through 2014 is around 3 times higher than that through 2008. In the first half of 2015, the amount of publications reached 200, predicting a new climax of publications at the end of 2015.

The majority of publications about nanoemulsions focus on their application in the preparation of polymeric nanoparticles or action as a nanoreactor due to their small droplet size, long-term stability, and high relative surface areas (Solans et al., 2005). Nevertheless, among the publications of nanoemulsions, food-related items are still fewer (Fig. 1.1b). But it displays a prospect for high-sustained growth. Utilizing emulsion technology to create broad applications, such as milk, soft drinks, nutriceuticals fortification, has been developed in the food industry (McClements and Rao, 2011; McClements, 2010). The application of emulsion technology in the food field can design new structures of products and

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00001-5

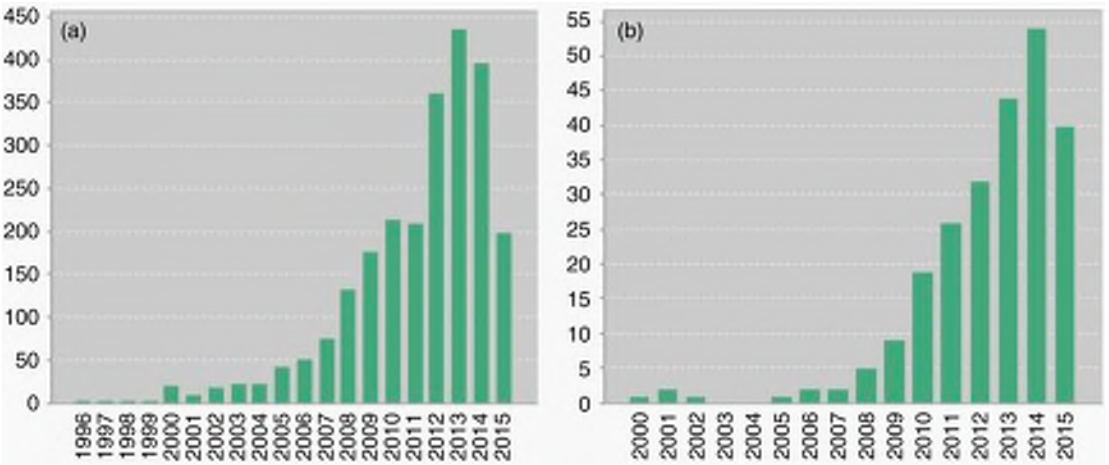


**Figure 1.1. Number of published items per year including nanoemulsion (a) and food-related nanoemulsion (b) using statistics database from "Web of Science" (Thomson Reuters).**

control physicochemical properties, such as texture, taste, flavor, and stability. Furthermore, nanoemulsions have been proven to be considerable delivery systems for encapsulating, protecting, and enhancing bioavailability of lipophilic bioactive nutriceuticals, drugs, and so on (Huang et al., 2010; Silva et al., 2012).

Currently, nanoemulsions are popular to be utilized in functional food systems to encapsulate, protect, and deliver nutraceuticals, which has aroused much attention from the scientific community. Massivenatural phytochemicals extracted from food-based materials have proved to have exciting benefits or to improve health problems. Fig. 1.2 displays a summary of some lipophilic functional compounds that have been studied comprehensively and commonly incorporated in foods or cosmetic nanoemulsions. Unfortunately, most of them possess poor water solubility. Their hydrophobicity always causes negative influences on their absorption by human digestive systems and applications in food products (McClements, 2010; McClements and Rao, 2011).

The term of "lipid soluble vitamin" refers to a group of oil-soluble vital nutrients that have diverse biochemical functions, such as vitamin A and vitamin D, serving as protectors of eyesight and regulators of mineral metabolism respectively (Jenning et al., 2000b; Guttoff et al., 2015; Ziani et al., 2012). To protect from deficiency diseases and maintain health, foods and beverages are fortified with vitamins A, D, and E. Nanoemulsions are particularly suitable for the encapsulation and delivery of those lipophilic components. The $C_{max}$ of natival vitamin E obtained in the


**Figure 1.2. Common lipoophilic bioactive compounds encapsulated into foods nanoemulsion delivery systems.**

pharmacokinetics study of nanoemulsions-loaded systems has high value, as compared with market soft capsules using noncampartment models. Besides, the plasma concentration time profiles in rats are 1.6-fold enhancement (Gong et al., 2012).

Polyphenols, for example, curcumin (Yu and Huang, 2012; Ahmed et al., 2012), resveratrol (Sessa et al., 2011, 2014), carotenoids, for example, lutein (Mitri et al., 2011; Vishwanathan et al., 2009), β-carotene (Qian et al., 2012b), and flavonoids, for example, tangeretin (Ting et al., 2015b), nobiletin (Chen et al., 2015), and qucertin (Pool et al., 2013a) are proposed for health benefits due to the capacities of preventing oxidative damage, protecting the heart from disease and losing weight. However, poor water solubility, high melting point, chemical instability, and low bioavailability limit their applications. It was reported that lecithin-based nanoemulsions are able to effectively transport resveratrol through Caco-2 cell monolayer and improve its bioavailability (Sessa et al., 2014). The degradation rates of β-carotene were slowed down by encapsulating into β-lactcoglobulin-stabilized nanoemulsions (Qian et al., 2012a,b). Oral bioavailability of tangeratin has been evaluated using in vitro TNO gastrointenstinal model and in vivo mice pharmcokinetics tests. The results revealed that the bioavabilability of nanoemulsion-delivered tangeratin is 2.6-fold in vitro and 2.3-fold in vivo models higher than that of unformulated one (Ting et al., 2015a,c).

Essential oils, usually used as food additives due to their excellent flavors, are found to exhibit good antibacterial activities. An essential oil is defined as a group of concentrated hydrophobic

liquids containing volatile aroma compounds from plants, such as eugenol, D-limonene (Burt, 2004). Nanoemulsions are used for protection of volatile compounds against environments and improvement of their antimicrobial capacities (Donsì et al., 2011a; Li et al., 2015). The increases of the eugenol, which are loaded in SDS and Tween-80–stabilized nanoemulsions, antimocrobial activities are observed by lowering minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) in *Staphylococcus aureus* and *Escherichia coli* cultures (Li et al., 2015).

More and more in vitro and in vivo studies have proven that the encapsulation efficiency, maintenance of bioactivity, and oral bioavailability are improved after formulating by nanoemulsions. According to the search results of nanoemulsions in vitro evaluations, the number of studies increases at a similar pace with that of nanoemulsions. However, in vivo studies present a significant lagging development. For most nanoemulsions delivery systems, in vivo biological efficiencies are still unclear. There are many of questions that should be answered (Huang et al., 2010). How could nanoemulsions enhance the oral bioavailability? What is the cell absorbed route of nanodelivery vehicles? How is the cellular signal transluction pathway of encapsulated compounds changed after they are absorbed in cells? Therefore, a growing amount of attention and effort is devoted to in vivo evaluation. More concerns are going to be addressed on the comprehensive understanding of the metabolism process of nanoemulsion-based delivery systems in the human body (Fig. 1.3).

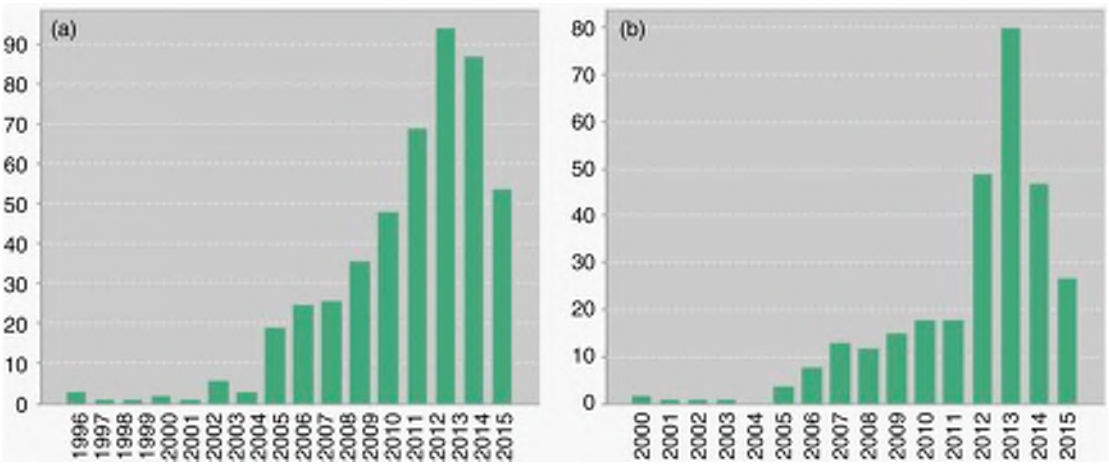


**Figure 1.3. Published items of (a) In vitro and (b) In vivo evaluation of nanoemulsion delivery systems using statistics database from "Web of Science" (Thomson Reuters).**

According to the literature reviews, nanoemulsions become the effective and potential delivery vehicles to protect the functional compound and to enhance their bioavailability. Hence, in this chapter, the basic knowledge of nanoemulsions is introduced. Furthermore, production methods, characterization techniques, and the applications of nanoemulsions in food fields are reviewed.

# 2 Basic Knowledge of Nanoemulsions

## 2.1 Definition and Physical Property of Nanoemulsions

Emulsion is a class of relatively stable mixtures consisted of immiscible liquids, which are commonly oil and water. Two major types of emulsions are oil-in-water (O/W) and water-in-oil (W/O). They are determined by oil or water phases that act as the dispersed or continuous phase. Because most food systems are made and utilized in water surrounding, O/W emulsions are hereby preferred to be discussed in this chapter. According to the stable mechanisms, physical properties, and thermodynamic stability, emulsions can be classified as macroemulsion, nanoemulsion, and microemulsion. The basic characters of those emulsions are listed in Table 1.1 (McClements, 2010). Compared with macroemulsions (conventional emulsions), nanoemulsions possess relatively small droplet sizes in the range of 20–200 nm (Huang et al., 2010). Nanoemulsions belong to kinetic stable systems, but they are thermodynamically unstable. Their appearances can be transparent, translucent, or have a creamy optical appearance, depending on the particle's size (Tadros et al., 2004b). When the droplet size is around or below 50 nm, the solutions are transparent and have a slightly bluish color. There is a noticeable turbid increase of nanoemulsion solutions when their particle size

**Table 1.1 Properties of Different Types of Emulsions**

| Emulsion Type | Diameter Range | Thermodynamic Stability | Appearance |
|---|---|---|---|
| Macroemulsion | 0.1–100 μm | Unstable | Turbid/opaque |
| Nanoemulsion | 20–200 nm | Unstable | Clear/translucent |
| Microemulsion | 5–50 nm | Stable | Clear |

*McClements, 2010*

## Table 1.2 Common Ingredients Used in Food Nanoemulsions

| Components | Examples |
|---|---|
| Oils | Soybean oil, sunflower oil, corn oil, fish oil, castor oil, coconut oil, mineral oil, olive oil, basil oil, essential oils |
| Emulsifiers | Sodium dodecyl sulfate, sorbitan monooleate, phospholipids, polysorbates, Tween-20–80, Span 20–80, whey protein, soy protein, casein |
| Cosurfactants | Ethanol, glycerin, PEG400, polyene glycol, propylene glycol, and sorbitol |
| Cosolvents | dimethylsulfoxide, ethanol, glycerol, propylene glycol, *N*-methyl pyrrolidone |
| Functional ingredients | carotenoids, curcumin, phytosterols, coenzyme Q, astaxanthin, Omega-3 fatty acids, vitamin E |
| Ripening retarder | Long-chain triglyceride, mineral oil, ester gum |

increases to 100~200 nm. Microemulsions have smaller particle sizes, and they are usually clear and thermodynamically stable.

## 2.2 Nanoemulsions Ingredients

The basic formulation of nanoemulsion can be divided into oil phase, emulsifier, and aqueous phase, which work together to modulate the formation of nanoemulsions. Common ingredients used in food nanoemulsions are presented in Table 1.2 and are discussed in detail.

### *2.2.1 Oil Phase*

Oil phase is one of the complex components in nanoemulsion systems because many kinds of oils with different polarity could be available and abundant lipophilic active ingredients might be loaded. Various oils are commonly used in the preparation of nanoemulsions, such as (tri-, di-, mono-) acylglycerols, free fatty acids, flavor oils, essential oils, mineral oils, fat substitutes, and waxes (Shah et al., 2010). In the food industry, triacylglycerol oils are often desirable as oil phase because of their low cost, nontoxicity, and abundant raw material source, including soybean oil, sunflower oil, corn oils, and canola oils. Due to the complex composition of bulk oils, their polarities, viscosities, and other physicochemical properties are different, which might affect the formation and stability of nanoemulsions. For example, the polarity of the plant oil is commonly very low, but the viscosity is relatively high as the

presence of long- or medium-chain triacylglycerol. Therefore, it is usually difficult to prepare nanoemulsions with those oils using methods of phase inversion temperature and high-pressure homogenization (Witthayapanyanon et al., 2006). Besides, bioactive compounds dissolved in the dispersed oil phase may also influence the formulation of nanoemulsions. Carotenoids, curcumin, phytosterols, vitamin E, and astaxanthin are frequently used as lipophilic active ingredients (Shakeel et al., 2010). The addition of active components often changes the physicochemical properties of nanoemulsions, such as the particle size and optical appearance.

### *2.2.2 Emulsifier/Surfactant*

Emulsifiers are a kind of surface-active molecules that are capable to adsorb on oil–water/air–water interfaces to reduce the interfacial tension and sustain droplets stability. Hence, it is crucial to choose one emulsifier or combined emulsifiers prior to the preparation of favorable nanoemulsions. The commonly used emulsifiers are small molecule surfactants that can effectively fabricate nanoemulsions by both high-energy approaches and low-energy approaches. Generally, the small molecule surfactants are classified as ionic, nonionic, and zwitterionic surfactants according to their electrical properties (Kralova and Sjöblom, 2009).

Compared with microemulsions, more kinds of emulsifiers are affordable to form nanoemulsions, including small molecule surfactants, proteins, and hydrocolloids. Due to differences of chemical structure, their arrangements and thickness on the interface are different. The thickness of the interface layer by small molecular surfactants is around 0.5–1 nm, proteins around 1–5 nm, and hydrocolloids around 5–10 nm (Dickinson, 2009). Schematic representations of relative thicknesses are displayed in Fig. 1.4. Proteins, kind of large amphiphilic molecules, are the

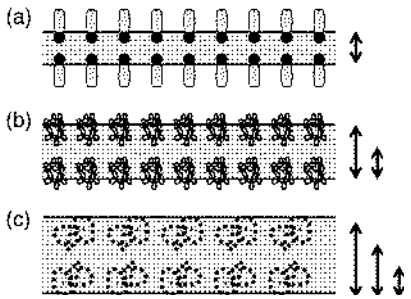


**Figure 1.4. Schematic representation of relative thicknesses of thin films between closely approaching oil droplets stabilized by (a) surfactants, (b) proteins, and (c) hydrocolloids.** (Dickinson, 2009)

most commonly natural emulsifiers in foods, such as whey protein, casein, β-lactoglobulin, ovalbumin, soy protein isolated, and bovine serum albumin. They all richly exist in the food systems such as beans, eggs, and milk. Most hydrocolloids are only regarded as the stabilizing agents of O/W emulsions except for gum Arabic, modified starch, and some kinds of pectin or galactomannan (Dickinson, 2003). The mechanisms of hydrocolloids' interfacial stabilization are explained by: (1) the hydrophobic modified groups on hydrophilic polysaccharide backbones, for example, modified starch; (2) the presence of protein segments are combined or physically mixed with polysaccharide, such as gum Arabic. But they are not effective enough as surfactants during the formation of fine nanoemulsions. Therefore, extensive interest in producing nanoemulsions with hydrocolloids is still present (Adjonu et al., 2014; Lam and Nickerson, 2013).

### 2.2.3 Aqueous Phase

Aqueous phase, usually water, is the irreplaceable component in the formation of nanoemulsions. On the one hand, the ratio of aqueous/oil is an important factor in the formation and the stability of nanoemulsions. On the other hand, the variety of other compositions may be contained in the aqueous phase, such as polysaccharides, proteins, cosolvents, salts, and nutrients (McClements and Rao, 2011; Bhatt and Madhav, 2011). These solutes in water might change the viscosity, pH, and ionic strength or the polarity of water, and then affect the interfacial tension and phase behavior of the emulsion systems. Therefore, dominating the aqueous phase composition could help regulate the physicochemical properties of nanoemulsions (Shafiq et al., 2007). The presence of pectin, guar gum, alginate, and other polysaccharides in the aqueous phase could help to stabilize nanoemulsions for their high viscosity (Choi et al., 2011). For protein dissolution in water, especially soy protein and casein, additions not just act as aqueous phase, but also serve as the surfactant and are associated with other surfactants contained in oil phase (Lee and McClements, 2010). Some cosolvents in water could change the polar of the aqueous phase and may play a similar role of cosurfactants.

### 2.2.4 Cosurfactants

Cosurfactants are amphiphilic molecules, usually not surface active enough to stabilize emulsions by themselves. They have an affinity for oil phases and water phases, commonly possessing hydrocarbon chain and hydroxyl groups, such as ethanol

and glycerin. They can further reduce the surface tension on the basis of emulsifiers, which sometimes are required for the formation of nanoemulsions with smaller droplet sizes (Flanagan and Singh, 2006).

### 2.2.5 Other Components

Other components in nanoemulsions might involve cosolvents, ripening retarders, and functional ingredients. They are not the indispensable constituent parts, but sometimes change the properties of the emulsions. Cosolvents (such as alcohols and polyols) are polar molecules, but not surface active themselves. However, they may induce slight changes in the physicochemical properties of oil phase or aqueous phase, such as density, refractive index, and interfacial tension (Saberi et al., 2013a). Ripening retarder is an important agent to prevent nanoemulsions from Ostwald ripening. It is common in highly hydrophobic material, such as long-chain triacylglycerol, mineral oil, and ester gum (McClements, 2011). Functional ingredients are always encapsulated in nanoemulsions for the production of nutraceutical-fortified foods. But their solubility in oil phase might cause adverse effects on the property of nanoemulsions.

## 2.3 Physical Stability of Nanoemulsions

Due to small droplets of nanoemulsions in the range of 20–200 nm, nanoemulsions are relatively stable against gravitational separation, flocculation, and coalescence. When the particle radius is smaller than 90 nm, Brownian motion is sufficient to overcoming gravity force (McClements, 2012), which is also helpful for the prevention of flocculation and coalescence. Compared with microemulsion, the relatively thick interfacial emulsifier layer would improve the stabilization of nanoemulsions (Tadros et al., 2004b).

Ostwald ripening is reported to be the main destabilization factor of nanoemulsions (Solans et al., 2005). This process easily occurs between small and large droplets because of molecular diffusion of oil between droplets through the continuous phase. Theoretically, for two independent droplets of radii $r_1$ and $r_2$ (where $r_1 < r_2$),

$$\left(\frac{RT}{V_m}\right)\ln\left[\frac{c(r_1)}{c(r_2)}\right]=2\gamma\left(\frac{1}{r_1}-\frac{1}{r_2}\right) \tag{1.1}$$

where $c(r)$ is the solubility surrounding a particle of radius $r$, $V_m$ is the molar volume of the dispersed phase and $\gamma$ is interface tension

(Tadros et al., 2004a). In Eq. (1.1), it is obvious to see that the higher rate of Ostwald ripening occurs with the larger difference between $r_1$ and $r_2$.

The quantitative calculation of the rate of Ostwald ripening is described by Lifshitz–Slesov–Wager (LSW) theory,

$$r^3 = \frac{8}{9}\left[\frac{c(\infty)\gamma V_m D}{\rho RT}\right]t \tag{1.2}$$

where $c(\infty)$ is the bulk phase solubility, $D$ is the diffusion coefficient of the disperse phase and $\rho$ is the density of disperse phase.

Therefore, there are several ways to reduce Ostwald ripening according to Eq. (1.2). One is the decrement of bulk phase solubility, which can be achieved by adding long chain triglyceride with lower solubility and diffusion coefficient in the oil phase (Wooster et al., 2008). The second is the addition of a small amount of components that are poor or insoluble in continuous phase (Solans et al., 2005). The third is the selection of the surfactants, which play an important role in lowering Ostwald ripening due to significant decrease of interface tension ($\gamma$). Finally, increasing interfacial elasticity is supposed to suppress the Ostwald ripening (Meinders and van Vliet, 2004). In food nanoemulsions, proteins and hydrocolloids can provide higher stability of nanoemulsions, because they possess the more rigid viscoelastic interfacial networks. Whereas the occurrence of Ostwald ripening is still difficult to be inhibited except for those are stabilized by particles (Wooster et al., 2008).

## 2.4 Advantages of Nanoemulsions

The attractions of nanoemulsions for studies and applications in food areas are due to a number of potential advantages.

*Long-term stability.* The very small droplet size does not only reduce the gravity induced phase separation (creaming or sedimentation), but also prevents the flocculation and coalescence due to a relatively thick surfactant film that compared with microemulsion (Tadros et al., 2004b).

*Natural surfactants.* Unlike microemulsions, which require a high amount of surfactants (20% or even higher), the surfactant concentration for preparing nanoemulsions is commonly used in the range of 5–10%. The high amount of synthetic surfactants is limited during the commercial application. Hence, exploitations of new surface-active compounds have aroused interest to displace the synthetic surfactants. Food-grade proteins and polysaccharides are promising natural surfactants applied in forming nanoemulsions (Dickinson, 2008, 2009).

*High surface area.* Because of small sizes, nanoemulsions possess large surface areas. Large surface areas will increase the bioaccessibility of endogenous surfactants, such as lipase or bile salt in human digestion, or it can transport through the cell membranes easily and enhances penetration of bioactive encapsulated compounds (Tadros et al., 2004b).

*Improvement of food physicochemical properties.* Nanoemulsions are always used for designing new structured food or regulating food textures by altering rheological properties, such as possessing high viscosity or forming gel-like characteristics. Moreover, nanoemulsions could be applied for the controlled releases of flavor or taste compounds (Silva et al., 2012; Huang, 2012).

# 3 Production of Nanoemulsions

Although nanoemulsions are thermodynamically unstable systems, they can be regarded as kinetically stable solutions due to their small droplet size and long-term stability. It means that the preparation of nanoemulsions needs to input energy. Nanoemulsions can be prepared using a number of methods, which can be commonly classified low-energy approaches and high-energy approaches simply depending on the energy input. The optimum particle sizes can be obtained by those methods and also rely on materials used for preparation.

## 3.1 Low-Energy Approaches

Low-energy approaches rely on the spontaneous formation of fine droplets when the environmental conditions of incompatible oil/water/emulsifier systems are altered. Membrane emulsification, spontaneous-emulsification methods and phase inversion are the commonly used low-energy approaches (Table 1.3).

*Membrane emulsification* is a low-energy process and was developed several decades ago by Suzuki and others (Suzuki, 1981). The continuous flow systems with emulsification devices could produce stable nanoemulsions with better controlling of droplet size distributions. And they allow forming emulsions without high mechanical stress at lower energy input ($10^4$–$10^6$ J/m$^3$) (Ribeiro et al., 2005). Many natural products contained in systems were commonly fabricated by the simply methods (Laouini et al., 2012). It was regarded as a promising alternative method for nanoemulsions formation, although the droplet size is usually higher than that of other methods prepared. However, it also needs to expend effort to optimize appropriate processing parameters. Oh et al.

## Table 1.3 Nanoemulsions Cases Prepared by Low-Energy Approaches

| Preparation Method | Oil Phase | Bioactive Component | Surfactant/ cosurfactant | Diameter | References |
|---|---|---|---|---|---|
| Membrane emulsification | MCT | Vitamin E | Tween-80 | 76–105 nm | Laouini et al. (2012) |
| Membrane emulsification | Methylene chloride | Flurbiprofen | Tween-20/ Tween-80/polyvinyl alcohol | 60–98 nm | Oh et al. (2011) |
| Spontaneous emulsification | MCT | Vitamin E | Tween-20, 40, 60, 80 | 54–200 nm | Saberi et al. (2013c) |
| Spontaneous emulsification | Castor oil/ MCT | Carbamazepine | Polysorbate 80 | 148–153 nm | Kelmann et al. (2007) |
| PIC | *n*-Dodecane | — | Sodium dodecyl sulfate/hexanol | 20–160 nm | Sole et al. (2012) |
| PIC | Hydrogenated polyisobutene | — | Polyethylene glycol-400 monoisostearate | 100 nm | Sonneville-Aubrun et al. (2009) |
| PIT | Cremophor/ paraffin oil | — | Cremophor ® A6 | 330–500 nm | Fernandez et al. (2004) |
| PIT | Orange oil | β-Carotene | Tween-20 | 78–100 nm | Qian and McClements (2011) |

(2011) studied effects of process parameters on particle sizes of nanoemulsions using SPG membrane emulsification and 10 kinds of surfactants. The results showed that the type of surfactant, agitator speed (150–1200 rpm), feed pressure (15–80 kPa), stabilizer concentration, and the temperature of the continuous phase strongly influenced the z-average diameter and size distribution of the emulsion droplets.

*Spontaneous emulsification method* is the simplest method for preparing nanoemulsions. The nanoemulsions are formed spontaneously when the right proportions of oil, water, surfactant, and/or cosurfactant mixed together. No expensive equipment is required no matter how the two phases and prepared conditions (pH, stirring speed, and ionic strength) varied. Fine oil droplets can be formed when an oil/surfactant mixture is added to water. The sequence of addition is not critical because this kind of

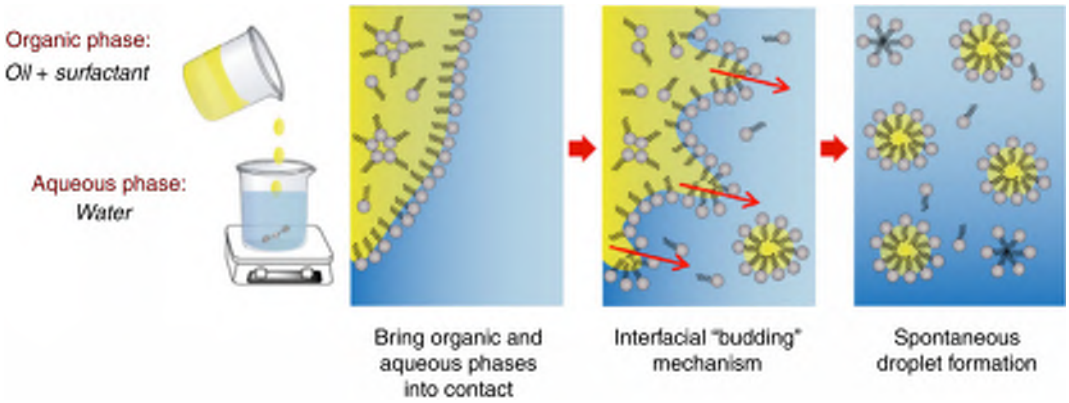


**Figure 1.5. Schematic representation of proposed mechanism for spontaneous emulsification** (Saberi et al., 2013b).

nanoemulsion is formed spontaneously. But the system compositions (oil-to-emulsion ratio content, the surfactant-to-emulsion ratio) and preparation conditions (stirring speed) may influence emulsions properties (Kelmann et al., 2007; Saberi et al., 2013c). Some tentative mechanisms have been proposed to account for spontaneous emulsification, including diffusion of solutes between two phases, interfacial turbulence, surface tension gradient, dispersion mechanism, and condensation mechanism (Silva et al., 2012). Fig. 1.5 shows a representative schematic for spontaneous emulsification. The surfactant moves from the organic phase to the water phase, leading to interfacial turbulence. Then the spontaneous O/W droplet formation may result from the increase of entropy and the decrease of the Gibbs free energy of the system.

*Phase inversion composition (PIC)* is a method that optimizes the curvature by changing the composition at certain temperature (commonly at room temperature). The practical operation is progressive dilution with water or oil phase. This phase change is driven by Gibbs free energy of the emulsions resulting in spontaneous inversion of the surfactant's curvature between positive and negative (Sonneville-Aubrun et al., 2009; Roger and Cabane, 2012). The classical PIC is used to produce O/W emulsion by water diluting W/O emulsion. As Fig. 1.6 shows, the dilution may operate differently. The W/O microemulsion and water could be added in different procedures and steps (Solè et al., 2012).

*Phase inversion temperature (PIT)* is similar with PIC to a certain degree. But the mechanism is optimum curvature of surfactant by altering temperature instead of changing the composition. It confirms that curvature of surfactant changes at different

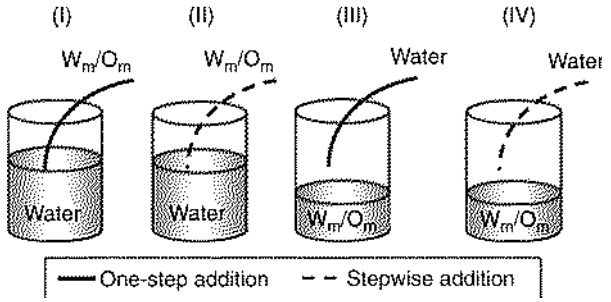


**Figure 1.6. Scheme of PIC to produce O/W emulsion by diluting W/O emulsion.** (Solè et al., 2012)

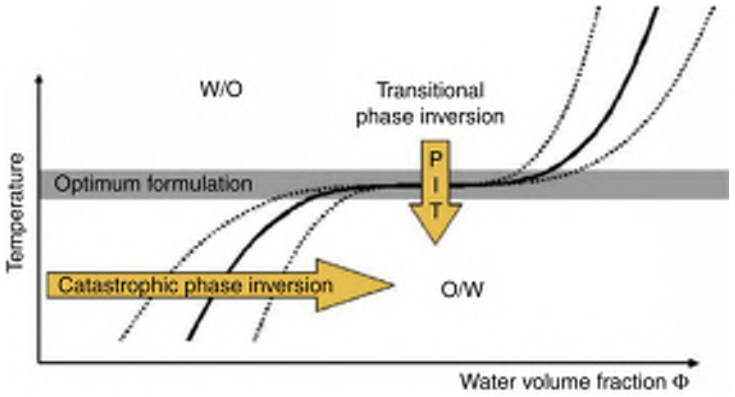


**Figure 1.7. Phase inversion is a function of temperature** (Fernandez et al., 2004).

temperatures (Fig. 1.7). Phase inversion occurs at a critical temperature. Fernandez et al. (2004) reported the curvature spontaneously reached the zero point during rapid cooling process after critical temperature treatment resulting in stable nanoemulsions formation. The inversed temperature was dependent on the composition of the emulsions as well as external conditions (Qian et al., 2012c). For example, higher surfactant concentration would make a inversed temperature (Izquierdo et al., 2004).

## 3.2 High-Energy Approaches

High-energy approaches rely on different mechanical devices, such as high-speed homogenizer, high-pressure homogenizers, microfluidizers, which could provide intensive energy to disrupt oil and water resulting in fine droplets. Energy inputs modulate the properties of the nanoemulsions and excellently prevent

## Table 1.4 Nanoemulsions Cases Prepared by High-Energy Approaches

| Preparation method | Oil Phase | Bioactive Component | Surfactant | Diameter | References |
|---|---|---|---|---|---|
| High pressure homogenization | MCT | β-Carotene | Tween-20–80 | 132–184 nm | Yuan et al. (2008) |
| | Corn oil | — | SDS, Tween-20, β-lactoglobulin, sodium caseinate | 123–245 nm | Qian and McClements (2011) |
| | MCT | — | Tween-80 | 150–250 nm | Liedtke et al. (2000) |
| | Sunflower oil | | Tween-80, SDS | 90–190 nm | Donsì et al. (2011b) |
| Microfluidizers | Lauroglycol 90 | Aspirin | Cremophor EL | 150–170 nm | Tang et al. (2013) |
| | Croton oil | — | Tween-80 | 42–758 nm | Kuo et al. (2008) |
| | Soybean oil | Aspirin | Tween-80 | 70–123 nm | Subramanian et al. (2008) |
| Ultrasonic homogenizers | D-limonene | — | Polyoxyethylene oleyl ether | 20–100 nm | Li and Chiang (2012) |
| | Basil oil | — | Tween-80 | 20–50 nm | Ghosh et al. (2013a) |
| | MCT | PLA, PEG-PLA | OSA | About 150 nm | Preetz et al. (2010b) |
| High-speed devices | Liquid lipid | — | Tween-80 | 210–290 nm | Yilmaz and Borchert (2005) |

droplet disruption and coalescence. Recent researches have been summarized in Table 1.4.

*High-pressure homogenization* causes the emulsion mixture first to suffer high pressures and then pass a controlled valve. The homogenization prevents the droplets from disruption and forms fine droplets. The emulsion compositions and high-pressure homogenization parameters, including pressure, temperature, and cycle, directly affect the properties of final formed nanoemulsions (Yuan et al., 2008; Donsì et al., 2011b; Lee and Norton, 2013). The stability of this kind formed nanoemulsions usually decreased with the increase of temperature, but increased with pressure and homogenization cycle. The size commonly decreases with the increase of pressure and cycles. Qian and McClements found that the mean droplets size and pressure has a linear log–log relationship, but the temperature of temperature is not involved (Qian and McClements, 2011; Liedtke et al., 2000). McClements further

optimized and proposed the theory for droplets size during homogenization (McClements, 2004).

*Microfluidizer* is a mixing technique, which is somewhat similar in the high-pressure homogenizer. A high-pressure displacement pump is used in the device to force the product through the interaction chamber composing of many small channels called microchannel. Prior to the homogenizer by microfluidizer, the aqueous phase and oil phase were combined together to yield a coarse emulsion, usually obtained by high-speed homogenizer. The coarse emulsion then passed through a microfluidizer to produce stable nanoemulsions (Koroleva and Yurtov, 2012; McClements, 2011, 2012). It has been considered to be a simple and scalable process for nanoemulsion formation. Many researchers have examined the major factors that determine the size of the droplets produced by microfluidizers. They found the relationships were similar with the high-pressure homogenization method (Kuo et al., 2008; Subramanian et al., 2008). Nanoemulsions produced by microfluidizer usually share the high stability for a long time (Tang et al., 2013).

*Ultrasonic homogenizers* utilize high-intensity ultrasonic waves to produce strong disruptive forces. The strong disruptive forces could transform the oil-and-water immiscible solutions with surfactants to fine and stable droplets (Ghosh et al., 2013a; Preetz et al., 2010b). Many publishers have reported in this area although seldom practical applications in food field (Ghosh et al., 2013a). As compared with other devices, batch and flow-through ultrasonic homogenizers could prepare monodispersed and more stable emulsions (Li and Chiang, 2012; Izquierdo et al., 2005).

*High-speed homogenization* mainly provides the shearing force to form the nanoemulsions. Rotor/stator devices, that is, Ultra Turrax, are typical high-speed devices. Emulsions produced by these devices always have large particle sizes and wide particle size distribution. Researchers could prepare stable nanoemulsions by combining these devices with other high-energy devices including high-pressure homogenization and ultrasonic homogenizers (Yilmaz and Borchert, 2005; Meleson et al., 2004). High energy has been taken to disperse oil-and-water phase, but most of them have been to transform heat or dissipated (Anton et al., 2008).

Overall, the formation and properties of nanoemulsions depends on numerous factors, principally including compositions, environmental conditions, and emulsification techniques. According to literature reports, high-energy approaches are the more common methods used to prepare nanoemulsions than low-energy methods in industrial food operations (Anton et al., 2008).

# 4 Techniques for the Characterizations of Nanoemulsions

The physiochemical properties of nanoemulsions are well known to affect the texture, taste, flavor, and stability of emulsion-based products. Therefore, it is important to characterize the properties of nanoemulsions, such as physical properties, stabilities, rheological property, and microstructure. Up to now, various techniques have been developed and applied in the characterization of nanoemulsions.

## 4.1 Basic Physical Properties

The typical and basic physical properties of nanoemulsion are particle size, particle size distribution, particle charge, and lipid crystallinity.

### *4.1.1 Particle Structure and Size Distribution*

*Light scattering* is a variety of analytical instruments that are available to measure the particle size and particle size distribution of nanoemulsions. Most of these instruments are fully automated and are capable to analyze thousands of particles every second. It is possible to obtain aerodynamic particle size distributions in a few seconds. Different instruments have their own principles to characterize the particle size and its distribution. Consequently, nanoemulsions with different droplet sizes and concentrations should choose the suitable measurements.

Dynamic light scattering (DLS) technique is used for rapidly determining the size distribution of small particles or droplets in suspensions. Currently, the suitable particle range characterized using commercial DLS instrument is about 3 nm to 5 μm. Due to Brownian motion of small particles, the change of relative spatial location will induce the intensity fluctuation. So, DLS is based on the record of the intensity fluctuations that occurred over time when light is scattered by particles (Joosten et al., 1991; Mason et al., 2006; Fryd and Mason, 2012). This noninvasive technique provides a direct characterization for investigating the size and evaluating the size distribution of nanoemulsions (Yun Zhang, 2003). For nanoemulsions, the size distribution profiles displayed relative mono-disperse, which is usually the single and narrow peak. Qian et al. (2012c) prepared β-carotene-enriched nanoemulsions that possess a mono-modal particle size distribution with the majority of particles being less than 100 nm and mean particle size being 79 nm. To compare the particle size distribution

under different external conditions is also a good way to evaluate the stabilities of nanoemulsions.

Static light scattering instruments utilize some mathematical models (ie, "Mie theory") to predict the scattering pattern of an emulsion from the characteristics of the droplets that it contains (refractive index ratio, absorption coefficient, and diameter) (McClements, 2007b). The particle size distribution is obtained using the software based on mathematical model, which can figure out the best-fit relationship between the measured scattering pattern and the theoretically predicted one. Then it reports the data as the format of a table or a plot of particle concentration (number or volume) versus particle size (diameter or radius). But it is commonly used to measure larger droplets with the value of size ranged from 100 nm to 1000 μm.

*Small-angle X-ray scattering (SAXS)/small-angle neutron scattering (SANS)* are fundamental tools applied for analyzing the microstructural characterization of colloidal particles (Gradzielski, 2008). In the SAXS experiment, the typical SAXS pattern, which is a function between intensity and scattering vector, is obtained at low angles (typically 0.1–10°). After analyzing and fitting curves with the suitable models, it could obtain the information about shape, size, and nanostructure. Similarly, differences in neutron scattering length density of nanoemulsions are used for collecting SANS data. Using short wavelengths ($\lambda < 10$Å), SANS data is possible to gain accurate data of structure factor (Mason et al., 2006). Those methods have advantages of nondestructive measurements and the minimum amount of samples. Zhao et al. used SAXS to study the structures of double nanoemulsions. They found that the typical SAXS pattern was displayed and ordered structure existed in the emulsion system. To obtain the detailed information of emulsion droplet shape and size, they used mathematical model to fit the curves and found that core-shell models could fit them well (Zhao et al., 2011). In addition, SAXS/SANS can be combined with other techniques, such as near infrared spectroscopy (Balakrishnan et al., 2008) and DSC measurements (Jenning et al., 2000c).

### 4.1.2 Particle Charge

The electrical surface charge of emulsion droplets is usually characterized using zeta potential (ζ-potential). ζ-potential is a scientific term for describing the electro-kinetic potential in nanoemulsions. When particle carrying surface charges are dispersed in a liquid phase, ions of opposite charge (called counter-ions) in this suspension are attracted to the surface of particles and form a firm attachment layer that is called "Stern layer." The combination

of Stern layer and diffuse layer is assigned as the electrical double layer, the thickness of which depends on the type and concentration of the ions in the suspension as well as on the particle surface. The electrical charge on the droplet surface affects the interactions between emulsion droplets and plays an important role in the stability of nanoemulsions. Colloids with high absolute value of zeta potential (negative or positive, commonly above 30) are electrically stabilized, while colloids with low zeta potentials tend to coagulate or flocculate (McClements, 2007b; Mohanraj and Chen, 2007).

### 4.1.3 Lipid Crystallinity

*Differential scanning calorimetry* (DSC) is a thermo-analytical technique, which was used to study the influence of oil crystallization on the stability of the nanoemulsion systems. It works based on the crystalline temperature differences of pure oil and oil contained in emulsion (Rafanan, 2013; Thanasukarn et al., 2004). Nucleation in bulk oils is an unstable factor of nanoemulsions. It is initiated by the presence of impurities, which first leads to oil crystal partial formation and rapidly propagate throughout the whole oil. During the storage of oil products or emulsions, fully or partially oil crystallization has side effects on their stabilities. Using the DSC instrument, the percentage of destabilized fat in the emulsions is calculated from measuring the area under the nonemulsified enthalpy peaks, which are measured during a cooling cycle, and divide it by the total area under all of the enthalpy peaks. When the oil is dispersed into small droplets, interfacial layer limits the nucleation growth of oil crystal. Thanasukarn et al. found that protein stabilized emulsion present more stable than Tween-20 stabilized ones under the temperatures where the oil phase was partially crystalline. The results attributed that the protein-stabilized emulsion possessed the relative thick interfacial layer (Thanasukarn et al., 2004). Besides, the polymorphic form of fat crystals can also be identified by measuring the temperature at which phase transitions occurred and the amount of heat absorbed/released using DSC (McClements, 2007a).

### 4.1.4 Nuclear Magnetic Resonance

Recently, nuclear magnetic resonance (NMR)-based techniques have attracted more attention for the characterization of nanoemulsions. They are used to study different emulsion components, types, structures, diffusion properties of components, and relaxation behavior (Acharya and Hartley, 2012; Hathout and Woodman, 2012). A lot of researchers applied the NMR in

microemulsion and nanoemulsion, which showed that a large number of information can be gained via NMR techniques (Furó, 2005; Balinov et al., 1994; Hollingsworth and Johns, 2003; Lingwood et al., 2012; Hughes et al., 2013). In particular, the Fourier transform pulsed gradient spin echo (FT-PGSE) technique can separately measure self-diffusion coefficients of oil, water, and surfactant molecules in the one experiment (Gradzielski, 2008). This technique is easily used to discern signals in the spectra that are associated to a single one type of molecule, such as surfactant, oil, or water. Therefore, it is easy to get the diffusion of the different constitutive components of the emulsion. Besides, this technique is capable of testing the connectivity in emulsions, a question that is often very difficult or impossible to address by other methods (Gradzielski, 2008). The phase transient between a droplet emulsion phase and a bicontinuous emulsion can be investigated very elegantly through the PGSE-NMR (Gradzielski, 2008).

In addition, NMR peak attenuation is also utilized to determine the displacement (translational diffusion) of spins because of specific nuclei in the lipid/surfactant and water over a defined period in a magnetic field gradient. Iseult Lynch et al. combined the characterization methods of NMR and SANS to study the structural changes of emulsion as a function of temperature (Stubenrauch et al., 2008). However, the application of NMR in nanoemulsions is still a promising area that needs to be exploited. Jenning et al. (2000a) used $^{1}$H-NMR to exploit the oil phase components of nanoemulsions. They developed NMR method to determine the faction ratios of medium-chain triglycerides and glyceride behenate that are incorporated into preparing nanoemulsions. The characterization of nanoemulsions involves measuring the molecular relaxations of component molecules can be realized via NMR relaxation technique. It can provide useful information about shape and size of droplets, and it can easily pick up subtle changes of that information without any interference from droplet interactions at high-volume fractions (Acharya and Hartley, 2012).

## 4.2 Rheology

Rheological properties are characters of reflecting the flow behavior and deformation during the producing or delivering processes. The rheological parameters are important to the performance of nanoemulsion in coating or concentrated drying. The commonly used instruments for measuring the rheology of nanoemulsions are shear device for obtaining the value of apparent viscosity and the dependent relationship between apparent viscosity and shear stress. The viscoelastic properties of emulsions

are tested using dynamic oscillation methods. They are presented as the values of loss modulus and storage modulus that are dependent on frequency. When the emulsions were colloidal stable, the flow curves usually exhibited a constant value of apparent viscosity at low shear rates ($\sim 0.01 s^{-1}$). But a strong shear thinning follows at high shear rates. However, discontinuous emulsions might exhibit Newtonian behavior over a wider range of shear rates (Sharma et al., 2010). The type of surfactants, shape, and number density of the droplets, as well as the interactions between these droplets affect the rheological properties of nanoemulsions (Acharya and Hartley, 2012). Howe and Pitt investigated the effects of surfactants on the emulsion rheology. They found that some cosurfactants could weaken the interactions between gelatin and the anionic surfactant, leading to reduced viscosity and enable emulsions to be concentrated (Howe and Pitt, 2008). Besides, a rheology test has relative higher sensitivity than some macroscopic properties to detect subtle structural changes. So it is usually worked with other techniques that were applied in the characterization of nanoemulsions. For example, SANS equipment and rheometer have been used together to measure the percolation threshold and nanostructure of emulsions containing a telechelic polymer and silica nanoparticles (Puech et al., 2008).

## 4.3 Microstructure Characterization

Microscopy was a powerful imaging technique for nanoemulsions. Electron microscopy (EM) techniques offer the direct visualization of the microstructure of nanoemulsions with a high resolution (<5 nm) (Acharya and Hartley, 2012). It is easily to get the information of droplet size, shape, and aggregation state of the nanoemulsions. Some of the useful methods are discussed as following (Silva et al., 2012).

### *4.3.1 Transmission Electron Microscopy*

Transmission electron microscopy (TEM) is an effective imaging technique capable of imaging at a significantly higher resolution (0.2 nm) than light microscopes, owing to the small de Broglie wavelength of electrons (Luykx et al., 2008). It is used extensively in the study of materials for science/metallurgy and biological sciences (Silva et al., 2012). Researchers studied the morphology and structure of the nanoemulsions using TEM (Fig. 1.8) (Bouchemal et al., 2004; Vyas et al., 2008; Shafiq et al., 2007; Inugala et al., 2015). Kanafusa et al. (2007) observed the morphology of nanoemulsion droplet using TEM. It exhibited spherical shape and relative smooth surface of emulsion droplets, which was consistent with

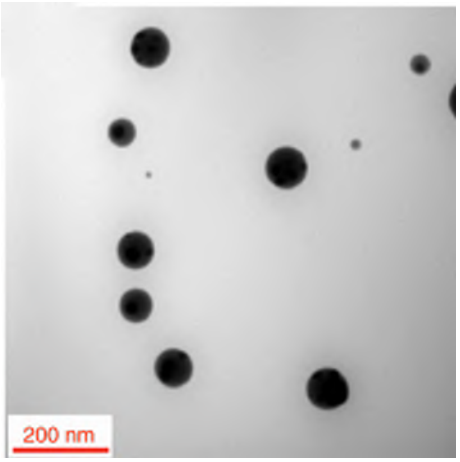


**Figure 1.8. Transmission electron microscopy image of the reconstituted nanoemulsions** (Inugala et al., 2015).

the results obtained by DLS. However, TEM has some drawbacks (Luykx et al., 2008). On the one hand, it requires extensive sample preparation to produce a sample thin enough to be electron transparent. On the other hand, the structure of the materials may change during the preparation or ruined by the high-energy electron beam. This has led to the development of cryogenic preparation (cryo-TEM) and freeze-fracture (FFEM) techniques.

Cryo-TEM is successfully used to characterize the structure of microemulsions. Spernath et al. (2009) studied the evolution of the supramolecular structure of the nanoemulsions containing polymerized monomer as the oil phase. In FFTEM, samples are cleaved under vacuum. The fracture plane typically follows the interior (hydrophobic) domains of membrane-like structures providing a "face on" view of samples (Acharya and Hartley, 2012). It generally gets a clear image of the network structure of bicontinuous nanoemulsions and/or droplets within discontinuous nanoemulsions (Krauel et al., 2007).

### *4.3.2 Scanning Electron Microscope*

Scanning electron microscope (SEM) was another indispensable tool for the characterization of materials from nanometer to micrometer scale (Goldstein et al., 2003). It is one of the most versatile instruments that is available for the examination and analysis of the microstructure morphology and chemical composition (Fig. 1.9) (Ferreira et al., 2015). SEM image reflects the surface structures. Due to the very narrow electron beam, SEM

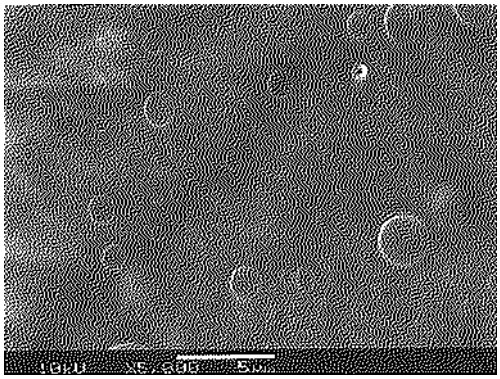

**Figure 1.9. Scanning electron microscopy image of ketoprofen-loaded pomegranate seed oil nanoemulsions stabilized by pullulan** (Ferreira et al., 2015).

micrographs have a great depth of field obtaining a characteristic three-dimensional appearance that is useful to understand the surface structure of oil droplets (Silva et al., 2012). At relative lower magnification, SEM is able to obtain a large amount of sample at one time, while at higher magnification, it can get high-resolution images of local structures. Several benefits of SEM make it one of the most extensively used methods (Luykx et al., 2008). Except for observation of droplets, SEM is also used for studying the coarse crystalline of β-carotene after encapsulated. From SEM images, the original coarse crystalline β-carotene displayed irregular shapes and sizes, as opposed to the nanodispersions (Silva et al., 2012; Tan and Nakajima, 2005).

However, it also has some drawbacks, such as expensive cost, requirement of high vacuum, and relatively high sample conductivity. Moreover, the presence of surfactants is sometimes adverse to the SEM characterization due to the formation of a smooth camouflaging coating on the particle surfaces (Luykx et al., 2008).

### *4.3.3 Atomic Force Microscopy*

Atomic force microscopy (AFM) is a developed microscopy technique, which is used to detect the changes of force between a shape probe and a sample that has been immobilized. The images generate raster by scanning the sample beneath the probe, resulting in a high-resolution 3-D profile of the sample surface (Fig. 1.10) (Silva et al., 2012; Preetz et al., 2010a). AFM acts as a complementary technique to analyze the structural characterization of nanoemulsion. It was utilized to distinguish the nanoemulsion and nanocapsules by comparing the shapes and thickness of capsule shell (Preetz et al., 2010b).

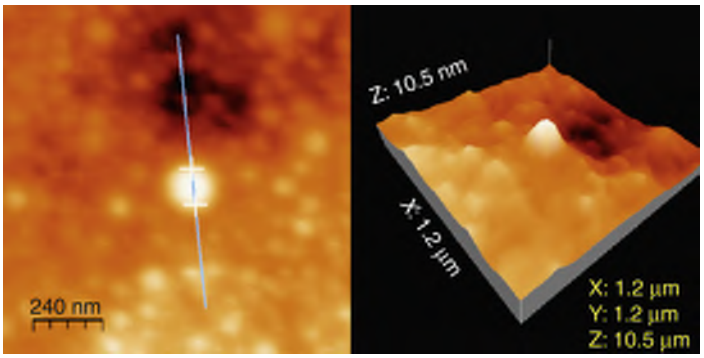


**Figure 1.10. Atomic force microscopy image of nanoemulsions** (Preetz et al., 2010a).

# 5 Cases Review of Nanoemulsions Applications in Food

Due to the potential advantages of nanoemulsions, they have attracted more and more interests in the food, medical, and pharmaceutical industries. But unfortunately, the practical applications of nanoemulsions are not well reported. The review reported that application of nanoemulsions in the food industry is much less than that in cosmetic and pharmaceutical industries (Maali and Mosavian, 2013), which is due to the stability of nanoemulsions (Gutiérrez et al., 2008). Hereby, some applications of nanoemulsions in food industries are listed in the next sections.

## 5.1 Delivery Systems for Hydrophobic Nutraceuticals

Fig. 1.2 displays various natural hydrophobic functional compounds (pigments or nutraceuticals) with excellent physiochemical and biological properties. However, their incorporation into food systems is still a critical challenge due to their poor water solubility, high melting point, crystallization, chemical instability, and low bioavailability. Meanwhile, the poor water solubility will cause lots of problems during food processing. Encapsulation by nanoemulsions has been found to possess obvious advantages.

### *5.1.1 Chemical Stability Enhancement*

β-carotene, a natural antioxidant found in a variety of fruits and vegetables, has been proposed to account for the health benefits,

such as preventing oxidative damage. Unfortunately, their chemical degradation limits the incorporation into food and beverage products. Qian et al. (2012a,c) has studied the impact of chemical stability of β-carotene encapsulated in nanoemulsions. The results displayed nanoemulsions stabilized by β-lactoglobulin were more stable against color fading than those stabilized by Tween-20. Besides, addition of some water-soluble or oil-soluble antioxidants decreased the rate of β-carotene degradation effectively. It infers that the emulsions can be used to increase the chemical stability of encapsulated components, but the effects also are relied on for the composition or structure of emulsions.

### *5.1.2 Physical Stability Improvement*

More and more hydrophobic crystalline bioactive compounds have been exploited, which possess considerable excellent health benefits and therapeutic efficacies. Unfortunately, it is found that those compounds after dissolving are apt to separate out from solvents as crystals. Nanoemulsions have been used to encapsulate them in order to improve the hydrophobicity and absorption during gasticintestinal tract (Pool et al., 2013b; Karadag et al., 2013; Tran et al., 2014). Chen et al. (2015) has developed a nanoemulsion that contained HPMC to improve the retention of nobiletin. The individual crystals of nobiletin are a needle-like structure and tend to associate with each other to form large clusters. But after added 0.05% HPMC at 37°C storage for 1 week, the results revealed that the crystals of nobiletin disappear. Moreover, it is found that addition of HPMC could prolong the metastable supersaturated state of the nobiletin in the nanoemulsions with initial concentration below 4.5 mg/mL.

### *5.1.3 Bioavailability Increment*

Solubility and hydrophobicity of compounds mainly affect the bioavailability of compounds. Tangeretin, one of typical PMFs, exhibits poor oral bioavailability due to its hydrophobic chemical structure. Ting et al. (2015a–c) has tried to build up a viscoelastic emulsion system to encapsulate tangeretin and enhance the bioavailability and efficacy. Those results indicated that emulsion-delivered tangeretin could be digested faster than tangeretin-MCT suspension and possess 2.6-fold higher bioaccessibility using in vitro lipolysis and TNO gastrointestinal models. The in vivo pharmacokinetics results on mice analysis confirmed that the oral bioavailability of tangeretin in the emulsion-based system was increased 2.3-fold when compared with its suspension. What is more, the antiproliferative activity of tangeretin was evaluated by in vitro MTT analysis of colonic carcinoma cell lines and in vivo

oral efficacy of AOM/DSS-induced colitis related colon tumorigenesis model. The tumor incidence and multiplicity were significantly reduced after the encapsulation of nanoemulsions.

Lack of intake amount of polyunsaturated fatty acids has been attributed to coronary heart disease (CHD) and cerebrovascular disease (CVD). Their absorption is limited by the water-solubility. Lane et al. (2014) have prepared an omega-3 algal oil nanoemulsion and fed with 11 subjects to complete single blind and randomized crossover trial experiments. Results demonstrated that the absorption of polyunsaturated fatty acids in nanoemulsions was significantly higher than that in the bulk oil.

Otherwise, the bioaccessibility of phytochemicals in the nanoemulsion-based delivery systems is still influenced by oil type, droplet size, encapsulated compounds, and so on. There are studies that found that β-carotene bioaccessibility decreased in the order long chain triglycerides (~66%) ≫medium chain triglycerides (~2%) > orange oil (Qian et al., 2012b). Meanwhile, the bioaccessibility increased with decreasing mean droplet diameter (small~ medium ≫large) (Salvia-Trujillo et al., 2013). In contrast, the bioaccessibility of curcumin decreased in the order medium chain triglycerides > long chain triglycerides ≫ short chain triglycerides (Ahmed et al., 2012). For coenzyme Q10 systems, the results of an in vivo rat feeding digestion model showed that the bioavailability in small intestine tissues was increased in the corn oil encapsulated emulsion system (Cho et al., 2014). In addition, the rate and the extent of lipid digestion decreased with increasing the surfactant concentrations, but concentration of surfactant showed no appreciable influence on the bioaccessibility of vitamin E (Mayer et al., 2013).

### *5.1.4 Bioactivity Maintenance*

Reduction of bioactivities of functional compounds during processing is adverse for the application. Wang et al. (2008) found that nanoemulsions with the droplet particle size 79.5 nm could successfully enhance antiinflammation activity of curcumin. The inhibition effect of TPA-induced edema of mouse ear reached 85%. Junyaprasert et al. (2009) prepared Q10-loaded nanoemulsions with high stability and in vitro permeation studies stated that the amount of Q10 release and occlusiveness are the two major keys to promote the deep penetration of Q10 into the skin.

## 5.2 Antimicrobial Nanoemulsions

Antimicrobial activity of nanoemulsion has been widely reported in the food field to prevent equipment, packaging, or products

from contamination for their nonthermal preservation methods, which could reserve the textural properties and nutritional values in the maximum level. The commonly used antimicrobial nanoemulsions were O/W style with the average droplet size between 200 nm and 500 nm (Saranya et al., 2012). It has been confirmed that nanoemulsions possess a wide antimicrobial activity against various food pathogens (McClements and Rao, 2011; Karthikeyan et al., 2011; Hamouda et al., 2001; Sugumar et al., 2013), including bacteria (*Lactobacillus delbruecki*, *E. coil*, *S. aureus*, *Vibrio cholera*, and so on), viruses (herpes simplex, influenza A, vaccinia viruses, and so on), fungi (*Candida albicans*, *Dermatophytes*, and so on), and spores (*Bacillus anthracis*, *Bacillus cereus*, and so on).

Essential oils, including clove essential oil, cinnamon oil, mandarin essential oil, lime oil, cinnamon oil, basil oil, are commonly used ingredients as natural antibacterial agents. They can endow the nanoemulsion powerful antimicrobial activity due to the components, including α-pinene, benzaldehyde, carvacrol, carvone, eugenol, eugenyl acetate, geraniol, limonene, menthol, terpineol, thymol, and vanillin (Ghosh et al., 2013b). The content and kind of oil in the nanoemulsion most determined the antimicrobial activity of nanoemulsions largely depends on the content of essential oils (Chang et al., 2012), which is theoretically reasonable. Particle size of nanoemulsions and microorganisms also affect the antimicrobial activity. Donsì et al. focused on the encapsulation of essential oils into nanoemulsion in order to enhance their antimicrobial activity while minimizing the impact on the quality. Three different classes of microorganisms (*L. delbrueckii*, *Saccharomyces cerevisiae*, *E. coli*) were taken to antimicrobial activity. They found that the antimicrobial activity depended on the target microorganism and nanoemulsions with smaller diameters showed higher antimicrobial activity because of the quicker transport through the cell membrane of the target microorganisms (Donsì et al., 2011a). This fusion between the emulsion and the anionic charge on the pathogen could result in the antimicrobials' lysis and death. Strong electrostatic attraction could improve the fusion and then nanoemulsions with positive charge exhibited higher antimicrobial activity (Hamouda and Baker, 2000).

Synergistic effect between different antimicrobial agents is always taken into account to improve the antimicrobial activity of nanoemulsions. Nisin is a kind of natural antibacterial compound commonly contained in nanoemulsions to combine with some essential oils. It was found that the combination with D-limonene displayed the positive effect (Zhang et al., 2014). The emulsifier also confirmed affected antimicrobial activity. Emulsifiers significantly affected the formulation of the nanoemulsions, including

stability and size. Additionally, they determined the capability to solubilize the antimicrobials (Donsì et al., 2012). Teixeira et al. (2007) found that nanoemulsions with nonionic surfactants were effective to inhibit bacterial growth in biofilms. Therefore, rational design of nanoemulsions with essential oils, emulsifiers, and other components need to be synthetically taken in consideration when the desired function of antimicrobial activity is achieved.

## 5.3 Modulation of Products Shelf-Life

Contamination of fresh products by food-borne bacteria reported to lead nearly half (46%) of food-borne illnesses from 1998 through 2008 in the United States (Painter et al., 2013). So, prevention of contamination and control of bacterial growth on fresh produce is an imperative task to public health. The current strategy applied in industry is washing of the product using dilute chlorine aqueous. However, due to the potential health concerns and environmental-friendly problems of chlorine by-products, exploring natural bioactive compounds with strong antimicrobial capacity is an urgent need. Due to the predominant properties, nanoemulsion can be a candidate to avoid food spoilage. Bhargava et al. (2015) reported that an oregano oil nanoemulsion was applied to the control of food-borne bacteria on fresh lettuce. Different kinds of lettuce were first contaminated by three microbials, including *Listeria monocytogenes* ATCC 19115, *Salmonella Typhimurium* ATCC 19585 and *E. coli* O157:H7 ATCC 700927. Then the contaminated lettuces were immersed in oregano oil nanoemulsions for 1 min following by drying 30 min. The treatment of oregano oil nanoemulsion was able to inhibit microbial growth significantly. The growth of *S. aureus* in orange juice was controlled by eugenol-loaded antimicrobial nanoemulsion against microbial spoilage (Ghosh et al., 2014). Eugenol-sesame oil nanoemulsion were prepared by ultrasound emulsification and then added into fresh orange juice. The shelf life of juice was evaluated and compared by treatment of eugenol nanoemulsion and a common preservative (sodium benzoate). S3E3 (Sesame oil: Eugenol: Tween 80: Water = 3:3:18:74) nanoemulsion showed enhanced in situ antibacterial activity over the same concentration of sodium benzoate (ie, 3% of stock solution and 0.3% sodium benzoate concentration in orange juice), and better results were obtained at storage temperature of 4°C. Sunflower oil–based nanoemulsions were investigated to be able to extend the shelf life and maintain the quality of king mackerel steaks stored at 20°C (Joe et al., 2012). Nanoemulsions prepared with d-limonene and nisin were used to control the stability of chicken broth and vegetable cream (Mate et al., 2015).

## 6 Summary

This chapter reviews the basic knowledge of nanoemulsions and a number of case studies are used to highlight the characterization and application of nanoemulsions. Nanoemulsions have numerous advantages, such as relatively high physical stability and high surface area. It can be produced by high- and low-energy emulsification methods. The formulation selection for nanoemulsion formation is broader than microemulsion. Physiochemical properties of nanoemulsion always have considerable influences on the property of products, such as texture, taste, flavor, and stability. Hence, various analysis techniques have been exploited and applied for the nanoemulsion characterization. Overall, nanoemulsion technology has potential applications in the food industries.

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# 2

# PREPARATION OF NANOMATERIALS FOR FOOD APPLICATIONS USING MEMBRANE EMULSIFICATION AND MEMBRANE MIXING

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## 1 Introduction

Membrane processes are major tools in the food processing industry. Several important processes are developed in the dairy industry, for example, for milk standardization, and for the separation and/or fractionation of fat globules from whole milk, in the beverage industry for the treatment of wine, beer, and fruit juices, and in the egg products industry (Dauphin et al., 2001). Membrane processes are also increasingly used for the treatment of fruit and vegetable juices and concentrates, waste streams, and coproducts like the recovery and recycling of blood plasma in abattoirs. Usual membrane processes used in the food industry are microfiltration, ultrafiltration, nanofiltration, reverse osmosis, and electrodialysis. These membrane processes are usually recognized for the following advantages: operational simplicity, low energetic requirements, good stability under a wide range of operative conditions, high ecocompatibility, easy control and scale-up, and large flexibility (Drioli and Romano, 2001).

Several new membrane processes have emerged during the past two decades, such as membrane emulsification and membrane mixing. In a membrane emulsification setup, the dispersed phase is forced to pass through the pores of a membrane to detach in the form of droplets into the continuous phase. The pressure

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00002-7

used is typically in the range of some bars. The resulting droplet size depends on several parameters, including the membrane pore size, the pore size distribution, the nature of the dispersed and continuous phases, and is not controlled by the generation of turbulent droplet breakup like in other emulsification systems such as high-pressure homogenizers. A large range of colloids have been prepared using such technique, these include oil-in-water (O/W, water-in-oil (W/O), multiple emulsions like water-in-oil-in-water (W/O/W) or oil-in-water-in-oil (O/W/O), polymeric microspheres, core-shell microcapsules, and hollow polymeric microparticles (Vladisavljević and Williams, 2005). In a membrane mixing reactor, microporous membranes are employed as dispersion device. The first solution or suspension flows through the membrane pores to mix at the outlet of the membrane pores into another solution or suspension. The method is appropriate to obtain high micromixing efficiency like in microfluidic devices (Jia and Liu, 2013). The membrane mixing method has been widely reported for the preparation of a large range of materials such as nanoparticles, nanocapsules, liposomes, synthesis of polymers, parallel, and consecutive reactions. The final aim of the technique is to improve the nanoparticles size and size distribution, the molecular weight distribution of polymer in case of polymeric reactions, or the selectivity of other complex reactions (Jia and Liu, 2013). In both processes, the membrane pores act as parallel capillaries for the introduction of one solution into another solution, which flows tangentially to the membrane surface. In membrane emulsification, there is no reaction between the two phases, and in membrane mixing, a reaction may occur between the two solutions.

Membrane emulsification and membrane mixing could be both of interest in the food industry for the preparation of several products in the nanometer or micrometer range, including nanoemulsions, multiple nanoemulsions, nanocapsules, liposomes, etc. This state-of-the-art review summarizes the main principles of these techniques, as well as several food applications that have been reported so far.

# 2 Preparation of Food Nanomaterials by Membrane Emulsification

## 2.1 Membrane Emulsification

### *2.1.1 Previous Reviews*

Several reviews on membrane emulsification have been published to show the principles of the method, the influence of

process parameters, the comparison with other techniques, and the large range of applications. Membrane emulsification was first presented at a conference by Nakashima et al. (2000). These authors summarized the main characteristics of membrane emulsification in this state of the art paper. They underlined the advantages of the method for the preparation of emulsions and colloids in the food, pharmacy, and chemistry fields. Recently, Piacentini et al. (2014) presented a review on membrane emulsification, including the different configurations and the large range of dispersions prepared, including O/W, W/O, multiple emulsions and particles. A special attention was devoted to patents, with 60 patents referring to membranes, apparatus, methods, and a broad range of applications. The patents came mainly from Japan (60%).

Other state-of-the-art papers focused on more specific aspects. In addition to the main aspects of membrane emulsification, Gijsbersten-Abrahamse et al. (2004) described an industrial case of membrane emulsification for the production of food emulsions. In particular, it was shown that a microsieve membrane with a low porosity gave the best results in terms of droplet size. van der Graaf et al. (2005a) focused on the production of double W/O emulsions by means of membrane emulsification (as the second emulsification step). Double emulsions have several very interesting potential applications, for example, for the production of low calorie food products, encapsulation of medicines and other high value products. The advantages and disadvantages of membrane emulsification in relation to the production of stable double emulsions were summarized and the performance compared to those of classical methods.

Vladisavljević and Williams (2005) focused on the manufacturing of emulsions and particulate products using membranes. For the first time, the large range of products that can be obtained by this technique was listed in a very comprehensive manner. Membrane emulsification was used for the production of simple O/W and W/O emulsions, multiple emulsions W/O/W and O/W/O, and colloids like polymeric and lipidic micro(nano)spheres or capsules. Charcosset (2009) focused on membrane emulsification process for food applications. The review presented the main principles of the membrane emulsification technique, and then focused on the preparation of several products used in the food industry, such as simple emulsions, multiple emulsions, and encapsulated materials. The review also discussed limits, advantages, and perspectives of the technique in the food industry. For emulsions with higher disperse phase fractions, premix membrane emulsification is an interesting alternative, based on passing a preemulsion through a microporous membrane or porous bed of particles (dynamic

membrane). Nazir et al. (2010) provided an overview of principles of premix membrane emulsification, including mechanisms and the preparation of various products, as well as further improvement of the process.

### 2.1.2 Principles

Food emulsions are usually produced by colloid mills, rotor–stator systems, and high pressure homogenizers, these processes produce emulsions with small droplet size but with a relatively high droplet size dispersion. Furthermore, they require a high energy input and high shear forces to produce emulsions. The membrane emulsification process is schematically shown in Fig. 2.1a for some systems configurations. W/O and O/W emulsions are produced using hydrophobic and hydrophilic membranes, respectively. In a typical crossflow emulsification membrane, the dispersed phase flows under pressure through the micropores of a membrane, while the continuous phase circulates tangentially to the membrane surface (Vladisavljević and Williams, 2005; Charcosset, 2012). Droplets grow at pore openings until they detach under the effect of the shear rate created by the crossflow. Surfactant molecules in the continuous phase stabilize the newly formed interface, to prevent droplet coalescence immediately after formation. As a result, the resulting droplet size distribution depends mainly on the membrane properties (pore size distribution, pores shape, hydrophobicity, etc.) and not by the breakup of droplet on the generation of turbulence like in high-pressure homogenizers. The apparent shear stress is lower than in classical emulsification systems because small droplets are directly formed by permeation of the dispersed phase through the micropores, instead of disruption of large droplets in zones of high energy density. Besides the possibility of using shear-sensitive ingredients, emulsions with narrow droplet size distributions can be produced. Furthermore,

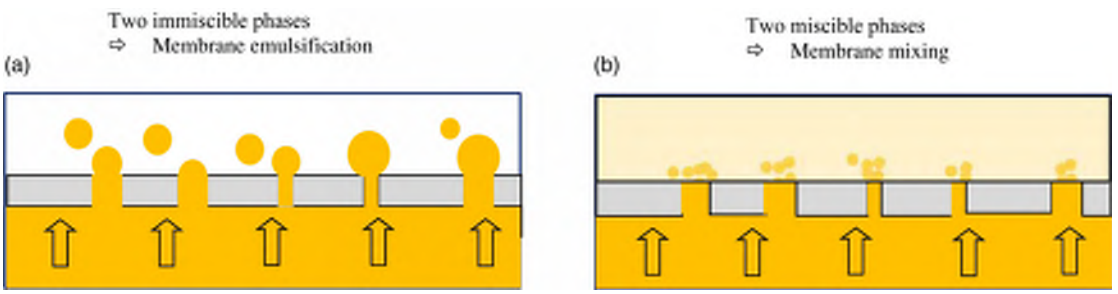


**Figure 2.1. Schematic drawings of (a) membrane emulsification and (b) membrane mixing.**

membrane emulsification processes allow the production of emulsions at lower energy input ($10^4$–$10^6$ J/m$^3$) compared to conventional mechanical methods ($10^6$–$10^8$ J/m$^3$) (Altenbach-Rehm et al., 2002).

Theoretical data for droplet formation are obtained by calculating the overall forces, assuming a rigid and spherical droplet (De Luca et al., 2008). The main forces that act on the forming droplets are (Fig. 2.2):

- Interfacial tension force, $F_\gamma$, which represents the effects of dispersed phase adhesion around the edge of the pore opening;
- Static pressure difference force, $F_{SP}$, due to the pressure difference between the dispersed phase and the continuous phase at the membrane surface;
- Drag force, $F_D$, created by the continuous phase flowing past the droplet parallel to the membrane surface;
- Dynamic lift force, $F_L$, which results from the asymmetric velocity profile of the continuous phase near the droplet;
- Buoyancy force, $F_B$, due to the density difference between the continuous phase and the dispersed phase;
- Inertial force, $F_I$, is the linear momentum force associated with a mass of the fluid flowing out from the opening of the pore.

Comparison between the results obtained with forces based models and experimental data are scare. A good agreement is usually obtained for a small range of parameters. For example, De Luca et al. (2008) concluded that to obtain a good agreement between theoretical data and experiments, in terms of mean size diameter, the wall shear stress has to be equal or larger than 7 Pa and the membrane pore diameters below 1.5 μm. In addition to

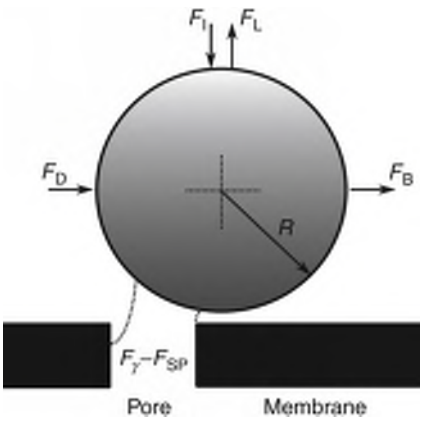


**Figure 2.2. Forces acting on a droplet at a pore outlet.** From Charcosset (2009), with permission.

models based on forces balances, several numerical studies have been reported to simulate droplet at the membrane pore outlets (Rayner et al., 2004). Visualization of droplet formation using high-speed camera systems or optical microscopy is also interesting to better understand the membrane emulsification process using single pores, microengineered membranes, and microporous membranes (van der Graaf et al., 2005b).

### 2.1.3 Configurations

Several configurations are available to conduct a membrane emulsification experiment. Some of these techniques are shown in Fig. 2.3. Crossflow or dead-end membrane emulsification were the first techniques introduced. Fine droplets are formed at the outlet of the membrane pores when the dispersed phase is pressed through the pores. The shear stress generated by the crossflow must be high enough to induce droplet break up and/or droplet aggregation. Insertion of static turbulence promoters is an alternative method of increasing shear stress at the membrane surface while maintaining a low shear in the recirculation loop (Koris et al., 2011).

In addition, some systems use a moving membrane under rotation or vibration. In these devices, the droplet detachment from

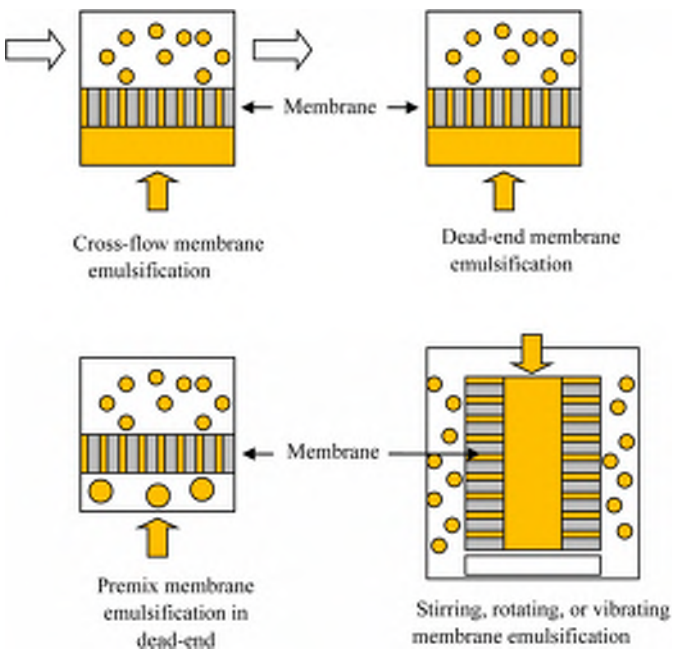


**Figure 2.3. Different emulsification methods and systems.**

the pore outlets is improved by the movement of the stationary continuous phase. In these devices, optimal results are obtained when using fast adsorbing emulsifiers in the continuous phase (Pawlik and Norton, 2013). The shear stress is generated by the rotation of the membrane and the phase to be dispersed passes centrifugally through the pores of the membrane and forms droplets moving into the continuous phase. The emulsion droplet size depends on the operating parameters. It decreases with increasing rotational velocity, due to higher detaching drag force acting on the droplets, and increases with increasing transmembrane pressure, due to higher interface formation rate.

In the premix membrane emulsification technique, a coarse premix emulsion is pushed through a microporous membrane leading to a fine emulsion having smaller and uniform droplets; the operation can be repeated several times (Nazir et al., 2010). The emulsion droplet size depends on several parameters like the number of cycles, the dispersity being improved by increasing the number of cycles. Premix membrane emulsification is necessary in particular in case of dispersed phase with high viscosity, emulsions that have to be prepared at high dispersed phase fraction, and also for shear sensitive ingredients as the shear stress can be lowered in case of premix emulsification. With increasing the number of passes, the droplet monodispersity is improved, and the permeate flux may increase, probably as a result of the decreased viscosity related to droplet size reduction. However, components in the premix emulsion may have negative side effects like fouling of the membrane and therefore decreasing permeate flux.

### *2.1.4 Membranes*

A large range of membranes have been investigated for the preparation of emulsions (Fig. 2.4). The first membranes used in the beginning of the 2000s were produced by a Japanese company (Ise Chemical Co., Japan). Due its narrow pore size distribution, the Shirasu porous glass (SPG) membrane has been the first membrane used for emulsification and is still very attractive for a large range of applications (Nakashima et al., 1991). This membrane is synthesized from $CaO\text{-}Al_2O_3\text{-}B_2O_3\text{-}SiO_2$ type glass, which is made from "Shirasu," a Japanese volcanic ash. The SPG membrane has a uniform internal microstructure, characterized by interconnected cylindrical pores with a tortuosity factor around 1.3 (Fig. 2.4a). On scanning electron microscopy (SEM) images, the pores have a noncylindrical shape because they extend in all directions and pore junctions can also be seen on the surface (Vladisavljević et al., 2005). A wide spectrum of mean pore sizes is available from 0.1 to 20 μm and a high porosity from 50 to 60%. The properties

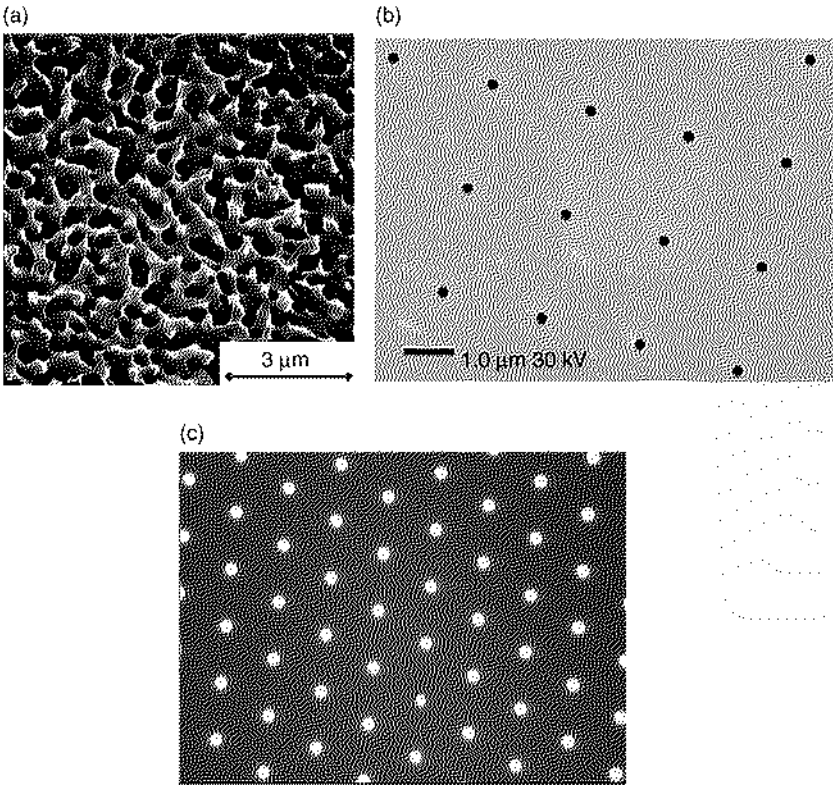


**Figure 2.4. Scanning electron micrograph (×3)**. (a) The surface of a hydrophilic SPG membrane with 0.262 μm pore size; (b) the surface of a micromachined silicon nitride membrane; (c) the surface of a membrane with a 30 μm regular pore size. Part a, SPG Technology Co., Vladisavljević et al. (2005); part b, Aquamarijn Microfiltration, Zhu and Barrow (2005); part c, Micropore Technologies Ltd., Stillwell et al. (2007), with permission.

of the membrane surface (eg, hydrophilicity and hydrophobicity) have a strong influence on the results of the membrane emulsification technique. Indeed the properties of the SPG membranes can be changed by chemical reaction with organosilane compounds such as chlorosilanes or physical coating with silicone resin to make it more hydrophobic. For the preparation of O/W emulsions, the membrane has to be hydrophilic, so the dispersed phase (oil) does not spread at the membrane surface when coming out of the membrane pores. For W/O emulsions, the opposite effect has to be taken into account. The membrane has to be hydrophobic, so the dispersed phase (water) coming out of the membrane pores do not spread at the membrane surface and thus form well defined water droplets.

In addition to SPG membranes, other membranes have been successfully used like silicon and silicon nitride microsieves (Aquamarijn Microfiltration BV, The Netherlands) (Zhu and Barrow, 2005). The main advantage of these membranes is that they can be obtained with very regular pore size, which is not the case of SPG membranes. This property allows the preparation of very monodispersed emulsions. The main limitation of these membranes is that their pore size is higher than a few μm, therefore they are well suited for the preparation of emulsions with droplet size in the range 5–10 μm or higher; for finer emulsions, SPG membranes are preferred. Microsieve membranes are commercialized with different pore geometries, the main geometry being a circular shape (Fig. 2.4b).

Microengineered membranes are supplied by Micropore Technologies Ltd. (Hatton, Derbyshire, United Kingdom) (Stillwell et al., 2007; Vladisavljević et al., 2012). They have been reported for a large range of preparations, including emulsions, double emulsions, nano- and microparticles, liposomes and micelles. The membranes used are nickel microengineered membranes containing uniform cylindrical pores arranged at a uniform spacing. The membranes are available with diameter pores of 5, 10, 20, or 40 μm and higher and spacing of 80 or 200 μm. The membranes are fabricated by the UVLIGA process, which involves galvanic deposition of nickel onto the template formed by photolithography (Vladisavljević et al., 2012). A perfect hexagonal array of pores with a pore at the center of each hexagonal cell can be seen on SEM pictures (Fig. 2.4c). The flat disc membrane can be fitted in a stirred cell under a paddle blade stirrer.

Other commercial microfiltration membranes are attractive because of their availability in very large surface area, and their high flux through the membrane pores like ceramic aluminum oxide ($\alpha$-$Al_2O_3$) membranes, α-alumina and zirconia coated membranes, and polytetrafluoroethylene (PTFE) membranes (Charcosset, 2012). W/O emulsions can also be successfully prepared using polytetrafluoroethylene (PTFE), polyamide hollow fibers membrane, and homemade silica-based monolithic membrane.

### *2.1.5 Effect of Parameters*

The effect of process parameters on droplet generation has been the subject of several reviews (Joscelyne and Trägårdh, 1999; Charcosset et al., 2004; Vladisavljević et al., 2012; Charcosset, 2012). The main factors affecting the drop size are wetting properties and microstructure of the membrane (pore size distribution, pore shape, spatial distribution of the pores, pore tortuosity, etc.), but other parameters also play an important role, such as

transmembrane flux, shear stress on the membrane surface, viscosity of the continuous and dispersed phase, surfactant type and concentration.

The membrane properties have a major role on the process. It is usually observed that the average droplet diameter, increases with the average membrane pore diameter, the constant of proportionality being typically 2–10. Differences in operating conditions (type of dispersed and continuous phase, type of membranes, pressure, and crossflow velocity) can explain the large range of ratio obtained. For membranes other than SPG like ceramic membranes, higher values have been reported, typically between 3 and 50. The pore size distribution of the membrane has also a major effect on the emulsification result, and monodispersed emulsions can be produced only if the membrane pore size distribution is sufficiently narrow. The porosity at the membrane surface has also a major effect on the emulsification result, as two adjacent pores may lead to coalescence between forming droplets at the pore outlets. Therefore, a sufficient distance between adjacent pores is necessary to avoid droplets coalescence between the droplets coming at the outlet of the membrane pores.

Operational parameters are the applied transmembrane pressure and the shear stress at the membrane surface. An increasing transmembrane pressure leads to an increasing permeate flux, which is highly needed for industrial production. Indeed, several factors can limit the dispersed phase flux through the membrane, like the high viscosity of the dispersed phase and/or membrane fouling (internal or surface fouling), and make an industrial production unrealistic. Moreover, too high fluxes are undesirable, as jets can formed at the outlet of the membrane pores rather than droplets. Also, high fluxes could lead more easily to the coalescence of adjacent droplets. The shear stress applied on the membrane surface has a similar effect. The shear stress is needed to detach droplets forming at the pore outlets; however, too high shear stress can lead to coalescence of adjacent droplets.

The influence of the type of surfactant in the membrane emulsification process is a key parameter. Surfactants have a key role for the formation of emulsions, first the interfacial tension between the dispersed and continuous phase is decreased, and emulsions with better distribution can be obtained. Second, surfactants stabilize the droplets and make coalescence of droplets more difficult. The rules applying to conventional emulsification methods are suitable to the membrane emulsification process, like the Bancroft rule, which states that: “The phase in which a surfactant is more soluble constitutes the continuous phase.” As a consequence, the type of emulsion (O/W or W/O) is dictated by the emulsifier and

the emulsifier should be soluble in the continuous phase. Like in conventional emulsification, a large range of surfactants can be applied to the membrane emulsification process.

The viscosity of the dispersed phase is also a very important parameter, as the dispersed phase has to pass through very small pores. Too high viscosity would make the membrane emulsification technique unrealistic at industrial scale because of very low flux through the membrane. In this case, premix emulsification is an interesting alternative, as the coarse emulsion has a much lower viscosity than the initial phase to be dispersed.

## 2.2 Preparation of Food Nanomaterials

Due to the increasing interest for micro- and nanomaterials for food products, membrane emulsification has been used for the preparation of a large range of products, including simple emulsions, multiple emulsions, nanocapsules and microcapsules, liposomes, and nano- and microaerated food gels (Fig. 2.5). In the following sections, we will detail some of the findings in this field.

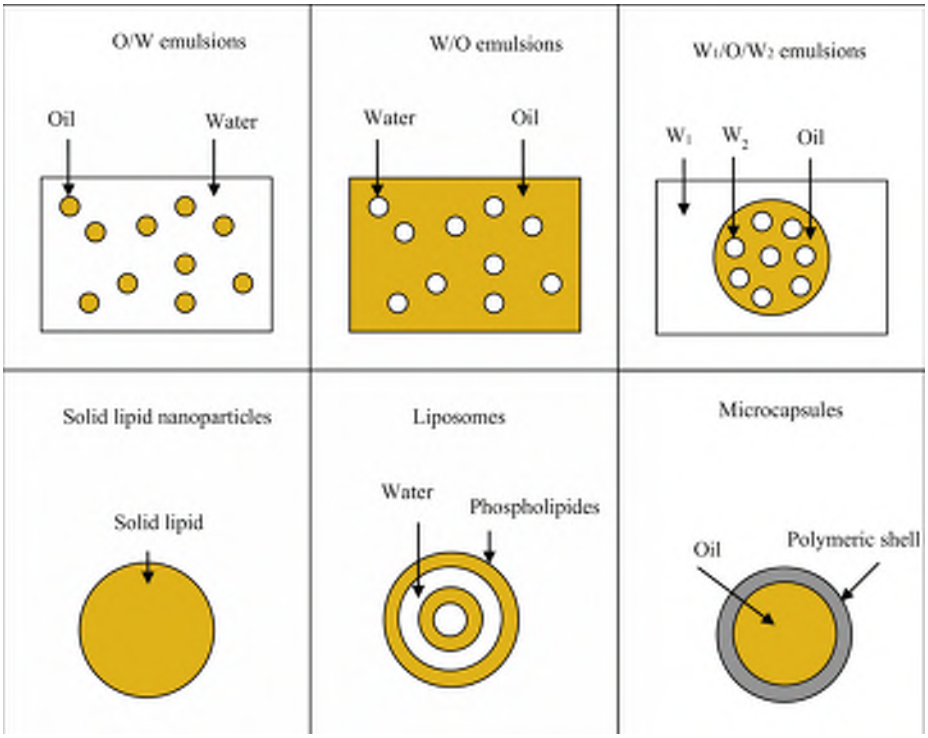


**Figure 2.5. Different nanomaterials prepared by membrane emulsification and membrane mixing.**

### 2.2.1 Simple Emulsions

Both O/W and W/O are found in many food applications (Friberg et al., 2004). O/W emulsions are the most used emulsions for food and exist in many forms, such as mayonnaises, cream liqueurs, creamers, whippable toppings, and ice-cream mixes. Their properties can be changed by choosing different emulsifiers and other components in the aqueous phase. Examples of W/O emulsions used in the food industry are butter, margarines and fat based spreads. Fewer parameters can be varied to control their properties than in the case of O/W emulsions.

Various food emulsions have been prepared by membrane emulsification. As mentioned previously, the formulation has to be carefully selected. Due to the specificity of the process, which makes use of membrane pores of very small size, membrane pore fouling may occur during preparation. For example, lecithin is a negatively charged food grade emulsifier that is widely used in the food industry and can produce small oil droplets during conventional homogenization processes. It can be extracted from a variety of sources such as soybeans, rapeseed, and egg. In addition, it can be totally biodegraded and metabolized. However, the lecithin emulsifier, in spite of its net negative charge, tended to foul the SPG membrane by blocking the membrane pores because the positive groups on the lecithin molecules could interact with anionic silanol groups on the SPG membrane surface (Surh et al., 2008). Moreover, large populations of relatively large oil droplets were found in all lecithin stabilized emulsions. O/W emulsions with very complex interfacial structures can also be produced. Berendsen et al. (2014) used whey protein isolate (WPI), which is a by-product in cheese-making and has excellent emulsifying properties, and carboxymethyl cellulose (CMC), which is widely used in food for its physical properties and its low cost. In addition, CMC can form electrostatic complexes with WPI over a range of pH and ionic strength. Using premix membrane emulsification with SPG membranes, different interfacial structures were obtained made of whey protein isolate WPI and CMC. Emulsions were stabilized by one interfacial layer, made of WPI (monolayer emulsion) or 0.5wt.% WPI–0.25wt.% CMC complex (complex emulsion), or by two interfacial layers: one layer made of WPI and the second made of CMC (bilayer emulsion) or WPI–CMC complex (sequential emulsion).

In the dairy industry, emulsification processes need to control the drop size and distribution drop size of fat globules because a change in these factors may alter rheological and organoleptic properties of the final product. Membrane emulsification has been demonstrated to able to produce emulsions for the dairy industry.

Using SPG membranes, Scherze et al. (1999) and Muschiolik et al. (1997) prepared O/W emulsions with a continuous phase containing milk proteins, the dispersed phase consisted of liquid butter fat or sunflower oil. The O/W emulsions were characterized by their particle size distribution, creaming behavior and protein adsorption. The low shear forces of the membrane technique are a major advantage compared to high pressure homogenization and can reduce the degradation of the physicochemical and molecular properties of the proteins. Katoh et al. (1996) also reported the preparation of W/O food emulsions with an oil content of 25% (v/v). The technique was evaluated for large-scale applications, especially the flux through the membrane was optimized and was found to be considerably increased (100 times) when the hydrophilic membrane was pretreated before use by immersion in the oil phase.

Using premix membrane emulsification, Gutiérrez et al. (2009) confirmed the emulsifying capacity of milk proteins, which were able to stabilize the milk emulsion without the use of any external agents as surfactants. In the dairy industry, the reduction of the use of food additives is hardly needed both to save money and increase consumer acceptance. Using tubular SPG membrane, they obtained a stable final emulsion of 30% w/w oil, at dispersed phase fluxes of 5 or 50 L/h $m^2$. However, at the higher flux and at higher oil concentrations, the fat globule size distribution was found more bimodal, which may limit the flux value in the industrial process.

O/W emulsions prepared by membrane emulsification can be loaded with functional food ingredients, for example, with antioxidant properties, like astaxanthin and lutein. The applications of these molecules in food and cosmetic formulations are limited by their instability when exposed to oxygen, light or high temperatures, and hence oil droplets are used as carriers. Astaxanthin is a natural carotenoid product with antioxidant properties. Ribeiro et al. (2005) used the premix membrane emulsification technique by passing several times a preemulsion through a hydrophilic or hydrophobic membrane. Palm oil containing dissolved astaxanthin and water containing a combination of two emulsifiers, to stabilize the droplets, were used. Each O/W passed the membrane 3 times under pressure of 5–15 bar and disperse phase fraction from 10 to 40 wt.%. The other molecule, lutein is a natural carotenoid with antioxidant activity. It prevents UV-induced erythema and inflammation, and has a protective role against skin cancer. However, lutein application in food and cosmetic formulations is limited by its instability when exposed to oxygen, light or high temperatures, and hence oil droplets are used as lutein carriers.

Matos et al. (2015a) prepared highly concentrated O/W emulsions containing lutein, using a two-stage process: membrane emulsification and further vacuum evaporation. Monodisperse emulsions with controlled droplet size, a water volume fraction ($f_w$) of 0.25, and encapsulation efficiency up to 97% were obtained. Emulsions were formulated with two different food-grade oils (soybean oil and miglyol) and a nonionic surfactant Tween-20, 2% (w/w).

### 2.2.2 Multiple Emulsions

W/O/W multiple emulsions consist of aqueous phase droplets dispersed within larger oil droplets, which are themselves dispersed in an aqueous phase (Vladisavljević et al., 2014). A major advantage of W/O/W multiple emulsions is their ability of loading hydrophilic actives for controlled release. Double emulsions (eg, W/O/W) can be produced by membrane emulsification (van der Graaf et al., 2005a). The primary emulsion is obtained by means of a conventional method or by membrane emulsification. In contrary to conventional emulsification methods, it becomes possible to produce small and monodisperse droplets without using high-shear stresses that cause escape of the internal droplets.

For food applications, several actives have been loaded in multiple emulsions using membrane emulsification, for example, a hydrophilic model compound of a bioactive substance (1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt) (Shima et al., 2004), procyanidin (Berendsen et al., 2015), resveratrol and vitamin B12 (Matos et al., 2015b), and *trans*-resveratrol (Matos et al., 2014). For all these active substances, encapsulation overcomes the drawbacks of sensitivity to high temperature, chemical degradation and limited bioavailability. Shima et al. (2004) investigated W/O/W emulsions to protect bioactive compounds from stomach acid and intestinal digestive fluids. W/O/W emulsion were used as a carrier system for the daily uptake of a hydrophilic model compound of a bioactive substance (1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt). Berendsen et al. (2015) used premix membrane emulsification to produce procyanidin-loaded $W_1/O/W_2$ emulsions with narrow droplet size distributions. Procyanidins are secondary metabolites of plants that can be found in a number of plant-based foods and beverages. Regular intake of foods rich in procyanidins can lead to a reduction of risk in cardiovascular diseases, diabetes, and certain types of cancer. Matos et al. (2014, 2015b) used cross-flow or dead-end membrane emulsification for the encapsulation of resveratrol, vitamin B12, and *trans*-resveratrol in food-grade double emulsions. Resveratrol is a natural polyphenol found in a wide variety of plants that has beneficial effects on human health

because of its antioxidant properties, and antiinflammatory, cardioprotective and anticancer activity, is often used as a functional food ingredient. Vitamin B12 is an essential nutrient for normal cell function, human growth, and blood formation. *Trans*-resveratrol is a natural occurring polyphenol found in a wide variety of plants. It has reported beneficial effects for human health, such as antioxidant, antiinflammatory, cardioprotective, and antitumor properties.

Several membrane emulsification devices can be used for the preparation of multiple emulsions, such as the classical cross-flow membrane emulsification technique (Matos et al., 2015a), the membrane oscillating device (Vladisavljević et al., 2014) and the premix emulsification method (Shima et al., 2004; Berendsen et al., 2015). All these devices are suitable for the production of food grade multiple emulsions loaded with active agents having interesting properties. Vladisavljević et al. (2014) used an electroplated nickel membrane oscillating in 2 wt.% aqueous Tween-20 (polyoxyethylene sorbitan monolaurate) solution for the preparation of food-grade W/O/W multiple emulsions by injecting a W/O emulsion into an aqueous phase. The droplets size depended on the amplitude and frequency of membrane oscillation. Premix membrane emulsification was used, for example, by Shima et al. (2004). In this method, membrane filtration of a coarse W/O/W emulsion prepared with a rotor/stator homogenizer produced a fine emulsion with a mean oil-droplet diameter below 1 μm and encapsulation efficiency higher than 90%. However, the included water-phase disappeared during the membrane filtration of the coarse emulsion when preparing the fine emulsion.

### *2.2.3 Encapsulation*

Nanoencapsulation involves loading bioactive agents within carrier systems with a dimension in nanoscale. In food processing, numerous applications have emerged these past years with the purpose to produce functional foods with higher nutritional value, lower dose of synthetic preservatives and better organoleptic properties (Fathi et al., 2014). Such applications are aimed to enhance the stability of sensitive compounds during production, storage and ingestion (eg, fortifiers like vitamins and minerals), decrease evaporation and degradation of volatile bioactives, (eg, natural and synthetic flavors), mask unpleasant tastes, (eg, polyphenols), or limit exposure to oxygen, water or light (eg, unsaturated fatty acids).

Carbohydrates, proteins or lipids are possible encapsulating carrier materials as they are food grade, biodegradable, and stable

in food systems during processing, storage, and consumption (Fathi et al., 2014). Polysaccharide-based delivery systems are suitable for many industry applications because they are biocompatible, biodegradable, and possess a high potential to be modified to achieve the required properties. In contrary to the lipid carriers, carbohydrate-based delivery systems can interact with a wide range of bioactive compounds via their functional groups, which makes them versatile carriers to bind and entrap a variety of hydrophilic and hydrophobic bioactive food ingredients (Fathi et al., 2014). Various carbohydrates can be used including starch, cellulose, pectin, guar gum, chitosan, alginate, dextrin, cyclodextrins, and new sources of native gums. Methods for fabrication of carbohydrate-based delivery systems include coacervation, spray drying, electrospinning, electrospray, supercritical fluid, emulsion-diffusion, reverse micelle, salting-out, ultrasonication, and high pressure homogenization.

Membrane emulsification is a possible method for the production of food grade nanoencapsulated products. Some examples are given, including the encapsulation of fish oil omega-3 and omega-6 and the encapsulation of probiotics. These systems rather refer to microencapsulation as their size is in the micrometer range. However, similar methods could be applied to the production of carrier systems with a dimension in nanoscale. Fish oils omega-3 (ω-3) and omega-6(ω-6) are well known to contain fatty acids, which are among the most important functional food ingredients (Chatterjee and Judeh, 2015). They improve the cardiovascular activity, enhance long-term memory and normal brain function. However, ω-3 fatty acids are prone to degradation releasing unhealthy products such as secondary oxidation products of polyunsaturated fatty acids, aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds. Encapsulation can provide stability and protection, confer targeting, and release characteristics, mask unpleasant odor and taste, extend the shelf-life and enhance the bioavailability and palatability of the encapsulated materials. For example, Chatterjee and Judeh (2015) prepared fish oil–loaded microcapsules from O/W emulsions using chitosan as shell material. The emulsions were prepared by both membrane and ultrasonic emulsification processes. The microcapsules obtained by membrane emulsification had larger diameter compared to those from the ultrasonic emulsification. However, the microcapsules obtained by membrane emulsification process gave better loading capacity and encapsulation efficiency. The results obtained confirmed that encapsulation using chitosan significantly increased the thermal stability of the encapsulated fish oil.

The second application is related to the encapsulation of probiotics. There is considerable recent interest in probiotics for promotion of human health (Song et al., 2003). The probiotic bacteria most commonly studied include members of the genera *Lactobacillus* and *Bifidobacterium*. Microorganisms used as probiotic adjuncts are commonly delivered in the food system. However, when these microorganisms are injected, their activity and viability are reduced under highly acidic conditions. Hence, there is a need for lactic acid bacteria that are resistant to the stressful conditions of the stomach and the upper intestine, which both contain bile. SPG membrane emulsification has been used successfully for sodium alginate microencapsulation of *Lactobacillus casei* (Song et al., 2003). In this method, the cell concentrate suspension was prepared in an alginate solution containing preservatives. This cell suspension was then used as the dispersed phase for the preparation of a W/O emulsion using hydrophobic SPG membranes with pore size of some microns.

### *2.2.4 Solid Lipid Nanoparticles*

Solid lipid nanoparticles (SLNs) have been initially developed in the pharmaceutical industry to deliver lipophilic bioactive compounds. They were introduced more than 20 years ago as an alternative to solid nanoparticles, emulsions, and liposomes in cosmetic and pharmaceutical preparations. For food applications, SLNs can incorporate bioactive compounds, such as carotenoids, omega-3 fatty acids, and phytosterols (Weiss et al., 2008). These functional foods are designed to improve the long-term health and well-being of consumers. SLNs may thus provide physical stability, protect ingredients against chemical degradation, and allow for precise control over the release of encapsulated components during mastication and digestion to maximize adsorption.

SLNs can be produced using methods like high-pressure homogenization, microfluidization, and emulsification–solvent evaporation (Aditya and Ko, 2008). Various lipids with different physicochemical properties (melting point, composition, chain length, etc.), which can be incorporated into food products can be used for fabrication of SLNs. These solid lipids include triglycerides, fatty acids, waxes, steroids, partial glycerides, and hard fats. Also, surfactants play a key role in determining the physicochemical properties of SLNs such as size, surface charge, stability, and polymorphic transition. Various types of surfactants, which are acceptable for addition into food products, can be used to stabilize SLNs. These surfactants include proteins and polyoxyethylene sorbitan monooleate (Tweens).

Membrane emulsification is also a possible method for SLNs production (Charcosset et al., 2005). The principle is identical to that used for the production of emulsions (Fig. 2.1a), except that the lipid phase is heated at a temperature above the melting point of the lipid before being passed through the membrane pores. Like in classical emulsification, several membranes are available for SLNs production including tubular ceramic membrane and flat microengineering membranes. The membrane emulsification method was used successfully for the production of SLNs loaded with vitamin E (Charcosset et al., 2005). The advantages of the membrane method for the preparation of SLNs are the control of the SLNs size by an appropriate choice of process parameters and scaling-up obtained by increasing the membrane surface. The limits are related to membrane fouling, with the consequence of a decreasing permeate flux versus time. Fouling is potentially high as the lipid solutions are very viscous, even at temperature above the melting point of the lipid. Alternatives have to be tested, such as premix membrane emulsification which could reduce membrane fouling.

### *2.2.5 Aerated Food Gels*

Food gels are soft solids containing a high amount of an aqueous phase (ie, >80%) that have received much attention these last years (Zúñiga and Aguilera, 2008). Gel-like structures are present among most high-moisture foods like jellies, yogurt, and processed meats. Membrane emulsification can be used for the production of aerated food gels (Bals and Kulozik, 2003). In this case, the dispersed phase is a gas, which is pressed through the pores of a microporous membrane into the continuous phase. Bubbles are covered with surface-active substances of the continuous phase and they are detached from the membrane surface by the shear forces exerted by the phase flowing along the membrane surface. Bals and Kulozik (2003) investigated the influence several operating parameters (pore size, foaming temperature, and viscosity of the continuous phase, etc.) on the properties of foams produced by membrane foaming using whey proteins as surfactants. An important aspect is that the added amount of gas must be stabilized as completely as possible in the foam. Raising the foaming temperature increased the quantity of stabilised gas. The whey proteins then diffused faster to the bubble surfaces and stabilize these by unfolding and networking reactions to prevent the coalescence of the bubbles. A different configuration of membrane foaming used a dynamically enhanced membrane emulsification system consisting of two cylindrical cylinders: the inner cylinder is rotated, the outer cylinder being fixed membrane

(Müller-Fischer et al., 2007). The gas was pressed through the membrane and was detached as small bubbles by the flow shear stress. Using this technique, the foam microstructure was found to be significantly improved, with smaller mean bubble sizes and narrower size distributions.

Microbubbles and nanobubbles have also been produced successfully using membranes (Kukizaki, 2009). Although the results obtained are not directly related to food products, the technique could be useful in the field of beverages (bottled water, flavored water, etc.), dairy related (milk, cheese, butter, etc.), alcohol-related, ice-related, meat-related products, and so on. In this method, compressed air flows under pressure through the micropores of a SPG membrane. Several configurations have been tested including flat membranes, tubular membranes with crossflow or dead-end with internal or external pressure. Small microbubbles or nanobubbles formed at the outlet of the membrane pores and stabilized in the aqueous phase containing sodium dodecyl sulfate (SDS) as the surfactant. Bubbles with sizes 8–9 times larger than the membrane pores were obtained with a rather low stability (a few hours). However, it can be foreseen that the choice of more appropriate surfactant and gas would give bubbles with higher stability. As the gas phase is not viscous, high fluxes through the membrane can be obtained with no or very limited fouling. In addition, asymmetric SPG membranes can be employed, which can increase the flux through the membrane thanks to their larger pores within the supporting layer (Kukizaki, 2009). For the preparation of nanobubbles, SPG membranes with pores in the manometer range are needed (Kukizaki and Goto, 2006). These membranes are not yet commercially available and were produced by the authors. Using these membranes and under optimal conditions, nanobubbles with mean size in the range 400–700 nm were obtained (around 8–9 times the mean diameter of the membrane pores).

# 3 Preparation of Food Nanomaterials by Membrane Mixing

## 3.1 Membrane Mixing

In a membrane mixing reactor, one reactant solution permeates through the micropores of an ultrafiltration or microfiltration membrane into another solution (Fig. 2.1b). The droplet size at the outlet of the membrane pores is comparable with that of the pores that is to say around 5–10 nm for an ultrafiltration membrane, and around 100–200 nm for a microfiltration membrane. This results

in a decrease in characteristic diffusion time and therefore improvement of micromixing between both solutions. Compared to the membrane emulsification technique, fewer studies are devoted to membrane mixing. A very complete review summarizes the progress of the membrane mixing reactor in homogeneous liquid processing including features, applications, advantages, and limits (Jia and Liu, 2013). Membrane mixing reactor is also termed "membrane micromixing reactor," or "membrane dispersion reactor," or "membrane contactor."

In many industrial processes, micromixing (mixing at molecular scale) has a crucial role as it may influence selectivity, yield, and quality of final products. Such processes include reaction, gas absorption, emulsification, foaming, and blending. To intensify mixing, various reactors are used such as stirred tank reactor and T-tube or Y-tube (Bénet et al., 2002). The common characteristics of micromixing and microreactors is small channels with dimensions below 1 mm. One first reported application of membrane mixing was the synthesis of polyaluminum chloride and preparation of nanoparticles using hollow fibers membranes (Jia and Liu, 2002). The applications were further expanded to a larger range of products, including a large range of nanoparticles, nanocapsules, and liposomes, synthesis of polymers, parallel and consecutive reactions. The technique may improve the nanoparticles size and size distribution, molecular weight distribution of polymer in case of polymeric reactions, or the selectivity of complex reactions (Jia and Liu, 2013).

Various membrane configurations have been tested, such as flat membranes, tubes, capillaries, hollow fibers, or monolithic multichannels (Jia and Liu, 2013). The membrane configuration influences the specific surface/volume ratio, and the hydrodynamic inside the reactor, and therefore micromixing. The performance of the mixing membrane reactor also depends on other operating conditions (ie, concentrations, pressure, temperature, and cross-flow velocity) and process configuration (ie, constant permeation rate, bidirection flow, pulse flow).

Numerical simulations have been used to predict velocity and concentration profiles inside a membrane mixing reactor. For example, Kieffer et al. (2008) simulated velocity and concentration profiles inside a tubular or hollow fiber membrane using computational fluid dynamics (CFD). The authors showed that mixing between two components flowing respectively from the membrane pores and at the membrane surface was obtained by diffusion along the streamlines separating both components. Fig. 2.6 shows a hollow fiber of length L and radius R (2D geometry) in a cylindrical coordinate system. The membrane may be a tubular

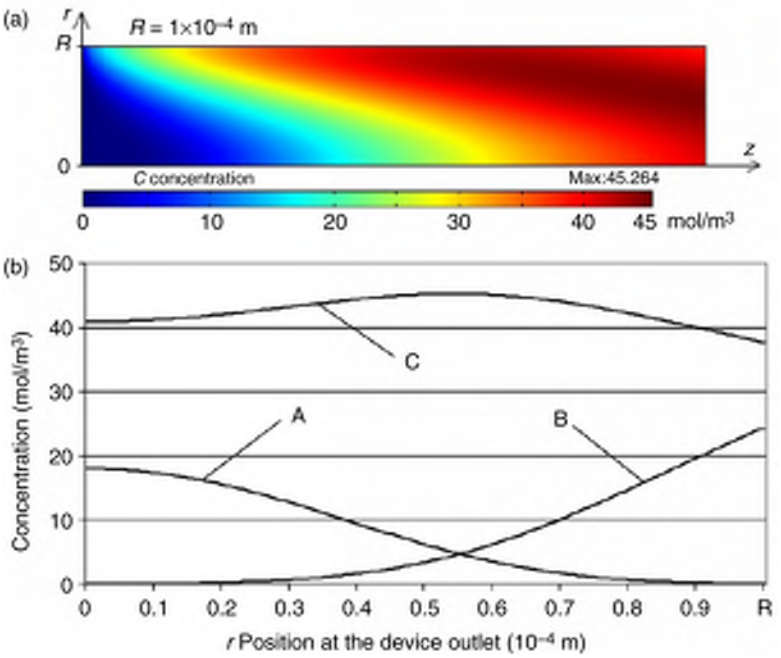


**Figure 2.6. Numerical simulation of mixing in a hollow fiber membrane.** (a) Two-dimensional concentration profiles of C, and (b) concentration profiles of A, B, and C at the outlet section of the lumen side ($z = 0.4$ m). Parameters values: $v_{in,L} = 0.1$ m/s, $v_{in,S} = 1.25 \times 10^{-5}$ m/s, $1 \times 10^{-4}$ m inner radius, $C_{A,in} = C_{B,in} = 100$ mol/m$^3$, $D_A = D_B = D_C = 2.13 \times 10^{-9}$ m$^2$/s, $k = 1$ m$^3$/mol s. From Kieffer et al. (2008), with permission.

or a hollow fiber membrane device, according to the inner radius data. For a hollow fiber membrane, the mixing zone width is close to the inner membrane diameter data, therefore mixing and reaction occur inside all the lumen side of the membrane. Radial mixing in porous hollow fiber is significant because the time required for the permeate to be convectively transported to the hollow fiber centerline is 1–2 times smaller than the mean residence time inside the device (Zarkadas and Sirkar, 2006). The membrane process thus leads to a new method of mixing, characterized by low energy input and the absence of mechanical components inside the mixing device (ie, stirrers and motors).

The advantages of membrane mixing may be summarized as follows: micromixing efficiency, economical, high production capacity, and easy scale-up (Jia and Liu, 2013). The scale-up ability of membranes is well known in classical processes like ultrafiltration and microfiltration, and is easily obtained by increasing the membrane area (membrane length and number of channels). However,

applications of membrane mixing reactors are still limited as the membrane price, robustness and fouling may limit large scale commercialization. To overcome these limits, the membrane mixing reactor should demonstrate its advantages in product quality, productivity and cost over classical techniques. As membrane processes are well integrated in the food industry with traditional processes like ultrafiltration, microfiltration, reverse osmosis and electrodialysis, membrane mixing could find its own place for food applications.

## 3.2 Food Applications

Nanomaterials for food that can be prepared using a membrane mixing reactor include liposomes, nanoemulsions, nanocapsules, and nanospheres (Fig. 2.5).

### *3.2.1 Liposomes*

Liposomes are spherical particles with size in the nanometer to micrometer range formed by polar lipids (Laouini et al., 2012b). The vesicular particles may consist of one or several bilayer membranes. Because of their structure, liposomes can entrap hydrophilic molecules in their internal aqueous compartment or lipophilic molecules within the lipid membrane. Due to their biocompatibility, biodegradability, and low toxicity, potential applications of liposomes as pharmaceutical carriers for efficacy enhancement and toxicity reduction are well recognized. Several commercial products are commercialized, mainly for cancer treatment. They were developed at laboratory scale, tested clinically and then commercialized. Some of these liposomal preparations are Doxil, Myocet, and Visudyne. One of the first liposome preparation methods was reported by Bangham in the early 1960s. Since then, several techniques have been reported in the literature including the thin-film hydration method, reversed phase evaporation, solvent injection techniques, and membrane extrusion.

Potential applications of liposomes for the food industry were discussed recently in detail by Liu et al. (2013). The applications concern mainly the protection of sensitive ingredients and increasing efficacy of an additive by confining undesirable activities to the liposome core. Liposomes for food applications can be prepared from egg, soy, or milk phospholipid. Flavors and aroma can be incorporated in the liposome bilayer to remain protected against degradation during storage, and to be released in the mouth, triggered by an increase in temperature. Liposomes can be employed in food also to encapsulate vitamins, like ascorbic acid and vitamin A. These applications of enhancing natural

preservatives, including antioxidants such as vitamin E and C, will undoubtedly become very important due to recent dietary trends, which tend to reduce the addition of artificial preservatives and increase portion of unsaturated fats in the diet (Laouini et al., 2012b). Liposomes have also been proposed as carriers of minerals in foods. In dairy products, several applications could be possible, and liposomes could be used to control the release of minerals during heat treatment, to minimize unwanted aggregation.

In the membrane extrusion technique, the dispersed phase containing large liposomes is forced to flow through a membrane with a uniform pore size distribution generating a homogenous population of small vesicles (Mui et al., 2003). Membrane extrusion for the preparation of liposomes is reported to be an easy, reproducible method, producing no detectable degradation of the phospholipids, and a high encapsulation efficiency of the liposome preparation. Multilamellar vesicles have mean diameters approaching the pore diameter of the polycarbonate membrane through which they are extruded. Other parameters such as the temperature and the applied pressure across the membrane also influence the characteristics of the liposomes obtained.

The ethanol injection method is commonly used in the industry because of its simplicity and also for its scale-up ability (Charcosset et al., 2015). Other advantages include its good reproducibility and the absence of degradation of lipid and active molecules when loaded into the liposomal structures (lipid bilayer or aqueous core). In this technique, appropriate lipids are dissolved in ethanol. By injection of this lipid phase into an aqueous phase, the immediate precipitation of the lipid molecules occurs, which assemble into bilayer fragments. These fragments then form close spherical structures of bilayer membranes (liposomes) as they tend to reduce the surface of their hydrophobic parts in contact with the aqueous phase, when submitted to stirring or ultrasonication. The theory of the bilayer fragments was presented by Lasic (1995) and is still admitted.

Membrane mixing is an appropriate technique for the preparation of liposomes based on the ethanol injection method (Charcosset et al., 2015). In this technique, the lipid(s) and active molecule(s) are dissolved in ethanol, this organic phase is then passed through the pores of the microporous membrane using a pump or a pressurized vessel. Bilayer fragments form and precipitate in the aqueous phase circulating at the membrane surface. Several membranes can be employed such as hollow fibers or tubes, made of organic or inorganic materials. By increasing the membrane surface, a large volume of liposomes can be prepared; meanwhile, the flux of liposomes suspension is increased.

In addition, hydrophilic or hydrophobic active molecules can be incorporated like in other techniques of liposomes preparation. The more adequate active molecules are hydrophobic as they give higher encapsulation rate and are loaded within the bilayer membrane. Hydrophilic molecules, which have to stay mainly in the aqueous phase, give usually lower encapsulation rates.

More complex structures can be obtained using the ethanol injection method (Sherry et al., 2013). In drug-in cyclodextrin-in-liposomes-in-complexes, liposomes are associated with cycodextrin to obtain higher encapsulation rates when loading hydrophilic or hydrophobic molecules. The method has been proved useful for the encapsulation of several essential oils like clove, to limit oxidation and for antibacterial and antifungal effects. Encapsulation can circumvent the drawbacks of essential oils like light sensitivity, volatility, and poor water solubility for food applications.

### *3.2.2 Nanoemulsions*

Nanoemulsions have droplet size in the nanometric scale (typically in the range 20–200 nm) (Laouini et al., 2012a). Their very small droplet size causes a large reduction in the gravity force and the Brownian motion, which may be sufficient for overcoming sedimentation or creaming. Their high physical stability makes them attractive for a large range of applications in pharmaceutical, cosmetic, food industries, and so on. Another advantage of nanoemulsions is that they can be prepared using surfactant concentration less than 10%, unlike microemulsions, which require a high surfactant concentration usually around 20% and higher. In addition, their optical clarity is important in food applications where the final product should appear clear, such as gelatin desserts. This property can be attributed to the relatively small size of the droplets they contain compared to the wavelength of light (Walker et al., 2015). Finally, nanoemulsions are suitable for efficient delivery of lipophilic bioactive agents, due to their large surface area.

Nanoemulsions can be produced using either high or low energy techniques (Walker et al., 2015). High-energy methods rely on the application of mechanical energy to disrupt the separate O/W phases, mix the two phases together, and form tiny oil droplets. These methods require specialized equipments such as high-pressure valve homogenizers, microfluidizers, and sonicators. Low-energy methods rely on changes in the environment or solution conditions to promote the spontaneous formation of tiny oil droplets. They depend on the type and amount of surfactant, oil and water present. Several low-energy emulsification techniques are available, including the spontaneous emulsification, phase

inversion temperature, phase inversion composition, and emulsion inversion point. In the spontaneous emulsification method, one phase (eg, an organic phase containing surfactant and oil) is slowly added to another (eg, water) to spontaneously form a nanoemulsion.

In recent years, there has been a considerable interest in the utilization of nanoemulsions as delivery systems for lipophilic bioactive agents in foods and beverages because of their high physical stability, ability to increase bioavailability, as well as high optical clarity (McClements and Rao, 2011). Nanoemulsions can be used as delivery systems for lipophilic bioactive components, such as oil-soluble vitamins, nutraceuticals, flavors, and antimicrobials. For example, they may offer a promising way to incorporate omega-3 fatty acids into liquid food systems, to protect the oil from oxidation, mask undesirable off-flavors, and increase oral bioavailability (Walker et al., 2015). They have potential for the encapsulation, protection, and release of omega-3 fatty acids in liquid food systems like beverages, dressing, sauces, and dips. These delivery systems could be used in the food industry to fortify foods and beverages with these bioactive lipids. Nanoemulsions may also incorporate essential oils having in vitro antimicrobial activity. For example, Salvia-Trujillo et al. (2015) obtained nanoemulsions containing essential oils (lemongrass, clove, tea tree, thyme, geranium, marjoram, palmarosa, rosewood, sage, or mint) by microfluidization of coarse emulsions. The average droplet size was found in the range of a few nanometers. The in vitro antimicrobial activity against *Escherichia coli* of nanoemulsions was assessed and compared with that measured with conventional emulsions. Lemongrass, clove, thyme, or palmarosa-loaded nanoemulsions had a higher in vitro bactericidal action against *E. coli*. In addition, a faster and enhanced inactivation kinetic was observed in the case of nanoemulsions containing lemongrass or clove essential oils in comparison with their coarse emulsions. Donsì et al. (2011) also reported the production of nanoemulsions of essential oil for incorporation into fruit juices, in order to enhance their antimicrobial activity while minimizing the impact on the quality properties of the final product. A terpenes mixture and D-limonene were encapsulated into nanoemulsions based on food-grade ingredients, prepared by high-pressure homogenization. An increase in the antimicrobial activity resulted, which was dependent on the formulation and mean diameter of the delivery systems as well as on the microorganisms class.

Membrane mixing has been rarely applied to the preparation of nanoemulsions, although the high micromixing efficiency of this technique was reported as very attractive for this application

(Laouini et al., 2012; Oh et al., 2011). For example, Laouini et al. (2012a) selected nanoemulsion components (MCT oil and surfactant mixture Tween-80/Brij 35) after solubility studies, and concentration range by construction of ternary phase diagrams. For nanoemulsions preparation, a SPG membrane was used in a crossflow mode. Several parameters influenced the nanoemulsion characteristics. Small droplets and narrow size distribution were obtained at low transmembrane pressure, high continuous phase flow rate and high agitation speed. Under optimal conditions, nanoemulsions were obtained with a span factor of 0.25 ± 0.01, which means good monodispersity, and an average size of 78 ± 3 nm. Oh et al. (2011) used a SPG membrane to produce an O/W nanoemulsion of flurbiprofen consisting of methylene chloride as the dispersed phase, polyvinyl alcohol as the stabilizer and a mixture of Tween-20 and Tween-80 in water as the continuous phase. Emulsion droplets with a mean droplet size of 25 times smaller than the mean pore size and a narrow droplet size distribution were produced successfully.

### *3.2.3 Nanocapsules*

Nanoparticles (nanospheres and nanocapsules) are ranging in size from about 10 to 1000 nm (Couvreur et al., 2002; Charcosset and Fessi, 2005). Nanospheres have a matrix type structure with bioactive agents adsorbed at their surface, entrapped in the particle or dissolved in it. Nanocapsules have a polymeric shell and an inner liquid core, the bioactive agents being dissolved in the inner core, or adsorbed at their surface. Nanoparticles have been investigated for the entrapment of a wide variety of bioactive agents. Several methods for the preparation of nanocapsules are available, involving either a dispersion of preformed polymers or a polymerization of dispersed monomers. Nanocapsules prepared by dispersion of preformed polymers involve the use of purified natural molecules or preformed synthetic polymers. It is based on the interfacial deposition of a polymer following displacement of a semipolar solvent miscible with water from a lipophilic solution. The organic phase (solvent, polymer, oil, and bioactive agent) is added dropwise under moderate stirring into the aqueous phase (water, and surfactant). Interfacial polymerization is another method to prepare nanocapsules in which two monomers, one oil-soluble and the other water-soluble, are employed and a polymer is formed on the droplet surface. In this method, the organic phase (solvent, monomer, eventually oil, and bioactive agent) is added into the aqueous phase (water, comonomer, and surfactant).

Nanocapsules are very attractive in food development as their oily core can be active. Nanocapsules can also be used for the

encapsulation of actives substances loaded in the oily core or polymeric shell, like flavors, probiotics, sweeteners, nutraceuticals, pigments, antimicrobials, antioxidants. In addition, nanocapsules that have a polymeric shell surrounding their oily core can serve as a protective barrier capable of preserving the functionability and bioavailability of different food additives. Several applications of nanocapsules have been proposed in food products development. For example, Zambrano-Zaragoza et al. (2011) prepared polymeric nanocapsules containing food ingredients by the emulsification–diffusion method. The method was found very successful and applied to the encapsulation of DL-α-tocopheryl acetate and β-carotene. The authors used an optimization method to find the best particle size, polydispersion index, density, and zeta potential. The influence of three parameters: shear rate, polymer-wall concentration (poly-3-caprolactone), and stabilizer concentration (polyvinyl alcohol) was investigated.

The membrane mixing technique is a possible method to prepare nanocapsules in an efficient way due to is high efficiency (Charcosset and Fessi, 2005; Khayata et al., 2012; Jia and Liu, 2013). For example, Charcosset and Fessi (2005) investigated two methods for the formation of nanoparticles: the nanoprecipitation and the interfacial polymerization methods using a ceramic tubular membrane for micromixing. The influence of process parameters (membrane pore size, flowrate, and organic phase pressure) on organic phase flux and on nanocapsules size was investigated. Nanocapsules as small as 260 nm were obtained with a nanofiltration membrane, a transmembrane pressure of $3 \times 10^5$ Pa (3 bar) and a crossflow rate of 1.7 m/s. The advantages of the membrane mixing reactor compared to other processes for nanoparticles preparation were underlined to be its scale-up ability, and the possibility to control nanoparticles size by an appropriate choice of the membrane.

### *3.2.4 Nanoparticles*

In the food industry, several types of nanoparticles have been developed to encapsulate, protect, and/or release active food active principles (Joye and McClements, 2013). Other useful effects of nanoparticles are reported, for example, to overcome undesirable effects on food quality, which may be due to fat, sugar, or salt reduction. Nanoparticles can also act as sensors to detect contaminants or microbial spoilage in food products. The techniques used to produce nanoparticles have been classified in two main classes, including top-down methods that breakdown bulk materials or larger particles into nanoparticles (such as high-pressure homogenization) and bottom-up methods used to prepared

nanoparticles by assembling molecules or smaller particles together (like spontaneous emulsification and antisolvent precipitation) (Joye and McClements, 2013).

To produce the nanoparticles, the antisolvent precipitation method uses addition of a nonsolvent to a solvent solution containing biopolymer(s) or compound(s) (Joye and McClements, 2013). Nanoparticles with adequate properties (size distribution, morphology, encapsulation rate, delivery efficacy, etc.) can then be obtained. The two most common types of biopolymers that can be used to produce nanoparticles using the antisolvent precipitation method are proteins (eg, albumin and gelatin) and polysaccharides. After precipitation of the nanoparticles it is also possible to include a hardening step (eg, addition of a chemical hardening agent such as glutaraldehyde) in which the proteins are crosslinked and the nanoparticle matrix is fixed.

Mechanical stirring applied to the antisolvent and antisolvent solutions has a major impact on the particle properties as stirring determines the rate and degree of supersaturation, and therefore strongly influences the nucleation rate and particle growth kinetics. In this point of view, membrane mixing is a very attractive method as it provides excellent micromixing conditions as well as scale-up properties, which make it suitable for industrial applications. Two main applications of nanoparticles preparation using membrane mixing have been reported, which could be suitable in the food industry. The first application concerns the preparation of bovin serum albumin (BSA) nanoparticles using a combination of the membrane mixing method and the desolvation method (Yedomon et al., 2013). In this technique, BSA was dissolved in ethanol and passed through the membrane pores. As a result, BSA and albumin coacervates were formed at the outlet of the membrane pores in the aqueous solution circulating at the membrane surface. BSA nanoparticles were then obtained by glutaraldehyde reticulation of the BSA coacervates. Two parameters can be controlled using the membrane mixing method: the ethanol pressure and the crossflow rate of the circulating phase. Larger volumes can be easily prepared by increasing the membrane area and keeping other parameters the same. In addition, the process is rather simple and presents low membrane fouling (the flux through the membrane are high and do not decrease vs time during at least some hours).

The second application of membrane mixing is the preparation of chitosan nanoparticles. Chitosan nanoparticles can be prepared by membrane mixing based on ionic gelation using sodiumtripolyphosphate (TPP) as a crosslinking agent (Hassani et al., 2015). In this method, the TPP solution passed through a

microengineered membrane into the chitosan solution stored in a stirred cell. At optimal conditions, the technique was shown to give nanoparticles with small mean size (in the range 90–100 nm), a rather small polydispersity index (around 0.22), and the zeta potential (+31 mV) obtained was high enough to suggest high stability. The preparation of chitosan nanoparticles depends on mixing conditions (homogenizer or magnetic stirring) and in this point of view membrane micromixing creates on top of the membrane surface favorable conditions to obtain well-characterized chitosan nanoparticles.

## 4 Conclusions

Membrane emulsification and membrane mixing are two different techniques using membrane pores to deliver one solution into another one. In membrane emulsification, the use of appropriate surfactant(s) stabilizes the droplets formed at the membrane pore outlets. In membrane mixing, the membrane provides a controlled feeding: the size of permeate at the outlet of the membrane pores is on the nanoscale or micron-scale, which favors very efficient micromixing. Although both techniques are based on the permeation of a phase through the membrane micro- or nanopores, the mechanisms involved are rather different. In membrane emulsification, the surfactant plays a major role to stabilize the droplets formed at the pore outlets; in membrane mixing the technique is aimed at providing optimal micromixing conditions at the membrane surface.

A major advantage of both methods is related to the scale-up ability of the membrane devices. The flux may easily be increased by increasing the membrane area, for example, by adding more tubes or fibers, increasing the tube or fiber length, and using devices in parallel, to achieve the desired productivity. Also, for both techniques, low shear is particularly attractive as shear sensitive ingredients can be used. Both techniques behave like microreactors with small channel diameters. Compared with conventional microreactors, the membranes provide a large number of feeding points, the specific area/volume is very high, and therefore very high production capacity can be obtained.

However, industrial applications of membrane emulsification and membrane mixing are still limited. For both methods, limitations are associated to low fluxes when using highly viscous to-be-dispersed phase, and to fouling phenomena. These disadvantages may be solved by a proper choice of the formulation, and by using configurations such as premix membrane emulsification, and

rotating or vibrating membrane devices, especially in case of membrane emulsification. To overcome these limits, the membrane processes should show advantages in product quality, productivity, and cost over classical techniques. No doubt, optimization of the operating conditions and design of the device would further increase the performance of membrane emulsification and membrane mixing, especially for the food processing industry.

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# 3

# NANOEMULSIONS CONTAINING UNSATURATED FATTY ACID CONCENTRATES

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## 1 Introduction

Nanotechnology was first discussed in 1959 by the physicist Richard Feynman. In the lecture, it was discussed as the manipulation of very small scales, the molecules and atoms level: the nanoscale (Chau et al., 2007; Poole and Owens, 2003). The term nanoparticle (NP) includes nanocapsules and nanospheres, which differ according to the composition and structural organization. Nanocapsules are constituted by a casing around an oily core. The active agent can be dissolved in the core and/or adsorbed to the capsule wall. On the other hand, the nanospheres are formed by a polymeric matrix, without oil in its composition (Reis et al., 2006). Structures at this scale have unique functional properties, not found in the macro scale (Poole and Owens, 2003). This fact becomes a challenge for scientists, but is also a great opportunity for the development of new materials with properties and functionalities previously impossible to reach (Kane and Stroock, 2007).

In this context, the interaction of the natural sciences and engineering has grown rapidly due to a common interest in small structures. Most nanotechnology research focuses on developing applications in bioscience and engineering. Strategies for applying nanoscience in the food industry are quite different in relation to the more traditional applications of nanotechnology. Food processing involves a wide variety of raw materials and technological processes (Moraru et al., 2003). The food industries are constantly

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00003-9

looking for ways to improve the production efficiency, food safety, and food characteristics, in order to increase competitive advantages and market share. Nanotechnology has impact on all stages of the food production, initiating in agriculture, after, in the processing (where the creation of emulsions and encapsulation evolved to nanoscale) and in the final product (where the packaging area has gained numerous improvements by incorporating nanoparticles) (Cushen et al., 2012).

In the food industry, nanotechnology has given focus to research in packaging, food monitoring, additives controlled release, and bioactive compounds. Besides assisting in the nutrients release, the nanoparticles can be used in foods to alter their functional and/or rheological properties (Moraru et al., 2003). The demand for functional foods with high nutritional value, low dosage of synthetic preservatives, and better organoleptic characteristics, leads to the applications of nanoemulsions and nanoencapsulation in food processing. This technology can be used to increase the stability of sensitive compounds during production, storage, and ingestion, reducing evaporation and degradation of volatile bioactive or limit exposure to oxygen, water, or light (Fathi et al., 2014).

Among the functional foods that have gained prominence, nutraceutical products for supplementation of polyunsaturated and monounsaturated fatty acids can be cited (PUFA and MUFA). Due to the high unsaturation degree present in MUFA and PUFA, these molecules become more susceptible to oxidation phenomena (Belhaj et al., 2010). Several authors have studied the production of nanoemulsions containing unsaturated fatty acids concentrates, from different sources, in order to prepare nanoemulsions with the capacity to act against oxidants factors, such as temperature, exposure to light and oxygen, thereby improving its quality (Cavazos-Garduño et al., 2015; Esquerdo et al., 2015; Walker et al., 2015a; Cho et al., 2014; Salminen et al., 2014; Staszewski et al., 2014; Deshpande et al., 2013; Dey et al., 2012; Belhaj et al., 2010).

# 2 Nanoemulsions

## 2.1 Fundamental Aspects

An emulsion can be defined as a material that contains small oil droplets dispersed in an aqueous mean or, small water droplets dispersed in a lipid medium, in which, at least, the phases are immiscible. A system that consists of oil droplets dispersed in an aqueous phase is called an oil-in-water (or o/w) emulsion,

whereas, a system that consists of water droplets dispersed in an oil phase is called a water-in-oil (or W/O) emulsion. The droplets are named disperse, internal or discontinuous phase, while, the envolving liquid is called external or continuous phase (McClements and Weiss, 2005).

Food, pesticides, and pharmaceutical industries have shown great interest in the use of emulsions to encapsulate, protect, and deliver active lipophilic components (such as unsaturated fatty acids concentrates). For certain applications, it is desirable colloidal dispersions in nanoscale (nanoemulsions), once they offer advantages, such as higher stability, are optically clear and can increase the availability of certain bioactive compounds (McClements, 2012).

## 2.2 Nanoemulsions and Nanocapsules

Lipid nanocapsules are colloidal systems transporters developed to encapsulate, protect, and distribute functional lipophilic components (Fathi et al., 2014). Encapsulation can be defined as a method for retaining a substance (active agent) inside another (wall material) (Nedovic et al., 2011).

One of the most traditional methods for the nanocapsule production is the nanoemulsions formation. Once a simple and convenient method for encapsulation has been developed, various wall materials can be used. The appropriate wall material should have high emulsifying activity, high stability, and the lipids must not separate from the emulsion during dehydration (Matsuno and Adachi, 1993). Furthermore, for food purposes, it is necessary for the GRAS certificate for the wall materials. The regulations for food additives are much more stringent than for pharmaceutical or cosmetic products. Therefore, some compounds that are accepted for drugs encapsulation cannot be approved for use in the food industry. The definition of the encapsulation purpose is an important criterion for the material selection. The effect may be, for example, increase shelf life, taste masking, or controlled release (Wandrey et al., 2010).

The effects of PUFA and MUFA encapsulation are closely related to the functions and properties of lipids. Unsaturated fatty acids are essential nutrients for humans. Lipids encapsulation delays the auto-oxidation of unsaturated fatty acids and improves the stability (Matsuno and Adachi, 1993). Due to the sensitivity of unsaturated fatty acids regarding to the oxidative degradation, food production enriched with PUFA and MUFA concentrates is a difficult task. The encapsulation of these concentrates is an alternative to slow oxidation and prevent the metabolic rate loss (Zimet and Livney, 2009).

## 2.3 Nanoemulsions Properties

The nanoemulsions thermodynamic properties are similar to those of the conventional emulsions. The main difference between them is that the nanoemulsions have slower kinetics destabilization, which makes them more stable, having high suspension stability and a long shelf life, mainly due to the droplet size and the high Brownian motion (Dey et al., 2012). An important application of the nanoemulsions is the incorporation of lipophilic active ingredients in water-based foods or drinks that must remain transparent, such as certain kinds of water, enriched drinks, sauces, and juices (Velikov and Pelan, 2008). In general, traditional emulsions are formed by droplets with radius ranging from 100 nm to 100 mm, and tend to be optically opaque. Nanoemulsions are formed by very small droplets, with radius ranging from 10 to 300 nm, which means that they tend to be transparent or only slightly cloudy (Fig. 3.1). Due to the small droplets size, the nanoemulsions have higher stability to gravitational separation or aggregation.

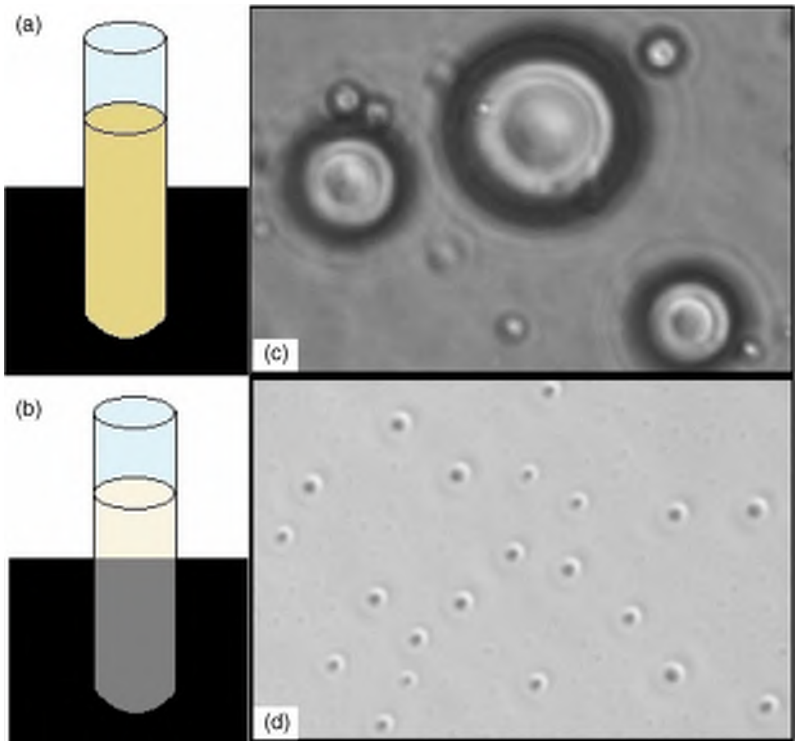


**Figure 3.1. Schematic diagram comparing the appearance and particle size of the emulsions and nanoemulsions containing nanocapsules.** Appearance of (a) emulsion and (b) nanoemulsions; and difference in emulsions particle size of (c) microcapsules and (d) nanocapsules.

The main factors that determine the optical properties of nanoemulsions are given by the relationship between refractive index, oil concentration and particle size in colloidal distribution. This relationship is relevant to the development of products that must be translucent or opaque. Studies demonstrated that the optical appearance of O/W emulsions can be modulated by changing the size and concentration of droplets. Translucent nanoemulsions should have a droplet diameter less than 50 nm. Above 50 nm, the emulsions begin to become turbid. The efficiency of the oily droplets dispersion determines how much oil can be incorporated until there are alterations in turbidity. For turbid products, most droplets should be between about 200 and 400 nm of diameter (Piorkowski and McClements, 2014).

Zimet and Livney (2009) noted that there is a market for nutraceutical foods and beverages enriched with essential oils, including omega-3 fatty acid. One of their purposes was to form small nanoparticles of omega-3 emulsion, which would allow the enrichment of clear acidic drinks. They used beta-lactoglobulin as a natural nanomolecular carrier, and for hydrophobic molecules was provided as evidence to a spontaneous connection of docosahexaenoic acid (DHA) and beta-lactoglobulin. This allowed the formation of transparent nanoparticle dispersions, containing 0.05% of beta-lactoglobulin and DHA, with good colloidal stability and average particle size of 100 nm. Then, they proposed one way to encapsulate long chain polyunsaturated fatty acids, such as DHA.

Ilyasoglu and El (2014) used the fish oil as a source of EPA and DHA to enhance fruit juices. They studied the impact of pH and polymer concentration on the physicochemical characteristics of fish oil nanocapsules with sodium caseinate and gum arabic complex. The different formulations showed that the addition of gum arabic in the caseinate dispersion avoided the self-aggregating of molecules. Zeta potential analysis showed that the pH 4 was the more suitable to prepare these nanocapsules.

Komaiko and McClements (2015) examined the possibility of incorporating nanoemulsions ($d < 100$ nm) on a gelatin dessert. The study measured the nanoemulsions turbidity as a function of temperature. Gelatin incorporated with nanoemulsion had maintained rheological characteristics similar to the standard gelatin. The temperature increase affected the optical properties of the emulsions, which had a small increase in turbidity; however, the samples remained translucent. Helgason et al. (2015) investigated the formation of nanoemulsions with very small particles and their influence on the emulsion appearance. The nanoemulsions particle size decreased with the increase in homogenization pressure. These nanoemulsions were optically transparent, and this

fact was attributed to the particle size, which was much smaller than the light wavelength. Furthermore, these results indicated that the particle size has influenced the change in nanoemulsions appearance, after lipid phase solidification.

Another important feature of the nanoemulsions is its stability. The small size of the nanoparticles leads to the prevention of flocculation and coalescence droplets. The destabilization kinetics of nanoemulsions is so slow (~months) that they are considered kinetically stable, and Ostwald ripening regulates the destabilization process (Anton and Vandamme, 2011). It is practically impossible to stop Ostwald ripening, which makes it of great importance for maintaining the emulsion stability in the long-term. It is a coarsening process, where the dispersed droplets aggregate to larger droplets, due to pressure differences between the different size particles (Meinders and Vliet, 2004). Ostwald ripening or molecular diffusion arises from the polydispersity of the emulsion and of the solubility difference between small and large droplets (Solans et al., 2005; Tadros et al., 2004). In theory, Ostwald ripening leads to the condensation of all the droplets counted as a single droplet or a phase separation. However, this does not occur in practice because the growth speed of the droplet size decreases with increasing globules size (Tadros et al., 2004). The Ostwald ripening decrease can be obtained by adding a small amount of a second oil having low solubility in the aqueous phase, as well as the addition of a second surfactant with the same alkyl chain length and ethoxylation degree higher than the principal surfactant, in the case of systems stabilized with ethoxylate nonionic surfactants (Solans et al., 2005).

The influence of the surfactants on the stability of physical and chemical properties of nanoemulsions containing omega-3 was investigated by Salminen et al. (2013). The nanoparticles had average diameters from 131 to 168 nm and PDI approximately of 0.2, indicating that they were stable to particle aggregation, which can be attributed to electrostatic repulsion. The long-term stability was measured after 50 days, and the results showed that the emulsions were physically stable. The physical stability of the lipid nanoparticles was highly dependent on the surfactants, which can control the crystallization process during the preparation, as well as during storage.

Belhaj et al. (2010), in the study of different nanoemulsion formulations composed of salmon oil, verified that the minimum size can be achieved as a function of the material viscosity and homogenization parameters. They found that the nanodroplet size depends on physical parameters, as also on the oil composition and on the surfactant properties. The results showed that the use

of marine phospholipids as emulsifiers provided a considerable increase in the oxidative stability of salmon oil, with an increase in the PUFA availability. Awad et al. (2009) demonstrated that the fish oil nanoparticles may be produced, and these systems can be used to encapsulate, stabilize, and deliver omega-3 fatty acids. No significant changes occurred in the average particle diameter (147 nm) over time, indicating that the suspensions were stable to droplet aggregation and Ostwald ripening, during storage. The results showed that fish oil, rich in polyunsaturated fatty acids, can be successfully incorporated in solid lipid nanoparticles (SLN) suspensions.

## 2.4 PUFA and MUFA Concentrates in Nanoemulsions

Currently, importance has been given to the sources of unsaturated fatty acids, due to changes in the human diet and the onset of diseases related to low consumption of these compounds, as well as their recognized therapeutic significance, especially those of the omega-3 family. Sources of unsaturated fatty acids can be from animal and vegetable, both terrestrial and aquatic (Kralovec et al., 2012).

Fish oil is the most abundant and the cheapest source of EPA and DHA (Kralovec et al., 2012). Both EPA and DHA are in a larger category of unsaturated fatty acids (UFA). Compared to saturated fats, unsaturated fatty acids are more easily utilized for energy production when ingested. The unsaturation degree increase leads to an increase in the relative mobility of stored fat, making PUFA and MUFA more available to the body. PUFA have been associated with numerous health benefits, including prevention of cardiovascular disorders, diabetes, arthritis, cancer, brain, and eye development, and gene expression. DHA is a primary component of the lipids in the brain, and it is critical for brain development. The fish oil consumption decreases the production of very low density lipoprotein (VLDL) and triglycerides, by inhibition of the synthesis of hepatic triglycerides (Shahidi 2008; Young, 2001; Storlien et al., 2000). However, the presence of methylene bis-allylic and double bonds with *cis*-configuration in unsaturated fatty acids makes these molecules susceptible to structural changes, particularly oxidation, isomerization, and polymerization (Kralovec et al., 2012).

The great potential that the market provides for dietary supplements of unsaturated fatty acids, and products in which these acids are incorporated, led to research in order to obtain these concentrates. Fish oil is an important source of unsaturated fatty acids, and it is preferably used as a raw material for preparing concentrates of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Crexi et al., 2010, 2012; Kralovec et al., 2012).

Esquerdo et al. (2015) performed nanoemulsions containing nanocapsules of unsaturated fatty acids concentrates using chitosan as the encapsulating agent. The research demonstrated that the capsules were able to retard the primary oxidation of the unsaturated fatty acids concentrates.

Enriched fish oil containing omega-3 to obtain stable lipid nanocarriers was studied by Lacatusu et al. (2013). The lutein loaded lipid nanocarriers and unloaded lipid nanocarriers were synthesized by using the melting emulsification coupled with the high shear homogenization technique. The authors explained that the presence of lutein slowed the oxidation reactions, leading to more stable lipid particles.

In Deshpande et al. (2013), using linseed oil as a source of unsaturated fatty acids concentrates evidenced the importance of preparing nanoemulsions as drug delivery for the treatment and prevention of vascular diseases. In this nanodelivery system were incorporated an encapsulating agent (linseed oil) rich in omega-3 polyunsaturated fatty acids (PUFA) and α-linolenic, and a bioactive lipid, which improves endothelial function and cardiovascular health. Beneficial responses were found for the use of a nanoemulsion system omega-3-PUFA/17β-estradiol/ceramide.

Despite the growing number of studies indicating numerous health benefits associated with consumption of PUFA and MUFAs dietary intake remains low (Jin et al., 2008; Kris-Etherton et al., 2000). The growth of the functional food market resulted in the development of food products enriched with fish oil and other omega-3 sources, such as algae and flax. Among fortified foods mentioned: bread, cereal, milk, yogurt, juices, pasta, and cheese.

## 3 Preparation Methods

Nanoemulsions have a particular interest as colloidal delivery systems because they can be easily fabricated from food grade ingredients using relatively simple processing steps, such as mixing, shearing, and homogenizing (Rao and McClements, 2011). To prepare an emulsion, oil, water, surfactant, and energy are needed (Tadros et al., 2004).

The oil phase influences the curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. The fatty acids increase the permeability by disrupting densely packed lipids and fill the extracellular spaces of stratum corneum. Among unsaturated fatty acids, oleic acid is an effective skin penetration enhancer (Saini et al., 2014)

The surfactant selected should be able to reduce the interfacial tension to a very small value. This facilitates the dispersion during preparation and provides a flexible film that can readily deform around the droplets. This adsorption behavior can be attributed to the solvent nature and chemical nature of the surfactant that combines both polar and nonpolar groups in a single molecule. Low HLB values (hydrophilic lipophilic balance) are generally used for the formulation of W/O emulsions. Surfactants having high HLB value (>12) favor the formation of O/W emulsions (Glatter et al., 2001; Talegaonkar et al., 2008).

The preparation techniques and the manufacturing material are responsible for the final characteristics, quality, and application of the prepared nanoemulsions. The nanodroplets quality is generally dependent on the type of material used, that is, oil and emulsifier, while the droplets size is determined by extent of the disruptive forces applied to prepare the nanoemulsion (Abbas et al., 2015).

There are a limited number of food-grade surfactants available for the preparation and stabilization of these colloidal systems. Many of these are synthetic surfactants, which can be applied in different countries or that can be used at low levels due to regulatory, economic, or sensory issues. There is a demand for the use of starches and proteins in conventional emulsifiers. Sugar esters can be used as surfactants in the food and pharmaceutical industries due to their pleasant taste and aroma, low toxicity, and high biodegradability compared with petrochemical-based surfactants (Abbas et al., 2015; Rao and McClements, 2011).

Besides the emulsifier addition, another way to maintain the most stable nanoemulsions is the addition of other stabilizers, such as texture modifiers, maturation inhibitors, or weighting agents. The kinds of solvent that may be added influence directly the emulsion characteristics (Zeeb et al. 2014; Huynh et al., 2009). Some authors studied the alcohol addiction on the emulsions formation and its stability. Zeeb et al. (2014) demonstrated that the addition of short chain alcohols for emulsions stabilized by protein, before homogenization, can reduce the droplets size, thereby, facilitating the nanoemulsion formation. Esquerdo et al. (2014) studied microcapsules of PUFA and MUFA concentrates from fish oil, and they compared the influence of ethanol and hexane as solvents in the nanoemulsion preparation. The ethanol was the most suitable solvent for the microcapsules preparation.

The nanoemulsions formation, also called dispersion or emulsification, is generally achieved through methods such as agitation, homogenization, sonication, or high pressure. These methods are called high energy and provide enough energy to increase oil/water interfacial area. Low-power methods also allow

the nanoemulsions formulation without needing of any device or energy. Low-energy methods take advantage of the physicochemical properties of intrinsic components in order to generate submicron droplets (Anton and Vandamme, 2011; Solans et al., 2005). The preparation method has great influence on the physicochemical properties, which, in turn, have a direct consequence on the thermodynamic instability of the nanoemulsions (Pey et al., 2006).

## 3.1 High Energy

### *3.1.1 High Pressure Homogenizers*

Smaller particle sizes are achieved when the equipment supplies the power in the shortest time with the most homogeneous flow. High-pressure homogenizers are suitable to these requirements, so they are widely used to prepare nanoemulsions. Generally, conventional high-pressure homogenizers operate at pressures between 50 and 100 MPa (Solans et al., 2005).

A coarse emulsion is usually produced using a high shear mixer and then it is fed into the input of the high-pressure homogenizers. The homogenizer has a pump that draws the emulsion into a chamber and then forces it to pass through a valve. As the emulsion passes through the valve, it undergoes a combination of intense disturbing forces (turbulence, shear, and cavitation), then the larger droplets are split into smaller parts. A variety of different types of nozzles are available to enhance the efficiency of droplet break in the homogenizer (McClements, 2011).

Microfluidizers are similar to high-pressure homogenizers. However, the design of the channels through which the preemulsion flowing in the device is different. The microfluidizer divides an emulsion into two streams, which collide in the interaction chamber. Intense destructive forces are generated in the interaction chamber, where the two emulsion streams collide, leading to highly efficient droplet break. High-pressure systems and microfluidizers have been used in the literature for the preparation of MUFA and PUFA nanoemulsions from different sources (Deshpande et al., 2013; Belhaj et al., 2010).

### *3.1.2 Ultrasonication*

Ultrasonic emulsification is very efficient in reducing the droplets size, but is only suitable for small batches. The efficiency of the dispersion process is strongly dependent on the ultrasonic time in different ranges. The more hydrophobic the monomer, the longest sonication time is required (Solans et al., 2005). This kind of homogenizer uses high-intensity ultrasonic waves to generate severe disruptive forces (mainly generated by cavitation) forming

small droplets by breaking of the oil and aqueous phases. The type and amount of emulsifier used in the emulsion formation and the oil phase viscosity affects directly the homogenization efficiency. Ultrasonic homogenizers are particularly suitable for low viscosity fluids, and less suitable for more viscous systems (Piorkowski and McClements, 2014). The use of ultrasound as a high energy source has many advantages, such as lower power consumption, less use of surfactant and smaller droplet size. More homogeneous batches generally are obtained in comparison with the conventional mechanical methods (Tabibiazar et al., 2015).

Dey et al. (2012) prepared a nanoemulsion with fish oil rich in EPA and DHA, and they compared their intestinal absorption in vivo. The emulsions were successfully prepared using the high speed and ultrasonication as homogenization methods. Nanoemulsions rich in omega-3 were also prepared using an ultrasound device by Cavazos-Garduño et al. (2015). In the preparation of betulinic acid nanoemulsions stabilized by omega-3 enriched phosphatidylcholine, they verified that the nanoemulsion characteristics were dependent on the oil type and ultrasound amplitude.

Strunz et al. (2008) studied if the consumption of Brazil nut (rich in PUFA and MUFA) could affect the plasma lipids, a polipoprotein and some functional properties of the antiatherogenic high-density lipoprotein (HDL). The lipid nanoemulsion was prepared by prolonged ultrasonic irradiation in aqueous media and a two-step ultracentrifugation of the crude emulsion.

## 3.2 Low Energy

### *3.2.1 Spontaneous Emulsification*

The spontaneous emulsification process occurs by adding a hydromiscible solvent solution containing a small concentration of O/W. The oil droplets are formed with diameter dependent on the solvent and excess of oil ratio (Solans et al., 2005). The main characteristic of this technique results from the state of imbalance of two liquids with different physicochemical properties, placed in contact each other under stirring. This kind of emulsification occurs just in specific conditions, and in this process there is an entropy increase and a Gibbs free energy reduction. The energy source used is mainly originated from interfacial turbulence, which is closely connected to the surface tension gradient induced by the two phases of solute diffusion (Anton et al., 2008). According to Anton and Vandamme (2009), nanoemulsions generated by this method are kinetically stable for months. For the nanosized droplets formation in spontaneous emulsification method, it is necessary that a high ratio of organic solvent and oil used are in this

phase, before mixing with the aqueous phase. Consequently, the organic solvent diffusion is faster and the generated turbulence leads to the formation of nanoparticles.

Davidov–Pardo and McClements (2015) used the spontaneous emulsion method to produce nanoemulsions containing resveratrol in grape seed oil capsules. Grape seed oil is rich in unsaturated fatty acids, which represent more than 89% of the total oil composition. The effect of droplet size on the chemical stability of encapsulated resveratrol was examined. It was shown that resveratrol can be encapsulated within delivery systems and protected from degradation.

### *3.2.2 Phase Inversion Methods*

Nanoemulsions can be obtained using physicochemical system properties. These methods alter the spontaneous surfactant curvature. This can be achieved for nonionic surfactants by changing the system temperature, forcing a transition from a W/O emulsion at low temperatures to a W/O emulsion at higher temperatures (transition phase inversion, TPI). During the cooling, the system passes a point of zero curvature and minimal spontaneous surface tension, promoting the formation of finely dispersed oil droplets. However, besides the temperature other parameters such as concentration or the pH may be considered (Fernandez et al., 2004).

Spontaneous transition in the curvature radius of the surfactant molecules may be obtained by changing the volume fraction of the phases in the method known as the emulsion phase inversion (EPI). The successive water addition in the oily phase forms water globules in the continuous phase (bicontinuous microemulsion W/O). Increasing the water volume fraction, a spontaneous change in curvature of the surfactant molecules occurs (reverse catastrophic phase), leading to the emulsion reversal from W/O to O/W. This emulsification method occurs at constant temperature, not requiring heating the sample at a given temperature. The inversion can be induced also by addition of substances to the formulation, which are able to alter the system phase transition point, like an electrolyte, a surfactant, an alcohol or oil (Fernandez et al., 2004; Salager et al., 2004).

## 4 Nanoemulsions Characterization

Among the environmental factors that can affect the stability of nanoemulsions containing unsaturated fatty acids concentrates are cited temperature, light, and oxygen. These factors may result in chemical and physical reactions, such as changes in the size and distribution of particles, precipitate formation, oxidation, and hydrolysis. The stability study should include the final product characterization and stability evaluation, in relation to

## Table 3.1 Characterization Techniques for Nanoemulsions and Their Respective Purposes

| Characterization Technique | Finality |
|---|---|
| Peroxide index, p-anisidine value, TBA and volatile compounds | • Verify changes by oxidation. |
| Gas chromatography | • Identification of fatty acids profile<br>• Sample preparation, purification and quantification<br>• Determining oxidation compounds. |
| Fourier transform infrared spectroscopy (FT-IR) | • Qualitative and quantitative information<br>• Oil oxidation rate<br>• Demonstration of the presence and interaction type between the phases. |
| X-ray diffraction (XRD) | • Polymorphic structure characterization<br>• Differentiation of crystalline and amorphous materials. |
| Differential scanning calorimetry (DSC) | • Temperature effect and thermal stability<br>• Temperature and fusion heat. |
| Droplet size distribution | • Particle size<br>• Changes in droplets size distribution during storage. |
| Polydispersity | • Measure of the particles size distribution. |
| Scanning electron microscopy (SEM) | • Particle morphology and aggregation state<br>• Composition and topology<br>• Detect bacterial contamination. |
| Transmission electron microscopy (TEM) | • Structure to the atomic level<br>• Size distribution<br>• Particles internal morphology. |
| Atomic force microscopy (AFM) | • Topographic analysis<br>• Magnetic resonance submicron sized<br>• Three-dimensional information. |
| Zeta potential | • Changes in the nanoparticles surface by estimating the surface potential, functional groups density or increasing the surface hydrophilicity. |

the storage time of the formulation containing nanoemulsions. Besides the droplets size and size distribution aspects, the macroscopic evaluation, the qualitative and quantitative composition, zeta potential, pH, rate and form of the drug combination, release kinetics, and others, are important to attest to the quality of the final product (Schaffazick et al., 2003). Table 3.1 presents the main characterization techniques for nanoemulsions containing unsaturated fatty acids concentrates.

## 4.1 Oxidative Stability of Nanoemulsions Containing PUFA and MUFA Concentrates

The oxidation reduces the lipid nutritional quality and produces undesirable flavor and aroma. The fatty acid structure affects directly its susceptibility to oxidation. Polyunsaturated fatty acids are much more susceptible to oxidation than saturated fatty acids (Kralovec et al., 2012). The oleic acid with a single double bond reacts approximately 10 times faster than their saturated counterparts, stearic acid. Linoleic acid (two double bonds) reacts more than 100 times faster, and linolenic acid (three double bonds) reacts nearly 200 times fastest. EPA (five double bonds) and DHA (six double bonds) are extremely susceptible to oxidation (Pokorny et al., 2001).

A source of unsaturated fatty acids is microbial oils, which are generally less complex than the fish oils, displaying a simple fatty acid profile. These oils may also have different ratio of EPA and DHA and often have natural antioxidants that can help to protect the oil from oxidative damage during processing. Some microbial sources of omega-3 oils can be used for preparing compositions as the ratio AA:EPA:DHA in milk (2.0:0.2:1.0, w/w) for use in infants, which is preferred high DHA and low EPA (Kralovec et al., 2012). In fish oils, it is possible to find quantities of eicosapentaenoic acid (20:5 n–3), ranging from 3% to 18% of total lipids depending on the fish. The same occurs with docosahexaenoic acid (22:6 n–3), which can be found in up to approximately 13% of total lipids (Shahidi, 2008). The primary omega-3 sources most commercially used are anchovy oil (*Engraulis ringens*) and sardine oil (*Sardinops sagax sagax*) containing respectively, 15–22% EPA and 9–15% DHA (Kralovec et al., 2012).

The obtainment of omega-3 concentrates can be accomplished by techniques, such as crystallization by cooling, supercritical fluid extraction, molecular distillation and concentration by lipases (Liu et al., 2006; Wanasundara and Shahidi, 1999). Another efficient way to obtain PUFA concentrates is the urea complexation method. This separation method is based on the separation by unsaturation degree, where the more unsaturated acids are less complexed with urea (Crexi et al., 2012).

Crexi et al. (2012) studied the chemical hydrolysis reaction of oil carp (*Cyprinus carpio*) to obtaining unsaturated fatty acids concentrates by urea complexation. They found that after the hydrolysis, the concentrates had 31.4% more MUFA and PUFA than the bleached oil. In addition, there was a reduction of 75% of saturated fatty acids. A total of 89% of PUFA and MUFA were found in concentrates, compared to approximately 68% in the bleached oil.

The nature of unsaturated fatty acids is critical to its operation in terms of health benefits. However, this same property also makes them highly susceptible to oxidative deterioration. The oxidation is a free radical process and can be initiated by a variety of factors including light, heat, enzymes, and metals. The oxidation process occurs in three phases; initiation, propagation, and termination. During initiation, a hydrogen atom is captured from a lipid molecule giving rise to a lipid radical. This lipid radical reacts with oxygen to form peroxide. Subsequently, the peroxide can abstract a hydrogen atom, producing hydroperoxide and a new lipid radical (Kulas et al. 2006; Shahidi and Zhong, 2005; AOCS, 1992).

The highly unsaturated nature of long chain fatty acids (omega-3), such as EPA and DHA, affects the oxidation rate and produces highly complicated results in terms of generated products. These compounds can lead to products unacceptable in terms of sensory attributes, and some compounds produced by oxidation have negative health effects. Each double bond is a portal for hydrogen abstraction, allowing the formation of more than 16 EPA hydroperoxide isomers and 20 DHA hydroperoxide isomers. This large number of possible isomers during the initiation phase leads to generating a large number of possible secondary oxidation products (Kulas et al., 2006; Shahidi and Zhong, 2005; AOCS, 1992). The parameters related to oil oxidation can be quantified based on the determination of specific compounds, such as peroxides, anisidine, volatile compounds, and malonaldehyde (Roby et al., 2015).

Numerous analytical methods are routinely used to measure lipid oxidation in foods. This lipid oxidation can be evaluated by oxygen absorption, loss of initial substrate, free radicals formation, and the formation of primary and secondary oxidation products. Among the analysis to classify these oxidations may be mentioned: chromatographic analysis to verify changes in compounds; iodometric titration or FTIR for peroxide value; 2-thiobarbituric acid (TBA) value, p-anisidine (p-An) value, and carbonyl value (Shahidi and Zhong, 2005).

Moomand and Lim (2014) selected the hydroperoxide method as an indicator of early stage oxidation in encapsulated fish oil. The primary oxidation products are unstable and tend to decompose into radical species, which are contributing to the secondary products formation. The p-An value was determined to control the extent of secondary oxidation products formation. The oxidative stability of fish oil encapsulated and not encapsulated was monitored over 14 days. The results showed that the fibers with the capsules provided greater oxidative stability compared to non-encapsulated fish oil.

The structural modification effect of the soy protein on the emulsion properties and oxidative stability of fish oil microcapsules has been reported by Zhang et al. (2014). The emulsions were freeze dried and the lipids oxidation of the powder was verified by peroxide value and headspace propanal. To assess the oxidative stability of encapsulated fish oil, it was carried out a storage test (4 or 8 weeks, at 35°C). It was demonstrated that fish oil without encapsulation was highly oxidized, with rapid lipid oxidation rate. The oxidation products formation rate decreased in the microcapsules coated by hydrolysates. The results demonstrated that the modified wall material combined after hydrolysis had protective action favorable for the core material.

Salminen et al. (2013) determined the impact of tensoative properties on the lipids oxidation of nanoparticles incorporated with omega-3 fish oil. The primary oxidation was evaluated by hydroperoxides and the secondary oxidation was evaluated by oxidation products, for 50 days. According to the authors, the heating used for the nanoemulsions preparation caused no increases in the oxidation reactions, suggesting that the heating time was short to start the degradation of fish oil rich in omega-3. The results showed that the nanoparticles stabilized with high-melting lecithin presented high oxidative stability, enabling the formation of lipid nanoparticles physically and oxidatively stable.

Staszewski et al. (2014) also used the hydroperoxides to evaluate the oxidative stability of nanoemulsions containing PUFA rich oil. Green tea polyphenols and b-lactoglobulin nanocomplex were used, respectively, as emulsifiers and antioxidants in the preparation of emulsions containing fish liver oil rich in omega-3. The hydroperoxide concentrations in the nanoemulsions were measured. After 30 days, the emulsions formed by phenol-B-lactoglobulin nanocomplex proved to have an excellent performance as an antioxidant, even in combination with a protein. The low hydroperoxide level in the emulsions indicated that fish liver oil was relatively stable to oxidation during storage.

The oxidative stability of fish oil nanoemulsions produced by spontaneous emulsification was studied by Walker et al. (2015a). The authors evaluated the oxidative stability by the peroxide values and thiobarbituric acid-reactive substances, for 14 days. The surfactant concentration and particle size effects on the oxidative stability were evaluated. The particle size and the surfactant concentration did not have a large impact on lipid oxidation rate in the fish oil emulsions. It was possible to use a low-energy homogenization method to produce emulsions, which may be suitable to fortify food systems or transparent beverages. However, the

authors noted that although simple, this method requires relatively high levels of synthetic surfactants.

## 4.2 Identification of PUFA and MUFA Concentrates by Chromatography

The most used technique to analyze the fatty acids profile of lipids is gas chromatography (GC). The process to determine the levels of PUFA and MUFA in oils starts with a liquid extraction to obtain oil samples. Subsequently, the oil content is determined gravimetrically and then the volatile oil is converted into methyl ester derivatives followed by gas chromatography analysis (Vongsvivut et al., 2012). The chromatography is a separation technique, primarily used in chemical analysis. It can be used for the preparation, identification, and quantification. Chromatography is a powerful and versatile technique, which can separate a mixture into its individual components, and simultaneously provide a quantitative estimation of each constituent (Scott, 2003).

Dillon et al. (2013) used chromatography methods (silver (I)-mercaptopropyl stationary phase), otherwise known as silver-thiolate chromatographic material for the purification of omega-3 (EPA and DHA) present in fish oil. According to the authors, the traditional methods for isolation of omega-3, normally requires multiple and complicated steps to obtain high purity products (>95%). The analysis of the fatty acid ethyl esters products from a fish oil supplement, by GC-FID, demonstrated that the sample had more than 20 different fatty acids, ranging from C14:0 to C22:6. The sample had 18% (w/w) of EPA and 12% (w/w) of DHA. The employed phases were effective for the purification of omega-3 fatty acids, which showed purities higher than 95% of EPA and 99% of DHA.

The preparation of foods enriched with unsaturated fatty acids need knowledge about the composition of the active compound, in order to establish a correlation between the material added and the food. Nanoemulsions offer a viable alternative to disperse the lipophilic compounds and improve its dissolution, permeation, absorption, and bioavailability. Enzyme modified phosphatidylcholine with omega-3 fatty acids was used by Cavazos-Garduño et al. (2015) as an emulsifier to stabilize nanoemulsions. The oil fatty acid profile was analyzed by chromatography and showed that the sample had the total of 74% of EPA, DPA, and DHA. The authors used these results to calculate the concentrate molecular weight, and thus to establish correlations for the nanoemulsions preparation.

Furthermore, by chromatographic analysis, it is possible determine the formation of oxidation compounds present in

enriched foods. Gökmen et al. (2011) examined the effects of the nanoparticle amount on bread characteristics for the development of functional breads enriched with omega-3 nanoparticles. The degradation of unsaturated fatty acids and the contaminants formation after heat treatment were investigated in buns and toasted bread. The oxidation products of breads were analyzed by GC-MS after cooking, and after 7 days of storage. The formation of lipid oxidation compounds was more pronounced in the bread containing oil in free form than in encapsulated form. This demonstrates that the nanoencapsulation reduced the oxidation products formation in the bread enriched with omega-3 fatty acids.

## 4.3 Identification of PUFA and MUFA Concentrates by FT–IR

The chromatography technique may be considered an invasive indirect method, requiring intensive labor. Furthermore, the sample preparation requires use of solvents and chemicals products, which may be prejudicial to the environment and increase the analysis cost. On the other hand, the attenuated total reflection Fourier transform infrared (ATR–FTIR) spectroscopy is a rapid technique that requires practically no sample preparation (Vongsvivut et al., 2012).

FT–IR analysis has proven to be a fast and effective technique for obtaining information about the oxidation rate of edible oils. Belhaj et al. (2010) prepared different nanoemulsion formulations composed of salmon oil and lecithin with or without the antioxidants addition. The oxidative stability of the samples was accompanied, among other methods, by infrared spectroscopy (FT–IR). It allows for the quantitative or qualitative determination of organic compounds in samples, and the bands intensity in the spectrum is proportional to the concentration. The oxidation increase was monitored by the growth of the absorption band in the hydroxyl region. The results showed that the raw salmon oil was well protected by its own natural antioxidant. The use of marine phospholipids as an emulsifier in nanoemulsions increased the oil salmon stability against oxidation with an increase in availability of long chain PUFA, especially DHA.

Through multivariate statistical analysis of spectra obtained by FT–IR, it is possible to obtain qualitative and quantitative information on the PUFA levels present in the analyzed oil. Vongsvivut et al. (2012) conducted a study to compare the use of gas chromatography techniques and ATR–FTIR in the quantitative analysis and of fatty acids composition in fish oil supplements microencapsulated with gelatin shells. The model accuracy was tested

using an independent validation set of samples in order to evaluate their ability to serve as a quality control method. Spectral data showed a good fit to a linear calibration model ($R^2 = 0.99$). Subsequently, the independent validation set provision, including a part of the standard set and a set of tests has been shown to be highly satisfactory.

FT–IR spectra can demonstrate the presence and the interaction type between the phases. Aghbashlo et al. (2012) examined the effect of wall material composition and the presence of Tween-20 in an emulsion of fish oil microcapsule. For this, the authors obtained the FT–IR spectras of fish oil and of wall materials (skim milk powder–lactose and skim milk powder–maltodextrin), and they produced microcapsules, using these wall materials. From the results, it can be concluded that there was a change in the intensity of oil and wall material bands after capsule production. A physical interaction between the oil and the wall material was assumed, showing good encapsulation characteristics. Banerjee et al. (2013) prepared microcapsules in the multiple emulsion systems. FT–IR was performed to detect the presence of some chemical interactions between the oil and the polymer. Through the spectra, the interactions were stable, and there was no presence of chemical interactions between the microcapsules and the wall materials used.

## 4.4 X-Ray Diffraction (XRD) and Differential Scanning Calorimetry (DSC)

Lipids may present different polymorphic forms. Polymorphism is observed due to the alkane chains organization in packings α (hexagonal), β' (orthorhombic), and β (triclinic). The hexagonal packing α is the most disorderly manner. The orthorhombic form β' is less cluttered and the triclinic β is the most organized. Thus, for industrial applications, it is necessary the control this polymorphism (Bunjes et al., 2007; Allais et al., 2003). The polymorphic structure has direct influence on the encapsulation efficiency and the active expulsion during the storage process; it is very important to their characterization. One of the techniques used to characterize the polymorphic structure of solid lipid nanoparticle is the X-ray diffraction, which determines the length spacing of lipid reticulum. Furthermore, the X-ray diffraction allows checking the amorphous or crystalline material, and serves to evaluate the influence of oil constituent on the nanocrystals spacing of the nanostructured lipid carriers. However, the association of this technique with differential scanning calorimetry technique (DSC) is important. DSC allows to differentiate amorphous solids

and liquids (Ruktanonchai et al., 2008; Mehnert and Mader, 2001; Müller et al., 2000).

Besides the analysis of composing substances of the oils rich in unsaturated fatty acids, much research has also been conducted in order to verify the fortified foods quality. Among these researches, the temperature effect on the processing of these foods can be cited. DSC monitors heat effects associated with phase transitions and chemical reactions as a function of temperature. The reference is an inert material. The temperatures of the sample and of the inert material are increased to the constant rate. During the sample heating, for example, from room temperature to its decomposition temperature, peaks of enthalpy variation can be obtained; each peak corresponding to a heat effect associated with a specific procedure, such as crystallization or melting. The DSC technique can be used to acquire quality information on the physical state of the unsaturated fatty acids concentrates present in the nanoemulsion. DSC curves can be successfully used for obtaining information about the temperature stability of unsaturated fatty acids dispersed in the nanoemulsion liquid phase. The thermograms obtained by DSC analysis provides the sample temperature and fusion heat, and this technique can be used to determine the crystals morphology, which also can be verified by X-ray diffraction (Banerjee et al., 2013; Rey et al., 1993).

Awad et al. (2009) examined the effect of the lipid phase composition containing omega-3 in the crystallization of solid liquid nanoparticles suspensions. The nanoemulsions were cooled to induce crystallization and solid nanoparticles formation. The differential scanning calorimeter was used to study the crystallization, melting, and polymorphic behavior of different emulsion formulations containing different proportions of fish oil and tripalmitin. DSC measurements suggest that crystallization, melting, and polymorphic transitions were influenced by the amount of fish oil added. The crystallization and melting temperatures decreased with the increase in fish oil content, which may be attributed to the formation of less ordered crystals. The results showed that the fish oil, rich in polyunsaturated fatty acids, can be incorporated into the solid liquid nanoparticles suspensions, which can be used for stabilizing fish oils against oxidation.

Salminen et al. (2014) studied the surfactant composition influence on the physical stability of lipid particles with encapsulated fish oil. The differential scanning calorimeter was used to determine the melting and crystallization of the samples. The authors found melting and crystallization temperatures lower for nanostructured lipid carriers containing 20% fish oil, compared to solid liquid nanoparticles with tristearin. The results were

explained by the formation of less ordered crystals. Furthermore, the results indicated that the most likely reason for the weak effect of some super cooling systems was the interfacial heterogeneous nucleation. Surfactants may initiate crystallization interface and subsequently induce crystallization of lipid carriers via heterogeneous nucleation.

Yang et al. (2014), to better understand the polymorphic transformation rate, performed DSC graphs . O/W emulsion with 10% (weight/volume, w/v) of lipid (oil and tristearin) and 2% (w/v) of surfactant were prepared by mixing lipid and aqueous phases. The studies suggested that the polymorphic transformations began during 12 h of cooling. Moreover, the authors found that the oil addition accelerated the polymorphic transformation rate. The crystallization temperature, melting temperature, and melting enthalpy decreased linearly with the oil content increase.

## 4.5 Droplets Size Distribution

The most important physical property is the nanoparticles size, which must be accurately estimated. It may also correspond to a particles size distribution. Light scattering is the most common technique used for size determination of particles in food (Brar and Verma, 2011).

The droplets size distribution present in the nanoemulsions has a great impact on its physical stability and optical properties. Thus, immediately after the manufacturing process, it is very important to determine the droplets size distribution to ensure that the final product met the quality criteria expected. Measurements can be performed by light scattering instruments. It is also important to measure changes in product droplets size distribution during storage or following an accelerated storage test to predict its long-term stability (Piorkowski and McClements, 2014; McClements, 2007). Variations in the dynamic light scattering instrument (DLS) and the adopted analytical procedures can often limit the accurate information about the particles size, which makes it difficult to understand the nanoparticles size dependence. The DLS measures the Brownian motion and relates this movement with a mean hydrodynamic equivalent diameter. Through applying the autocorrelation function and subsequent calculations of the exponential decay, the mean particles size can be calculated from the time dependent fluctuations in light intensity (Lim et al., 2013; Brar and Verma, 2011).

The particle concentration in a size class is usually presented as percent by volume or number. The particle size is normally presented as the particle radius (midpoint, or diameter). The same

particle size distribution may look different if plotted, as volume versus particles size, or particle size versus number. Emulsions for commercial beverages, for example, are always polydisperse systems, which can be characterized as unimodal, bimodal, or multimodal depending whether there are one, two or more peaks in the particles size distribution. The ideal is to obtain a narrow monomodal distribution, which usually provides the best long-term stability. For example, it may be possible to detect a small population of large particles that can cause problems with creaming during storage long-term (Piorkowski and McClements, 2014; Lim et al., 2013). The size and the nutraceutical composition of nanoemulsions containing long chain fatty acids was studied by Cho et al. (2014). The authors demonstrated the importance of defining the exact size of the particles dispersed in nanoemulsions because smaller droplets are digested more rapidly than larger ones. This difference in absorption rate was attributed to the greater surface area exposed to lipid intestinal juices. In their study, they also noted that the bioavailability of fatty acids was higher in nanoemulsions with smaller diameters.

Vyas et al. (2008) developed nanoemulsions containing Saquinavir dissolved in different types of oils rich in polyunsaturated fatty acids. The nanoemulsions were prepared to examine the oral bioavailability and the protease distribution in vital organs, including the brain. The hydrodynamic diameter of oil droplets in the control nanoemulsion and the nanoemulsion containing Saquinavir/PUFA was measured using a light scattering method. The average diameter in nanoemulsions ranged from 100 to 200 nm.

A second important number is the polydispersity, which is a breadth measure of the particle size distribution. The polydispersity is a calculated parameter of the auto-correlation function analysis of dynamic light scattering measurements. In this analysis it is assumed that the particles have one size and a simple exponential fit is applied to the autocorrelation function. The polydispersity values range from 0 to 1. A higher value indicates a less homogeneous distribution in the nanoparticle size. Typically, the diameters measured with DLS varies between 100 and 300 nm and have a polydispersity index lower than 0.3. It is important to know how to interpret intensity, volume, and number. Polydispersity index less than 0.1 is typically referred to as monodisperse. If this is not the case, the sample is polydisperse and has varying particles sizes. Since each size has a different correlation function, broader is the distribution and, worse is the fit. The distributions are multimodal, showing multiple peaks, if the polydispersity is greater than 0.3. This is an additional signal that the sample is polydisperse or contaminant particles are mixed with the sample

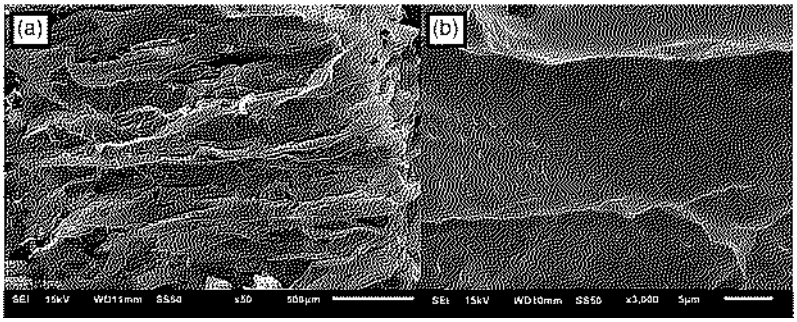


**Figure 3.2.** (a) Highly porous structure obtained by lyophilization of nanoemulsion containing nanocapsules of unsaturated fatty acids concentrates and chitosan (b) nanocapsules of unsaturated fatty acids concentrates present in the microstructure.

(Nanocomposix, 2015; Lim et al., 2013). Nanoemulsions containing unsaturated fatty acids have been studied in the literature, and the particles size and the polydispersity (intensity-based size distribution) are key features in the characterization of these nanoemulsions (Esquerdo et al., 2015; Walker et al., 2015a; Staszewski et al. 2014; Deshpande et al., 2013; Dey et al., 2012).

Staszewski et al. (2014) verified the size and polydispersity of nanocomplexes form of nanoemulsions containing oil rich in omega-3. The initial droplets size and emulsions stability have been improved in the nanocomplexes presence. The droplets had monomodal size distribution. The polyphenols presence decreased the droplets size of emulsions, which was beneficial to the stability. According to Walker et al. (2015a), the oil in the nanoemulsion stability during storage depends on at least of three factors: the initial particles size, the total surfactant concentration, and the preparation method.

## 4.6 Particles Morphology

The particles morphology and aggregation state can be checked using some form of microscopy. The conventional optical microscopy can only be used to study large microstructures ($d > 1000$ nm), being insufficient to study the nanoparticles. In this case, electron or atomic force microscopes are more appropriate. Both techniques have the resolution needed to study nanometric particles (Joye and McClements, 2014).

Scanning electron microscopy (SEM) can provide information on surface characteristics, such as composition and topology. In SEM analysis, the samples should be frozen, dried, or fractured and subsequently coated with metal compounds, allowing that

the sample structure is not altered before the observation (Joye and McClements, 2014). SEM images can verify physical characteristics of nanoparticles after certain storage period. Gallardo et al. (2013) examined linseed oil rich in omega-3 microcapsules for functional food application. The emulsion was dried by a spray dryer to obtain SEM images of the individual microcapsules, recently prepared and after storage of 10 months. It was concluded that no signs of physical damage occurred in the aged samples. Moreover, the SEM images can be used to view the morphology of lyophilized microcapsules, which after drying, in general, presents a porous surface (Fig. 3.2) (Karaca et al., 2013), and also to check the nanoparticles integrity after food addition. Gökmen et al. (2011) included omega-3 nanoencapsulated in breads and verified that the crust particles have been extensively damaged due to more severe thermal conditions during cooking; however, in the bread center, the particles remained intact.

SEM can also detect contamination by bacteria in foods. Nanoemulsion preparations can be used to inactivate the microorganisms growth, for example assess the food-borne bacteria inactivation in fresh lettuce. The lettuce was inoculated with bacteria, and after immersed in the nanoemulsion. The bacteria morphology was examined by SEM, and it could be seen that the bacteria cell surface was remarkably blown, having a reduced amount compared to the control (Bhargava et al., 2015).

Transmission electron microscopy (TEM) is one of the most powerful analysis tools available, which can provide information on the structure and size of the nanoparticles in emulsions. Through the use of short wavelengths with high power achievable electrons, researchers are able to investigate the nanoparticles structure in the atomic level. By analyzing the image supplied by TEM micrograph, it is possible to obtain quantitative results on the nanoparticles size distribution (Lim et al., 2013). The particles inside morphology can also be studied with TEM, following the steps of fixation, dehydration, and sectioning (Chen and Subirade, 2005).

Deutch–Kolevzon et al. (2011) used Cryo-TEM photomicrographs to view microemulsion prepared and loaded with two types of omega-3 fatty acids. Nanostructured lipid carriers (NLC) and solid lipid nanoparticle (SLN) samples had their morphological properties evaluated by Cryo-TEM (Yang et al., 2014). Vyas et al. (2008) used TEM analysis to evaluate the morphology and view the precipitation of the drug upon addition of the aqueous phase. The micrographs were performed to observe the physical properties of the Saquinavir with oils rich in PUFA droplets in nanoemulsions. The droplets sizes were confirmed by DLS analysis.

The Atomic Force Microscopy (AFM) provides the usual topographical analysis, and it can also be performed in submicron-sized magnetic resonance imaging. The resolution is in the range of nanometers and 3-dimensional information can be obtained. The samples for atomic force microscopy are typically subjected to relatively mild preparation procedures, which reduce the risk of damage or alter the sample properties before measurement. Despite all the recent advances, sample preparation, and artifacts for observation are still limiting to the wider use of this technology (Joye and McClements, 2014; Lim et al., 2013).

According to Abbas et al. (2015), AFM and DLS are useful techniques, relatively rapid and noninvasive for the analysis of the size, size distribution, and morphology of polymeric nanoparticles. Such images are useful to enhance the contrast surface and edge characteristics in biological samples and make it easier to distinguish the particle morphology of the background noise. Allied to this, the authors used TEM images to observe the shell thickness and core material morphology.

## 4.7 Droplets Charge and Interfacial Properties

Changes on the nanoparticles surface can be measured by estimating the surface potential, density of functional groups or by the increasing of the surface hydrophilicity. One of the methods for measuring these changes is the determination of the zeta potential of suspensions (Soppimath et al., 2001). The zeta potential reflects the composition of the nanoemulsions interface, in relation to surfactant or the presence of molecules with charge at interface. Zeta potential determination is generally performed using specific electrophoretic techniques. High zeta potential values (above 20 mV), positive or negative, suggests nanocapsules suspensions are more stable because the repulsion between the particles prevents the aggregation. The zeta potential can be an effective way to control the nanoparticles behavior because it indicates changes in the surface potential and repulsive forces between the particles (Piorkowski e McClements, 2014; Roland et al., 2003; Benita e Levy, 1993).

For sterically stabilized emulsions, the droplet charge may not be important in terms of their physical stability, but it may still be important in systems where chemical reactions occur (Piorkowski and McClements, 2014). The zeta potential can be determined in formulating solid-nanoemulsions and nanoemulsions rich in PUFA to indicate the desired stability and surface charge (Ahmad et al., 2014; Vyas et al., 2008). Ahmad et al. (2014) justified the high negative charge due to the presence of the fatty acids anionic

group and glycols present in the oil, surfactant, cosurfactant, and solid carrier.

The phase boundary between the oil and water emulsion is formed in a narrow area surrounding each oil droplet, and contains a mixture of oil, water, and emulsifiers molecules, as well as possibly other molecular species. The interfacial region may affect many important physical and chemical properties and sensory properties of nanoemulsions, including stability, rheology, mouth feel, and flavor. Among the most important properties of the interfacial region are: composition, structural organization, thickness, rheology, interfacial tension, and charge. The electric charge on the droplet interface affects its interaction with other charged molecules, as well as its stability to aggregation. The thickness and rheology of the interfacial region influence the emulsions stability, gravitational separation, flocculation, and coalescence. For this reason, it is important to have an understanding of the droplets interfacial properties in emulsion, and to establish the main affecting factors (Piorkowski and McClements, 2014).

About the rheology, the thinning behavior of emulsions containing unsaturated fatty acids concentrates can be attributed to the formation of droplets agglomerates or aggregates. The flocculated droplets suspensions tend to have a pronounced shear thinning behavior. At low shear rates, the hydrodynamic forces possibly are not large enough to break the bonds holding the particles, and thus the flakes may act as droplets with a fixed size and shape, resulting in constant viscosity. When the shear rate is increased, hydrodynamic forces could become large enough to deform and eventually disrupt the flakes formation. The flakes deformation leads to a reduced viscosity (Dey et al., 2012; McClements and Weiss, 2005). The incorporation effect of nanoemulsion in the rheological properties of gelatin gel was evaluated by Komaiko and McClements (2015). The results showed that the introduction of nanoemulsion droplets in hydrogels had little effect on its rheological characteristics, probably due to the droplets low concentration and the smaller particles fit easily inside the gel pores.

The investigation of the bonds between the wall material and the lipid droplets, and also the influence on the emulsion physicochemical properties can reveal useful information for the formulation of new foods, with different rheological properties (Yuan et al., 2013). Aghbashlo et al. (2012) found flow indexes smaller than 1 for emulsions containing fish oil, showing a shear thinning (pseudoplastic) non-Newton character. The emulsion prepared with 100% skim milk powder and without surfactant showed highest viscosity, which can be assigned to this greater protein content in the aqueous phase. The surfactant incorporation in the

emulsion decreased the interfacial tension between the oil and water phase, which in turn reduced the emulsion viscosity.

The interfacial behavior is an important aspect for the nanoemulsion formation and stability. Proteins, for example, are substances with surface activity used to stabilize emulsions by adsorption to the oil–water interface, thereby reducing interfacial tension and forming a mechanical energy barrier against coalescence at the interface in the emulsion system. Most emulsion systems used in foods and beverages exhibit a shear-thinning behavior, which is important to decrease the viscosity under reflux for consumption (Zhang et al., 2014).

## 5 Advantages and Risks

The nanoparticles size provides numerous advantages compared to microparticles, including high intracellular uptake. In terms of intestinal absorption, besides particles size, the nanoparticles nature, and properties apparently have influence on the absorption by the intestinal epithelium (Reis et al., 2006). Nanoemulsions have great potential to overcome challenges associated with the development of foods and beverages enriched with omega-3 (Walker et al., 2015b).

Lipids-based nanocarriers have the possibility of industrial scale production, and feature advantage of a high encapsulation efficiency and low toxicity. A nanoemulsion containing unsaturated fatty acids concentrates has some interesting physical properties that can be applied to distinguish them from the microemulsion. For example, typically the microemulsions have multiple visible light scattering, and therefore have opaque appearance. In contrast, the droplets sizes in nanoemulsions are much smaller than the visible wavelength, which means that they are optically transparent. This is a very favorable characteristic for nanoemulsions, having application as omega-3 carriers in food and beverage (Fathi et al., 2014).

The rheological properties of nanoemulsions are different than the microemulsions. Nanoemulsions have higher stability against gravity than microemulsions, due to the Brownian movement of the nanosized droplets, caused by entropic driving forces. An interesting characteristic of the nanoemulsions is that they are metastable, and it can be diluted with water, without change in droplets size distribution (Fathi et al., 2014; Gutieirrez et al., 2008; Tadros et al., 2004). Some obstacles must be overcome before the nanoemulsions enriched with omega-3 can be incorporated in commercial food products. Among the obstacles that need be

overcome, the susceptibility to lipid oxidation is one of the most important. This will ensure the physical stability of the system, aiding to distribute a nutritionally beneficial amount of a bioactive in a bioavailable form, and providing a tasty product that would be acceptable to the consumers (Walker et al., 2015b).

Despite the advantages of nanoemulsions containing unsaturated fatty acids concentrates, there are potential risks to human health and environment. Currently, few systematic studies have been conducted dealing specifically the potential toxicity of lipid nanoparticles that may be present in foods and beverages. However, some insights into the nanoparticles potential toxicity can be obtained considering the influence of particles characteristics on their biological destiny. The use of nanotechnologies in the food industry can be hazardous due to the use of new materials in new ways, making important identify and quantify these risks (Cushen et al., 2012).

To improve the food functionality and/or nutritional value, functional ingredients are designed to be smaller than the traditional. If the bioactive component has a very low bioavailability, the absorption by the body can be increased by encapsulating it within the lipid nanoparticles. However, toxic effects, which could not be predicted from the same material on a macroscopic basis, can occur. This can happen, if the bioactive component is incorporated in a product which is consumed regularly in large volumes, such as sodas or drinks. It is possible that the nanoparticles interact with other food materials, being unknown how this would affect the nutrients absorption. In addition, the use of nanoforms may request a review of recommended daily unsaturated fatty acids amount (Cushen et al., 2012).

## 6 Applications in the Food Industry

The small droplets with very high kinetic stability and optical transparency of the nanoemulsions, compared to conventional emulsions, gives advantages for their use in many technological applications (Solans et al., 2005). New farming systems and food security, disease-treatment delivery methods, pesticides, and packaging materials are examples of areas where nanotechnology has been applied (Moraru et al., 2003).

Systems based on emulsions with various structures have been used for delivery of lipophilic food ingredients (eg, omega-3 oils). The emulsions can be provided in liquid, gelled, or powdered forms (Augustin and Hemar, 2009). The most widely delivery systems used for the omega-3 oils incorporation in foods and beverages are the bulk oils, emulsions, and powders. These

powders are typically formed by spray drying emulsions. There are considerable challenges to incorporate omega-3 in many types of functional food products, due to its low water solubility, low chemical stability, and variable bioavailability (Walker et al., 2015b). Microencapsulated powders can be used for blending with other dry ingredients, or incorporated into a food product at different processing stages (for example, omega-3 oil encapsulated powder in infant formulas, breakfast bars, milk, eggs, pates, and yogurt) or reconstituted prior to incorporation in liquid products (Augustin and Hemar, 2009).

Emulsions, such as milk, yogurt drinks, and sauces are ubiquitous in food. The consumption of oil rich in omega-3, incorporated in conventional emulsions, is not always well received by consumers, mainly due to its characteristic smell and tendency to produce an unpleasant reflux. Studies to develop new oral vehicles with better acceptance by consumers and greater bioavailability would be highly beneficial (Dey et al., 2012). The omega-3 nanoemulsions as release systems can be used in the food industry to fortify foods and beverages with these bioactive lipids. They could be used in the pharmaceutical industry to increase the therapeutic bioactivity of fatty acid omega-3. The digestion, bioavailability, and ability to avoid oxidation of the unsaturated fatty acids omega-3 has been studied using nanoemulsions (Esquerdo et al., 2015; Walker et al., 2015a; Cho et al., 2014; Salminen et al., 2014; Staszewski et al., 2014; Deshpande et al., 2013; Dey et al., 2012; Belhaj et al., 2010; Awad et al., 2009; Vyas et al., 2008). Among the foods that can be fortified with omega-3 nanoemulsions and nanocapsules can be mentioned, bread (Gökmen et al., 2011), gelatin (Komaiko and McClements 2015), and yogurt (Chee et al., 2005). Table 3.2 presents some authors who have studied the preparation of emulsions and nanoemulsions with unsaturated fatty acids rich in omega-3 for food purposes.

## 7 Futures Perspectives and Challenges (Main Trends in Nanoemulsions Field)

The food industry has been confronted with a growing demand for food with a low content or without fat. A fat reduction diet can provide inadequate hydrophobic micronutrients amounts, including essential fatty acids. The low omega-3 consumption shows the need for alternative food supplies on the market that provide these fatty acids. The polyunsaturated and monounsaturated fatty acids (MUFA and PUFA) have an important role in the body, preventing disease, being essential in human nutrition.

## Table 3.2 Incorporation Studies of Emulsions and Nanoemulsions of MUFA and PUFA in Foods and Beverages

| Omega-3 Source | Product | References |
|---|---|---|
| EPA and DHA rich fish oil | Oil-in-water nanoemulsion and emulsion. | Dey et al. (2012) |
| Flax seed oil | Omega-3 polyunsaturated fatty acid containing nanoemulsion system for combination C6-ceramide and 17β-estradiol delivery. | Deshpande et al. (2013) |
| Salmon oil and salmon lecithin | Coenzyme Q10 PUFAs nanoemulsions. | Belhaj et al. (2010) |
| Fish oil concentrate | Betulinic acid nanoemulsions stabilized by omega-3 enriched phosphatidylcholine. | Cavazos-Garduño et al. (2015) |
| Flaxseed oil | Functional bread containing nanoencapsulated omega-3 fatty acids. | Gökmen et al. (2011) |
| Fish oil | Fruit juice enriched with nanocapsules of EPA/DHA with sodium caseinate and gum arabic complex. | Ilyasoglu and El (2014) |
| Algae oil | Strawberry flavored yogurt supplemented with an algae oil emulsion. | Chee et al. (2005) |

Coupled with this, there is a growing interest in the research and development, regarding nanoemulsions for foods and beverages applications, due to its advantages, such as transparency, stability, and bioavailability. Nanoemulsions are a promising way to add oils rich in omega-3 to food systems, aiming to protect the oil from oxidation, mask undesirable flavors, and odors and increase the oral bioavailability. Therefore, further studies in this area are clearly needed to improve the nanoemulsions production and to obtain formulations that add high stability, higher shelf life, and satisfactory physical characteristics. Toxicological studies should be also conducted to ensure that the new technologies are safe for widespread use in food and beverages business.

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# 4

# NANOFORMULATIONS OF POLYPHENOLS FOR PREVENTION AND TREATMENT OF CARDIOVASCULAR AND METABOLIC DISORDERS

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## 1 Introduction

Polyphenols are found naturally in fruits, vegetables, cereals, and beverages. Fruits such as, grapes, apples, pears, cherries, and berries contain 200–300 mg/100 g (fresh weight) of polyphenols. The process products of fruits also contain significant amounts of polyphenols. Cereals, dry legumes, and chocolate contribute largely to the polyphenolic intake (Manach et al., 2005; Spencer et al., 2008). Kuhnau (1976) advised the total dietary intake of polyphenols to be about 1 g/d in the United States; nevertheless, the uncertainty in the polyphenol intake persists and hence, the variations remain.

Polyphenols are secondary metabolites produced in plants to protect them from ultraviolet radiation and attack of pathogens (Williams et al., 2004). They are used as food supplements and in the cosmetic industry. As food ingredients, they contribute to the bitterness, astringency, color, flavor, odor, and oxidative stability. They have received considerable scientific interest because of their possible beneficial effects on human health. Chemically, this is a group of natural compounds with phenolic structures; and is a collective term for a number of subgroups of phenolic compounds. Studies have reported that polyphenols differ significantly in their stability, bioavailability, and physiological functions.

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00004-0

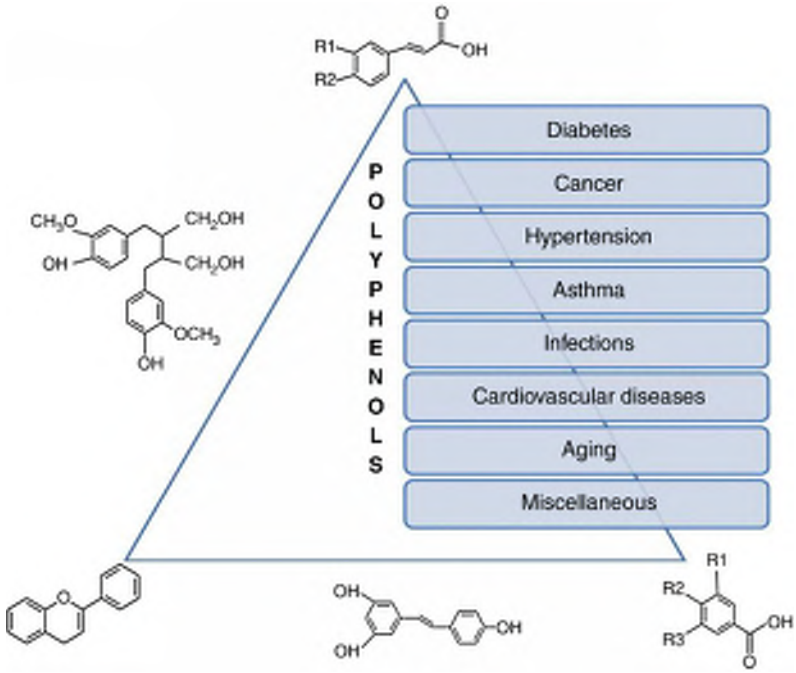


**Figure 4.1. Health benefits of dietary polyphenol.**

Studies have strongly supported the role for polyphenols in preventing different types of cancers, cardiovascular, and neurodegenerative diseases, and inflammation (Hodgson, 2008; Kumar and Pandey, 2013) (Fig. 4.1). Polyphenols are strong antioxidants and both; in vitro and in vivo studies have indicated that its antioxidant property is about 10 times higher than that of vitamin C and 100 times higher than those of vitamin E and carotenoids. These complement and add to the antioxidant function of vitamins and enzymes and prevent oxidative stress caused by reactive oxygen species (ROS). Modulatory effects of polyphenols on cell signaling pathway may aid in explaining the mechanism of the action of polyphenol-rich diets (Williams et al., 2004).

## 1.1 Distribution, Classification, and Structure of Polyphenols

More than 8000 polyphenolic structures have been currently identified and among them more than 4000 are flavonoids (Bravo, 1998; Cheynier, 2005). They range from simple to highly polymerized phenolic structures with molecular weights >30 kD.

Polyphenols are traditionally considered antinutrient due to the inhibitory action of tannins (one type of polyphenol) on protein digestibility. However, owing to the antioxidant properties of dietary polyphenols, the interest in these compounds has increased greatly in recent years (Bravo, 1998).

Several different kinds of polyphenols have been identified in food (Manach et al., 2005). They are present in fruits, vegetables, cereals, chocolate, whole grains, and beverages like coffee, tea, and wine. Polyphenols exist as aglycone as well as glycosides. Glycosides have either neutral and acylated sugars at different positions. Generally, polyphenols are classified according to their chemical structure. The polyphenols are classified broadly into flavonoids and phenolic acids. The former comprise of flavones, flavonols, flavanols, flavanones, isoflavones, proanthocyanidins, and anthocyanins (Fig. 4.2). A few examples of most commonly found flavonoids are:

- Quercetin: a flavanol abundantly found in onion, tea, and apple
- Catechin: a flavanol found in tea and fruits

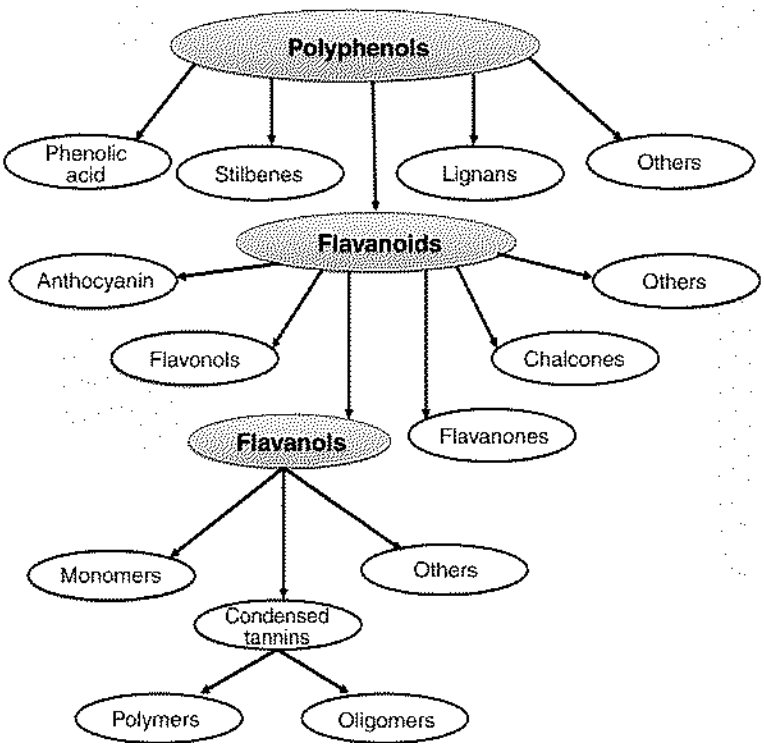


**Figure 4.2. Different classes of dietary polyphenols.**

- Hesperetin: a flavanone seen in citrus fruits
- Cyanidin: an anthocyanin imparting color to fruits, such as blackcurrant, raspberry, strawberry
- Daidzein: an isoflavone found in soybean
- Proanthocyanidins: common in many fruits, such as apple, grape, or cocoa and are responsible for their characteristic astringency or bitterness
- Caffeic acid: present in fruits and vegetables that are esterified with quinic acid to form chlorogenic acid, as seen in coffee
- Ferulic acid: present in cereals and is esterified to hemicelluloses in the cell wall.

## 1.2 Phenolic Acids

These polyphenolic compounds are categorized into derivatives of benzoic and cinnamic acid. Benzoic acid derivatives have C1–C6 backbone whereas cinnamic acid derivatives have C3–C6 backbone (Fig. 4.3). Both of these originate from aromatic amino acid, L-phenyl alanine. Fruits, vegetables, and grains contain phenolic acids that are distributed throughout the plant including seeds, leaves, roots, and stems. The majority of these are linked through ester, ether, or acetal bonds to the structural components of the plant (cellulose, proteins, and lignin), to larger polyphenols (flavonoids), smaller organic molecules (glucose, quinic, maleic, or tartaric acid), or other natural products (terpenes) (Clifford 1999b; Lam et al., 2001); and could be hydrolyzed upon acid or alkaline hydrolysis, or by enzymes.

## 1.3 Stilbenes

These contain two phenyl moieties connected by a methylene bridge and they are found in fewer amounts in diet. Most stilbenes in plants are antifungal phytoalexins that are synthesized in response to injury or infection. Resveratrol (3,4',5'-trihydroxystilbene) is the most common stilbene found in grapes and red wine (Fig. 4.3).

## 1.4 Lignans

These are diphenolic compounds with 2,3-dibenzylbutane structure formed by the dimerization of two cinnamic acid residues (Fig. 4.3). Secoisolariciresinol, a lignin, is considered as an estrogen. Linseed has high amounts of secoisolariciresinol (3.7 g/kg dry weight) and low amounts of matairesinol.

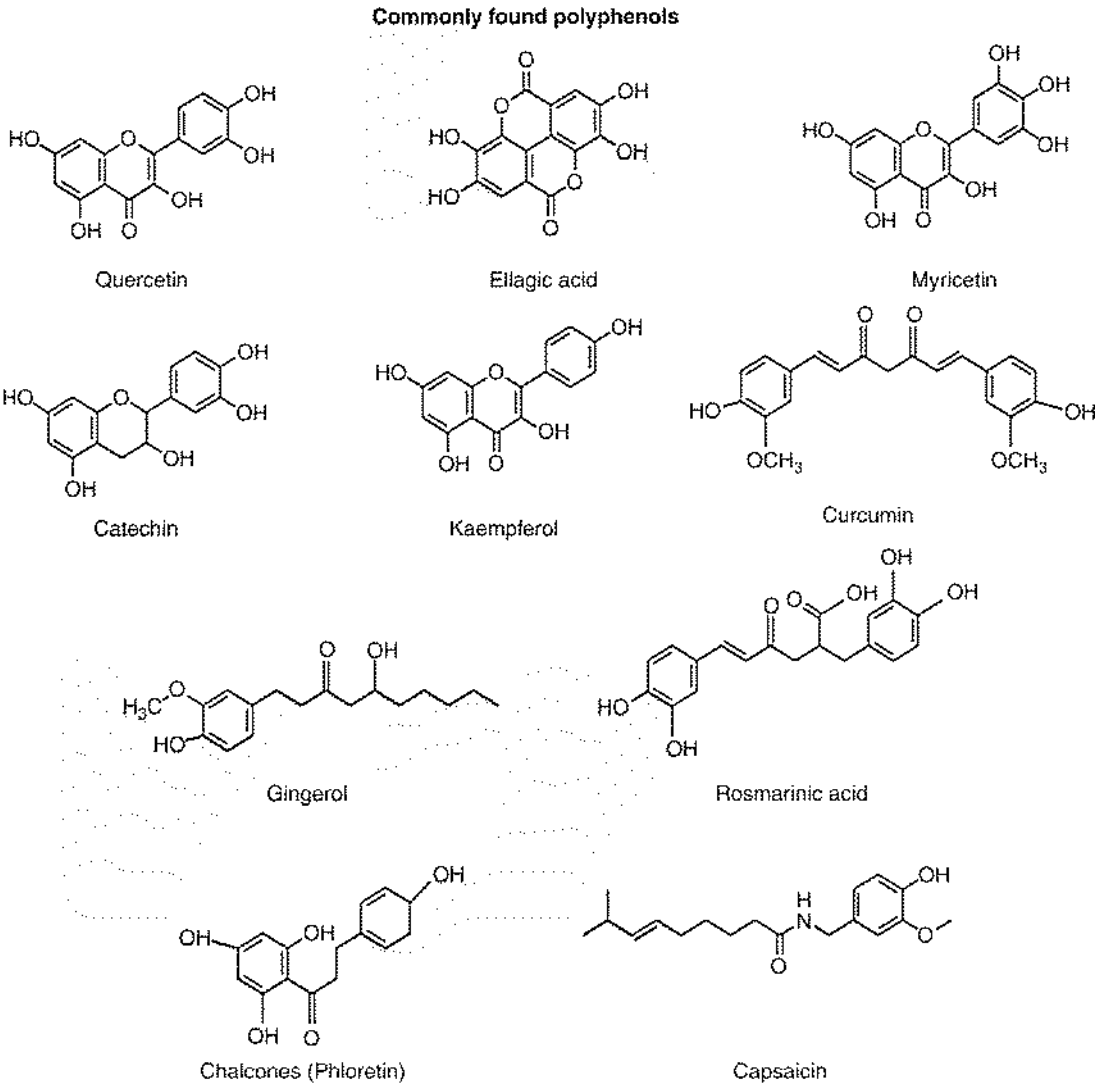


**Figure 4.3. Chemical structure of common polyphenols.**

## 1.5 Flavonoids

These are 15 carbon skeleton compounds having C6–C3–C6 structural backbone. Both the C6 units are phenol rings while C3 unit is heterocyclic. Flowers, fruits, and leaves are a rich source of flavonoids and more than 4000 varieties have been identified to date. Flavonoids are divided into six subclasses, namely, anthocyanins, flavanols, flavones, flavanones, isoflavones, and flavonols, depending on variations in the heterocyclic ring. The general

structure of flavonoids have phenol ring (ring B) attached to the C2 position of heterocyclic ring (ring C), while isoflavones and neoflavonoids have phenol ring attached to C3 and C4 positions of heterocyclic ring, respectively. Chalcones, another subgroup of flavanoids, are basically aglycones that exist as glycosides in plants. Quercetin, myricetin, catechins, and so on, are commonly found flavonoids (Fig. 4.3).

### 1.5.1 Isoflavones, Neoflavonoids, and Chalcones

Isoflavones are most commonly found in the leguminous family of plants having phenyl ring B attached to the C3 position of ring C. Soybean isoflavones play a major role in human health. Genistein, daidzein, glycetein, biochanin A, and formononetin are different isoflavones found in soy and red clovers. They are generally present as glycosides in plant. Genistein, daidzein, and glycitein are found at a concentration of 1:1:0.2 ratios in soy and its process products. Neoflavonoids are rarely present in food material. Dalbergin is an example of neoflavone found in plants (Coward et al., 1998). Chalcones have different structure than other flavonoids. The C3 unit is open-ring and they are usually found in apples and hops (Tsao et al., 2003; Zhao et al., 2005).

### 1.5.2 Flavones, Flavonols, Flavanones, and Flavanonols

Flavones and their 3-hydroxy derivatives, flavonols, form the largest subgroup of polyphenols and have glycosides, methoxides and acylated products on all their three rings. Quercetin and kaempferol, commonly seen flavonols, have a minimum of 279 and 347 different glycosidic combinations, respectively. Flavanones have unique substitution patterns (prenylated flavanones, furanoflavanones, pyranoflavanones, benzylated flavanones) and they form the largest group with substituted derivatives. Taxifolin is a commonly seen flavanonol in citrus fruits (Tsao, 2010).

### 1.5.3 Flavanols, Proanthocyanidins, and Anthocyanidins

Flavanols (flavan-3-ols or catechins) have no double bond between C2 and C3 and no carbonyl at C4 in ring C. So, there are two chiral centers (C2 and C3), and hence the molecule exists in four diastereoisomers. Common examples of flavanols are catechin (*trans*) and epicatechin (*cis*), which exists in two steroisomeric forms. (+)-Catechin and (−)-epicatechin are often found in food plants. Skins of grapes, apples, and blueberries are also rich in flavanols (Tsao et al., 2003). These monomeric flavanols and their derivatives (gallocatechins) are found abundantly in tea leaves and cacao beans (chocolate) (Prior et al., 2001). Catechin

and epicatechin can form polymers (proanthocyanidins), which produce anthocyanidins in the presence of an acid catalyst.

Proanthocyanidins are oligomers (2–7 monomers), often referred to as condensed tannins. These are strong antioxidants with potential health benefits. Based on the interflavanic linkages, proanthocyanidins could be classified into type-A (C2–*O*–C7 or C2–*O*–C5 bonding) and type-B (C4–C6 or C4–C8). Examples of proanthocyanidin commonly found in nature are procyanidin (trimer) and theaflavin (dimer), which are formed due to fermentation of tea flavanols.

Anthocyanidins, principally found in the red, blue, and purple pigments of flower petals, fruits, vegetables, and certain grains (black rice), are glycosides that include cyanidin, delphinidin, and pelargonidin and more than 24 other monomeric anthocyanidins (Total-31 anthocyanidins). Most of the anthocyanins are based on cyanidin, delphinidin, and pelargonidin with methylation, methoxylation, hydroxylation, and glycosylation. The most significant physical parameter of anthocyanin is color, which is pH dependent. It turns into red in acidic and blue in basic conditions. Degree of hydroxylation, methylation and glycosylation also affect the color. However, anthocyanins are chemically stable in acids (Clifford, 2000).

## 1.6 Polyphenolic Amides

Polyphenolic amides have substitution at N- of the amide linkage and two such polyphenolic amides with health benefits are capsaicinoids (capsaisin found in chili pepper) and avenanthramides (oats) (Davis et al., 2007). Capsaicin has shown strong antioxidant and antiinflammatory properties; and modulates the oxidative defense mechanism in cells. Avenanthamides are known for their antioxidant properties and they inhibit LDL oxidation.

## 1.7 Others

In addition to the aforementioned polyphenols, there are some nonflavonoid polyphenols that are included in this category, which have significant health benefits. These are commonly seen in various foods, such as grains, nuts, berries, and turmeric. Ellagic acid and its derivatives (berry fruits and in the outer shell of various nut fruits), lignans (flax, sesame, and several grains), curcumin (turmeric), and rosmarinic acid (caffeic acid) are some of the recommended nonflavonoid polyphenols for its health benefits. Rosmarinic is a dimer of caffeic acid and ellagic acid is a dimer of gallic acid. These polyphenols are known for their antioxidative

properties and few of them may possess antinutritive properties (Tsao, 2010).

## 2 Biological Application and Health Effect of Polyphenols

Polyphenols are secondary metabolites produced in plants as a defensive mechanism against other organisms and have shown potential health benefits against several noncommunicable diseases. Epidemiological reports indicate that intake of polyphenol containing food in high amounts reduces the risk of carcinogenesis, inflammatory, and degenerative diseases, and cardiovascular diseases (CVD) (Checkoway et al., 2002). Polyphenols have been shown to act as antioxidants, anticancer, antitumor, antiproliferative, and antiinflammatory agents. Flavonoids have been strongly linked to health benefits in human, animal, and in vitro studies (Schroeter et al., 2001). In CVD, they have been reported to alter lipid metabolism, inhibit low-density lipoprotein (LDL) oxidation, minimize the formation of atherosclerotic lesions and platelet aggregation and the expression of vascular cell adhesion molecules (Ludwig et al. 2004; Jeong et al. 2005; Hubbard et al. 2006). They also reduce blood pressure but improve endothelial function (Hubbard et al., 2006). Flavonoids also exert beneficial cognitive effects and invalidate specific age-related neurodegeneration (Joseph et al., 1999). They also show anticarcinogenic effects by inducing apoptosis (Fabiani et al., 2002; Fini et al., 2008), inhibiting cell proliferation (Corona et al., 2009) and preventing angiogenesis and invasion of tumor cells (Piao et al., 2006) (Fig. 4.4).

### 2.1 Polyphenol in the Prevention of Cellular Oxidation

Recently, noncommunicable diseases such as cancer, cardiovascular diseases, chronic inflammation, and other degenerative diseases have been related to oxidative stress due to reactive oxygen and nitrogen species. Polyphenols as strong antioxidants are reported to neutralize free radicals either by donating an electron or a hydrogen atom. These suppress the generation of free radicals and deactivate the active species and their precursors. The 3-hydroxy flavonols are considered as important antioxidants due to their highly conjugated system and hydroxylation patterns. These also act as direct radical scavengers in the lipid peroxidation chain reaction process and donate electrons to the free radical, thus neutralizing the latter and making them stable

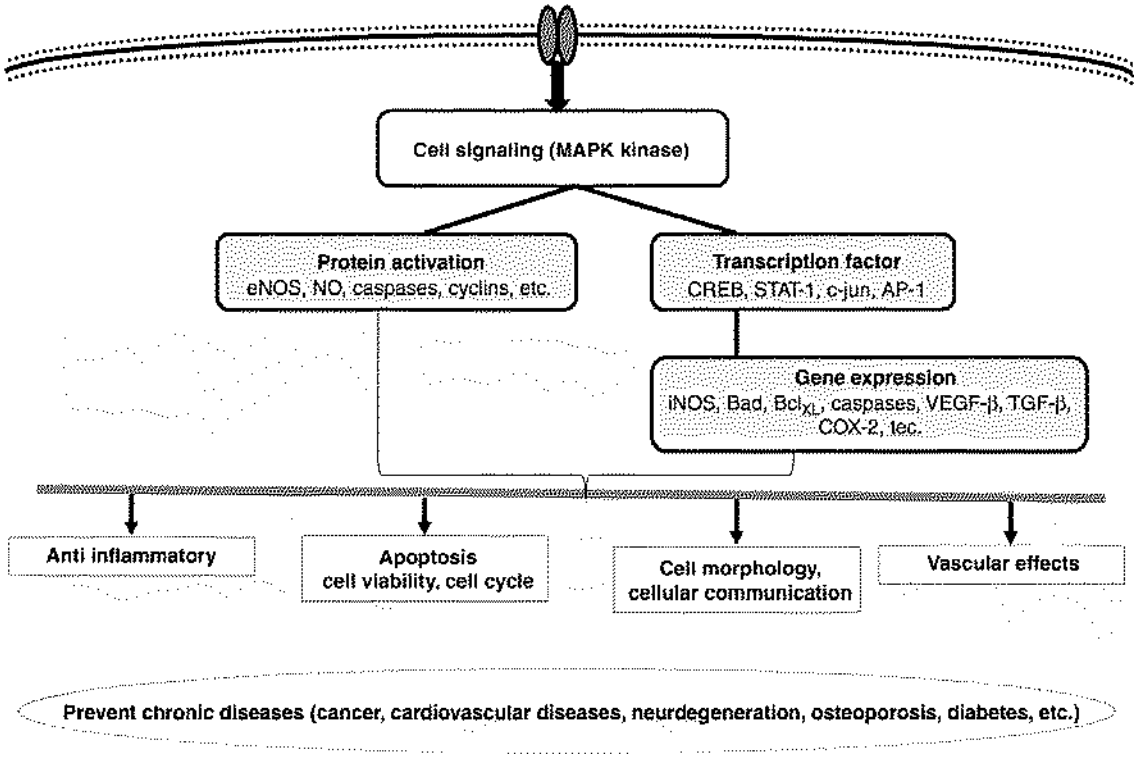


**Figure 4.4. Polypheols avert major diseases such as, cancer, cardiovascular diseases, neurodegenerative diseases, diabetes, osteoporosis.**

(Rice-Evans et al., 1996; Guo et al., 2009). Polyphenols also act as metal chelators and aid in chelation of transition metal, $Fe^{2+}$, that is involved in the reduction of the Fenton reaction and thus prevents oxidation by hydroxyl radicals (Pietta 2000; Perron and Brumaghim, 2009). Polyphenols also act as coantioxidant and aid in the regeneration of essential vitamins (Zhou et al., 2005).

Polyphenols are reported to promote levels of endogenous antioxidants such as, glutathione peroxidase, catalase and superoxide dismutase that degrade hydroperoxides, hydrogen peroxide, and superoxide anions, respectively. They are also known to inhibit xanthine oxidase (Du et al., 2007). Apart from the conventional mode of antioxidation through hydrogen- or electron-donation, polyphenols have been hypothesized to affect protein kinase and lipid kinase signaling pathways and exert beneficiary effects on cells (Williams et al., 2004). Due to their smaller particle size, flavonoids are absorbed directly through the gastrointestinal tract and metabolized in the tissues; however, their concentration in the plasma are observed to be low (<1 μmol/L) (Manach et al., 2005).

At this concentration, they may not exhibit antioxidant activity, hence attention is directed beyond the usual antioxidant activities in vivo (Williams et al., 2004; Halliwell 2008).

## 2.2 Polyphenols in the Treatment of Cancer

Cancer is a heterogeneous and multifaceted disease involving an unbalanced control of cellular proliferation. Major types of cancer include breast, lung, colorectal, and prostate cancers, which accounts for more than 50% of all the cases. Case-control, epidemiological, and cohort studies have shown an inverse relationship between the regular intake of fruits and vegetables and the development of cancer (Benetou et al., 2008). However, this was not same for the cancer incidences in bladder, pancreatic, and stomach (Benetou et al., 2008; Boffetta et al., 2010). Despite this, polyphenols found in fruits and vegetables have been reported to exert protective effects against gastrointestinal cancer (Manson, 2003). Polyphenols from tea, red wine, cocoa, fruit juices, and olive oil have influenced carcinogenesis and tumor development at cellular level, possibly by interacting with the reactive intermediates and activated carcinogens and mutagens, and by modulating the activity of proteins (Plaumann et al., 1996; Middleton et al., 2000) and expression of cancer-associated genes (Van Erk et al., 2005). Intake of green tea flavanols has also been reported to minimize the cancer risk in biliary tract (Zhang et al., 2006), bladder (Rieger-Christ et al., 2007), breast (Leong et al., 2008), and colon (Larsen and Dashwood, 2010). This could be due to the high amounts of epigallocatechin gallate that induces apoptosis (Khan et al., 2006).

Phenolic alcohols, lignans, and secoiridoids (found in olive oil) have shown to inhibit initiation, promotion, and metastasis in human colon adenocarcinoma cells and downregulate the expression levels of COX-2 and Bcl-2 proteins in vivo (Llor et al., 2003; Gill et al., 2005). Polyphenols aid in the removal of carcinogens (Owen et al., 2000), modulate the cancer cell signaling pathway via MAPK kinase and PI3K (Khan et al., 2006; Corona et al. 2007) and cell cycle progression (Corona et al., 2009), promote apoptosis (Fabiani et al., 2002; Fini et al., 2008) and modulate the activities of the enzymes including glutathione peroxidase, catalase, NADPH-quinone oxidoreductase, glutathione S-transferase, and cytochrome p450 (Adams and Chen, 2009), thus preventing carcinogenesis. Polyphenols also inhibit p38/CREB signaling pathway leading to the decrease in the expression of COX-2 levels and arrest of cell cycle at G2/M phase (Corona et al., 2007). Further, epicatechin and its dimmer, and hydroxytyrosol are reported to

hinder ERK1/2 phosphorylation and lessen the expression levels of cyclin D1, leading to cell cycle arrest (Guichard et al. 2006; Fantini et al., 2015). Epigallactocatechin gallate and hydroxytyrosol also inhibit COX-2 expression and minimize the colorectal neoplasia in colorectal cancer (Chell et al., 2006). Certain polyphenols thwart the advanced glycation end product (nonenzymatic glycation of proteins due to high glycolysis and glucose intake) formation in vivo and in vitro and limit the carcinogenesis and tumor formation (Lo et al., 2006; Sang et al., 2007).

## 2.3 Polyphenols in the Treatment of Cardiovascular Diseases

CVD is chronic and multifactorial that involves genetic and environmental factors. Epidemiological and human intervention studies suggest that regular intake of fruits, vegetables, cocoa, tea, and wine may exert cardio-protective effects due to the presence of high amounts of polyphenols in them (Arts et al., 2000; Mink et al., 2007). Intake of flavonols, flavones, flavanols, anthocyanin, and flavanone has been associated with the reduced risk of CVD (Mink et al., 2007). A review on soy and cocoa flavonoids indicated that these aid in reducing cardiovascular risk (Hooper et al., 2008). Various in vitro and in vivo studies suggest that polyphenols exert antioxidant properties on the vascular system (Rein et al., 2000); thus lower blood pressure (Desch et al., 2010), improve endothelial function (Grassi et al., 2009), inhibit platelet aggregation (Pearson et al., 2002), oxidize LDL (Mathur et al., 2002) and reduce inflammatory responses (Mao et al., 2002). Hypertension and CVD incidence are found to be considerably reduced in the Kuna Amerinds of the San Blas Island in Panama due to regular intake of dietary polyophenols (Hollenberg et al., 1997). Meta-analysis studies have also confirmed the blood pressure–lowering capacity of flavanol-rich cocoa (Desch et al., 2010). Similarly, consumption of black tea was also reported to decrease blood pressure (Yang et al., 2009).

It has been suggested that polyphenols would modulate the level and activity of endothelial nitric oxide synthase (eNOS) and bioavailability of nitric oxide (NO) (Leikert et al., 2002; Appeldoorn et al. 2009). Aortic ring experiments supplemented this view, where polyphenols at various physiological concentrations have shown to induce endothelium-dependent relaxation (Chin-Dusting et al., 2001; Woodman and Chan, 2004) due to the regulation of vascular nitric oxide owing to its interaction with PI3-kinase/Akt pathway and intracellular $Ca^{2+}$ on eNOS phosphorylation leading to NO production (Lorenz et al., 2004; Stoclet et al., 2004).

Apart from these, polyphenols also induce the production of prostacyclin, inhibit endothelin-1 and endothelial NADPH oxidase (Steffen et al., 2008). It also restrains angiogenesis, migration, and proliferation of vascular cells, and activates matrix metalloproteinase (MMP) (Stoclet et al., 2004). Inhibition of platelet activation and aggregation is another mechanism of action exhibited by polyphenols in preventing CVD (Freedman et al., 2001). Flavanols and flavonols regulate MAPK signaling and NF-κB leading to the suppression of NADPH oxidase, thus preventing vascular injury (Peppa and Raptis, 2008; Kim et al. 2010).

## 2.4 Polyphenols in the Treatment of Neurodegenerative Disease

Neurodegenerative disorders (Parkinson's and Alzheimer's diseases) represent a growing threat to society and are prompted by neuroinflammation, glutamate excitotoxicity, oxidative stress, iron and/or depletion of endogenous antioxidants (Jellinger, 2001; Barzilai and Melamed 2003). Epidemiological studies recommended that moderate consumption of wine may reduce the incidence of Alzheimer's disease (Lindsay et al., 2002). Flavonoids have been associated positively to suppress dementia, Alzheimer's disease, and Parkinson's disease in the aged groups and improve cognitive performance (Dai et al., 2006; Letenneur et al., 2007). Hesperetin, naringenin, and their metabolites traverse the blood-brain barrier (BBB) in vitro and in situ models (Youdim et al., 2004). Similarly, availability of anthocyanins is also reported in the cortex and cerebellum of rat and pig (Kalt et al., 2008; Milbury and Kalt, 2010). This movement of polyphenols through BBB with no structural variations enables them to act as neuroprotective and neuromodulatory agents.

The mechanism of action of flavonoids could be primarily due to the protection of vulnerable neurons, enhancement of the existing neuronal function and stimulation of neuronal regeneration (Youdim and Joseph, 2001). Polyphenols, due to their high antioxidative properties, protect neurons against oxidative stress (Innami et al., 1998) and Aβ-induced neuronal damage (Luo et al., 2002). Polyphenols from *Ginkgo biloba* has neuroprotective properties and protect hippocampal neurons from NO- and beta-amyloid-induced neurotoxicity (Bastianetto et al., 2000). Anthocyanins and isoflavones also thwart neurodegeneration caused by the accumulation of advanced glycation end products (AGE) during normal and abnormal brain ageing (Ramasamy et al., 2005). Citrus flavanone (tangeretin) serves as a potential neuroprotective agent against Parkinson's disease by maintaining nigro-striatal integrity

and functionality (Datla et al., 2001). Caffeic acid and tyrosol protect against 5-*S*-cysteinyl-dopamine and peroxynitrite neurotoxicity in vitro (Vauzour et al., 2010). Flavonoids also interact with neuronal signaling to protect against neurotoxicity induced by AGEs (Lee and Lee, 2007). Further, polyphenols are reported to improve memory, learning, and general cognitive ability (Rendeiro et al., 2012). Fruit and vegetable polyphenols have also shown to influence memory and depression in human subjects (Krikorian et al., 2010). Blueberries and strawberries were reported to improve spatial working memory (Williams et al., 2008), object recognition memory (Goyarzu et al., 2004), and modulate inhibitory fear conditioning (Barros et al., 2006). Quercetin, rutin, and fisetin invalidate neuronal and behavioral aging (Pu et al., 2007); and *G. biloba* polyphneols promote inhibitory avoidance conditioning in vivo (Topic et al., 2002).

Another mechanism of action of polyphenols on cognition and against neurodegenerative diseases could be due to their interaction with neuronal and glial signaling that influence gene expression and cell death. Flavonoids modulate protein and lipid kinase signaling pathways by altering MAPK signaling cascades possibly by downregulating nuclear factor-Kappa B (NF-κB) that respond to p38 signaling and is involved in iNOS induction (Wang et al., 2005; Spencer 2007). Dietary polyphenols, thus demonstrate a beneficial role on the cell signaling pathways, transcription factors and cytokines in the improving neural responses and preventing neurodegenerative diseases.

## 2.5 Polyphenols in the Treatment of Diabetes

Diabetes mellitus is a metabolic syndrome that in the long term may cause severe health effects and they are of two types: insulin dependent diabetes mellitus (IDDM, type 1 diabetes mellitus) and noninsulin dependent diabetes mellitus (NIDDM, type-2 diabetes mellitus). The latter is most common and is related to family history, age, obesity, and lack of exercise. Administration of polyphenols in animals has been reported to influence glycemia. Caffeic acid and isoferulic acid are reported to reduce the fasting glycemia and attenuate plasma glucose in vivo (Hsu et al., 2000). Acyl substituted anthocyanin also reduce glycemia induced by maltose in rats (Matsui et al., 2002). Prodelphinidins and 4-hydroxybenzoic acid also reduce the fasting glycemia in rats and lower their plasma glucose level (Geetha et al., 1994; Peungvicha et al., 1998). Catechin improves the glucose tolerance while similar observations are reported for fermented tea extract in vivo (Shenoy, 2000). *P*-hydroxybenzoic acid, which shows hypoglycemic effects in vivo,

were reported to have no effects on insulinemia and hepatic glycogen (Peungvicha et al., 1998).

The mechanism of action of polyphenols could be through the inhibition of glucose absorption in the gut and peripheral tissues. The hypoglycemic effects of acyl-substituted anthocyanins are due to the inhibition of α-glucosidase in the gut mucosa. α-Amylase and sucrase inhibition by catechin are reported to cause improved glucose tolerance in rats (Matsumoto et al., 1993). Inhibition of intestinal glycosidases and glucose transporter by polyphenols are reported in vitro (Matsui et al., 2002). Quercetin inhibits the glucose transport by GLUT2 in a transfected oocyte model and subdues the glucose absorption in Zucker obese rats (Perez-Vizcaino and Duarte, 2010). Quercetin derivative (quercetin 3-*O*-glucoside), tannic acid, and chlorogenic acid at higher concentrations inhibit the $Na^+$-dependent hexose uptake in everted gut sacs (Gee et al., 2000). Glucose absorption is not only limited in the small intestine but also in the kidney by phlorizin. Caffeic acid increases glucose uptake in rat adipocytes and mice myoblasts indicating the role of peripheral tissues in glycaemic regulation (Cheng and Liu, 2000). Tea extracts and EGCG also contribute similarly in rat epididymal adipocytes in both control and insulin treated cells (Anderson and Polansky, 2002). Soleus muscle cells from streptozotocin-induced diabetic rats also show similar performance in the presence of isoferulic acid (Liu et al., 2000). However, quercetin and genistein exhibit contradictory behavior in vitro possibly by inhibiting GLUT1-mediated glucose transport (Strobel et al., 2005). These contradictory behaviors could be explained by the different nature and functionalities of polyphenols, which include inhibition of gluconeogenesis, adrenergic stimulation of glucose uptake, or the stimulation of insulin release by pancreatic β-cells. Flavonoids act as insulin secretagogues or insulin mimetics (Cazarolli et al., 2008). Some polyphenols hinder GLUT1 glucose transporter and insulin response, thus interfering with glucose uptake by peripheral tissues (Strobel et al., 2005).

In humans, the effect of polyphenols on glycemia and diabetes are still vague. Decaffeinated coffee does not affect glycemic content or insulinemia in humans when it is ingested along with the glucose. However, it decreases the secretion of glucose-dependent insulinotropic polypeptide and increases the glucagon-like peptide 1 in order to maintain a consistent delay in the intestinal glucose absorption (Johnston et al., 2003). Polyphenols reduce the risk of diabetic complications, as AGE products generate oxidative stress. It also reduces renal damage as observed by curcumin in diabetic rats (Giacco and Brownlee, 2010). Chlorogenic acid has

been associated with a diminished risk of type-2 diabetes (Van Dam and Feskens 2002). Decaffeinated coffee lowers postprandial level of glucose-dependent insulinotropic-polypeptide (GIP) and enhances glucagon-like peptide-1 (GLP-1), suggesting the significance of chlorogenic acid in decreasing the rate of intestinal absorption of glucose (Johnston et al., 2003). Hydroxycinnamic acid, ferulic acid, p-coumaric acid, and eugenol alone and in combination with commercial hypoglycemic drugs (thiazolidinedione and metformin) were reported to be beneficial in the treatment of diabetes mellitus. Chlorogenic and cinnamic acids increase the expression of PPARγ, whereas hydroxycinnamic acids enhanced the expression of PI3K. These also reduce the expressions of the fatty acid synthase and HMG CoA reductase genes; thus reducing the secondary complications caused by lipid accumulation (Prabhakar and Doble, 2009).

## 2.6 Polyphenols in the Treatment of Osteoporosis

Bone loss and osteoporosis are frequently seen in postmenopausal women. Hormone replacement therapy is often chosen; yet, the patients are reluctant to follow it for its possible side effects and long-term risks. Isoflavone due to its weak estrogen-like activity has been used as an alternative for osteoporosis treatment in menopasuse women and has been investigated in vivo (Kuiper et al., 1998). It is observed that genistein, daidzein, or their glycosides are able to prevent the loss of bone mineral density and trabecular volume in ovariectomy animals (Nakajima et al., 2001; Picherit et al., 2001). Soy protein with normal or reduced isoflavone content also shows osteoprotective effects in ovariectomized rats (Genant et al., 1989; Wei et al., 2012). However, it is observed that isoflavones may restore the bone mineral density after continuous treatment but not osteopenia in these animals after ovariectomy (Picherit et al., 2001). The mechanism of action of isoflavones on osteoporosis has been scanty. Daidzein inhibits the differentiation of osteoclasts developing on dentine slices and reduces the dentine resorption in vitro, while daily subcutanous injection of genistein to ovariectomized rats increases the number of osteoblasts but has no effects on bone resorption (Rassi et al., 2002). Rutin (glycoside of quercetin) restores the bone mineral density in ovariectomized rats and is more efficient than isoflavones (Horcajada-Molteni et al., 2000). It also improves the bone resorption and osteoblastic activity. Catechins have adverse effects on bone mineral density and bone metabolism (Hegarty et al., 2000).

Human intervention and observational studies indicate that soybean consumption is associated with high bone mineral

density among Japanese women (Tsuchida et al., 1999). Intake of soy-rich diet in postmenopausal women stimulates bone osteoblastic activity indicating the functionality of isoflavones on bone health (Alekel et al., 2000; Chiechi et al., 2002). Furthermore, isoflavone supplementation (37–62 mg/d) for long periods of time significantly improves the urinary excretion level of several biomarkers of bone resorption (Morabito et al., 2002). In addition, the femur and lumber spine mineral density also show significant improvement with the intake of dietary polyphenols.

The health benefits of polyphenols are enormous and these vary with their structure. Further, these benefits also depend on their bioavailability and mode of administration.

## 3 Dietary Intake of Polyphenols

Polyphenols, being the secondary plant metabolites, are not synthesized in the human body, and hence are obtained through the dietary components including beverages (coffee, tea, beer, and red wine), fruits (apples, pomegranates, citrus fruits, and grapes), onions, chocolates, vegetables, and soybean derived products (El Gharras, 2009). The daily consumption of total flavonoids through the diet in the United States is calculated to be 1 g. It consists of 45% biflavones, 20% catechins, 17% anthocyanins, and 16% flavonols, flavones, and flavanones (Manach et al., 2004; Scalbert and Williamson, 2000). The intake of polyphenols varies greatly due to the dietary habits and preferences, and availability of particular food in a locality. Flavanone intake is more in southern Europe where citrus fruits are produced. The dietary intake of isoflavones is greater in Asians when compared to Europeans and Americans. This is due to the high consumption of soy products (10–35 g/d), which chiefly contain isoflavones. The intake of flavonols is in the range of 5–125 mg/d in Italy. In Germany, the consumption of phenolic acids ranges 6–987 mg/d (Manach et al., 2004). Coffee is a rich source of phenolic acids. Phenolic acid intake is comparatively more in heavy coffee drinkers. The intake of hydroxycinnamic acids can go up to 800 mg/d (Clifford, 1999a).

Accurate measurement of intake of a dietary polyphenol is necessary for the assessment of its health benefits. Methods such as food-frequency questionnaires, 24-h dietary recalls, and food diaries are generally used to estimate the dietary intake of polyphenols (Tucker, 2007). Accurate measurement with these methods is difficult as it is based on self-reporting by participants and data on the polyphenol content in a food (Zamora-Ros et al., 2014).

Polyphenol content in a food is obtained from food-composition tables that are built from data extracted from scientific literature. The analytical methods used for polyphenol estimation sometimes give erroneous results. Estimation of total polyphenol content in a food product by Folin-Ciocalteu colorimetric assay gives false positive results for nonphenolic reducing agents, such as ascorbic acid (Lester et al., 2012). Therefore, careful evaluation of data should be done before adding it to the food-composition tables. Variation in polyphenol content also results from differences in plant variety, season of harvesting, processing methods, and storage conditions (Manach et al., 2004). Errors may also arise from the imprecise reports from the participants in the study. There is a need to develop a method to estimate polyphenol intake, which is independent on food intake and polyphenol content in a food, so that accurate assessment of polyphenol intake can be done for its health benefits. A correlation between dietary intake and plasma concentrations or urinary excretion of metabolites has been found for flavonols, flavanones, and isoflavones (Chen et al., 1999; Noroozi et al., 2000; Radtke et al., 2002). These polyphenol biomarkers could provide more reliable estimates of polyphenol intake as they are independent of error-prone factors.

## 4 Pharmacokinetics of Polyphenols and Its Effect on Bioavailability

It is important to have the knowledge of pharmacokinetics of polyphenols because these compounds are extensively metabolized and the metabolites need not have the same biological effect as that of the parent molecule. Bioavailability is the proportion of an active ingredient that enters the systemic circulation and becomes available at the site of action. Although polyphenols exhibit a wide range of health benefits, most of them have poor bioavailability. Despite the fact that polyphenols are present in many dietary components, their plasma concentration rarely exceeds 1 μM. Even if a polyphenol is administered as a pure entity, it hardly makes it to 1 μM of plasma concentration (Scalbert and Williamson, 2000). The levels of polyphenols that appear in vivo study are much lower than the concentrations that are effective in vitro conditions, which makes them inefficient for systemic therapy. The factors that are responsible for poor bioavailability of polyphenols include poor intestinal absorption due to low water solubility and poor permeability, and extensive metabolism by liver and gut enzymes and colon microflora (Fig. 4.5) (Fang and Bhandari, 2010; Hollman, 2004).

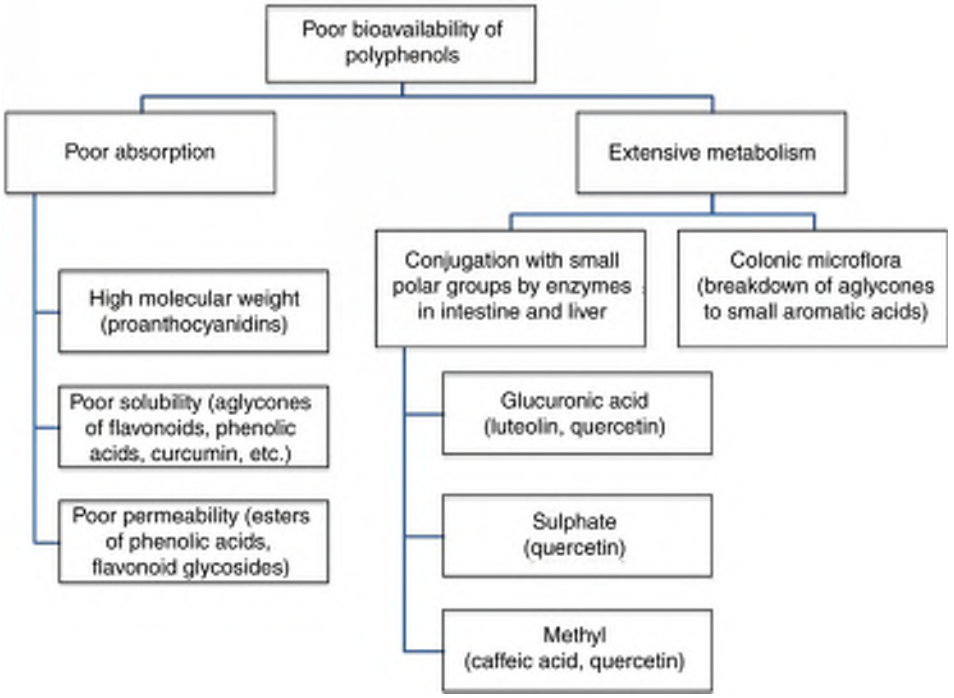


**Figure 4.5. Reasons for poor bioavailability of polyphenols.**

## 4.1 Gut Absorption

### *4.1.1 Effect of Gastric Acid*

After consumption, polyphenols are exposed to strong acidic conditions in the stomach. The polyphenols, such as quercetin, resveratrol, and catechin are reported to be stable at low pH conditions (Tagliazucchi et al., 2010). Procyanidins from cocoa beverage are found to be stable in gastric juice in healthy volunteers (Rios et al., 2002). This is contradictory to the results of in vitro study wherein breakdown of proanthocyanidins to monomers is shown in simulated gastric juice (Spencer et al., 2000). This could be due to the more exposure time in vitro conditions than gastric residence time.

### *4.1.2 Solubility and Permeability*

For a compound to reach systemic circulation and become bioavailable, it must get dissolved first in intestinal lumen followed by permeation through intestinal epithelial cell layer. Polyphenols in plants are present as aglycone as well as glycosylated forms. A compound with water solubility <100 μg/ml is considered as

poorly soluble (Kaur and Kaur, 2014). The majority of the polyphenols in their aglycone form are poorly water soluble. The commonly occurring polyphenols including curcumin, ellagic acid (Bala et al., 2006), quercetin (Cadena et al., 2013), genistein, daidzein (Stancanelli et al., 2007), apigenin (Zhang et al., 2012), and hesperetin (Mishra et al., 2009) have water solubility lower than 10 μg/ml. The glycosylated forms of polyphenols are more hydrophilic than their aglycone forms due to the presence of sugar moiety. But in most of the cases they are too polar or too large to cross the intestinal epithelial layer. The glycosides, for which the specific membrane transporters are present in enterocytes, are absorbed into systemic circulation (Hollman, 2004; Manach et al., 2004).

### 4.1.3 *Effect of Molecular Weight*

Molecular weight is a determining factor for the bioavailability of high molecular weight polyphenols, such as proanthocyanidins. Chemically, they are oligomers of flavanols. Most of the compounds of this class are not absorbed in the small intestine. They reach the colon unaltered and are broken down by microflora resulting in metabolites that are absorbed (Santos-Buelga and Scalbert, 2000; Williamson and Clifford, 2010). Because these compounds are not absorbed, they can reach at high concentrations in the colon and may exert local antioxidant activity against oxidizing agent (Halliwell et al., 2000). Dimeric and trimeric compounds of this class are able to cross the intestinal epithelium as shown in permeability assay in Caco-2 cell line (Deprez et al., 2001), but a very low plasma concentration of procyanidin dimer B2 is reported in humans after consumption of cocoa beverage (Holt et al., 2002).

### 4.1.4 *Effect of Glycosylation*

All types of polyphenols, except flavanols, are found to be conjugated to one or more sugar moieties (glycosylation). Conjugation with various organic acids and cell wall components is also reported. Glycosylation has an important role in the absorption of flavonols such as quercetin, rutin, and so on. While aglycone is absorbed by passive diffusion, glycosylated compounds are too hydrophilic to penetrate the intestinal wall by passive diffusion (Hollman, 2004; Manach et al., 2004). The partition coefficient of quercetin is found to be 3 times higher than its glycoside, quercetin-3-O-rhamnoglucoside (Brown et al., 1998). Due to this fact, most of the glycosides are absorbed only when they reach the colon where they are deconjugated by colonic microflora. This causes delayed and inefficient absorption. Colonic microflora also

causes ring opening of heterocycle in aglycone moiety resulting in the formation of aromatic acid metabolites (Manach et al., 2004).

Glucosides of quercetin, however, have been reported to have rapid and efficient absorption than other glycosides. Plasma concentration of quercetin reaches its maximum concentration very fast after the consumption of onions than that reached after the consumption of apples. Onions are rich in quercetin glucosides whereas apples contain different types of quercetin glycosides such as, arabinosides, xylosides, rhamnosides, galactosides, and glucosides (Hollman et al., 1997). Absorption of quercetin glucosides occur in the small intestine and it is proposed that sodium-dependent glucose transporter SGLT1 is involved in the transport of these hydrophilic molecules into enterocytes (Hollman et al., 1995). The glucoside is then hydrolyzed by cytosolic β-glucosidase to form aglycone, which is absorbed into the systemic circulation (Day et al., 1998). Even when administered in pure form, the absorption of quercetin 4'-glucoside is shown to be rapid and extensive than rutin (quercetin-3-rutinoside). The bioavailability of latter is only 20% of that of the former (Hollman et al., 1999). The hydrolysis of rutinose from rutin occurs only when it reaches colon (by microflora) as there is no transport/hydrolase available in the small intestine for rutinose moiety causing delayed and inefficient absorption.

Contradictory results are reported for the effect of glycosylation on the absorption of isoflavones. Glucosides of daidzein and genistein show a higher area under the curve of the plasma concentration when compared to their corresponding aglycone forms when administered in healthy volunteers (Setchell et al., 2001); whereas, in another study, aglycones present in fermented soy products show better bioavailability than glucosides from soybeans (Hutchins et al., 1995).

### *4.1.5 Effect of Esterification*

Phenolic acids such as ferulic acid and caffeic acid are present in their esterified form with organic acid, lipids, and sugars. Chlorogenic acid, a component of coffee, is formed by the esterification of caffeic acid with quinic acid. The esterification has marked effects on the bioavailability of phenolic acids. In general, free forms of phenolic acids are rapidly absorbed than the esterified forms (Olthof et al., 2001). Humans do not have esterases in the small intestine that are capable of hydrolyzing esters of phenolic acids. Their hydrolysis occurs only when they reach the colon where colonic microflora hydrolyzes them to release free phenolic acid (Plumb et al., 1999). So, absorption of a phenolic acid gets delayed if it is in the esterified form. Caffeic acid is found to be rapidly and

extensively absorbed than chlorogenic acid in patients who underwent colonic ablation (Olthof et al., 2001). The concentration of metabolites after the administration of caffeic acid in rats was found to be 100 times higher than that obtained after administration of chlorogenic acid (Azuma et al., 2000).

Ferulic acid is esterified with polysaccharides such as arabinoxylans present in the plant cell walls. In cereals, ferulic acid is found in the esterified form in the outer husk. The bioavailability of ferulic acid in humans is higher from tomatoes when compared to that from cereals. Free ferulic acid is found in tomatoes whereas cereals contain ferulic acid in esterified form (Bourne and Rice-Evans, 1998; Mateo Anson et al., 2009). The absorption of polysaccharide-linked ferulic acid occurs mainly in the colon when enzymes such as xylanases from bacterial origin degrade linked polymer to small oligosaccharides followed by hydrolysis by bacterial esterases to release the free ferulic acid (Andreasen et al., 2001).

## 4.2 Phase 1 and Phase 2 Metabolic Reactions

Generally, phase 1 metabolic reactions include introduction of polar and reactive group on the compound so as to increase its polarity and make it a substrate for the phase 2 metabolic reactions. Hydroxylation catalyzed by cytochrome P-450-dependent mixed-function oxidase system is very common type of phase 1 metabolic reaction (Guengerich, 2006). Since polyphenols already have hydroxyl groups attached to them, this process is not important for them. So, they undergo type 2 metabolic reactions (conjugation reactions) faster than type 1 reactions (Lewandowska et al., 2013). The polyphenolic compounds consumed from diet are conjugated by methylation, sulfation, glucuronidation, or a combination. These reactions are catalyzed by a variety of broad specificity transferase enzymes in enterocytes and liver. The conjugation process (phase 2 metabolism) makes the use of the same pathway and enzymes as that of drug metabolism. But in the case of drug metabolism, free drug is also found in the plasma in addition to the drug conjugates (Scalbert and Williamson, 2000). In the case of polyphenols, free moiety is rarely found in the plasma. When quercetin is administered orally, only glucuronic acid, sulfate, or methyl conjugates are found in the plasma (Day et al., 2001; Justino et al., 2004). This could be due to the low concentration of polyphenols obtained from diet. In the case of drugs, high concentration of them saturates the conjugating enzymes and hence free form of the drug also gets absorbed and reaches the systemic circulation. When polyphenols are consumed as pure component,

significant amount of free form has been detected in plasma. Free catechin is detected in plasma after 30 min when pure catechin is administered orally (Bell et al., 2000). Conjugation has an important role in the assessment of health benefits of polyphenols as it may alter or diminish the biological properties of the molecules. Moreover, it makes these compounds more hydrophilic, which facilitates their urinary excretion and reduce plasma half life.

## 4.3 Metabolism by the Colonic Microflora

Colonic microflora plays an important role in determining the bioavailability of the ingested polyphenols. Many polyphenols are not absorbed in the small intestine and so eventually they reach colon where they are metabolized by gut microbes. The polyphenols, which are metabolized and secreted in the bile to the small intestine, also reach the colon but in their conjugated form. The colonic microflora has tremendous catalytic potential (Scalbert and Williamson, 2000). Quercetin-3-O-rhamnoside is not absorbed in the small intestine and it reaches colon unaltered where it is hydrolyzed to quercetin by α-rhamnosidase from *Bacteroides distasonis* (Bokkenheuser et al., 1987). But colonic microflora also causes breakdown of aglycone moiety in the polyphenols to more simple molecules leading to reduction in their bioavailability. The breakdown of flavonols by gut microbes produces hydroxyphenylacetic acid metabolites while flavanones produce hydroxyphenylpropionic acids (Manach et al., 2004).

# 5 Approaches for Enhancement of Bioavailability of Polyphenols

Extensive research has been carried out to enhance solubility and stability of polyphenols in order to improve their bioavailability. Various approaches are summarized in Fig. 4.6.

## 5.1 Nanoformulations

### *5.1.1 Liposomes*

Liposomes are colloidal particles prepared from naturally occurring phospholipids or from synthetic lipids. They are biocompatible and their physicochemical properties can be tuned by varying their lipid composition. The interaction between hydrophobic phospholipids and hydrophilic water molecules is the basis for the formation of liposomes. The vesicle of a liposome can be multilamellar or unilamellar depending on the method of

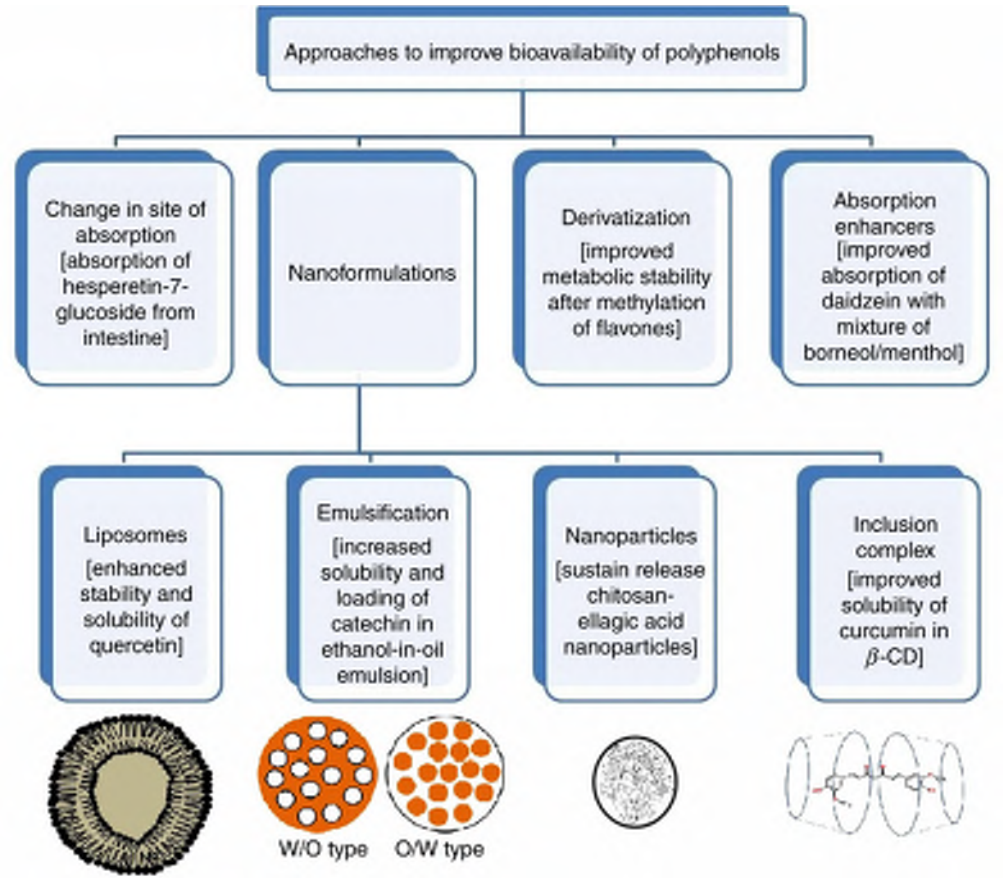


**Figure 4.6. Various approaches to improve bioavailability of polyphenols.**

preparation. Liposomes contain aqueous core whereas their wall is made up of lipids that make them suitable for encapsulation of hydrophilic as well as hydrophobic agents (Chang and Yeh, 2012; Fang and Bhandari, 2010). The bioavailability of encapsulated agents enhance as a result of solubility improvement, protection from gastric juice and enzymes. Liposomes can also be used to control the release rate and for targeted delivery of encapsulated agents (Paavola et al., 2000; Torchilin, 2007). Various commercial drugs such as amphotericin B, verteporfin, doxorubicin, daunorubicin, vincristine have been formulated in liposomes (Chang and Yeh, 2012).

The polarity of the polyphenolic compounds vary greatly and as mentioned earlier liposomes can be used to entrap both hydrophilic and lipophilic molecules. Therefore, liposomes have been proposed for encapsulation of polyphenolic compounds. The

hydrophilic compounds are encapsulated in the aqueous centre of liposomes. The hydrophilic components of tea extract, (+)-catechin and (−)-epicatechin, have very poor absorption efficiency. Both the isomers showed similar encapsulation levels and release rates. Another component of tea extract, (−)-epigallocatechin-3-gallate (EGCG), is found to have better encapsulation efficiency than (+)-catechin or (−)-epicatechin. It also showed increased skin deposition compared to (+)-catechin and (−)-epicatechin. This could be due to presence of lipophilic galloyl group in EGCG. Encapsulation efficiency of EGCG is further improved by the addition of 15% of ethanol to preparation hydration system (Fang et al., 2006). Improved bioavailability of (+)-catechin formulated in liposome is observed due to increased membrane permeability and protection from metabolic conversion. The encapsulation protects (+)-catechin from simulated gastrointestinal fluids (Huang et al., 2011). In addition to catechin, oral bioavailability of dehydrosilymarin (Chu et al., 2011), quercetin (Yuan et al., 2006), curcumin (Takahashi et al., 2009), and silymarin (El-Samaligy et al., 2006b) has also been found to be enhanced when formulated in liposomes.

Several factors are responsible for the enhancement of bioavailability by liposomes. Formulation of a polyphenol in liposomes increases its chemical stability in addition to solubility improvement. Increased chemical stability causes prolongation of residence time in biological systems. It is proposed that liposomes tend to accumulate in liver and acts as a slow releasing reservoir for polyphenol liposomes. This increases the plasma half life of a polyphenol resulting in improved bioavailability (Mignet et al., 2013). The liposomal form of quercetin is reported to be active at a dose where free quercetin is not active (Mandal et al., 2002).

Numerous in vitro and in vivo studies have shown better biological efficiency of polyphenol liposomes when compared to unformulated polyphenols. The encapsulation of curcumin in liposomes prepared from commercially available lecithin enhances its gastrointestinal absorption. The improved bioavailability causes enhancement of plasma antioxidant activity (Takahashi et al., 2009). Silymarin-loaded liposomes prepared from lecithin, cholesterol, stearyl amine, and Tween-20 show better hepatoprotective activity in carbon tetrachloride–induced rats when compared to silymarin suspension. The assessment of hepatoprotective activity is carried out using estimation of serum glutamic oxalacetate transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) in rat serum (El-Samaligy et al., 2006a). Intravenous administration of quercetin encapsulated in polyethylene glycol 4000 liposomes increases its circulation time.

The formulation inhibits tumor growth in immunocompetent C57BL/6N mice in BALB/c mice bearing CT26 colon adenocarcinoma and H22 hepatoma in a dose-dependent manner (Yuan et al., 2006). There are few reports wherein the formulation of polyphenols in liposomal system shows no difference in biological activity when compared to free polyphenols. $IC_{50}$ values of liposomes of fisetin and free fisetin are same in the murine Lewis lung carcinoma cells and colon 26 (CT26) carcinoma cells (Mignet et al., 2012).

### *5.1.2 Emulsification of Polyphenols*

Emulsions are a thermodynamically unstable mixture of two immiscible liquids. One liquid in the form of fine droplets (discontinuous phase) is dispersed into another liquid (continuous phase). In most of the cases emulsions are formed by water and oils. Emulsion is called oil-in-water (O/W) type when oil forms discontinuous phase whereas it is water-in-oil (W/O) type when water is in the form of droplets. The system is stabilized with the aid of various surface active agents known as emulsification agents (Khan et al. 2011). Multiple emulsion systems such as O/W/O or W/O/W have been developed to improve pharmacokinetic parameters of neutraceuticals (Benichou et al., 2004). Emulsions are commonly used formulations that offer unique advantages such as masking of bad taste and flavor, enhancement of solubility, protection from degradation, and enhancing the uptake of active component. They can be used for oral as well as topical applications. The lipophilic compounds that have very poor water solubility are commonly formulated in emulsions. Such components are solubilized in oils followed by homogenous dispersion in aqueous system using appropriate surface active agent. Emulsion improves bioavailability of an active component by increasing its solubility, extending gastric residence time and protecting against metabolic reactions (Ting et al., 2014).

Several polyphenols and extracts containing polyphenols have been formulated in the form of emulsion. Emulsification of polyphenols is done mainly to serve two purposes:

1. To improve their pharmacokoinetic properties.
2. Polyphenols, being strong antioxidants, protect oil from rancidity and thus enhance the stability of thermodynamically unstable systems.

Puerarin, an isoflavone found in kudzu plant, is reported to be effective against metabolic and cardiovascular diseases. Microemulsion of puerarin prepared using 20% of Tween-80, 20%

of glycerin, and 1.6% of ethyl oleate enhances the solubility of this poorly soluble isoflavone. The formulation shows better bioavailability in rabbits following intranasal administration (Yu et al., 2011). Many polyphenolic compounds such as catechin or anthocyanin have poor solubility in water or oil. A novel approach named "functional emulsions," is patented for emulsification of such compounds. The polyphenol is dissolved in ethanol to which 1% of polyglycerol oleic acid ester is added as an emulsifying agent. The mixture is then added to a vegetable oil and stirred at 3000 rpm for 10 min to obtain E/O (ethanol in oil) type of emulsion. A triple system, E/O/W, is prepared by dispersing E/O emulsion in water. High concentration of polyphenol can be emulsified in such systems, as polyphenols have good ethanol solubility. Such systems have applications in pharmaceutical, food, and neutraceutical industries (Nakajima et al., 2003).

Pure polyphenols and extracts containing polyphenols are reported to prevent the oxidation of lipids in emulsions, thereby, increasing the shelf life of emulsion. Such system provides dual advantage. Emulsification of polyphenol improves its pharmacokinetic properties and its antioxidant activity protects oil from rancidity. Fish oil is known for high therapeutic value but it is susceptible to lipid oxidation, which reduces its shelf life (Albert et al., 2015). An extract from grape containing flavanols, procyanidins, and glycosylated flavonols is shown to inhibit the oxidation of fish oil emulsion and frozen fish muscle. The O/W type of emulsion is prepared by mixing 1% of lecithin (emulsifier) and 10% of fish oil followed by sonication for 10 min. The extract is added at a concentration of 0.01% w/w. Different fractions of extract prepared by using column chromatography are checked for the activity. It is found that fraction containing oligomeric flavanols are the most efficient antioxidants in emulsion systems whereas monomeric flavanols are effective in bulk oils (Pazos et al., 2005). Polyphenol containing extracts of apple skin, pine, and witch hazel have also shown to inhibit lipid oxidation in fish O/W emulsion system (Rupasinghe et al., 2010; Iglesias et al. 2010). The antioxidant activity was found to be more in fractions containing galloylated polyphenols (present in witch hazel) when compared to their nongalloylated counterparts (present in pine) (Iglesias et al., 2010).

Antioxidant activity of polyphenols against lipid oxidation in vegetable oils is also reported. The oxidative stability of O/W type emulsion of sunflower oil (from which tocopherol was removed) was improved by the addition of caffeic acid and tea extracts. The emulsion systems consisted of Tween-20 (a synthetic surfactant) and bovine serum albumin as emulsifier. The emulsion was

homogenized by sonication. The emulsion system containing caffeic acid also contained ferric ion. Caffeic acid was found to protect emulsion from this prooxidant (Almajano et al., 2007a, 2008). Albumin has very less antioxidant activity of its own in O/W emulsion system consisting of Tween-20-acetate buffer-sunflower oil, but it increased antioxidant activity of catechins from green tea in a synergistic manner (Almajano et al., 2007b).

### 5.1.3 Self-Emulsifying Drug Delivery System

These are incomplete emulsions because they lack aqueous phase. They are also called self-emulsifying oil formulations. They are isotropic mixture of oils, surface active agents, and cosolvents. When they come in contact with aqueous environments in gastrointestinal tracts after ingested orally, they form emulsions due to gastrointestinal motility. They are physically more stable when compared to the conventional emulsion system. At the same time they retain all the merits of a conventional emulsion system. They have been proved to be excellent carriers of hydrophobic compounds (Neslihan Gursoy and Benita, 2004). Such a system has been developed for curcumin to enhance its solubility and subsequently improve its bioavailability. Curcumin given orally in Wistar rats by self-emulsifying drug delivery system showed better bioavailability when compared to curcumin suspension. The maximum plasma concentration value ($C_{max}$) increased by 8-fold in the case of self-emulsifying system when compared to the suspension. The formulation also showed better in vitro anticancer activity in human lung cancer cell line (Chopra et al., 2011).

### 5.1.4 Nanoencapsulation

Nanoparticles are becoming popular for the delivery of pharmaceuticals and neutraceuticals because of the unique properties they possess. Nanoencapsulation involves preparation of particles having at least one dimension in nanoscale and loading an active substance into or on the surface of the formed particles. Several biocompatible and biodegradable polymers are used for formulation of nanoparticles. Commonly used materials for their preparation include natural polymers such as starch, chitosan, and gelatin and synthetic polymers such as, polylactic acid, poly(lactic-*co*-glycolic acid), and polycaprolactone (Khushnud and Mousa, 2013). Compared to conventional materials, they provide a greater surface area to volume ratio. So increase the solubility of the compound results in improved bioavailability. They have also been used for controlled and targeted release of the actives.

Many formulations of polyphenol nanoparticles have been reported wherein improvement in pharmacokinetic and pharmacodynamic properties are observed (Jia, 2005).

Spherical nanoparticles of ellagic acid having average particle size of 176 nm are prepared by ionic gelation method using chitosan and sodium tripolyphosphate. The release of ellagic acid from the nanoparticle in phosphate buffer saline (pH 7.4) showed two-step release patterns. The release is rapid for the first 3 h followed by sustained release up to 48 h. It is proposed that the adsorbed ellagic acid on particles contribute to initial rapid release whereas entrapped ellagic acid in matrix contributes to sustained release. The cytotoxicity of ellagic acid nanoparticles is significantly higher than free ellagic acid as seen by MTT assay and DNA fragmentation analysis in human oral cancer cell line (Arulmozhi et al., 2013). Chitosan nanoparticles of yerba mate extract are prepared by ionic gelation method. It is a tea-like beverage rich in polyphenols. It is reported to possess antioxidant, antiinflammatory, hepatoprotective, antiobesity, and diuretic activity (Harris et al., 2011).

The nanoparticles of curcumin with poly (lactide-*co*-glycolide) (PLGA) and polyethylene glycol (PEG)-5000 are prepared using nanoprecipitation techniques. The technique involves solubilization of polyphenol and polymer in an organic solution followed by the addition of it to an aqueous solution containing a surfactant. Finally, organic solvent is vacuum evaporated and resultant dispersion is centrifuged to obtain the particles. The nanoparticles of curcumin showed improved cellular uptake and enhanced in vitro bioactivity when compared to free curcumin. The bioavailability is also found improved when compared to free curcumin in mice (Anand et al., 2010). The nanoparticles of quercetin prepared by precipitation technique showed better release and better antioxidant activity than the free quercetin. The release rate of quercetin is found to be increased by 74-fold when encapsulated in nanoparticles. It is proposed that entrapment of quercetin in polymer makes it amorphous, which increases its solubility (Wu et al., 2008).

Lipid nanocapsules of quercetin and epigallocatechin gallate are prepared using caprylic/capric triglyceride (oil phase), soybean lecithin, surfactant, and NaCl by phase inversion process. The polyphenols are mixed in oil phase, which is then added to a mixture of soybean lecithin, surfactant, and NaCl. The mixture is heated initially to form W/O emulsion, followed by cooling and addition of cold distilled water to form O/W nanocapsules. The encapsulated polyphenols are found to be more stable when compared to the free ones. The apparent water solubility of the

encapsulated quercetin is found to be 100-fold higher than the free form (Barras et al., 2009).

### 5.1.5 Inclusion Complexes

A complex in which one chemical moiety (host molecule) forms a cavity that can accommodate a guest molecule, is known as inclusion complex. Cyclodextrins (CDs) or their derivatives such as, methyl-β-cyclodextrin are generally used for the formation of inclusion complexes. CDs are naturally occurring cyclic oligomers derived from starch. They are made from six (α-CD), seven (β-CD) or eight (γ-CD) glucose residues linked by α-(1–4) glycosidic bonds to form a cylindrical structure. β-CD is the most commonly used among all the CDs for encapsulation purpose. The inner cavity of CD molecules is hydrophobic whereas the outer part is hydrophilic, which makes them suitable for encapsulating the hydrophobic molecules (Pinho et al., 2014).

Encapsulation in CDs improves the water solubility of the compound to a great extent. Also encapsulation protects compounds from light and oxidizing agents resulting in improved storage stability. These characteristics make CDs a suitable encapsulating agent for polyphenols, which are poorly water soluble and light-sensitive. Many polyphenols and extract containing polyphenols have been encapsulated in CDs. β-CD has been used to encapsulate oleuropein rich olive leaf extract (Mourtzinos et al., 2007), resveratrol (Lucas-Abellan et al., 2007), rutin (Haiyun et al., 2003), 3-hydroxyflavone, morin, and quercetin (Calabro et al., 2004). Various derivatives of β-CD are also reported for encapsulation of polyphenols. 2-hydroxypropyl-β-CD is used to encapsulate kaempferol, quercetin, myricetin (Mercader-Ros et al., 2010), hesperetin and hesperidin (Tommasini et al., 2005). Rosmarinic acid has been encapsulated in methyl β-CD (Celik et al., 2011). Encapsulation of polyphenols in all these cases improves their water solubility as well as antioxidant activity.

The encapsulation efficacy of a polyphenol is dependent on the type of CD used. Curcumin has shown different encapsulation efficiency with different CDs. Maximum encapsulation efficiency for curcumin, quercetin, and myricetin was obtained with 2-hydroxypropyl-β-CD (Lucas-Abellan et al., 2008; Tomren et al., 2007; Tonnesen et al., 2002) while that for rosmarinic acid was obtained with methyl β-CD (Celik et al., 2011). The nature of the core material also affects the efficiency of encapsulation. Low molecular weight, hydrophobic molecules possess great affinity toward CDs and hence give better encapsulation efficiency. For example, 3-hydroxyflavone showed better encapsulation than morin or quercetin (Calabro et al., 2004). Hesperetin

was found to be more effective than hesperidin (Tommasini et al., 2005).

Encapsulation results in improved the photo-stability of the polyphenol. Ferulic acid sunscreen lotion is approved in some countries for its photo-protective effect. But it has low stability under physical and thermal stress. Encapsulation of ferulic acid in α-CD improves its stability against UVB radiation. The inclusion complex is prepared by coprecipitation method. The complex exhibits slow and sustained release from a Strainer cell indicating improved bioavailability for skin applications. It is also shown that ferulic acid interacts with lipophilic interior of α–CD through carboxyl and α, β-unsaturated groups. A part of its aromatic moiety is also involved in the interaction (Anselmi et al., 2008). Hydroxytyrosol, a polyphenolic antioxidant component of olive oil when complexed with β-cyclodextrin, shows enhanced antioxidant activity and significant reduction in its degradation rate when compared to its free form when exposed to UV irradiation (Lopez-Garcia et al., 2010). Inclusion complex of flavonoid-rich St John's wort extract prepared by mixing it with β-CD at a mass ratio of 1:4, is more stable thermally than its free form. Differential scanning calorimetry thermograms indicate that thermal oxidation of the extract starts at 200°C while that of the inclusion complex is observed at 300°C (Kalogeropoulos et al., 2010).

In addition to CDs, hydrophobically modified starch has also been used for the preparation of inclusion complexes. An inclusion complex of curcumin and hydrophobically modified starch increases solubility of curcumin by 1670-fold. The anticancer activity of this complex is found to be better than that of the free form (Yu and Huang, 2010).

## 5.2 Derivatization to Improve Metabolic Stability and Transport

As discussed earlier, polyphenols are extensively metabolized by enzymes in gut and the liver and by gut microflora. The presence of hydroxyl groups makes them susceptible for metabolism. Methylation of the hydroxyl groups in the polyphenols results in improved metabolic stability against phase 2 conjugation reactions. Dimethylated forms of galangin (3,5,7-trihydroxyflavone) are relatively more stable toward glucuronidation in the liver fraction (Wen and Walle, 2006). Methylated (−)epigallocatechin is reported to be more stable than (−)epigallocatechin at neutral pH (Henning et al., 2008). Methylation of flavones results in improvement in their transport across Caco-2 transwell culture. A methylated flavone showed better bioavailabilty than its nonmethylated

counterpart in rats (Walle, 2007). In an attempt to protect quercetin from phase 2 conjugation reactions, its ester derivatives were synthesized and their transport across monolayers of canine MDCK-1, -2 and human Caco-2 cell lines was checked. Few of the synthesized compounds crossed epithelial monolayers without undergoing phase 2 conjugation reactions (Biasutto et al., 2007).

### 5.3 Changing Site of Absorption

Small intestine is the major site of the absorption for most of the compounds. Many polyphenols could not be absorbed from small intestine because of glycosylation. Hesperidin, a component of orange juice, is a glycoside of hesperetin having a rutinose sugar attached at the seventh position in ring A. Hesperidin passes through small intestine without getting absorbed and gets extensively metabolized by colonic microflora and thus have very poor bioavailability. The enzymatic conversion of hesperidin to hesperetin-7-glucoside in orange juice improves plasma concentration of hesperetin (Nielsen et al., 2006). Glucosides can be absorbed from small intestine due to the presence of transporters resulting in improved plasma concentration.

### 5.4 Absorption Enhancers

Bioavailability of a compound can be enhanced by coadministration with an absorption enhancer. For example, administration of an isoflavone, daidzein, with a eutectic mixture of borneol/menthol results in improvement of the solubility as well as permeability of the isoflavone in isolated rat intestinal membrane. Microemulsification of this system further improved the bioavailability (Shen et al., 2011). Coadministration of piperine, an alkaloid from black pepper, along with curcumin, resveratrol, epigallocatechin gallate resulted in enhanced bioavailability of these polyphenols. Piperine is reported to inhibit P-glycoprotein and CYP3A4 and thus coadministration of it along with polyphenols inhibits the metabolism of these compounds and hence improves their bioavailability (Lewandowska et al., 2013).

## 6 Concluding Remarks

Polyphenols, the superfamily of naturally occurring phytochemicals, are ubiquitously expressed in plants. These are gaining popularity because of their potential health benefits in minimizing the risk of cardiovascular diseases, inflammation, cancer, microbial and viral attack, and encourage immunomodulatory activities.

Their biological activities depend on their structural and physicochemical properties. Due to their ability to modulate the activities of multiple targets involved in CVD, these could be employed efficiently to reduce the risk of CVD and related metabolic diseases. However, the major setback related to the use of polyphenols is their poor bioavailability and stability, which hinder their use in in vivo. Polyphenols are absorbed readily in the small intestine and are subjected to digestion by gut and liver enzymes, and the enzymes released by the colon microbes. These have shown poor absorption and biodistribution, but often experience fast metabolism and excretion in vivo. The reasons for this could be their structure, molecular weight, solubility, inability to get absorbed to systemic circulation at gastric pH, etc. Fascinatingly, several recent studies have suggested that nanoencapsulation of polyphenols may aid in overcoming the limitations frequently observed with dietary polyphenols (bioavailability, pharmacokinetics, targeted treatment, efficacy, and safety), consequently improving their biological effects. Nanotechnology could play a pivotal role in CVD treatment and its prevention. Further, nanoparticles could be modified to enhance bioavailability, prolong circulation, enhance drug localization and efficacy, and lessen multidrug resistance. Nanotechnological applications to improve the in vivo functionalities of dietary polyphenols would include liposomal formulation, nanoemulsions, lipid nanocapsules, emulsions, inclusion complexes, and gels. These nanoformulations could be systemically administered by parenteral routes such as, intranasal, intravenous, and intraperitoneal, thus improving their effectiveness in the treatment and prevention of CVD and several other metabolic disorders.

## Acknowledgment

RPN acknowledges Indian Institute of Technology, Madras and MR acknowledges Women Scientist-A (WOS-A), Department of Science and Technology, Government of India, for their financial support.

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# NANOEMULSION: PREPARATION AND ITS APPLICATION IN FOOD INDUSTRY

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## 1 Introduction

Emulsion is a nonequilibrium heterogeneous colloidal dispersion consisting of two immiscible liquids. Nanoemulsions are isotropic, thermodynamically unstable, kinetically stable transparent colloidal dispersions consisting of oil and aqueous phase, surfactant and a cosurfactant with droplet size within the range 20–200 nm (Forgiarini et al., 2001; Gutierrez et al., 2008). Nanoemulsion was first prepared in 1940 (Bhosale et al., 2014). This type of emulsion is thermodynamically unstable because of the large positive interfacial tension between the oil and water phases. Very little scattering of the visible light takes place due to the smaller droplet size of nanoemulsions as compared to the wavelength of visible light. Therefore, nanoemulsions are appeared transparent in the visible light spectrum. They have much better kinetically stable to gravitational separation and aggregation than other emulsions (Tadros et al., 2004; Wooster et al., 2008). The optical transparency, kinetic stability of nanoemulsions are dependent on the thermodynamic conditions such as compositions, temperature, and pressure as well as on the preparation methods (Esquena and Solans, 1998). Therefore, it is possible to prepare nanoemulsions with reproducible properties and definite droplet sizes by utilizing different preparation techniques. Because of these advantages nanoemulsions have been achieving great interest for fundamental studies and applications in different fields such as of health care, cosmetics, food, agrochemicals, pharmaceuticals, and biotechnology.

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00005-2

Kinetically stable, optically transparent isotropic nanoemulsions have been used as a potential candidate in the field of food processing and packaging in encapsulation, protection and delivery of bioactive lipophilic components, such as nutraceuticals, drugs, vitamins, antimicrobials, and antioxidants within our body because of their excellent properties over the conventional emulsions such as high stability, high oil in water interfacial tensions, bioavailability, nontoxicity, nonirritant behavior (McClements, 2011). After ingestion within the body, bioactive materials should be protected during their storage of the bioactive ingredient, processing and storage of the functional food and also during gastrointestinal transit until they reach the desired site in the body. In this regard encapsulation of sensitive food ingredients is important to protect from heat, moisture, and pH. In the food industry, nanoemulsions are used for encapsulation of food ingredients, which includes incorporation, absorption, or dispersion of bioactive compounds within small capsules having diameters less than 100 nm. Encapsulation can improve the nutritional content of food without affecting the taste, texture of food, premature release of flavors, and improved stability and solubility of ingredients and final food products. Thus, encapsulation increases bioavailability and also increases the delivery of bioactive materials to cells and tissues within the body (Augustin and Hemar, 2009). Thus, nanoencapsulation provides high bioavailability, sufficient gastric residence time, high solubility, high stability, and protection from environment and promotes the activity and potential health benefits of nutraceutical molecules (Chen et al., 2006). This chapter deals with precisely on different synthetic routes of nanoemulsions, characterization as well as their applications in food industries.

# 2 Preparation of Nanoemulsions

## 2.1 Theory of Formation of Nanoemulsions

Nanoemulsion is a stable and clear multiphase colloidal dispersion. Generally several high-energy approaches are utilized for the reduction of the droplet size to nanorange. According to theory of emulsification, the Gibbs free energy for the formation of thermodynamically unstable emulsions is positive (Tadros et al., 2004). From the Gibbs free energy equation ($\Delta G = \gamma \Delta A - T\Delta S$), where $\gamma$ is the interfacial tension and $\Delta A$ is surface area, it is seen that if $\gamma$ is positive, a large amount of energy is required for expanding the interface. Again, due to the small positive value of the $T\Delta S$ of the system, Gibbs free energy, $\Delta G$ becomes positive. Therefore, the

nonspontaneous emulsion formation process requires high energy and presence of surfactants for the dispersion of one phase into another phase. Due to the presence of high interfacial tension between the outer and inner phase of a nanosize droplet, a high amount of energy is required for the formation of nanoemulsions. If the volume fraction of the dispersed phase is small, the droplet of the nanoemulsion is spherical.

The pressure exerted inside the droplets having curved interface is given by

$$p = 2\gamma/r$$

For nonspherical droplets, Laplace pressure value is represented as

$$p = \gamma(1/r_1 + 1/r_2)$$

where $r_1$ and $r_2$ are radii of curvature of the drops. From the relation it is seen that the Laplace pressure ($p$) is inversely proportional to $r$. Therefore, small size droplets experienced more pressure as compared to the large-sized droplets and as a result a smaller droplet requires much stress to undergo deformation than required by a large-sized droplet. Because of this a high amount of energy is required in the formation of nanoemulsions. With the addition of the surfactants and cosurfactants decreases the stress during the formation of nanoemulsions by reducing the interfacial tension and the Laplace pressure (Setya et al., 2014).

## 2.2 Components of Nanoemulsions

Nanoemulsions mainly consist of oil phase, surfactant, cosurfactant, additives, and aqueous phase.

*Oil*: Oil is an important component of nanoemulsions. It can easily change the properties of the nanoemulsions. Oils are used to solubilize the lipophilic substances. Long- and medium-chain triglycerides oils with different degree of saturation and semisynthetic medium chain derivatives possessing surfactant like properties are used as oily phase (Zhang et al., 2002; Jumaa & Mueller, 2002).

*Surfactants*: Surfactant plays an important role in the formation of the nanoemulsion, which reduces the interfacial tension between two immiscible liquids. It is classified as anionic, cationic, nonionic, and zwitterionic surfactants depending on the polar group present in the surfactant molecules. They reduce the Laplace pressure, the stress required to break the droplet to the nanometer scale and aggregation of particles of the

nanoemulsion. The formation of the W/O and O/W nanoemulsion also depend on the Hydrophile–lipophile balance (HLB) and critical packing parameter (CPP). Surfactants with low HLB are used to prepare W/O nanoemulsions whereas surfactants with high HLB used to prepare O/W nanoemulsions (Setya et al., 2014).

*Cosurfactants*: Cosurfactants are ampiphilic molecules and therefore they can distribute in large amounts at the surfactant interfacial monolayer. They help in the reduction of the interfacial tension between the two liquid layers by decreasing the viscosity of the interface and increasing the entropy of the system.

*Additives*: The stability of the nanoemulsion can also be achieved by addition of the additional additives, which enhance the longtime storage capacity of the nanoemulsion.

*Aqueous phase*: The nature of the aqueous phase also affects on the size of the droplets and stability of nanoemulsion. The size of the droplet present in the nanoemulsion mainly depends on the pH of the medium and presence of the electrolyte in the system.

When high concentrated surfactant is used during the preparation of nanoemulsion the interfacial tension of the oil–water interface shows very low and the interface is flexible, which is a necessary condition to obtain a stable nanoemulsion system. Different type of oils, surfactants, cosurfactants, and additives, which are used for the preparation of stable nanoemulsions are listed in Table 5.1.

## 2.3 Factors Affecting the Formulation of Nanoemulsions

Factors like the nature of the dispersed phase, dispersion medium, surfactant and cosurfactant, and emulsification conditions have considerable effect on the formation of stable nanoemulsions. The dispersed phase and the dispersion medium should be immiscible to each other to form an emulsion. The interface between the two phases, which is formed on mixing of the two liquids should be flexible. Water-soluble surfactants molecules are generally used to prepare nanoemulsions. Cosurfactants are used with the system containing short chain alkanes, alcohols, water, and surfactants. The selected surfactant molecules should lower the interfacial tension between the two liquid phases and it should form lyotropic liquid crystalline microemulsion phases. Large amounts of surfactants are used in order to coat particles of surface areas within the nanoscale range to inhibit the coalescence. To avoid precipitation the components are added in a slow rate during emulsification. In order to decrease the Laplace pressure a high amount of shear should be applied such that deformation of a large droplet to a smaller size droplet will become easy (Kim et al., 1992).

**Table 5.1 List of Oil, Surfactant, Cosurfactant, and Additive (Hari and Singh, 2012; Setya et al., 2014; Shah et al., 2010)**

| Components of Nanoemulsion | Examples |
|---|---|
| Oil | Captex 355 (glyceryl tricaorylate/caprate), Captex 200 (propylene dicaprylate/dicaprate glycol), Captex 8000 (glyceryl tricaprylate), Witepsol (90:10% w/w c12 glyceride tri: diesters), Myritol 318 (c8/c10 triglycerides), isopropyel myristate (myristic acid isopropyl ester). Castor oil, coconut oil, corn oil, fish oil, PEG-vegetable oil, wheat germ oil, etc |
| Surfactants | Capryol 90; Gelucire 44/14, 50/13; Cremophor RH 40; Imwitor 191, 308(1), 380, 742, 780 K, 928, 988; Labrafil M 1944 CS, M 2125 CS; Lauroglycol 90; PEG MW > 4000; Plurol Oleique CC 497; Poloxamer 407, 124, 188; Softigen 701, 767; Tagat TO; Tween-80; Labrasol, Cremophor EL; Tween-20; Tween-60, Brij 30 (polyoxythylene lauryl ether), Cremophor RH 40, Emulphor-620, Span 60, 80, 85 |
| Cosurfactants | Transcutol P, Transcutol HP; glycerin; ethylene glycol; polyethylene glycol, glycerine, lecithim, Lauroglycol 90, propylene glycol; ethanol; propanol; isopropyl alcohol; *n*-butanol; PEG 400; carbitol, etc. |
| Additives | Antioxidants (ascorbic acid, tocoferol, deforaxamine mesylate), tonicity modifiers (glycerol, sorbitol, xylitol), pH adjusting agents (solutions of NaOH and HCl), stabilizers (oleic acid, cholic acid, deoxy cholic acid, and their salts), preservatives (methyl paraben, propyl paraben, and benzalkonium chloride) |

## 2.4 Preparation Techniques

A large number of methods are reported in the literature for the fabrication of the stable nanoemulsion. The preparation of nanoemulsion is broadly categorized in two methods, namely high energy and low energy methods (Tadros et al., 2004). The details of each method are discussed in the following sections.

### *2.4.1 High-Energy Methods*

In high-energy emulsification methods, several devices are used to produce very high mechanical energy in order to form small droplet sized of nanoemulsions. In order to create intense disruptive forces, which are required to break up the oil and water phases to form nanosized droplets, mechanical devices are used.

The type of instruments used in the preparation method and the operating conditions like time, temperature, properties, and composition of the starting materials have adverse effects on the size of the particles of nanoemulsions. High-shear stirring, high-pressure homogenization technique, high-pressure homogenizer, or piston homogenizer and ultrasound are generally used to prepare nanoemulsions. Some techniques used in high-energy emulsification methods are discussed in the subsequent sections.

#### 2.4.1.1 High-Pressure Homogenization

In high-pressure homogenization techniques, high-pressure homogenizers, or piston homogenizers are used in the preparation of the nanoemulsion. Nanoemulsions with droplet size ~1 nm can be prepared by utilizing this technique. Both oil phase and aqueous phase are mixed under the influence of the force applied on their mixture through a small inlet orifice at very high pressure about 500–5000 psi. Due to the force applied on the liquid mixture, intense turbulence, and hydraulic shear produced and as a result very fine particles of emulsion are formed (Shakeell et al., 2008). The droplet of the prepared nanoemulsion is a liquid, lipophilic core, which is separated by a monomolecular layer of phospholipid from the continuous aqueous phase. Although the high-pressure homogenization process has great efficiency, this highly exothermic process requires high energy for the formation of nanoemulsion (Bhatt and Madhav, 2011). K.R. Khun and his coworkers prepared O/W nanoemulsion from New Zealand Milk Products (ALACEN 895, New Zealand) and the flaxseed oil purchased from Cisbra (Panambi, RS, Brazil) stabilized by whey proteins at 25°C by adopting high-pressure homogenization process (20–100MPa) with small droplet size and the stability of the prepared nanoemulsions was observed up to 3 months (Kuhn & Cunha, 2012). O. Kinawy et al. fabricated nanoemulsion from the mixture of palm oil, distilled water, vitamin E, and nonionic surfactant Tween-40 by using a high-pressure homogenizer at a pressure range of 200–500 bar at 60°C in three stages. They observed that the droplet size increases with increase in pressure, number of homogenization stage and temperature (Kinawy et al., 2012).

#### 2.4.1.2 Microfluidization

In this method microfluidizer device is used to mix the oil and the aqueous phase. Within the device the aqueous phase and oily phase are allowed to proceed in an inline homogenizer and results in the formation of an emulsion. After that the emulsion is processed into a microfluidizer and formed a stable nanoemulsion.

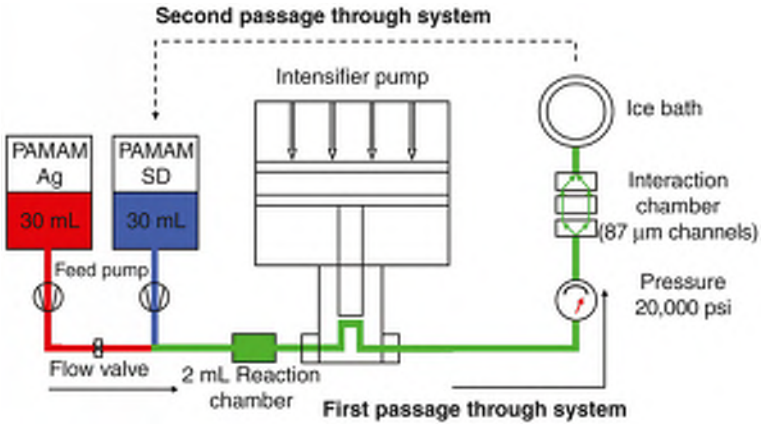


**Figure 5.1. Microfluidizer processor configuration.** Reprinted with permission from Strydom et al. (2013). Poly(amidoamine) dendrimer-mediated synthesis and stabilization of silver sulfonamide nanoparticles with increased antibacterial activity. Nanomed. Nanotechnol. Biol. Med. 9, 85–93.

The emulsion is allowed to pass through the interaction chamber of the microfluidizer for several times to form desired size particles. To get a uniform nanoemulsion, large droplets are removed by filtering the bulk emulsion through a filter under inert atmosphere (Bouchemal et al., 2004). A simple representation of the method is demonstrated in Fig. 5.1 in which the mixture of the oil phase and the water phase is forced to pass through some interaction chamber containing some microsized channels by applying pressure of about 500–20,000 psi with the help of a high-pressure positive displacement pump. On the flowing of the mixture through these microchannels on to impingement area very fine particles having size within the submicron range are prepared. In microfluidization technique, droplet size decreases with increasing homogenization pressure, increasing number of passes, increasing emulsifier concentration, and decreasing dispersed-to-continuous phase viscosity ratio.

J. Wang et al. prepared nanoemulsion from stearic acid, microalgae oil, water, and surfactant poloxamer 188 by using microfluidization technique and found droplet sized of lipid carrier about 318.2 nm after three times passes at pressure 1500 bar (Wang et al., 2014). Y.J. Jo et al. utilized microfluidization method to prepare β-carotene nanoemulsion having component β-carotene, medium chain triglycerides (MCT) oil, polyoxyethylene sorbitan monolaurate (Tween-20), polyoxyethylene monooleate (Tween-80), food protein like whey protein isolate (WPI), soybean protein isolate (SPI), and sodium caseinate (SC) (Yeon and Kwon, 2014).

#### 2.4.1.3 Sonication

In the sonication method high-intensity ultrasonic waves having frequency greater than 20 kHz are used in the formation of nanoemulsions having very fine tiny droplets (Leong et al., 2009). It is reported in the literature that bench-top sonicators are widely used by the researchers to prepare nanoemulsion with very fine droplet. These devices have an ultrasonic probe that contains a piezoelectric crystal that converts electrical waves into intense pressure waves. During the preparation of the nanoemulsions, the probe is dipped into the sample to be homogenized and intense disruptive forces at its tip is formed through combination of cavitation, turbulence, and interfacial waves. This is the main phenomenon of the sonication method for the preparation of nanoemulsions (Abismail et al., 1999; Kentish et al., 2008). Ultrasonic emulsification is preceded through two mechanisms (1) the applied sound field produces interfacial waves and the dispersion of the oil phase in the continuous phase takes place in the form of droplets, and (2) the applied ultrasound causes sound cavitations, which causes the formation and collapse of microbubbles by the pressure fluctuations of the simple sound wave and creates extreme levels of highly localized turbulence, which break the primary droplets into submicron size. Due to the inhomogeneous nature of the emitted sound field recirculation of the emulsions through the region of high power occurs and as a result all droplets experience the higher shear rate. The nanoemulsions produced by ultrasonication method have wider and bimodal size distributions. (Jafari et al., 2006, 2007). V. Ghosh et al. prepared nanoemulsion by using 20 KHz sonicator from basil oil, nonionic surfactant Tween-80, and water with droplet size 29.6 nm at low oil to surfactant ratio (Ghosh et al., 2013). Similarly R.D. Kale et al. prepared nanoemulsion of paraffin oil in presence of Tween-80 as surfactant using Leela Sonic 250 UPP ultrasonicator of power 250W (Kale et al., 2014). T. Delmas and his coworker prepared nanoemulsion consisting of a lipid core oil and wax, a mixture of insoluble surfactants phospholipids and hydrophilic surfactants PEG stearate adopting this ultrasonication technique (Delmas et al., 2011).

### *2.4.2 Low-Energy Methods*

The low-energy emulsification methods are cost effective in which nanoemulsions with the small-size droplet are prepared by using low amount of energy. The formation of the desired sized droplets of nanoemulsions are mainly dependent on the physical and chemical properties of the surfactants, cosurfactants, and oil used to prepare nanoemulsions as well as on the change of the interfacial tension on the transition of phases. On the basis of phase

behavior and constituent's properties, low-energy methods were modified to obtain very small size particles containing nanoemulsions. Usón et al. fabricated W/O nanoemulsions consisting of oil phase (isopropyl myristate), water, and surfactant with droplet size 60–160 nm by utilizing a low-energy method (Usón et al., 2004). Porras et al. also reported the formation of W/O nanoemulsions by using low-energy method with mean sizes 30–120 nm stabilized with mixtures of sorbitan ester surfactants (Porras et al., 2004). These low-energy emulsification methods include spontaneous emulsification (SE), phase inversion composition (PIC), phase transition, phase inversion temperature (PIT), emulsion inversion point (EIP) methods, which are described in detail in the next sections (Anton and Vandamme, 2009; Fernandez et al., 2004; Maestro et al., 2008). The low-energy method utilizes the stored energy of the system to form small droplets of nanoemulsions. This emulsification can also be carried out by changing the temperature, composition of the system, which affects the HLB of the system. But these complex methods require precise approach and synthetic surfactants are used. Some of widely used low-energy emulsification methods are discussed in the sunsequent section.

#### 2.4.2.1 Spontaneous Emulsification

In spontaneous emulsification formation of nanoemulsion takes place spontaneously when an organic phase and an aqueous phase are mixed together at a particular temperature (Anton and Vandamme, 2009). In this method the size of the droplet formed depends on the different parameters such as the compositions of the organic and aqueous phases, temperature, pH, and ionic strength and the mixing conditions such as stirring speed, rate of addition, and order of addition. When the organic phase consisting of nonpolar oil and a hydrophilic surfactant and water-miscible organic solvent is poured to water or water is poured to the organic phase containing nonpolar oil, water-miscible organic solvent and surfactant, formation of nanoemulsion takes place (Anton and Vandamme, 2009; Sonneville-Aubrun et al., 2009). A simple representation of the spontaneous emulsification is presented in Fig. 5.2.

The physicochemical mechanism of spontaneous emulsification method is the movement of a water-miscible component, which may be solvent or surfactant from the organic phase into the aqueous phase (Anton and Vandamme, 2009). On mixing the organic and aqueous phases, the water-miscible solvent or surfactant moves from the organic phase into the aqueous phase. Due to this movement a large turbulent force at the oil–water interface is generated and the oil–water interfacial area is also increased,

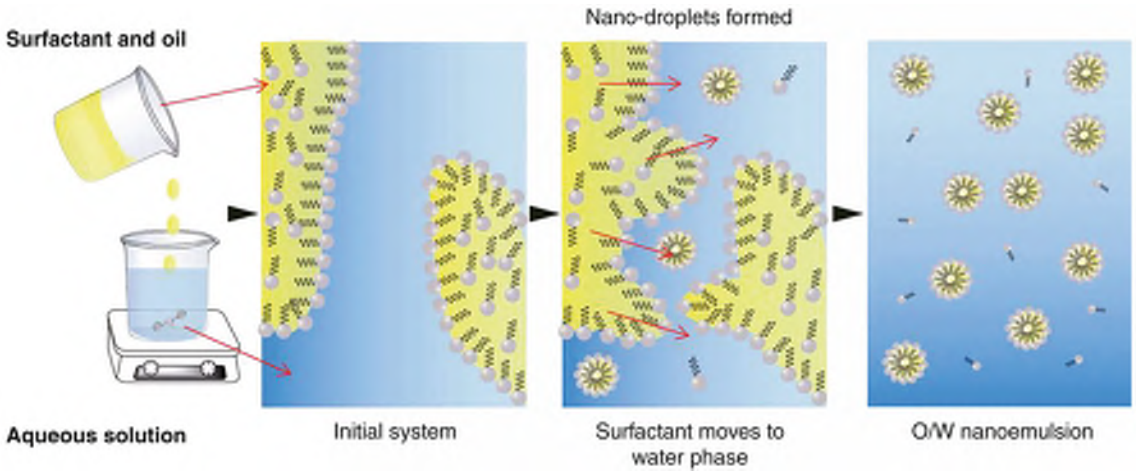


**Figure 5.2. Spontaneous emulsification method for preparation of nanoemulsion.** Reprinted with permission from McClements (2011). Edible nanoemulsions: fabrication, properties, and functional performance. Soft Matter 7, 2297–2316.

which causes the spontaneous formation of oil droplets surrounded by aqueous phase through a budding process. To prepare very small droplets containing nanoemulsions, large amount of miscible component should be added in the organic phase. The spontaneous emulsification method is widely used to encapsulate and deliver the lipophilic drug molecules in the pharmaceuticals industries (Narang et al., 2007; O'Driscoll, 2002; Pouton, 2000; Singh et al., 2009).

R.D. Kale et al. successfully prepared nanoemulsion with droplet size about 191.7 nm from coconut oil, Span 80, acetone in oil phase, distilled water and Tween-20 in aqueous phase by adopting this technique (Kale et al., 2014). C.B. Sainz and his coworkers also reported the preparation of nanoemulsion from purified water, acetem, and surfactant Tween-60 under agitation (Sainz et al., 2010). Y. Chang et al. prepared nanoemulsion by mixing an oil phase containing medium chain triglyceride (MCT), carvacrol, Tween-20, -40, -60, -80, and -85 and an aqueous phase containing citrate buffer pH 3.5 (Chang et al., 2013).

#### 2.4.2.2 Phase Inversion Technique

The phase inversion techniques may proceed through two types of mechanism which are (1) phase inversion temperature (PIT) and (2) phase inversion composition (PIC). The phase inversion temperature (PIT) method based on the principle of changes in the molecular geometry and the solubility of nonionic surfactants when the temperature of the nanoemulsion system is changed (Anton et al., 2007; Anton and Vandamme, 2009;

Gutierrez et al., 2008). Using the PIT method, nanoemulsions can be prepared by changing the temperature and time of stirring of certain mixtures of oil, water, and nonionic surfactant. In this technique one type of emulsion to another type, that is, W/O to O/W or O/W to W/O can be transformed in controlled manner through the formation of an intermediate bicontinuous phase (Fig. 5.3). With the change of temperature, the driving force arises at the oil–water interface is changed, which causes the change in the physicochemical properties of the surfactant (McClements, 2011). The solubility of the nonionic surfactant in oil phase and water phase is changed with the temperature of the system. At low temperature, the head group of the nonionic surfactant is more hydrated and the solubility of the surfactant in water phase is high whereas at higher temperature, solubility of the surfactant in water decreases because to the head group becomes dehydrated with increases in temperature. PIT is the particular temperature at which the solubility of the surfactant in oil and water phase is equal. Two types

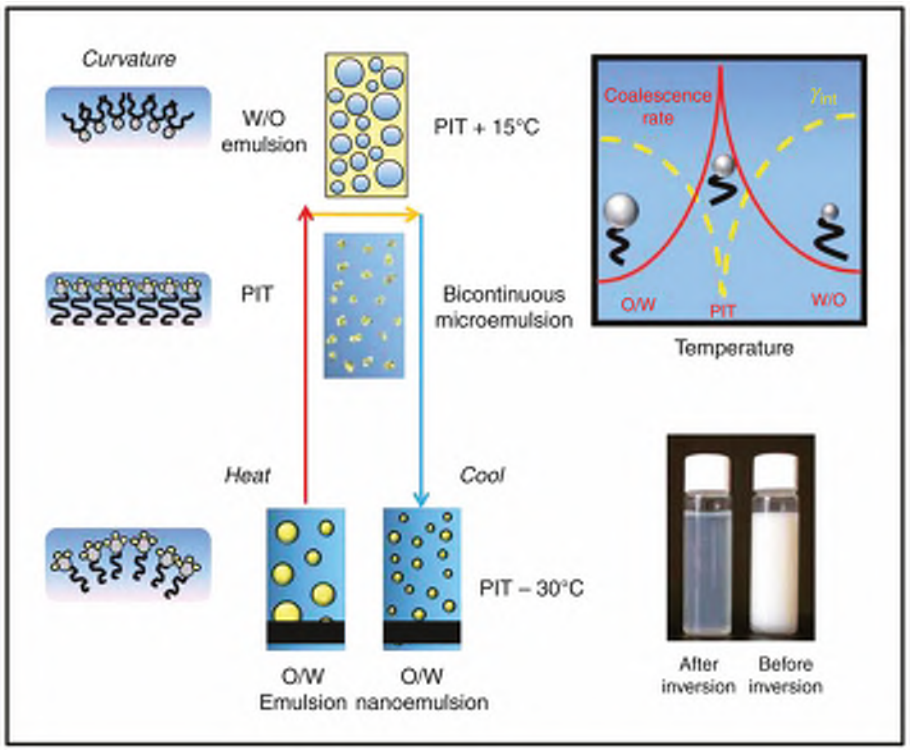


**Figure 5.3. Formation of nanoemulsions by the PIT method.** Reprinted with permission from McClements (2011). Edible nanoemulsions: fabrication, properties, and functional performance. Soft Matter 7, 2297–2316.

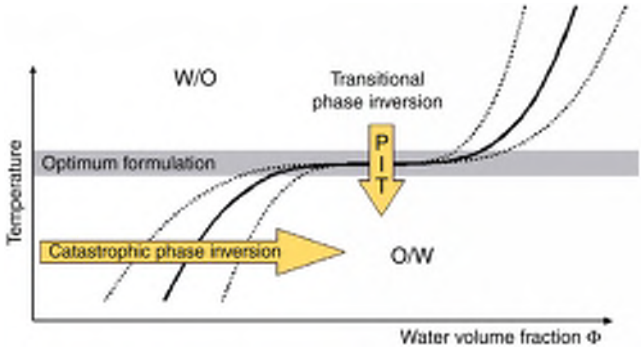


**Figure 5.4. Catastrophic and transitional phase inversion for the preparation of O/W emulsions.** Reprinted with permission from Fernandez et al. (2004). Nano-emulsion formation by emulsion phase inversion. Colloids Surf. A 251, 53–58.

of phase inversion emulsification method are used. These are (1) in the transitional inversion process the factors like temperature, electrolyte concentration, and hydrophile-lipophile balance the number of the surfactant and are changed to cause inversion of phase of the nanoemulsion, and (2) in catastropic inversion method, the volume fraction of the dispersed phase is increased. When an O/W-type emulsion containing nonionic surfactant is heated at the phase inversion temperature (PIT) the emulsion converts to W/O-type emulsion (Gascon et al., 2003) (Fig. 5.4). Catastropic and transition phase inversion technique adopt for the preparation of O/W emulsion is presented in Fig. 5.4.

The phase inversion composition (PIC) method is based on the principle of change of optimum curvature of the surfactant with the change in the composition of the system (Anton and Vandamme, 2009). In case of an O/W emulsion stabilized by an ionic surfactant, when a salt is added, it undergoes phase inversion and form a W/O emulsion. On the other hand, on dilution of a W/O emulsion containing a high salt concentration is converted into an O/W emulsion, because the ionic strength is decreased below some critical level (McClements, 2011). In this method formation of nanoemulsions do not require heat and presence of any organic phase. When water is added dropwise into oil phase containing the surfactant under constant stirring at a fixed temperature, a nanoemulsion having high kinetic stability is formed. The nanoemulsion is formed by spontaneous emulsification due to the phase transitions occur during the emulsification process (Patel et al., 2013).

P. Fernandez et al. prepared Water/Cremophor® A6/A25/paraffin oil emulsions compositions by adopting the phase inversion technique (Fernandez et al., 2004). They carried out the

**Table 5.2 List of Weight% of Water, Cremophor, and Paraffin Oil (Fernandez et al., 2004)**

| Water (%) | Cremophor (%) | Paraffin Oil (%) | Cremophor (%)/Paraffin Oil (%) |
|---|---|---|---|
| 75.8 | 2.5 | 21.7 | 0.12 |
| 73.9 | 5 | 21.1 | 0.24 |
| 71.9 | 7.5 | 20.6 | 0.36 |
| 70.0 | 10 | 20 | 0.5 |

experiment at 80°C with different surfactant-to-oil weight ratios. The compositions of oil-and-water phases they used are given in the Table 5.2.

It is mentioned that when the weight percent of Cremophor is less than 10%, O/W-type nanoemulsions were prepared by pouring water into oil phase. On the other hand, if the weight percent of Cremophor is more than 10%, O/W-type nanoemulsions were prepared by pouring oil in water phase (Fernandez et al., 2004).

#### 2.4.2.3 Membrane Emulsification Method

Membrane emulsification was introduced by Nakashima and Shimizu in 1980s in Japan (Nakashima and Shimizu, 1986; Nakashima et al., 1991). The principle of this method is the passing of the dispersed phase through the pores of a microporous membrane into a continuous phase under the effect of force (Nakashima et al., 1992). Formation and separation of the emulsified droplets at the end of the pores occurs through a drop-by-drop mechanism (Fig. 5.5). Adopting this method, emulsions having controlled and narrow droplet sizes can be prepared. This emulsification method requires less amount of emulsifier and energy. Because of the lower shear stress effect, some shear-sensitive components such as starch and proteins are used in the passing of dispersed phase through a membrane into a continuous phase (Vladisavljevic et al., 2000).

#### 2.4.2.4 Emulsion Inversion Point Method

In this technique one type of emulsion can be converted to another type through a catastrophic phase inversion (CPI) (Fernandez et al., 2004; Thakur et al., 2008). For example, a W/O emulsion with a high oil-to-water ratio is formed using a particular surfactant as a stabilizer. On increasing the amount of water

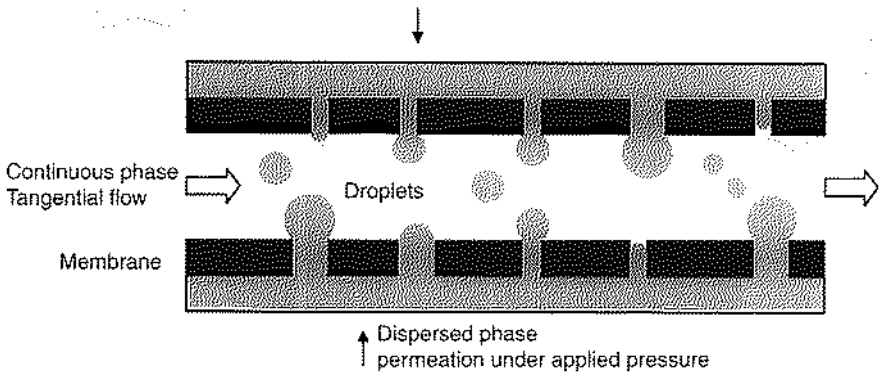


**Figure 5.5. Membrane emulsification process.** Reprinted with permission from Charcosset et al. (2004). The membrane emulsification process—a review. J. Chem. Technol. Biotechnol. 79, 209–218.

with continuous stirring, the water droplet concentration become very high at a particular point and the emulsion undergoes phase inversion and changes from a W/O to O/W system. The size of the nanoemulsion droplets formed in this method depends on the stirring speed and the rate of water addition (Thakur et al., 2008). Small surfactant molecules with the ability of stabilization for both O/W and W/O emulsions are used as the emulsifiers in this catastrophic phase inversion method. Cristina et al. prepared nanoemulsions with diameter in the range of 100–200 nm using CPI method (Sainz et al., 2010).

#### 2.4.2.5 Solvent Displacement Method

Nanoemulsions are formed spontaneously in the solvent displacement method. Diffusion of the oil phase to the continuous phase takes place when the oil phase is mixed with some organic solvents like low molecular weight alcohols and ketones, which are miscible with the aqueous phase. After the formation of the nanoemulsion the organic solvent is removed by simple vacuum evaporation technique. Nanoemulsion can also be formed spontaneously by adding an oil phase, which contains organic solvents in a small percentage to aqueous phase without any surfactant. In solvent displacement methods, nanoemulsions are formed at room temperature under simple stirring condition. But the disadvantages of this method are that the external input is required for removal of the organic solvents, which are used to prepare nanoemulsions. Moreover, high solvent-to-oil ratio value is required to prepare a nanoemulsion with desirable droplet and in such cases the solvent removal process may cause several difficulties (Lovelyn and Anthony, 2011).

# 3 Characterization of Nanoemulsions

Several characterization techniques are used to characterize a nanoemulsion, which includes size distribution, zeta potential, and crystallinity of the nanoemulsion. Some of the characterization techniques are discussed in this section.

## 3.1 Dynamic Light Scattering

Dynamic light scattering (DLS) technique or photon correlation spectroscopy (PCS) or quasi-elastic light scattering is used to determine the particle size, size distribution of nanoemulsions, and their fluctuations in terms of scattering intensity, which is caused by the Brownian motion of the particles within the nanoemulsions (Ruth et al., 1995). This technique is also used to measure polydispersity index of the nanoemulsion system, which is related to the broadness of the size distribution, which can be derived from the cumulative analysis of dynamic light scattering. Polydispersity index provides information of the homogeneity of the dispersion (Li et al., 2011).

Duartea et al. prepared nanoemulsion from the essential oil obtained from *Rosmarinus officinalis*, water and polysorbate 20 and they found that the mean diameter of the particle is less than 200 nm (Duartea et al., 2015). They observed from the particle size distribution analysis that different sizes of particles are present in the prepared nanoemulsions. They also studied the variation of particle size and polydispersity with time of storing the emulsion up to 30 days. They found that after 7 days the mean diameter increases whereas polydispersity decreases. But within 21–30 days polydispersity of the system is not changed with time. Finally they remarked that the micelle reached in a dynamic equilibrium within that time and the nanoemulsion became kinetically stable.

M. Jaworska et al. prepared cosmetically applicable O/W nanoemulsions using caprylic/capric triglycerides (GTCC), propylene glycol dicaprylate/dicaprate (PC), and oleic acid (OA) as oil phase, water, and polysorbate 80 as surfactant using phase inversion composition method at 25°C. The particles size distribution of the synthesized nanoemulsion was analysed by using DLS measurement technique by the Zetasizer NanoZS (Malvern Instruments, United Kingdom). They found that the droplet size of inert phase depends on the type of oil used for preparation of nanoemulsion. They found that the emulsion prepared by using crodamol GTCC had droplets size $r = 8$ nm, similarly crodamol PC nanoemulsion had droplet size $r = 22$ nm and the oleic acid-based

emulsion had droplet size $r$ = 339 nm at 10:90 oil/surfactant ratio. Further, emulsion with oleic acid oil is very highly polydispersed. The water insoluble nanoemulsions is formed by caprylic/capric triglycerides due to its most hydrophobic nature with the lowest value of HLB = 9.88, while the oleic acid formed water-soluble nanoemulsions with the highest HLB = 16.00. They observed that the lipophilicity of oil phase has significant influence on the size distribution and stability of nanoemulsions (Jaworska et al., 2013).

## 3.2 Zeta Potential

Zeta potential is the potential difference between dispersion medium and the stationary layer of fluid binding with the dispersed particle that separates low charged surfaces from highly charged surfaces (Preetz et al., 2010). Zeta potential value is related to the stability of colloidal dispersions. Generally for a stable suspension the value of zeta potential is ±30 mV. From the zeta potential value the degree of repulsion between adjacent, same charged particles within the dispersion phase can be verified. Highly stable small molecules and particles have high zeta potential value. When the zeta potential is low, attraction among the molecules is high and it causes flocculation of the system and the system becomes unstable. Thus, from the zeta potential measurement the surface charge properties and the physical stability of nanoemulsions can be investigated. A zeta PALS instrument is used to measure the surface charge of the particles. Its values are mainly dependent on the electrophoretic mobility of the oil droplets within the suspension under the influence of an external electric field (Yilmaz and Borchert, 2005).

The factors that affect the value of zeta potential of nanoemulsions are source of particles, types of surfactants, electrolyte concentration, or ionic strength, particle morphology and size, pH of the solution and state of hydration. The nanoemulsions with high zeta potential values are very stable. Because of the presence of highly charged particles droplet aggregation is inhibited (Araújo et al., 2011).

## 3.3 Conductivity

Conductivity of the nanoemulsion is measured to determine the nature of the continuous phase and phase inversion phenomena. Electrical conductivity is measured using a digital conductivity meter at ambient temperature. O/W Nanoemulsions have high conductivity whereas W/O nanoemulsions have low conductivity value (Rao and Shao, 2008).

## 3.4 Viscosity

The nature and concentration of the components of nanoemulsions including surfactants, water, and oil have considerable effect on the viscosity of the nanoemulsions. Viscosity decreases with the increase in the amount of water, on the other hand with increase in the amount of surfactants and cosurfactants viscosity also decreases due to the decrease in the interfacial tension between the aqueous and organic phases. The stability and the rheological properties of the nanoemulsion is measured from the value of viscosity. Patel et al. measured the viscosity of the nanoemulsion prepared from ketoconazole, coconut oil, Tween-80, and ethanol by using Brookfield Rheometer viscometer at 30°C with a CPE 61 spindle at 30 rpm and found the value of viscosity about 560.9 cp (Patel et al., 2013).

## 3.5 Interfacial Tension

The nanoemulsion formation mechanism and the properties can be easily determined from the value of the interfacial tension between the two liquid phases. For the system with surfactant molecules the values of interfacial tension are very low (Leong et al., 2009). Spinning-drop apparatus is used to measure the interfacial tension. Interfacial tension can be measured from the shape of the drop obtained by rotating a drop of low-density phase in a cylindrical capillary filled with high-density phase.

## 3.6 Differential Scanning Calorimetry

DSC is a very useful technique to gather the knowledge on the phase transitions phenomena of the materials such as melting, crystallization, glass transition, and decomposition of nanomaterial-bio conjugates. From the DSC measurements the structure and stability of nanoemulsion can be investigated based on the DSC data.

R.V. Tikekara and Nitin utilized DSC technique to determine the melting and solidification temperature of emulsified eicosane. They found that crystallization and melting temperatures for eicosane were 22.1°C and 37.2°C, respectively (Fig. 5.6) (Tikekara & Nitin, 2011).

## 3.7 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy provides the information of the different functional groups present in a nanoemulsion can be identified. Araújo et al. prepared THD

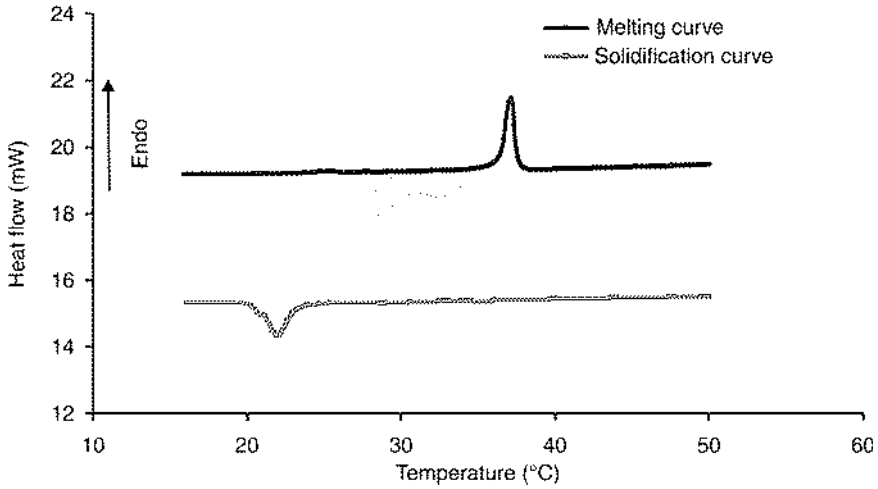


**Figure 5.6. Differential scanning calorimetry (DSC) thermograms for eicosane HML–bile salt emulsion.** Reprinted with permission from Tikekara and Nitin (2011). Effect of physical state (solid vs. liquid) of lipid core on the rate of transport of oxygen and free radicals in solid lipid nanoparticles and emulsion. Soft Matter 7, 8149.

nanoemulsions from castor oil, soybean lecithin, water, glycerol, polysorbate 80, and the β-THD crystals and characterized by FTIR spectra (Fig. 5.7). They found that both the polymorphic forms of THD show characteristic absorption bands in the FTIR spectra. The α-polymorphic form showed bands at 3200 $cm^{-1}$ (N-H stretching vibration), 3100 $cm^{-1}$(N–H stretching vibration), and 860 $cm^{-1}$

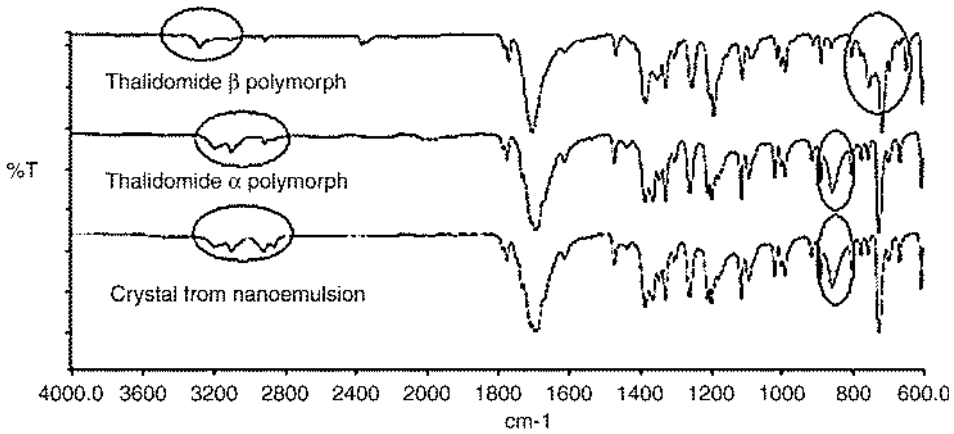


**Figure 5.7. FTIR spectra of thalidomide β-polymorph, α-polymorph, and nanoemulsion crystals.** Reprinted with permission from Araújo et al. (2011). Development and characterization of parenteral nanoemulsions containing thalidomide. Eur. J. Pharm. Sci. 42, 238–245.

(C–H stretching vibration), while β-polymorph showed bands in 3250 $cm^{-1}$ (N–H stretching) and 750 $cm^{-1}$ (C–H stretching) (Allen and Trotter, 1970; Carini et al., 2009; Lara-Ochoa et al., 2007; Reepmeyer et al., 1994). The crystals collected from nanoemulsion showed characteristic bands of α-polymorphic form at 3200 and 3100 $cm^{-1}$ corresponding to the N–H stretching vibration (Araújo et al., 2011).

## 3.8 X-Ray Diffraction

X-ray diffraction (XRD) technique gives the information about the crystallographic structure, chemical composition, and physical properties of materials. The sample can be analyzed on the basis of the scattered intensity of the X-ray beam, which is scattered when it is allowed to strike the sample to be investigated. The scattered intensity is plotted as a function of incident angle (Azároff et al., 1974).

Araújo et al. prepared THD nanoemulsions from castor oil, soybean lecithin, water, glycerol, polysorbate 80, and the β-THD crystals and is characterized by XRD analysis (Fig. 5.8). They observed that THD crystals are in α-polymorphic form in the nanoemulsion, which confirms the crystal growth as a different polymorphic from β- form that used for nanoemulsion preparation (Araújo et al., 2011).

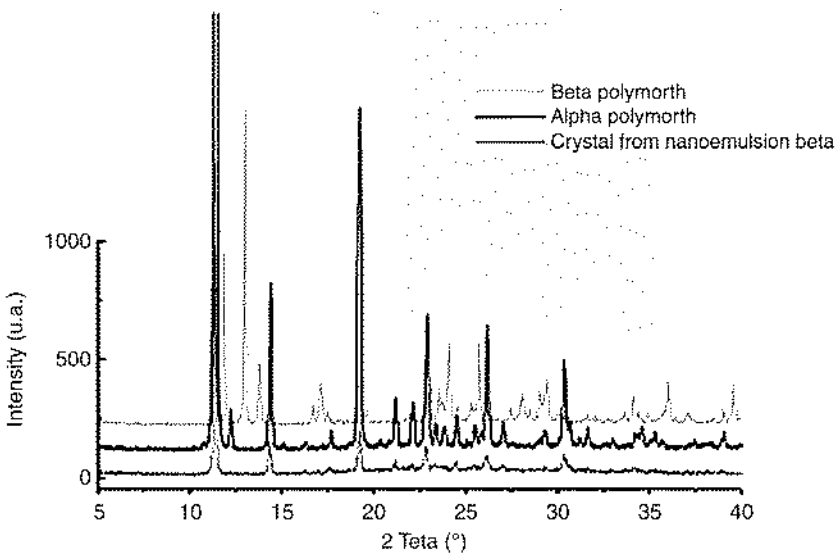


**Figure 5.8. XRD diffractograms of β-polymorph, α-polymorph and nanoemulsion crystals. (from top to bottom)** Reprinted with permission from Araújo et al. (2011). Development and characterization of parenteral nanoemulsions containing thalidomide. Eur. J. Pharm. Sci. 42, 238–245.

L. Cornacchia and Roos used O/W emulsion-based systems, in which the lipid carrier (hydrogenated palm kernel oil, HPKO or sunflower oil, SO) and dairy proteins were (whey protein isolate, WPI, and sodium caseinate) used for incorporation of β-carotene. The physicochemical properties of the encapsulating matrix was investigated by X-ray diffraction analysis. They reported that bulk HPKO showed two main peaks at ~21°C and ~23°C and the emulsified HPKO showed diffraction peaks between ~21°C and ~24°C, that confirmed that HPKO crystals were present in the systems stored at 20°C. The differences of XRD peaks were arisen due to the presence of different crystal structure due to the effect of the hydrophobic part of the proteinaceous emulsifier (Cornacchia and Roos, 2011).

## 3.9 Small-Angle X-Ray Scattering

Small-angle X-ray scattering (SAXS) is used to study the types of the structure of colloidal size particles. X-rays are scattered due to the elastic collision between the X-rays and the sample. The scattering of X-rays is recorded at the angles of range 0.1–10°C. The shape and size of macromolecules, structure, characteristic distances of partially ordered materials, pore sizes can be determined from this analysis. This method is nondestructive and a minimum amount of sample is required for analysis (Luykx et al., 2008).

Zhang et al. used this technique to analyse AOT/water/isooctane/$CO_2$ nanoemulsions. They reported that SAXS patterns show a single broad peak at $q \neq 0$ and a tail at high q range, where q is the scattering vector ($q = 4\pi \mathrm{Sin}\varphi/\lambda$, φ=scattering angle). Moreover, the position of the maximum moves to a lower angle at the higher pressure. The periodicity of the system was obtained at 48.9 (±0.5) A° for 2.92 MPa and at 50.4 (±0.5) A° for 3.08 MPa, respectively (Fig. 5.9). The periodicity at the higher pressure was larger due to the more solubilization of water in the reverse micelles at the higher pressure. The scattering angle is also increased due to the increase in the size of reverse micelles (Zhang et al., 2011).

## 3.10 Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) is very useful tool to investigate the structural formula, stereochemistry, and conformation of molecules. Both solid and liquid compounds can be characterized quantitatively by this technique (Rouessac and Rouessac, 2007). Jenning et al. utilized $^{1}H$ NMR for the characterization of incorporated medium chain triglycerides oil within a matrix of long-chain solid glyceride. The arrangement of the components and the environment of the oil molecules were investigated from the

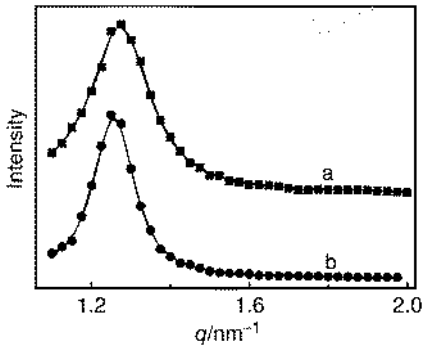


**Figure 5.9.** Small-angle X-ray scattering (SAXS) curves of AOT/water/isooctane/$CO_2$ nanoemulsions formed at (a) 2.92 MPa and (b) 3.08 MPa. Reprinted with permission from Zhang et al. (2011). Emulsion inversion induced by $CO_2$. Phys. Chem. Chem. Phys, 13, 6065–6070.

NMR study (Jenning et al., 2000). Casadei et al. also used $^1$H NMR technique to analyze incorporated ibuprofen and from the result obtained, they could determine the amount of unloaded ibuprofen and the trapping efficiency of the compound within solid lipid particles (Casadei et al., 2006).

## 3.11 Small-Angle Neutron Scattering

Small-angle neutron scattering (SANS) is used to obtain the information about the equilibrium stability and structure of the internal droplets of nanoemulsions. The scale of analysis of the SANS data is associated with the internal structure of nanoemulsion droplets within the range ~1–10 nm. From SANS the detailed structural information of the droplets of the various components can be obtained. Wang et al. prepared nanoemulsion from pentaoxyethylene lauryl ether (C12E5), dodecyldimethylammonium bromide, sodium bis (2-ethylhexyl) sulfosuccinate, sodium *n*-dodecylsulfatepentanol, and hexadecyltrimethylammonium bromide-pentanol with droplet radii about 15 nm. They investigated the nanoemulsion by SANS using the time-of-flight LOQ instrument at ISIS, United Kingdom. They examined the nanoemulsion with the volume fraction of the components ($\varphi_{decane} = 0.020$, $\varphi_{C12E5} = 0.010$) at different times and they obtained different data from SANS studies which are shown in Table 5.3 (Wang et al., 2008a).

## 3.12 Imaging Techniques

Based on the nature of the sample to be analyzed, different types of imaging techniques are used for nanoemulsion characterization.

**Table 5.3 SANS Data of Decane/$C_{12}E_5$ Nanoemulsions (Wang et al., 2008a)**

| Sample | $C_{drop}$ (μmol dm$^{-3}$) | $R_{fit}$ (nm) | $T_s$ (nm) |
|---|---|---|---|
| A, 30 min | 15 | 10.2 | |
| A shell, 30 min | | 10.1 | 1.1 |
| A, 120 min | 15 | 10.1 | |
| A, 240 min | 15 | 9.9 | |

Where $C_{drop}$ is the nanoemulsion droplet concentration, $R_{fit}$ is the average radius; $T_s$ is the apparent shell thickness.

These techniques are carried out to determine the size, shape, and orientation of the components within the sample of the nanoemulsions. Some of the imaging methods that are used for the characterization of nanoemulsions are discussed in the subsequent sections.

### *3.12.1 Transmission Electron Microscopy*

Transmission electron microscopy (TEM) is a technique in which a high-energy electron beam created from an electron gun is allowed to strike a very thin sample. When the electron beam is transmitted through the sample an image is produced bearing the information about the size, shape, crystalline, or amorphous structure and orientation of the components within the sample. TEM gives magnified image in various resolutions up to the range of 0.2 nm. In case of nanoemulsions, TEM is used to determine the morphology and size of emulsion droplet. Bouchemal et al. (2004) used TEM for the analysis of the morphology and structure of the nanoemulsions using TEM.

V. Ghosh et al. studied the morphology of nanoemulsion obtained from basil oil, nonionic surfactant Tween-80 with HLB-15, and water by transmission electron microscopy (Fig. 5.10). From the TEM image they observed that the emulsion contained spherical droplets with the size range 20–50 nm (Ghosh et al., 2013).

### *3.12.2 Scanning Electron Microscopy*

The morphology of nanoemulsions can be determined by scanning electron microscopy (SEM). SEM produces 3-dimensional high-resolution image of the sample and gives the information about the surface structure. (Fig. 5.11). Sometimes the surfactants used in the preparation of nanoemulsions can cause a smooth

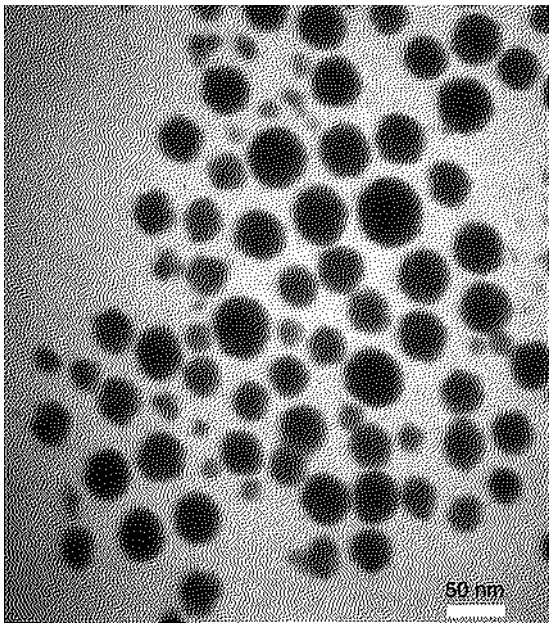


**Figure 5.10. TEM image of basil oil nanoemulsion.** Reprinted with permission from Ghosh et al. (2013). Ultrasonic emulsification of food-grade nanoemulsion formulation and evaluation of its bactericidal activity. Ultrason. Sonochem. 20, 338–344.

camouflaging coating on the particle surfaces and in such cases characterization of the nanoemulsion by SEM image is become impossible.

### *3.12.3 Atomic Force Microscopy*

Atomic force microscopy (AFM) technique is used to investigate the shape and size, physical properties of nanoemulsified coatings such as root mean-square roughness, average roughness, droplet size measurement, and surface morphology of nanoemulsions. This technique is used to explore the surface morphology at nano- to microscale and the influence of inhibitor on the generation and the progress of the corrosion at the metal/solution interface can also be studied by using this technique. The sample for the AFM is prepared by drop coating the diluted nanoemulsion onto a glass slide and drying. V. Ghosh et al. (2013) prepared nanoemulsion using basil oil, nonionic surfactant Tween-80 with HLB-15 and water. They determined the size and shape of nanoemulsion by AFM technique and found that the morphology of droplets was spherical in shape and the mean droplet diameter of the smooth surface was 20.1 nm (Fig. 5.12) (Ghosh et al., 2013).

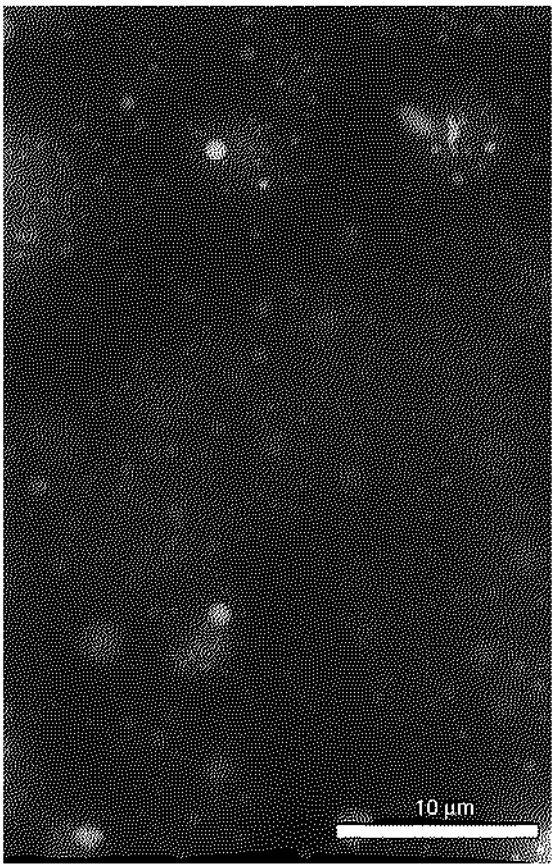


**Figure 5.11. SEM image of emulsion containing cinnamon oil in Acetem prepared by catastrophic phase inversion emulsification.** Reprinted with permission from Sainz et al. (2010). Nanoemulsions prepared by a low-energy emulsification method applied to edible films. J. Agric. Food Chem. 58, 11932–11938.

## 4 Properties of Nanoemulsions

Nanoemulsions have a much larger surface area-to-volume ratio than ordinary emulsions, this is because of the presence of fewer numbers of molecules of the dispersed phase than other ordinary emulsions and this difference is in the range from 100 to 1000, depending on the molecular weight of the particles (Mason et al., 2006). The physicochemical properties such as stability, rheology, and optical behavior of the nanoemulsions are related to the particle composition, concentration, size, physical state, encapsulation, and interfacial properties.

Nanoemulsions contain lipids, proteins, polysaccharide surfactant, minerals, and so on. The droplets in the nanoemulsion

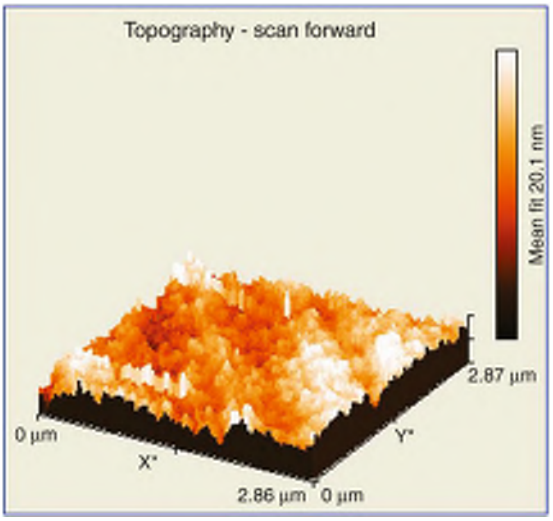


**Figure 5.12. Atomic force micrograph of basil oil nanoemulsion.** Reprinted with permission from Ghosh et al. (2013). Ultrasonic emulsification of food-grade nanoemulsion formulation and evaluation of its bactericidal activity. Ultrason. Sonochem. 20, 338–344.

consist of the lipophilic or nonpolar core bounded to a layer of adsorbed materials. The inner core is made up of triacyl glycerols, diacyl glycerols, flavor oils, mineral oils, fat substitutes, waxes, weighting agents, oil-soluble vitamins, and nutraceuticals. The outer shell or layer is consisted of surfactants, phospholipids, proteins, polysaccharides, minerals, and so on (McClements, 2005). The radius and thickness of the shell can effect on the particle composition of the nanoemulsions. The thickness of the shell layer varied with the type of the molecules adsorbed to the oil water interface (Mason et al., 2006; Tadros et al., 2004).

The particles concentration of the nanoemulsions depends on the amounts of emulsifiers or oil used to prepare nanoemulsion. The particles concentration is changed with changing the initial concentration of oil or with dilution or increasing concentration of the system after nanoemulsion formation. Due to the formation of particles with small diameter in the nanoemulsion, the particle concentration of nanoemulsion is different from the other conventional emulsion. The particle concentration of the conventional emulsion is also related to the oil concentration. In a nanoemulsion system thickness of the shell depends on the radius of the core. When core radius decreases in particles, shell thickness increases and as a result particle concentration increases (Mason et al., 2006; Tadros et al., 2004).

The rheological, optical properties, and stability of the nanoemulsions also depends on the size of the particles. Size of the particles varied with the condition of the nanoemulsion preparation such as temperature, pressure, miscibility of oil and water interface, type of emulsifier used, the interfacial tension, relative viscosities of dispersed and continuous phases, and concentration of emulsifiers (Jafari et al., 2006; McClements, 2005). The radius of the particles in the nanoemulsion system is identical to the sum of the radius of the core and shell layer of the droplets (Guzey and McClements, 2006). The mean radius of the droplets of nanoemulsion is in the range of 10–100 nm. Because of the small droplet size, nanoemulsions can scatter the visible light very weakly and therefore it does not show any color in the visible region and therefore nanoemulsions are transparent.

The charge of the particles effects the stability of the particles in the nanoemulsions system. Ionic emulsifier generally used for nanoemulsion formation, therefore droplet of the nanoemulsion adopted electrical charge. Stability of the nanoemulsion droplet depends on the mobility of these charged particles (McClements, 2005). Because of the presence of highly charged particles in the nanoemulsion system, they do not undergo aggregation and thus the system becomes kinetically stable. Droplets in the nanoemulsion system is stabilized by negative surfactant like lecithin having negative charge. Droplet with a positive charge is stabilized by positive surfactant such as arginate (Kralova and Sjoblom, 2009; Asker et al., 2009). Oil droplets are generally stabilized by protein, the charge of the protein depends on the pH of the solution with respect to the isoelectric point. Proteins have positive charge when pH of the solution is lower than isoelectric point, proteins have a negative charge when pH of the solution is higher than isoelectric point and have a neutral charge surfaces when pH of the solution equal to isoelectric point (Gu et al., 2005).

Interfacial characteristics are one of the important properties for determining the specific functional performance and designing the nanoemulsion system. The interfacial layer is a homogeneous layer around the droplet, contain certain molecules with particular structure. These molecules have specified interfacial properties like permeability, rheology, and environmental responsiveness (Mason et al., 2006; McClements, 2005). The selection of emulsifiers for the emulsion formation such as surfactant phospholipids, proteins, or polysaccharides is important to stabilize the interfacial characteristics of nanoemulsion droplet. Generally the spherical droplets of nanoemulsions have low value of interfacial tension.

Nanoemulsions have high encapsulation properties. It can encapsulate several lipophilic components such as vitamins, flavors, colors, preservatives, nutraceuticals, and drugs. These properties are utilized in several industries to carry out the encapsulation of lipophilic component for easy handling, utilization, and protection of the above lipophilic components (Acosta, 2009; Weiss et al., 2008).

Due to the aforementioned enormous properties, nanoemulsion possess several advantages such as:

- Nanoemulsions possess higher surface area compared to the other conventional emulsions, they can be used as template for the synthesis of nanoparticles.
- Nanoemulsions do not undergo the flocculation, coalescence, and sedimentation, which are commonly associated with microemulsion. Therefore, nanoemulsions are stable.
- Nanoemulsions can be used to encapsulate lipophilic component such as vitamins, flavors, colors, preservatives, nutraceuticals, drugs. So, nanoemulsions are widely used in food, pharmaceutical industries for storage and delivery of products.
- Nanoemulsion is nontoxic and nonirritant hence can be easily applied to the food preservation and encapsulation of bioactive compound.
- Nanoemulsions do not damage biological cells and therefore they can be used in pharmaceuticals and in cosmetics.
- Nanoemulsions can increase the bioavailability, shelf-life time, and solubility of the lipophilic compounds.
- Nanoemulsions can also be prepared by self-emulsification process, which requires a less amount of energy and its properties are independent on the experimental conditions.
- In encapsulation of both hydrophilic and lipophilic drugs, the same nanoemulsion can be used.
- Nanoemustions can mask offensive odors and bitter tastes of food.
- Nanoemulsions can protect the drug's molecules from oxidation and hydrolysis by air and water.

## 5 Application of Nanoemulsion in Food Chemistry

Nanoemulsions offer a wide range of applications due to their compositional flexibility in various fields including food, beverage, and pharmaceutical industries. Incorporation of bioactive molecules or other fluorescent dyes into nanoemulsion makes them a better system for exploring the properties of living cells and

for drug delivery. Nanoemulsions are liquid and can be easily deformed from large-size particles to smaller particles and because of these properties they can be used as efficient agents for cellular uptakes and dispersal phenomena. For pharmaceutical uses both oil-soluble and water-soluble drug molecules need to be entrapped into nanodroplets of direct and inverse nanoemulsions.

In cosmetic and food industries, nanoemulsions may provide rheological properties bearing transparent or soft solids. Due to the ability of nanoemulsion to block ultraviolet light, it is mostly used in sunscreen in cosmetics (Mason et al., 2006). Small size nanodroplets present within nanoemulsions provide enhanced transport efficiency of certain drugs or other bioactive molecules inside the droplets across biological membranes.

Besides food and pharmaceutical industries, nanoemulsions are also used in printing and data storage industries. Nanoemulsions can also be used in the form of ink in some thermally driven printers (Mason et al., 2006). However, colloidal dispersions of deformable nanodroplets, nanoemulsions have enormous applications in various fields, but herein we will focus only on the application of nanoemulsions in the food chemistry.

Due to the day-to-day development in technological aspects, research in the field of food packaging and processing has prolonged its range from microsized to nanosized particles. Nowadays, food nanotechnology is materializing with various innovative techniques in food packaging and nutraceuticals. A simple representation of the applications of nanotechnology in different areas of food science is shown in the Fig. 5.13. In recent years nanoemulsions are widely used for encapsulation of bioactive compounds and for the transport of probiotic and other functional health foods (Patel and Bhandari, 2014).

## 5.1 Nanoemulsions in Food Processing

Nanoemulsions have found a lot of applications in food processing compared to microemulsions due to its very small size, thermodynamic stability, transparency, continuous self-assembly with hydrophilic and hydrophobic portion and weak light wave scattering capacity, which eventually lead to their incorporation into optically transparent products such as fortified soft drinks and waters.

Unlike micro- or other conventional emulsions, nanoemulsions can be prepared to be more viscous or gel-like with very low droplet concentrations, which can be easily used to make products with novel texture and with low fats (McClements, 2011). Due to the stability of the droplet of the nanoemulsion stability

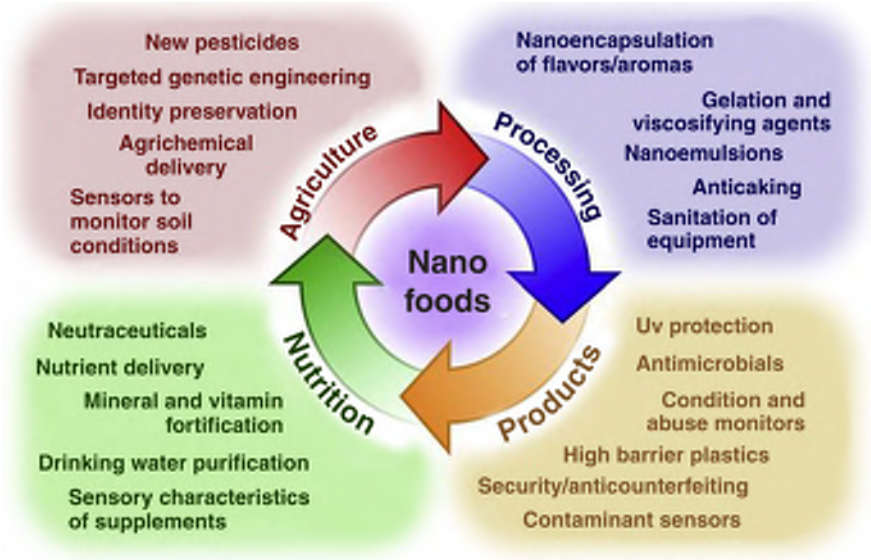


**Figure 5.13. Applications of nanotechnology in different food areas.** Reprinted with permission from Duncan (2011). Application of nanotechnology in food packaging and food safety: barriermaterials, antimicrobials, and sensors. J. Colloid Interf. Sci. 363(1), 1–24.

to particle aggregation and gravitational separation, nanoemulsions can enhance the shelf-life period of industrial products. Joe et al. used nanoemulsions prepared from sunflower oil for the processing of Indo-Pacific king mackerel steaks. They observed no microbial growth up to 12 h and the shelf life of the product was increased up to 48 h (Joe et al., 2012).

## 5.2 Nanoencapsulation

Nanoencapsulation comprises the inclusion, absorption, or dispersion of biologically active compounds into nanosized cells to increase the stability, solubility, and to protect the bioactive compounds against degradation. Moreover, nanoencapsulation also provides possibilities associated with the extension of gastrointestinal retention time which is caused by bio-adhesive enhancement in the intestinal epithelium (Bouwmeester et al., 2009). Nanoemulsions are also bearing the ability to enhance the bioavailability of encapsulated lipophilic substances (McClements, 2011). In nanocapsules the encapsulated bioactive compound is fitted into a cavity surrounded by a polymer membrane and in nanospheres the bioactive compound is uniformly dispersed in the matrix (Couvreur et al., 1995). O/W emulsion is used for

encapsulation of water insoluble hydrophobic compounds like water insoluble vitamins, minerals, aroma volatiles, flavor components, antioxidants, carotenoids, and lutein whereas W/O/W emulsion system is suitable for water-soluble bioactive compounds. Bioactive compounds are inserted in the aqueous core formulating in such nanoemulsions. Nanoencapsulation knowledge also demonstrated that the bioavailability of encapsulated bioactive compounds within lipid droplets increases with the decreasing the droplet size of the nanoemulsion. This is because of the facts that:

- Small droplets create a large surface area in the nanoemulsion system that can be digested by the enzymes more quickly, therefore their ingredients encaptulate in the nanoemulsion and are easily released and absorbed more.
- Small droplets present in the nanoemulsion penetrate into the mucous layer, which covers the epithelium cells within the small intestine, can increase their residence time and brings them to the site of absorption.
- Due to very small size, droplets may be directly transported by either paracellular or transcellular mechanism across the epithelium cell layer.
- Water-solubility of highly lipophilic components are also inversely proportional to the size of the droplet.

Recently, it has also been found that incorporation of curcumin (1.7-bis(4-hydroxy-3-ethoxyphenyl)-1.6- heptadiene-3.5-dione), a natural polyphenolic phytochemical within nanoemulsion, increases its oral bioavailability (Wang et al., 2008b; Huang et al., 2010). Curcumin is generally used as a natural food color and it exhibits antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities.

Day-to-day increasing applications of nanoemulsion basically in food chemistry is only due to its ability to entrap lipophililic compounds like flavors, antioxidants, and preservatives into transparent products. A number of studies have shown that for this kind of application, droplet radius is maintained to be small enough in comparison to the wavelength of light to resist strong light scattering. Wooster and coworkers remarked that nanoemulsion droplets should have radius less than 40 nm, which can process optically transparent systems and can be applied to food processing systems (Wooster et al., 2008). From these studies it is ensured that most of droplet size should fall below some critical range to prepare nanoemulsion for their applications in transparent food stuffs. For encapsulation, nanoliposomes, archaeosomes, and nanocochleates and natural biodegradable polymers like albumin, gelatin, alginate, collagen, chitosan, and

α-lactalbumin were used for encapsulation and as nanocarriers (Chen et al., 2006; Reis et al., 2006; Graveland-Bikker and De Kruif, 2006; De Kruif et al., 2004).

Nowadays, physiological effects of food ingredients affected by food processing, food matrix, and food digestion and metabolism have received considerable attention (Vos et al., 2006). The change of the food components at nanoscale and development of encapsulated ingredients can enhance the quality of traditional food, which leads to the improving the human health.

### *5.2.1 Various Techniques for Nanoencapsulation*

Due to the encapsulation, the physicochemical properties of the nanoemulsion droplet, like particle size, size distribution, surface area, shape, solubility, and encapsulation efficiency, and releasing mechanisms of the food ingredients are changed. Therefore, in food industries appropriate encapsulation techniques should be adopted to obtain the required size, physicochemical properties, nature of the core material, and wall materials. Capsules within the nanometer ranged in nanoencapsulation are prepared adopting various techniques namely nanoprecipitation, emulsification, emulsification-solvent evaporation, coacervation, inclusion complexation, and supercritical fluid. In nanoencapsulation either top-down or bottom-up approaches are utilized to develop materials in nanometer range. In the top-down approach, for a specific application small size materials with appropriate shape are prepared with the help of some particular instruments (eg, microfluidizer, high pressure homogenizer).

In the bottom-up approach, materials are produced when the precursors undergo self-assembly and self-organization. The mechanism of the materials formation process mainly depends on the several parameters like temperature, pH of the medium, concentration, and ionic strength (Augustin and Sanguansri, 2009). The top-down approaches like emulsification and emulsification–solvent evaporation techniques are used for encapsulation. Supercritical fluid technique, inclusion complexation, coacervation, and nanoprecipitation are used as the bottom-up approach (Sanguansri and Augustin, 2006; Mishra et al., 2010). Various hydrophilic and lipophilic bioactive compounds are encapsulated by emulsification, coacervation, and supercritical fluid techniques (McClements et al., 2009; Chong et al., 2009; Leong et al., 2011).

On the other hand inclusion complexation, emulsification–solvent evaporation, and nanoprecipitation techniques are mostly used to encapsulate lipophilic compounds (Reis et al., 2006). The

different techniques adopt for encapsulation of various hydrophilic and lipophilic bioactive compounds through the formation of nanoemulsions are discussed in the following sections.

#### 5.2.1.1 Emulsification Technique

To encapsulate the compounds, nanoemulsions are used either as dry powder or as colloidal suspension. After emulsification dry powder are prepared adopting drying techniques such as freeze drying or spray drying. Because of their high kinetic stability, nanoemulsionscan be used as a suitable agent for encapsulation and for retaining the surface oil content of the product (Jafari et al., 2008). The nonequlibrium nanoemulsion systems can be prepared by utilizing high-energy process, in which energy is produced from various mechanical devices. High-energy emulsification methods like high shear stirring, high-speed or high-pressure homogenizers, ultrasonicator, and microfluidizer processes are used for the formation of nanoemulsions. In these methods available energy required for production of nanoemulsions are produced within the mechanical device nanoemulsions with the smallest droplet sizes formed under the influence of the high pressure (Walstra, 1996). Jafari et al. (2008) utilized microfluidization technique for the encapsulation of fish oil in maltodextrin mixed with surface-active biopolymers containing Hi-Cap 100 and whey protein concentrate in a ratio of 3:1 and obtained the emulsion with droplet size of 210–280 nm. Wang et al. (2008) used high-pressure homogenization for the production of curcumin nanoemulsion with mean droplet size 80 nm and higher anti-inflammation activity (Wang et al., 2008b). Dupeyron and his coworkers used double emulsion (W/O/W) techniques and vacuum drying to encapsulate bovine serum albumin into methacrylic acid/ethylacrylate blended with PEG copolymer. They found that 90% nanoparticles had 77–78% encapsulation efficiency (Dupeyron et al., 2009).

Thus, it is seen that all the emulsification methods have the ability to reduce the droplet size to nanometer range and this technique can be used for encapsulation of products in food industries.

#### 5.2.1.2 Nanoprecipitation Technique

The principle of this technique is based on the spontaneous emulsification when the bioactive polymer containing organic phase is mixed with the aqueous phase. Precipitation of polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium takes place during nanoprecipitation (Galindo-Rodriguez et al., 2004). In this technique both nanocapsules

and nanospheres are formed. Ribeiro et al. utilized nanoprecipitation techniques along with freeze drying process to produce β-carotene loaded nanodispersions, in which β-carotene is encapsulated into PLA and PLGA. They used gelatin and Tween-20 as stabilizing hydrocolloids in the continuous phase. They reported that the prepared nanodispersions were more stable with the diameter of the droplets 80 nm (Ribeiro et al., 2008).

Nanoprecipitation is an effective technique for producing nanocapsules with high encapsulation efficiency, higher stability against degradation, and having size below 100 nm. But this technique can be used only for water-miscible solvents, in which the diffusion rate is enough to produce spontaneous emulsification and can be used to nanoencapsulate only lipophilic compounds.

#### 5.2.1.3 Emulsification–Solvent Evaporation Technique

This process involves the emulsification of the polymer solution into an aqueous phase and after that evaporation of polymer solvent takes place. The polymer is precipitated as nanospheres (Reis et al., 2006). Silva et al. utilized high-energy emulsification-evaporation technique for the production of nanoemulsions of β-carotene. They reported that the obtained β-carotene nanoemulsions had a volume surface diameter within the range 9–280 nm (Silva et al., 2011). The factors, like time and shear rate of the homogenization process, effects the particle size distribution and stirage stability. Nanoemulsions prepared through this method have good stability. But the stability of the emulsion is mainly depends on the emulsification technique and the other operating conditions. With changing these parameters the particle size of the emulsion can be changed. For producing nanocapsules suitable drying techniques must be used. The limitations of this method is that the emulsification-solvent evaporation technique requires high energy during emulsification. Moreover, only lipophilic core materials and food grade solvents can be used for the encapsulation process.

## 6 Conclusions

Nanoemulsions with droplet size less than 100 nm have drawn great attention in recent years because of their potential applications in cosmetics, pharmaceuticals, and food industries as a better delivery system due to their small droplet size, transparency, and high kinetic stability. Nanoemulsions are mainly used in the formation of transparent foods and beverages, to increase bioavailability, and to improve physical stability of the food in food industries. Due to the small droplet size nanoemulsions can

increase the bioavailability of encapsulated lipophilic substances. Therefore, nanoemulsions are used in products that need to be optically transparent or to increase bioavailability of an active component.

Further development in this field requires the identification of low-cost, acceptable and label-friendly food grade components and development of suitable operating techniques for preparation of nanoemulsions in which the existing equipments and manufacturing lines can be used so that the costs of manufacturing processes can be reduced. During encapsulation within the nanosize lipid droplet, the bioavailability and potential toxicity of the lipophilic substances can be changed. So the challenge involves the formation of suitable product compatible colloidal delivery systems, incorporation, and stabilization of the substances, and should have proper knowledge about the relation between product formulation and product functionality.

## Acknowledgment

The authors thank the director, CSIR-North East Institute of Science and Technology, Jorhat, India for the interest in this work and facilities. PB acknowledges DST, New Delhi, India, for DST-INSPIRE Fellowship grant.

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# 6

# FORMATION AND PROPERTIES OF NANOEMULSIONS

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## 1 Introduction

Colloids are an interesting class of soft materials due to their many interesting properties and specific applications. A colloidal dispersion is a heterogeneous system in which solid particles or liquid droplets with dimensions of order 100 nm or less are dispersed in a liquid medium. Typically, the sizes of the dispersed structures are not visible to the naked eye. Due to the small size of the dispersed phase the surface-to-volume ratio is large, which results in high interfacial energy (Walstra, 1996). In most colloidal dispersions, the sizes of the dispersed phases are very small and the difference in density between the continuous phase and dispersed phase is also small. As a result of this, the thermal energy is able to prevent the colloidal particles from sedimentation over a long period of time. This further highlights the importance of surface chemistry in colloid science. Some common examples of colloidal dispersions are milk, mayonnaise, ice cream, blood, paints, and haze.

The colloidal particles in dispersions undergo Brownian motion, and understanding their stability is a central issue in colloid and surface science. During Brownian motion when the colloidal particles approach one another the balance of attractive and repulsive forces determines the stability. A colloidal system is said to be unstable if the attractive van der Waals interactions are stronger than the repulsive forces. This leads to the formation of aggregation or flocculation (reversible aggregation) of colloidal particles. The structure of aggregates can range from compact flocs, which are in equilibrium exchange with a gas-like phase of single dispersed particles to tenuous fractal gels that are far from

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00006-4

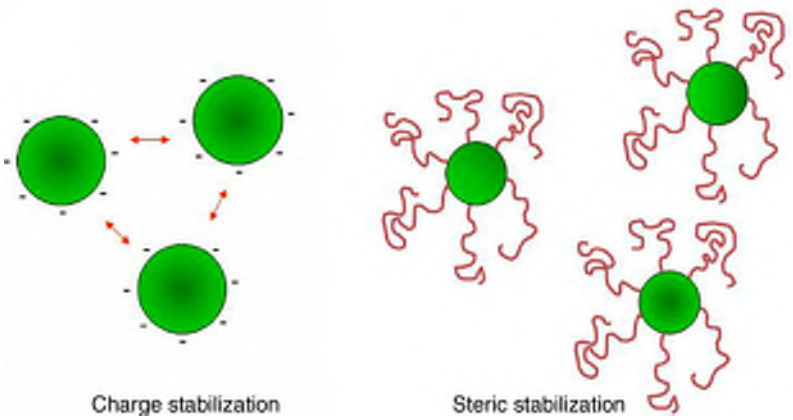


**Figure 6.1. Illustration of charge stabilization and steric stabilization of colloidal particles.**

equilibrium. If the repulsive forces are stronger than the attractive forces then the system is stable (Lin et al., 1990).

The van der Waals interactions between the colloidal particles can be manipulated to obtain stable dispersions. This is usually done by exploiting the electrostatic interactions in charge stabilization, or by modifying the interfaces by decorating them with long polymer chains or surfactants in steric stabilization (Mason et al., 2006; Myers, 1999). This is pictorially illustrated in Fig. 6.1.

## 1.1 Emulsions

Emulsions are dispersions of immiscible or partially miscible liquids that are made using mechanical forces such as stirring or ultrasonification (Becher, 1965; Mason et al., 1996). An interfacial tension is present between the two liquids due to differences in attractive interactions. The free energy required to disperse a liquid of volume $V$ into drops of radius $R$ is given by,

$$\Delta G = \gamma \frac{3V}{R} \tag{6.10}$$

where $\gamma$ is the interfacial tension. The stabilization of emulsions can be achieved by lowering the interfacial tension which reduces the free energy. The interfacial tension can be reduced by adding surface-active molecules or surfactants or amphiphiles in general. These molecules with a polar head group and a nonpolar hydrocarbon tail adsorbs at the interface between the two liquids.

The interfacial tension always acts to minimize the interfacial area between the two liquid phases and keeps the interface rather smooth leading to stability (Zemb, 2002). In the absence of any surfactants or other surface-active impurities, the system will always revert to the low energy thermodynamic equilibrium on prolonged storage. Thus, emulsions are not thermodynamically stable and tend to break up due to numerous processes including Ostwald ripening (Taylor, 2003).

In order to impart long-term stability to emulsions the addition of surfactants is highly essential. In general, the surfactant is usually soluble in the continuous liquid phase but not highly soluble in the dispersed phase. The activity of surfactants as emulsifiers is often quantified through hydrophile-lipophile balance (HLB) scale, which is an empirical range of numbers between 1 and 20. Using this scale the surfactants can be classified in terms of their relative solubility in aqueous and oil phases (Hamley, 2000). For example, the more hydrophilic the surfactant is, the higher its HLB.

## 1.2 Classification of Emulsions

Emulsions are classified based on composition and morphology. Emulsions in which small amount of oil is dispersed in large amount of water are called oil-in-water (o/w) emulsions or direct or water-based emulsions. In these emulsions, the surfactant is soluble in the aqueous phase and provides more stability to the water films. Emulsions in which small amount of water is dispersed in large amount of oil are called water-in-oil (w/o) emulsions or inverse or oil-based emulsions. In oil-based emulsions the surfactant is soluble in the oil phase and stabilizes the oil films.

The curved interface of droplets in emulsion exerts a pressure on the molecules inside the droplet and this pressure is termed the Laplace pressure. For spherical droplets at low volume fraction ($\phi$) the Laplace pressure is expressed by the following equation

$$\Pi_L = \frac{2\gamma}{a} \tag{6.11}$$

where $\gamma$ and $a$ are the interfacial tension and radius of droplet, respectively. Based on this equation the Laplace pressure increases with decrease in droplet size and as a result molecules in smaller droplets are subjected to high pressure than in larger droplets (Rallison, 1984). The smaller droplets thus tend to fuse into larger droplets. The most common example is the morning dew, which is formed from smaller droplets.

Various terms are commonly used to describe different types of emulsions based on droplet size and stability, and it is important to know these. The range of droplet size for each type of emulsion is defined in terms of physical and thermodynamic properties of the system. The different types of emulsions and their properties are as follows:

1. *Macroemulsions*: These are referred to as conventional emulsions with average droplet diameter between 100 nm and 100 μm. Due to the large droplet size it is thermodynamically unstable and is optically turbid. This type of emulsion is the most common form of emulsions used in food industry and is found in variety of food products such as milk, salad dressings, ketchups, margarines, and beverages.
2. *Nanoemulsions*: These systems consist of droplets in the size range from 20 to 100 nm with a surface-to-mass ratio in the range from 70 to 330 $m^2/g$. Due to the very small size of the droplets nanoemulsions are optically clear but thermodynamically unstable. A few years ago, the term "miniemulsion" was also used to refer to emulsions consisting of submicron droplets (Choi et al., 1985). Most miniemulsions are comprised of droplets in the size range from 100 nm to 1 μm. Even though a few miniemulsions systems have extended into the size range of nanoemulsion, their physical properties are not well understood due to the complexity involved in characterizing them. In practice it can be difficult to distinguish these systems.
3. *Microemulsions*: These are thermodynamically stable systems and are formed through spontaneous emulsification in the presence of an emulsifier (surfactant). The size of droplets in microemulsions ranges from 5 to 100 nm. These systems are formed by thermodynamic molecular self-assembly of emulsifier (lyotropic phases) without the use of shear force. The self-assembled phase exhibits interesting structures such as spherical micelles, cylindrical micelles, hexagonally packed cylinders, bilayers, vesicles, and sponges.

It is a general notion to think that nanoemulsions and microemulsions are essentially the same (Mason et al., 2006) as both contain droplets containing emulsifier, oil, and water. It has to be pointed out that the two systems are different. Nanoemulsions are formed by the application of shear and microemulsions are formed by spontaneous emulsification through self-assembly of emulsifiers. Microemulsions and emulsions can comprise of surfactants, oil and water (ternary systems). The main difference between metastable microscale emulsions and equilibrium microemulsions is the thermodynamic stability and not the composition of the system. Further, the emulsions and nanoemulsions are kinetically trapped systems.

## 1.3 Destabilization of Emulsions

The two main mechanisms by which emulsions can destabilize are (1) coalescence and (2) Ostwald ripening. During the process of coalescence the films of the continuous phase is ruptured, which leads to the fusion of two smaller droplets into a large single droplet. The coarsening or growth of droplets can be effectively controlled by using the right type of surfactant. This is true even at high-volume fractions provided the critical disjoining pressure $\Pi_d$ is not exceeded.

The emulsions can also be destabilized if the molecules of the dispersed phase have a relatively high solubility in the continuous phase. This forms the basis for Ostwald ripening. During this process of destabilization, the individual dispersed phase molecules from smaller droplets migrate by diffusion to large droplets with low Laplace pressure. In other words the larger droplets grow at the expense of smaller droplets. According to the Lifshitz–Slyozov law, the average drop size grows as one-third power of time as given next.

$$R(t) \sim (D\gamma t)^{1/3} \tag{6.12}$$

where $D$ and $t$ are diffusion coefficient and time, respectively. The rate of Ostwald ripening can be prevented by selecting molecules that have very low solubility in the continuous phase. By careful choice of materials and compositions the destabilization of emulsions can be prevented. The structure of emulsifier, concentration, and interfacial properties are known to influence Ostwald ripening in a complex way. Emulsifiers that greatly reduce the interfacial tension acts as diffusion barrier and retards the rate of Ostwald ripening. Adsorption of polymers of high molar mass at the interface is also known to greatly reduce mass transfer and decrease the rate of ripening process (Meinders and Vliet, 2004).

The fundamental study of rupture of one droplet in another immiscible liquid using shear stress was developed in the last century (Taylor, 1934). The typical size of the ruptured droplet can be estimated using the expression developed by Taylor as,

$$a \approx \frac{\gamma}{\eta_c \bar{\gamma}} \tag{6.13}$$

where $\eta_c$ and $\bar{\gamma}$ are the viscosity of the continuous medium and the shear rate, respectively.

This basic relationship gives the basis of emulsification; however, it does not satisfactorily describe the droplet failure at high-volume fractions. The rheological properties of surfactant

mesophases and polymeric liquids are also known to affect the capillary instability. The applied shear rate fundamentally determines the size and size distribution of ruptured droplets in an emulsion (Chandrasekhar, 1961; Mikami et al., 1975).

## 2 General Characteristics of Nanoemulsions

Nanoemulsions are dispersions containing droplets on nanometer length scale formed by the application of shear force. Although the exact definition of nanoemulsion and microemulsion is still controversial, the present convention for nanomaterials is materials that have structures with length scales in the range from 1 to 100 nm (Mason et al., 2006). Accordingly nanoemulsion consists of droplets in the size range from 1 to 100 nm. Miniemulsion refers to emulsions containing droplets of submicron size in the range from 100 nm to 1 μm (Hansen and Ugelstad, 1979). The nanoemulsions thus represent the lower limit of miniemulsion.

Nanoemulsions are interesting owing to their physical properties that are different from ordinary microscale emulsions. The equilibrium structures in nanoemulsions are much smaller than the wavelength of light as a result the nanoemulsions are optically transparent even at large volume fraction and for large difference in refractive index. However, this is not the case for microscale emulsions that exhibit strong multiple scattering of light and the emulsions appear turbid. Multiple scattering of light occurs due to the large size of emulsion droplets (Glatter, 2002).

For monodisperse droplets in nanoemulsion the optical transmission ($T$) is described by the following expression

$$T = \exp(-\tau l) \tag{6.14}$$

where $\tau$ is the turbidity and $l$ is the optical path length of light.

The specific turbidity ($\tau/c$) for the cross-section that scatters the light is given by,

$$\frac{\tau}{c} = \frac{3\pi}{4\rho_c}\frac{Q_{av}}{r_v} \tag{6.15}$$

where $c$ is the concentration of particles (in volume), $\rho_c$ is the density of the continuous phase, $Q_{av}$ is the mean light scattering efficiency, and $r_v$ is the volume-based mean radius. The $Q_{av}$ is determined by the size of particles, refractive indices of dispersed and continuous phases, and the wavelength of scattered light. For nanoemulsions, the size of droplets are much smaller than the wavelength of light ($r_v \ll \lambda$), and this leads to weak scattering of light. Thus, nanoemulsions are optically transparent.

Another important feature of nanoemulsions is that they have much larger surface-to-volume ratio owing to the very small droplets. Therefore, the phenomena related to droplets deformation or instability, such as modulus of elasticity, Laplace pressure is larger for nanoemulsions then ordinary emulsions. Depending on the molecular weight, the number of molecules of the dispersed phase in nanoemulsion droplets is much smaller than for ordinary emulsions (Miller, 2006).

Molecules such as ω-3 fatty acids, curcumin, phytosterols, limonenens that show biological and pharmacological effects are poorly soluble in aqueous solutions which limits their bioavailability (Huang et al., 1994). The nanoemulsions are widely used to improve the solubility and bioavailability of various lipophilic phytochemicals owing to their small droplet sizes and high kinetic stability.

The size of droplets in nanoemulsions also affects the rheological properties. The rheological properties strongly depend on the attractive or repulsive interaction between the nanodroplets (Mason, 1999). At low droplet volume fraction (dilute regime) the shear viscosity of nanoemulsions with repulsive interactions resembles that of hard spheres. According to Einstein, the viscosity of dilute hard sphere dispersion depends only on the viscosity of the continuous phase ($\eta_c$) and volume fraction (Russel et al., 1989). When the repulsive droplets deform at very high volume fractions the elastic shear modulus, $G'$, is proportional to the Laplace pressure of the nondeformed droplets. This indicates that large elastic moduli can be present in nanoemulsion droplets. The attractive interactions between droplets can lead to the formation of weak space-filling gels at volume fractions far below the maximum random jamming volume fraction.

The nanoemulsions also exhibit enhanced stability against creaming or sedimentation over conventional or miniemulsion at the same volume fraction. Due to Brownian motion the droplets are in a stable suspended state for extended period of time. The self-diffusion coefficient, $D$ of nanodroplets can be obtained using the Stoke–Einstein expression as,

$$D = \frac{k_B T}{6\pi\eta a} \tag{6.16}$$

where $k_B$ is the Boltzmann constant, $T$ is the temperature, $\eta$ is the viscosity of solvent, and $a$ is the diameter of the droplet.

The self-diffusion coefficient determined for nanoemulsion droplets of size range from 10 to 100 nm is in the range $10^{-7}$ to $10^{-8}$ cm$^2$/s. By using the colloidal law of atmospheres in nanoemulsions (Russel et al., 1989), it has been shown that repulsive nanodroplets stored in very tall cylinders are stable against creaming or

sedimentation. If the interaction between the droplets is attractive the droplets aggregate to from flocs that cream much rapidly leading to inhomogeneity in the system.

# 3 Characterization of Nanoemulsions

In order to explore the structure and behavior of liquid droplets of nanoemulsions, sophisticated characterization techniques are required. Optical microscopy using differential interference contrast or other phase contrast methods is generally not viable method for studying nanoemulsions. Nanoemulsions can be characterized based on nature and composition, size of droplet structures, and the magnitude of shear force. Advanced material characterization techniques such as static and dynamic light scattering (SLS and DLS) (Roshan Deen and Pedersen, 2011; Glatter, 2002; Pusey, 2002), small angle X-ray or neutron scattering (SAXS, SANS) (Pedersen, 2002) atomic-force microscopy (AFM) (Slayter and Slayter, 1992), and cryo-transmission electron microscopy (cryo-TEM) are typically used to study the structure and behavior of nanoemulsions (Johnson and Gabriel, 1981; Slayter and Slayter, 1992). Cryo-TEM provides only static real space images of droplets at very high magnifications. The dynamics of droplets cannot be studied by this method as the emulsion droplets are quenched into a vitrified solid phase (glassy phase).

The size and size distribution (size polydispersity) of nanoemulsion droplets can be obtained using DLS. Although DLS can provide the size distribution of droplets, this information alone is not sufficient to determine the spatial structure of droplets. The spatial structure of droplets and droplet curvature can be determined using SAXS and SANS measurements. The scattering profiles are fitted with appropriate models to obtain the spatial structure of nanoemulsion droplets (Pedersen, 2002).

## 3.1 Average or Mean Droplet Diameter

The average or mean diameter of droplets and their distribution in nanoemulsions can be determined based on number ($d_N$), mass or volume ($d_V$), and surface-area ($d_S$). The respective equations are given next:

$$d_N = \frac{\sum N_i d_i}{\sum N_i} \tag{6.17}$$

$$d_V = \frac{\sum N_i d_i^4}{\sum N_i d_i^3} \tag{6.18}$$

$$d_S = \frac{\sum N_i d_i^3}{\sum N_i d_i^2} \tag{6.19}$$

where $N_i$ is the number of droplets with diameter $d_i$.

If the nanoemulsion is monodisperse then $d_N = d_V = d_S$, and if the system is polydisperse in terms of droplet size then $d_N < d_S < d_V$. Depending on the type of experiment the average size of droplets can be determined by using any of the previous expressions. For food and pharmaceutical nanoemulsions the average droplet size is determined using the volume-based expression ($d_V$), which gives the size distribution in terms of small and large droplets. Such information is important in determining the stability of food and pharmaceutical nanoemulsions based products on shelf-life.

## 3.2 Laser Light Scattering

Laser light scattering is a powerful method for size characterization of nanoemulsions. When a beam of light passes through a colloidal dispersion the particles scatter the light in all directions. If the particles or droplets are small compared with the wavelength of light and/or low contrast, the intensity of the scattered light is uniform in all directions and is described by the Rayleigh–Debye–Gan (RDG) theory. If the refractive index difference between the solute and solvent is sufficiently small, RGD can be used for quite large particles. For large particles of diameter above 250 nm the scattered intensity is not uniform and is angle dependent and is described by Lorentz–Mie theory (Lorenz, 1898; Horvath, 2009). In this regime, refraction and reflections as well as a well-defined wavelength within the particles are different from that of the solvent.

Dynamic light scattering (DLS) or photon-correlation spectroscopy (PCS) or quasielastic light scattering is a widely used light scattering method to measure size of particles or droplets in the size range 1 nm up to 10 μm (Berne and Pecora, 2000). In this technique the fluctuation in intensity of scattered light is measured as function of time (intensity–time correlation function). The fluctuations in light intensity arise due to Brownian motion of droplets, and the constructive and destructive interference of scattered light produces a speckle pattern on the detector. The position of each speckle is constant in motion. This is due to continuous phase addition from the moving droplets, which forms new patterns. The rate at which the intensity fluctuations occur depends on the size of the droplets. The small droplets cause the intensity to fluctuate more rapidly than the large droplets.

The fluctuations in the scattering intensity of a particle can be related to an autocorrelation function, $g(\tau)$, as a function of time of

measurements, $\tau$. For a polydisperse suspension of particles, each particle size contributes to the autocorrelation function such that the function can be described as,

$$g(\tau) = \int G(\Gamma)\exp(-\Gamma\tau)d\Gamma \tag{6.20}$$

The desired distribution information is contained in $G(\Gamma)$, where $\Gamma$ is related to the relaxation of fluctuations by,

$$\Gamma = Dq^2 \tag{6.21}$$

where $D$ is the average diffusion constant of particles or droplets, and $q$ is the scattering vector. The scattering vector is given as,

$$q = \frac{4\pi n}{\lambda}\sin\left(\frac{\theta}{2}\right) \tag{6.22}$$

where $\lambda$ is the wavelength of light used, $n$ is the refractive index, and theta is the scattering angle.

The solution to $G(\Gamma)$ is nontrivial, and therefore a method of cumulant analysis is used to find an average of $\Gamma$. In order to do this, the equation is expanded as a Taylor's series about the mean value, and the series is integrated to give a polynomial in sample time. The first moment of the polynomial is the average and the second moment is the variance.

$$\ln[g_1(\tau)] = -\Gamma\tau + \frac{\mu_2(\Gamma\tau)^2}{2} - \frac{\mu_3(\Gamma\tau)^3}{6} + \ldots \tag{6.23}$$

where $g_1(\tau)$ is the electric-field correlation function, $\Gamma = -\ [d\ \ln(g_1(\tau)/d\tau]_{\tau\to 0}$ is the first cumulant, $\mu_2$ and $\mu_3$ are dimensionless quantities which describe the distribution of relaxation rates. For particles or droplets with diameters larger than $\lambda/20$ the reduced first cumulants, $\Gamma/q^2$, depends on the scattering vector $q$ and is written as,

$$\frac{\Gamma}{q^2} = D_z \cdot (1 + C \cdot \langle s^2 \rangle_z \cdot q^2 + \ldots) \tag{6.24}$$

where $D_z$ is the $z$-average translational diffusion coefficient, and $s$ is the $z$-average radius of gyration, and $C$ is a dimensionless quantity which depends on the structure and polydispersity of the sample (Ricker and Schmidt,1999). $D_z$ is related to the inverse $z$-average of the hydrodynamic radius, $<1/R_h>_z^{-1}$ of the particles by the Stokes–Einstein relation,

$$\frac{1}{\langle R_h \rangle_z} = \frac{k_B T}{6\pi\,\eta \cdot D_z} \tag{6.25}$$

where $k_B$, $T$, and $\eta$ the Boltzmann constant, temperature, and viscosity of the solvent, respectively.

The relative polydispersity of the system can also be calculated from the second moment $\mu_2$ of the second and third order fitting as,

$$\frac{\sigma}{R_h} = \frac{\mu_2^{1/2}}{\Gamma} \tag{6.26}$$

where $\sigma$ is the width of the number size distribution and $R_h$ is the average hydrodynamic radius.

Although DLS is a powerful technique for characterizing the size of nanoemulsion droplets, the shape of these droplets cannot be determined.

## 3.3 Small Angle Scattering Methods

Small angle scattering methods such as SAXS and SANS are highly sophisticated techniques for detailed characterization of nanoemulsion structures. In general, three methodologies are used to determine the microstructure (Zemb, 2002): (1) some important features of droplet microstructure can be determined directly, (2) fitting procedure with presumption of structures, and (3) the use of dilution lines to test microstructural models. Some of the models are the inhomogeneous solid model, fractal model, Talmon–Prager cell model and the cubic random cell model. Determination of volume fraction, specific area, and scattering lengths can be determined directly from the scattering curve. In addition, the spontaneous curvature of the emulsifier film as function of temperature, salinity, and chemical potential of water can be studied using scattering methods.

Other regular methods to characterize nanoemulsions are briefly mentioned in the next sections.

## 3.4 Zeta Potential

The long-term physical stability of nanoemulsion can be understood by measuring the zeta potential of droplets. This is a measure of surface charge of droplets and is usually measured as function of pH of the medium.

## 3.5 Dye Solubilization

A water soluble dye is solubilized within the aqueous phase of the w/o nanoemulsion droplets but is dispersible in o/w droplets. An oil soluble dye will have the opposite effect. Using this method the type of droplets present in nanoemulsions can be studied.

## 3.6 Electrical Conductance

Nanoemulsions of type o/w in which the continuous phase is water are electrically conducting. In contrast, w/o type nanoemulsions do not conduct electricity as the continuous medium is oil (hydrophobic). To determine the nature of continuous phase and to detect the phase inversion phenomena, the electrical conductivity measurements are very useful. Dielectric measurements are also useful in studying the structural and dynamic features of nanoemulsion systems.

## 3.7 Interfacial Tension

The formation and properties of nanoemulsions can be studied by measuring the interfacial tension. Ultralow values of interfacial tensions are correlated with the existence of lyotropic phases of the emulsifier and phase behavior of the nanoemulsion. The ultralow interfacial tension is measured using the spinning-drop method. In this method, the shape of a nanoemulsion drop rotating in a cylinder filled with high-density phase is measured from which the interfacial tension is derived.

## 3.8 Viscosity

The viscosity of nanoemulsions of several compositions can be measured in a Brookfield-type viscometer as function of shear rate and temperature.

## 3.9 Thermodynamic Stability

The stability of drug loaded nanoemulsions is characterized by three stress tests:

1. *Heating-cooling cycle*: The drug loaded nanoemulsions are subjected to six cycles of heating and cooling. The samples are cooled to 4°C and then heated to 45°C. The stability of the sample as a result of the heating-cooling cycle is evaluated by visual observation. The samples that remain optically clear after six cycles of heating and cooling are said to be stable. These stable samples are then subjected to centrifugation test (Mishra et al., 2014).
2. *Centrifugation test*: The stable nanoemulsion samples are centrifuged at a rate of 3500 rpm for 5 min. The samples that remain optically clear are subjected to the freeze-thaw test.
3. *Freeze-thaw test*: The samples are subjected to three freeze-thaw cycles between −20 and 25°C and observed for phase separation. The nanoemulsion formulation that remains optically

clear without any signs of phase separation after these tests are considered stable.

### 3.10 Skin Permeation Studies

Skin permeation studies (in vitro) of drug-loaded nanoemulsions are performed using Keshary Chian diffusion cell (Nicolosi et al., 2008). It is studied using abdominal skins of male rats, and the skins are placed between the donor and receiver chambers of vertical diffusion cells. The receiver chambers are filled with water containing 20 wt% ethanol and maintained at 37°C. The nanoemulsion formulation is placed in the donor chamber. At fixed time intervals of 2, 4, and 6 h, 0.5 mL solution from the receiver chamber is removed and analyzed using gas chromatography (GC). The aliquot removed from the receiver chamber is immediately replaced with an equal volume of water–ethanol mixture. The permeation rate of drug at steady-state through the skin is calculated from the slope of a plot of cumulative amount permeated versus time.

## 4 Formation of Nanoemulsions

A number of important factors must be considered for the preparation of stable nanoemulsion. These factors include, (1) appropriate composition based on phase diagram, (2) mixing order of components, and (3) the required sheer force for the formation of droplets. The other important criteria are (1) the dispersed phase molecules must be insoluble in the continuous phase to prevent destabilization of droplets by Ostwald ripening (2) the surfactants must not form lyotropic phases, (3) the presence of significant amount of surfactant excess in the continuous phase, and (4) use of large shear force for the formation of nanodroplets. Typically the stress level of 10–100 atm is suggested for the formation of droplets of desired size (Webster and Cates, 2001).

The nanoemulsion droplets have large surface-to-volume ratio, which requires a high concentration of surfactants for stability. As many surfactants form micelles above their critical micelles concentration, which can range from tens to hundreds millimoles, the continuous phase of nanoemulsion always contain excess of surfactant. This excess surfactants act as reservoir for coating the interfaces of new droplets. The nanoemulsion is said to be stable when the surface coverage of droplets by surfactants is complete. At low concentration of surfactant, the droplet coverage is also low, which can destabilize the nanoemulsion. However, stability can be achieved by either charge-stabilization or steric-stabilization of the droplets (Jones, 2000).

Based on the Taylor's equation for the radius of the ruptured droplet, the shear rates required for the formation of nanoemulsion can be estimated. To form water-based nanoemulsion containing droplet size of about 100 nm, the required shear rate is $\approx 10^8$/s. This shear rate is even higher when the size of droplets decreases. Such high shear rates are out of range of most common mixing devices. In order to overcome this and for the production of stable nanoemulsion droplets of desired sizes, ultrasonic devices and microfluidic devices are widely used.

A patented technology of using ultrasound for preparing emulsions dates back to 1960s. Since then a variety of ultrasonic devices has been developed for homogenization processes. From a premixed emulsion of droplets in microlength scale, a nanoemulsion can be prepared by agitation using ultrasonic devices (Landfester et al., 2000). In this method, the premixed emulsion is agitated using a vibrating solid surface at frequencies of 20 kHz or larger. This high frequency and power causes cavitation that breaks the large drops into nanodroplets. The two widely accepted mechanisms for ultrasonic emulsification are based on interfacial waves and acoustic cavitation.

In the first mechanism, the application of an acoustic field generates an interfacial wave, which finely disperses the oil phase in the continuous phase as small droplets (Li and Fogler, 1978a). In the second mechanism, acoustic cavitation caused by the ultrasound leads to the formation and subsequent collapse of microbubbles in a highly localized turbulence. The turbulent microimplosions break the primary droplets into very small (submicron size) droplets (Li and Fogler, 1978b). However, the droplet size distribution of nanoemulsion prepared using ultrasonic devices is always bimodal. If the emulsion is recirculated through the high shear region droplets of uniform size distribution can be achieved.

High-pressure homogenizers are the most commonly used devices to produce fine emulsions in the food and dairy industries. One of the applications is for homogenization of milk to reduce the size of fat globules, which otherwise tends to cream. A coarse emulsion is first produced using a high-speed mixer and fed into the high-pressure valve homogenizer. The flow velocity of the coarse emulsion is increased as it is fed between the valves. Here the emulsion is subjected to intensive disruptive forces that cause the rupture of larger droplets (by Eddie current) into smaller droplets. Turbulence of solution and cavitation of droplets are the two main mechanisms that control the formation of small droplets during the high-pressure homogenization process (Schultz et al., 2004). The nanoemulsion droplets of size ~100 nm can be produced by this method.

The high-pressure homogenization method of emulsification has advanced greatly in the last decade. A wide range of high-pressure homogenizers for laboratory and industrial production of nanoemulsions are now available in the market.

Microfluidic devices are also used for the preparation of nanoemulsion from concentrated emulsions. A stream of premixed concentrated emulsion is rapidly forced through microchannels of fixed geometry. Two jets of crude emulsions are accelerated at high velocities from two opposite channels. The jets of emulsion collide with each other creating a tremendous shear force. The inertial forces in the turbulent flow along with cavitation are responsible for the disruption of droplets. The combination of extreme shear rates and impact effects leads to the formation of fine droplets. O/w nanoemulsion of size ~50 nm, containing silicone oil and sodium dodecylsulfate (SDS) as surfactant has been prepared using high-pressure microfluidic method.

Microfluidizers are widely used in food and dairy industries for homogenization of products (Olson et al., 2004). Compared to conventional homogenization devices, the emulsions obtained using microfluidizers contain finely distributed small droplets. This shows that the droplet disruption in microfluidizers is highly efficient, and thus this method is widely used for the formation of nanoemulsions.

In recent years, a low-energy emulsification method has been developed by taking advantage of phase behavior to facilitate the formation of droplets of nanometer length scale (Rang and Miller, 1999). These low-energy methods include self-emulsification, phase transition, and phase inversion temperature (Pons et al., 2003; Forgiarini et al., 2000). To apply these low-energy emulsification methods, it is important to study the relationship between phase behavior of the components and the resulting nanoemulsions. Using the phase inversion temperature method, emulsions containing nonionic surfactants are obtained by rapidly increasing or lowering the temperature to pass through the hydrophilic-lipophilic balance (HLB) temperature.

If the initial emulsion system is located in a bicontinuous microemulsion region or a two-phase system at the HLB temperature, then the rapid change in temperature leads to the instant formation of a nanoemulsion. The phase instabilities caused during the emulsification process is thought to be the main factor for the formation of nanoemulsions (Wang et al., 2007). However, no direct relation has been documented. Therefore, further research is required to fully understand the mechanism of nanoemulsion formation by low-energy emulsification methods.

Nanoemulsions are also prepared by solvent evaporation technique. In this method, the drug is first dissolved in a solvent and it is emulsified in another liquid in which the drug is not soluble (nonsolvent). Evaporation of the solvent leads to precipitation of the drug. Particle aggregation and crystal formation can be controlled by using a high-speed stirrer.

## 4.1 Nature of Emulsifier and Properties

The low-energy methods of nanoemulsion formation require the use of emulsifiers to reduce the interfacial tension between the immiscible liquid phases. Emulsifiers are amphiphilic molecules containing a hydrophilic head and a hydrophobic tail. These emulsifiers adsorb at the interface of the droplet with the hydrophilic head group being oriented toward water and the hydrophobic tail toward oil. In order to form a stable nanoemulsion the choice of emulsifier is crucial (Hasenhuettl, 2008). The chemical structures of few emulsifiers that have used in the formation of nanoemulsions are shown in Fig. 6.2. The chosen emulsifier should be able

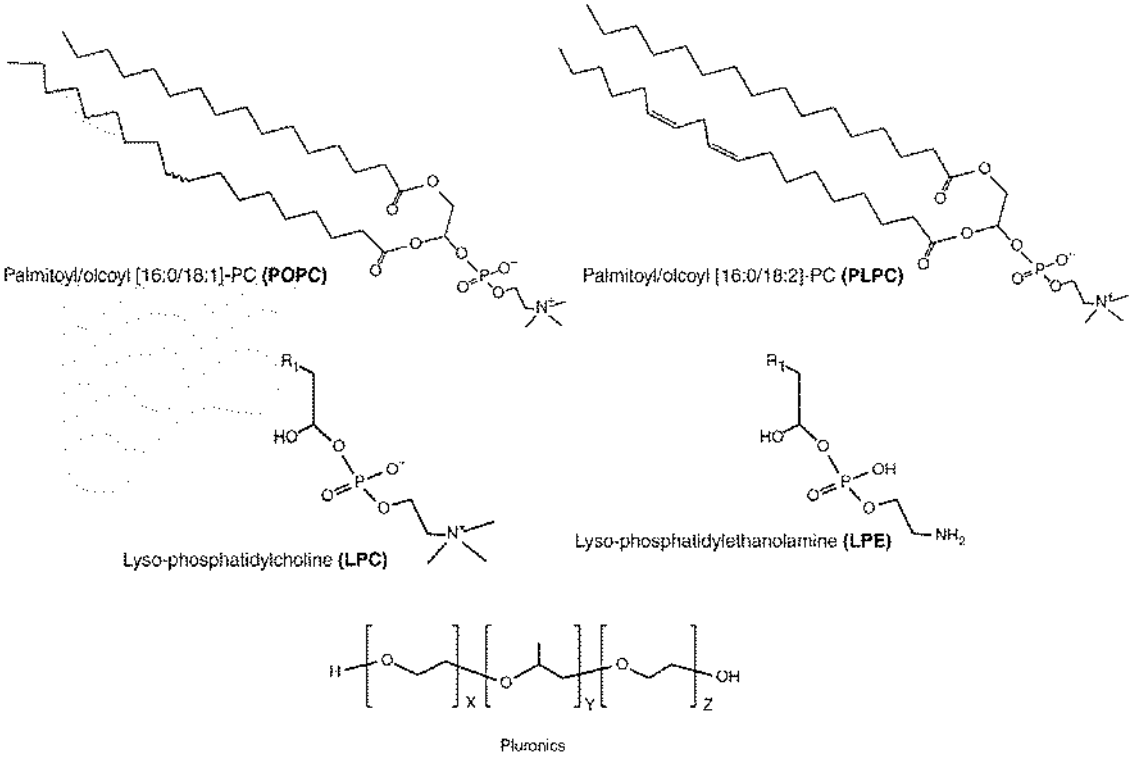


**Figure 6.2. Chemical structure of some emulsifiers.**

## Table 6.1 A Comparison of Fatty Acid Composition in Epikuron 200, Lipid S100, and Lecithin

| | Amount of Fatty Acid (%) | | |
|---|---|---|---|
| **Fatty Acid** | **Lipoid S100** | **Epikuron 200** | **Lecithin** |
| Palmitic and stearic acid | 14–22 | 16–20 | ~24 |
| Oleic acid | 11–15 | 8–12 | ~10 |
| Linoleic acid | 59–70 | 62–66 | ~59 |
| Linolenic acid | 3–7 | 6–8 | ~7 |

to rapidly adsorb at the oil-water interface and reduced the interfacial tension. The structure of the emulsifier molecule plays an important role in influencing the properties of nanoemulsions. In the food and pharmaceutical industries naturally available emulsifier based on fat-free soy lecithin (L-α-phosphatidylcholine with a mixture of other lipids) is widely sought after. This emulsifier is noncytotoxic and biodegradable. Compared with synthetic alternatives, lecithin can be fully biodegraded and metabolized. This is because phosphatidylcholine is an integral part of biological membranes and is nontoxic (Kriwet and Muller-Goymann, 1995). The types of the common soy lecithin used in pharmaceutical and food nanoemulsion formulations are presented in Table 6.1. Some common emulsifiers used in food, cosmetics, and pharmaceutical nanoemulsions are shown in Fig. 6.2.

This natural emulsifier readily forms bilayers and vesicles in water (Aboofazeli and Lawrence, 1993). The flat curvature of the bilayers, in principle, can be manipulated to spherical curvature by the use of coemulsifiers such as medium chain alcohols and certain nonionic emulsifiers (Jones, 2000). The small differences in the amount of various fatty acids in these emulsifiers as shown in Table 6.1 and this difference can have significant influence on the phase behavior and also on the stability of nanoemulsion droplets. The lipoid emulsifier shows high probability of forming bilayers in solution. This tendency can be explained in terms of packing parameters for surfactant self-assembly (Israelachvili, 2011).

These parameters are a strong function of optimal head group area of surfactant $a_0$, volume of surfactant tail $v_0$, and the critical chain length $l_0$. The critical packing parameter is given by,

$$p = \frac{v_0}{a_0 \times l_0} \tag{6.27}$$

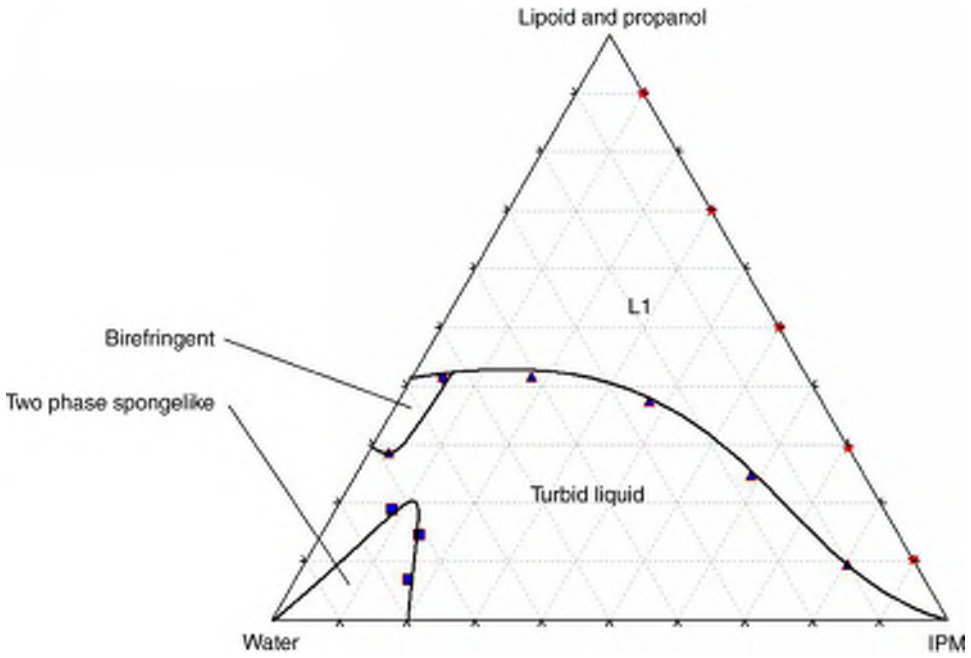


**Figure 6.3. Phase diagram for lipoid/propanol/IPM/water system at 23°C.** The ratio of lipoid-to-propanol is 1:1.

The following critical packing parameters and the formation of surfactant structures are well established (Hamley, 2000; Jones, 2000): $p < 1/3$: spherical micelles; $1/3 < p < 1/2$: cylindrical micelles; $1/2 < p < 1$: vesicles, flexible bilayers; $p \approx 1$: lamellae, planar bilayers; $p > 1$: inverse micelles.

For lipoid and lecithin the value of packing parameter is between 1/2 and 1, which indicates that these surfactants form lamellar phases in solution. The curvature of the surfactant aggregates in principle can be modified by using nonionic surfactants such as Brij700 and Brij65 or medium chain alcohols such as propanol, butanol, and pentenol. These cosurfactants change the critical packing parameter and thereby induce the formation of spherical micelles (Warisnoicharoen et al., 2000). The phase behavior of nanoemulsion based on lipoid, isopropyl myristate (IPM), 1-propanol, and water prepared by low-energy method is shown in Fig. 6.3.

In the ternary phase diagram the large clear region denoted as L1 corresponds to water-in-oil type nanoemulsion. The maximum water content of this nanoemulsion is 50 wt%, and beyond which the droplet curvature becomes distorted. The small birefringent region observed at 60–70 wt% water content is the region of bilayers. A sponge phase is also observed at very high water content.

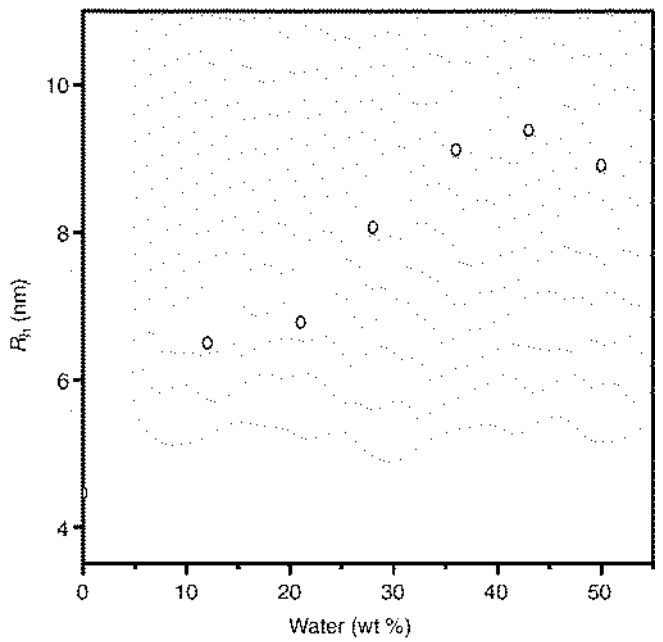


**Figure 6.4. Hydrodynamic radius of nanoemulsions of lipoid:propanol (1:1)/IPM/water system.**

The hydrodynamic radius of water droplets in the L1 phase measured using DLS is shown in Fig. 6.4. The droplets grow in size with increase in water content of the nanoemulsion, which is expected. At water content of 50 wt% the hydrodynamic radius decreases showing the onset of curvature distortion, which agrees with the results of the phase diagram Skovgaard (2007).

The complex phase behavior of this system clearly implies that additional coemulsifiers are required for the formation of o/w type lecithin nanoemulsions. The use of propanol and butanol as coemulsifiers is able to improve the stability of nanoemulsion due to their efficient incorporation in the emulsifier layer, which leads to the favorable formation of micelles. The partitioning of coemulsifiers (medium-chain alcohols) at high water content can lead to destabilization of nanoemulsion due to change in curvature of droplets as illustrated in Fig. 6.5.

The performance of emulsifiers is one of the important factors that governs the formation and stability of nanoemulsions. The emulsification and formation of nanoemulsion is thus greatly dependent on the nature of emulsifier and its rate of adsorption kinetics. The adsorbed emulsifier prevents droplet coalescence by providing charge stabilization. The adsorption kinetics of an

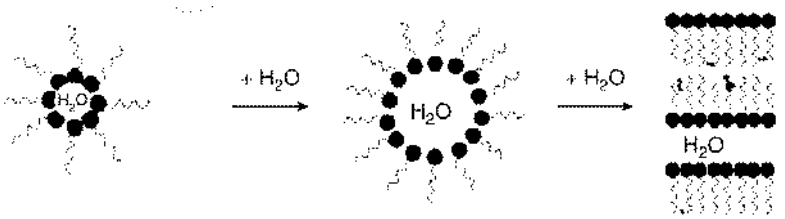


**Figure 6.5. Illustration of evolution of water-in-oil nanodroplets followed by change in curvature.**

emulsifier is related to its characteristic diffusion time ($\tau_D$) to the interface (Floury et al., 2003). Thus, the rate of adsorption of the emulsifier at the oil-water interface is a function of diffusion time. However, the diffusion time itself is dependent on many factors such as structure of emulsifier, temperature, solvent viscosity, pH. In general, low molar mass emulsifiers have higher rate of adsorption kinetics and enable the formation of nanoemulsion (Danov et al., 2001).

The adsorbed emulsifier at the oil-water interface lowers the interfacial tension to facilitate the rupture of large droplet under shear. The size of the droplets formed is dependent on the emulsifier's ability to reduce the interfacial tension. The greater the reduction of interfacial tension, the smaller the droplets formed during emulsification. The emulsifier Tween 20 is more effective in lowering the interfacial tension between corn oil and water in comparison to β-lactoglobulin (Vladisavljevic et al., 2006). The lowering of interfacial tension by emulsifiers between oil and water during emulsification requires less energy to rupture the droplets.

Another important factor that needs to be considered in the formation of nanoemulsion is the strength of interfacial membrane. During the homogenization process under high pressure the droplets collide frequently due to a squeezing force caused by turbulence (Narsimhan and Goel, 2001). This leads to coalescence of droplets and ripening of the system. This indicates that the strength of interfacial membrane of the droplets is critical for the formation of stable nanoemulsion. The strength of interfacial membrane of droplets to overcome coalescence can be enhanced by a few methods.

The droplet instability caused by coalescence can be prevented by surface coverage with emulsifier. During homogenization under high pressure the time scale is very small for the interface to be in equilibrium. The loading of emulsifier to the interface is given

by $\Gamma = N/\gamma$, where $N$ and $\gamma$ are the number of emulsifier molecules and interfacial tension respectively. Large values of $\Gamma$ indicates favorable lowering of interfacial tension and higher surface coverage of droplets. Therefore, the choice of emulsifier is crucial in the formation and stability of nanoemulsion. A high concentration of emulsifier with high rate of adsorption leads to better loading at interface which stabilizes the interfacial membrane.

Another method to minimize coalescence of droplets during homogenization is by electrostatic stabilization which includes electrical double layer forces (Jones, 2000). The presence of charges (ions) in the medium provides strong repulsion between the droplets and minimizes coalescence. The concentration of free ions in the medium should be low to ensure a large diffusive layer around the droplet. The dynamic simulation model of high-pressure homogenization also shows the influence of electrostatic forces in retarding the instability of nanoemulsion by coalescence (Hakansson et al., 2009).

During the process of high-pressure homogenization, the turbulence caused is much higher, which favors coalescence of droplets. The stabilizing electrostatic force is very weak (~100 times) against the strong turbulent squeezing force (Narsimhan and Goel, 2001) to provide stability. The electrostatic stabilization of nanoemulsion is therefore still open for debate (Walstra, 2003). This is due to many experimental factors that influence electrostatic stabilization of droplets such as type of homogenization devices, shear rate, type of emulsion systems, nature of emulsifier.

## 4.2 Viscosity of Phases

The Taylor equation Eq. 6.13 gives important information on the role of shear rate and rheological properties of the phases on the formation of emulsion. In practice, this equation is of significance for the formation of nanoemulsions, which can be achieved by tuning the viscosity of phases instead of extreme shear. It is well known that viscosity of phases play an important role in the formation and stability of droplets. The ratio of the viscosity of dispersed phase ($\eta_d$) to continuous phase ($\eta_c$) is crucial in the size control of droplets (Chanamai and McClements, 2000). If the ratio is very high, the droplets are more resistant to deformation under the flow field. The desired viscosity can be adjusted by many ways such as systematically varying the composition of oil and water, increasing the emulsifier content, introducing a cosolvent. In addition, the temperature during the homogenization process can also be increased to decrease the viscosity of the mixed phase.

## 5 Improving the Nanoemulsions

After the formation of nanoemulsion the size distribution and stability of the droplets can be improved by many physical methods. Polydisperse nanoemulsion droplets can be subjected to ultracentrifugal fractionation for separation into monodisperse droplets. Depletion interactions induced by surfactant micelles or polymers with known radius of gyration ($R_g$) can be used for the fractionation of large droplets (Jones, 2000; Bibette, 1991). This method of fractionation is not suitable for droplets whose size is similar to the size of micelles. Therefore, to fractionate nanoemulsions, ultracentrifugation is widely used.

In this method, the buoyant force and viscous drag force on the droplet is taken into consideration. At equilibrium, the buoyant force is opposed by the drag force, which leads to the following equation for terminal velocity $v$,

$$v = \frac{2a^2 \Delta\rho g}{9\eta_c} \tag{6.28}$$

where $a$, $g$, $\eta_c$, and $\Delta\rho$ are the radius of droplet, gravitational constant, viscosity of the continuous medium, and difference in density between the dispersed and continuous phase, respectively.

Based on this terminal velocity equation, larger droplets will cream or sediment faster than the smaller droplets since the velocity is proportional to $a^2$. After fractionation of droplets has been achieved the composition of the nanoemulsion can also be tuned. This can be done by adjusting the volume fraction and the number density of surfactant on the surface of the droplet. In addition, the concentration and the type of surfactant can also be changed to obtain stable nanoemulsion (Graves et al., 2005).

The volume fraction of nanoemulsion can also be increased by applying osmotic pressure using methods such as dialysis and ultracentrifugation. A nanoemulsion after ultracentrifugation usually contains a volume fraction gradient with higher volume fraction at the top of the tube. The time it takes for the volume fraction to equilibrate is long and therefore the nanoemulsion is stirred to speed up the equilibration process. In the dialysis method, the nanoemulsion is placed in a semipermeable dialysis tube and immersed in a large volume of the continuous phase which is usually water. The semipermeable membrane chosen must have the correct molecular-weight cutoff to avoid the diffusion of nanoemulsion droplets into the external continuous phase.

## 6 Droplet Structure

In order to understand the physical properties of nanoemulsions the droplet structure should be established first by various advanced characterization methods. The structure of nanodroplets is discussed using the concept of "nanoemulsion glass" introduced by Mason et al. (2006). The nanoemulsion glass is a disordered arrangement of concentrated nanodroplets that are very far from equilibrium. When the volume fraction of nanodroplets is high, significant deformation of droplets can take place leading to foam like structures where the droplets exhibit polyhedral morphology. At low volume fractions the droplets are spherical in morphology. The interaction between the droplets [structure factor $S(q)$] at high volume fraction can be studied using pair distance distribution function (PDDF), which is a statistical quantity that describes the relative positions of pairs of droplets in the nanoemulsion (Hansen and McDonald, 1990; Glatter, 2002).

Small angle scattering (X-ray and neutron) is an excellent method for investigating the structure of nanodroplets both at high and low volume. The correlation function and the PDDF in principle can be calculated from the scattered light intensity $I(q)$ as function of scattering vector $q$ by inverse transformation. When the volume fraction of nanodroplets is low the droplets are not deformed and the structure factor can be obtained as,

$$S(q) = \frac{I(q)}{F(q)} \tag{6.29}$$

where $F(q)$ is the form factor of nanodroplets.

The presence of interfacial interactions has significant influence in changing the structure of nanoemulsions. In the absence of any attractive interactions the nanoemulsions are homogeneous and stable dispersion of small droplets. When strong attractive interactions relative to thermal energy ($k_B T$) are present the droplets tend to aggregate and this causes large spatial inhomogeneity in the nanoemulsion. Attractive interactions are generally introduced by adding salt to the nanoemulsion and quenching the temperature over a small range (Wilking et al., 2006). The evolution of nanoemulsion droplet structure can then be measured using time-resolved small angle scattering methods.

Other than the scattering methods, the real-space determination of structure of nanoemulsion droplets is performed by continuous phase evaporation-TEM. In this method, the nanoemulsion is stained with depleted uranyl acetate and placed on a polymer coated (monolayer) TEM grid. Water is then evaporated

and the shapes of nanodroplets are observed at room temperature. This method of structural investigation gives accurate number-weighted size distribution.

Understanding of positional structure and deformation of droplets in fractionated nanoemulsions are beginning to emerge through scattering and real-space studies. Distribution of droplet size, interaction between droplets, and positional structures can be measured using small angle scattering methods (SAXS and SANS). Real-space structure of nanodroplets can be obtained using cryo-TEM and continuous phase evaporation-TEM.

# 7 Applications of Nanoemulsions

The interesting properties of nanoemulsions such as high kinetic stability, optical transparency, and low viscosity make them very attractive systems for many industrial applications. These include the pharmaceutical industries as targeted drug delivery systems (Wu et al., 2001), in cosmetics for the delivery of moisturizers and UV-blocking agents (Sonneville-Auburn et al., 2004), in agrochemicals for the active delivery of pesticides (Lee and Tadros, 1982), in the chemical industry as polymerization reaction media, and as nanotemplates for the preparation of nanoparticles (Liu et al., 2004).

The optically transparent droplets of very small size provide interesting non-Newtonian properties to the product. Due to the small size of nanoemulsion droplets many active drug molecules can be encapsulated and transported across biological membranes for targeted drug delivery applications. Thus, the bioavailability of drugs can be strongly enhanced by solubilization in the small droplets of nanoemulsion Forgiarini et al. (2001). We will discuss the application of nanoemulsions in various applications in the following sections.

## 7.1 Nanoemulsions in Food

The most significant concerns in the modern food industry are food safety and food quality (Rasooli, 2007; Lawrence, 2000). Minimally processed foods are vulnerable to microbial contamination and enzyme activity, which pose a great challenge to the food industry in regards to food safety and food preservation. Due to this challenge the food industries employ chemical substances to retard the growth of microorganisms and enzyme activity. The use of chemicals for food preservation also poses long-term toxicological implications (Mathias et al., 2015). In order to overcome this problem, new alternative technologies are being developed for replacement of chemical treatments of food.

Essential oils are currently used in the food industry for food preservation and to improve food quality. Essential oils are natural organic compounds and have antibacterial, antifungal, and antiviral properties. Some of the essential oils used in food industry are carvacrol, eugenol, carvone, citral, geraniol, terpineol, thymol, vanillin, and cinnamaldehyde (Burt, 2004). These essential oils prevent the oxidation of lipids and extend the shelf life of processed food products. Despite the numerous beneficial properties of these essential oils their wide application in the food industry is limited due to their poor water solubility, high volatility, and strong odor. Further, the incorporation of the oil phase in water-based food products is also a challenge due to physical and chemical instability. The use of nanoemulsions as promising systems for the application of essential oils in food matrix have been documented (Jo et al., 2015; Bhargava et al., 2015; Landry et al., 2015; McClements, 2004).

The nanoemulsions are kinetically stable and does not phase separate on prolonged storage. This property makes these systems as excellent carriers for hydrophobic bioactive substances. As mentioned in earlier sections, the choice of emulsifier is very critical in the formation of nanoemulsions. Some of the emulsifiers used in food emulsions are modified starches, sucrose esters, monoglycerides, proteins. Formation of nanoemulsions containing the essential oil in food products is well studied in recent years.

The oregano oil nanoemulsion (Bhargava et al., 2015) is reported to be effective against certain microorganisms such as *Listeria monocytogenes*, *Salmonella typhimurium*, and *Escherichia coli*. This emulsion is applied to fresh produce to control the growth of these microbes. Nanoemulsion containing the essential oil carvacrol (Landry et al., 2015) when applied to broccoli, alfalfa seeds, and radish is found to be effective against *Salmonella enterica* and *E. coli*. Nanoemulsions with lemongrass oil are effective in controlling the growth of microbes on plums without affecting the flavor and glossiness of the fruits. In addition, the applied nanoemulsion coating reduces ethylene production and controls the concentration polyphenolic compounds in plums (Donsi et al., 2015).

Nanoemulsions containing essential oils are thus emerging as alternative food preservation method to control the growth of pathogens on food products. The preservation and quality control of complex food matrices such as meat and meat products pose several challenges to the food industry (Sekhon, 2010). Thus, more intense research has to be conducted to understand the influence of essential oil nanoemulsions on meat and meat products preservation and quality.

## 7.2 Nanoemulsions in Cosmetics

Nanoemulsions have recently become potential vehicles/carriers for the controlled delivery of cosmetics and for the optimized dispersions of active ingredients to the skin. These dispersions are most suitable for the delivery of lipophilic compounds, which are generally insoluble in aqueous solutions. Due to the stability of nanoemulsions the transport of lipophilic compounds are encapsulated and transported better in comparison to vesicles, which are much large structures. Furthermore, the density of nanoemulsions does not allow phase-separation including sedimentation, creaming, flocculation, or aggregation, which makes them very attractive for application in cosmetics. The bioactive effects of nanoemulsions are also important for applications in cosmetics. This reduces the transepidermal water loss and strengthens the skin.

The nanoemulsions are generally prepared using high-energy methods such as high-pressure homogenization and microfluidization (Guglielmini, 2008). In these processes, the amount of surfactants used for the formation of nanoemulsions is generally low. Therefore, the use of surfactants that can cause skin irritation in cosmetics can be significantly reduced.

## 7.3 Nanoemulsions in Targeted Drug Delivery Systems

Camptothecin is a topoisomerase-I inhibitor that is effective against a broad spectrum of cancer cells. This drug is not water-soluble and therefore its clinical use is limited. Nanoemulsions containing pluronics and phospholipids were effective in loading this drug for effective controlled release (Chouksey et al., 2011).

Ubiquinone commonly known as Coenzyme Q10 (CoQ10) is an antioxidant and is involved in the production of energy within the cells. This compound is lipophilic and its oral bioavailability is limited. Encapsulation of CoQ10 in nanoemulsion results in enhanced bioavailability and controlled release. The application of CoQ10 has been improved further by the development of double emulsions. A double emulsion is an emulsion in another emulsion. Two main type of double emulsions are distinguished, water-in-oil-in-water (w/o/w) emulsion and oil-in-water-in-oil (o/w/o) emulsion. Double emulsion contain more interface and hence suitable for encapsulation of bioactive compounds.

Nanoemulsions as a drug delivery system for the prevention and treatment of cancer and inflammatory diseases have been developed (Ganta and Amiji, 2009). These nanoemulsion

formulations in general increase the bioavailability and improve pharmacokinetics. The emulsifiers used in such formulations are associated with adverse effects making these nanoemulsions not suitable for tunable drug delivery systems.

## 7.4 Nanoemulsion as Mucosal Vaccine

Nanoemulsions are also used to deliver recombinant proteins to a mucosal surface to induce an immune response. The nanoemulsion causes proteins applied to the mucosal surface to the adjuvant and it facilitates the uptake by antigen-presenting cells. The proof-of-concept in animals such as mice and guinea pigs for the delivery of hepatitis B and anthrax using carriers based on nanoemulsions is also reported. New o/w nanoemulsion for nasal delivery of vaccines for infectious disease such as smallpox and HIV has been developed (Arbor, 2008).

## 7.5 Antimicrobial Nanoemulsions

O/w nanoemulsions for antimicrobial applications contain droplets of size from 200 to 600 nm. The droplets are stabilized by surfactants and medium-chain alcohols, which act as cosurfactant. The cosurfactant concentration in these formulations is generally low and it helps to reduce the interfacial tension promoting the formation of small droplets. The nanoemulsion is active against a broad spectrum of bacteria, viruses, fungi, and spores. Some examples are *E. Coli*, Salmonella, HIV, Candida, anthrax. The nanoemulsion droplets with antimicrobial agents fuse with lipid containing organism thereby destroying them by various mechanisms.

The fusion between the nanodroplets is driven by the electrostatic attraction between the droplet charge and the charge on pathogens. When certain amounts of droplets fuse with the pathogens, the active ingredient from the nanodroplets is released to the lipid membrane causing lysis and death of pathogens. For pathogens like spores, germination enhancers are incorporated into the nanoemulsion in addition to other active ingredients.

## 7.6 Nanoemulsions in Cell Culture

Cell cultures are used for in vitro assays or to produce biological compounds such as recombinant proteins or antibodies. In order to optimize cell growth, the culture medium is usually supplemented with blood stream or with a number of defined molecules. It is still a challenge to supplement the media with

lipophilic substances, which limits the amount available for absorption by the cells. The lipophilic substances can be solubilized and transported to cell cultures using nanoemulsions. The nanoemulsion containing phospholipid emulsifier is effective in the delivery of lipophilic substances to mammalian cell cultures (Shakeel et al., 2008). The nanoemulsion droplets owing to their nanoscale size are easily taken up by the cells, leading to higher bioavailability of the lipophilic substance. The advantages of using nanoemulsions in cell culture technology are (1) better uptake of lipophilic supplements in cell culture, (2) improved growth and vitality of cultured cells, and (3) feasibility to conduct toxicity studies of lipophilic drugs in cell cultures.

## 7.7 Nanoemulsions for Delivery of Therapeutics

One of the highly promising applications of nanotechnology is the delivery of therapeutics in a cell-specific manner. Delivery vehicles composed of smart materials with tunable properties can greatly improve current therapeutic strategies by the method of encapsulation and site-specific delivery of the therapeutics. Emerging nanotechnologies that combine many physical and biological properties include polymeric nanocapsules, polymer microgels, mesoporous silica nanoparticles, liposomes, and nanoemulsions (Sainsbury et al., 2014). The features of nanoemulsions such as formulation stability, high loading capacity, and ease of manufacture make them attractive and well-suited for drug delivery applications. Nanoemulsions with advanced functionality for tunable biological interactions and the delivery of drugs are beginning to emerge in recent years Kim et al. (2008).

Parenteral administration (via the intravenous route) of drugs with limited solubility is a major problem in pharmaceutical industry. Formulations based on nanoemulsion have distinct edge over macroemulsion systems in parenteral delivery. This is due to the size of droplets in nanoemulsion, which permit longer residence time in the body. Both o/w and w/o type nanoemulsions can be used for this type of drug delivery applications (Devarajan and Ravichandran, 2011).

In oral drug delivery, nanoemulsion formulations offer several advantages over conventional oral formulations. The advantages are increased absorption of drug, improved clinical potency, and deceased drug toxicity. Nanoemulsions have been widely used for the oral delivery of drugs such as steroids, hormones, diuretic, and antibiotics. Pharmaceutical drugs of peptides and proteins are highly potent and specific in their physiological functions but cannot be administered orally. The bioavailability of such drugs in a

nonemulsion-based formulation is less than 10%. At this level the drugs are not therapeutically active by oral administration. Due to this limitation most protein-based drugs are only available as parenteral formulations. The peptide-drugs however, have an extremely short biological half-life when administered parenterally and requires multiple dosing. A nanoemulsion formulation of cyclosporine named as Neoral® is available. This is a replacement of Sandimmune® a crude o/w emulsion of cyclosporine formulation.

Topical administration of drugs is advantageous over other methods of drug administration. One of the main advantages is the avoidance of hepatic first metabolism of the drug and the related toxicity effects. The topical delivery route allows direct delivery and targeting of the drug to the affected area of the skin and eyes. The transdermal transport of indomethacin and diclofenac in lecithin-based nanoemulsion has been reported. The Fourier transform infra-red (FTIR) spectroscopy and differential scanning calorimetry (DSC) showed the disruption of lipid organization in the human stratum corneum after a day of incubation. The transdermal delivery of a lipophilic drug diphenhydramine hydrochloride from w/o nanoemulsion into human skin after exercise has also been studied. The nanoemulsion formulation contained a combination of emulsifiers such as Tween 80 and Span 20, isopropyl myristate and the active ingredient. The penetration characteristics of the drug can be modulated by varying the composition of the nanoemulsion (Attwood et al., 1992).

In ocular drug delivery, w/o type nanoemulsions are able to increase the absorption of drugs and prolong their release profile. The nanoemulsions containing the drug pilocarpine has been prepared using mixed surfactants such as lecithin, propylene glycol, and PEG 200 (polyethylene glycol). The low viscosity and refractive index of the nanoemulsion formulation was favorable for ophthalmologic applications. Other than ocular delivery, nanoemulsions based on fluorocarbon nonionic emulsifiers have been developed for pulmonary delivery (Tenjarla, 1999).

## 7.8 Nanoemulsion as Nontoxic Disinfectant Cleaner

A new nontoxic disinfectant cleaner based on nanoemulsion for use in commercial markets such as health care, hospitals, food processing, and military applications has been developed by Envirosystems Inc. This disinfectant is active against a wide spectrum of bacteria, viruses, and fungi in 5–10 min. The formulation consists of nanoscale oil droplets containing the active agent parachlorometaxylenol (PCMX) suspended in water. The oil droplets with PCMX carry surface charges that easily penetrate the cell

membrane of the microorganism rather than drowning the cells. This active agent is effective at low concentrations (1–2 orders of magnitude lower) than other disinfectants and therefore does not have any toxic effect on the user and environment.

Other microbial disinfectants require large doses of active ingredients to surround and disintegrate the pathogens (Rajalakshmi et al., 2011). The active ingredient is mostly nonflammable and safe to store and use in unstable conditions. It is nonoxidizing and does not corrode the casing material. The nanoemulsion disinfectant formulation can be applied to any hard surface.

### 7.9 Nanoemulsions in Biotechnology

Many biocatalytic and enzymatic reactions are conducted in pure organic or in a mixture of aqueous and organic media. These types of reactions are also conducted in biphasic media. Denaturation of biocatalysts occurs in pure polar media that causes a decrease in reaction rate. Enzymes in low water content have increased solubility in nonpolar reactants and can shift the thermodynamic equilibrium in favor of condensation. Enzymatic catalysis in nanoemulsions is widely used for a variety of reactions such as synthesis of esters, peptides, hydrolysis, and transesterification of sugar acetals (Tenjarla, 1999). Among the class of enzymes, lipases are the most widely used in microemulsion-based reactions.

## 8 Conclusions

The important aspects of nanoemulsions such as formation, behavior, properties, and applications are presented in this chapter. Due to many interesting properties of nanoemulsions such as high kinetic stability, optical transparency, and low viscosity these systems are attractive for many industrial applications including targeted drug delivery systems. In recent years, low-energy emulsification processes are widely used for the high-throughput production of nanoemulsions in food, cosmetic, and health-care products. In the future, nanoemulsions and self-nanoemulsifying systems will be widely used in the delivery of active molecules to the targeted sites thereby revolutionizing the field of food science and biomedical science.

## Acknowledgments

The authors thank Bente Olsen for her help in the preparation laboratory and the Danish Health Science Research Council for the financial support.

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7

# APPLICATION OF NANOEMULSION TECHNOLOGY FOR ENCAPSULATION AND RELEASE OF LIPOPHILIC BIOACTIVE COMPOUNDS IN FOOD

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## 1 Introduction

For many years, emulsions have been extensively used and employed in the pharmaceutical, cosmetic, and food industry as carrying systems of bioactive compounds. The emulsification technology is defined as the process in which a liquid is dispersed in another immiscible one; therefore an emulsion consists in two immiscible liquids (commonly water and oil) in which one of them is dispersed as small droplets inside the other one. Generally, in the food industry, the droplet diameter of the emulsion-based products ranges between 0.1 and 100 μm. Emulsions are classified according to the distribution of their lipophilic and aqueous phases; a system in which the lipophilic particles are dispersed in an aqueous phase is called oil-in-water emulsion (o/w), meanwhile, a system in which the water droplets are dispersed in an oil phase is called water-in-oil (w/o) emulsion (McClements, 1999).

Several researches have shown that emulsions formation is a technology that offers solutions to the food market, which needs to be continuously improved in order to cover the consumers' needs such as reduction in fat content but maintaining palatability

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00007-6

and nutritional value without compromising the food and texture attributes, among others (De Hoog, 2011; Mun et al., 2010; Hemar et al., 2010).

One of the main limitations during emulsions formation is the need to comply diverse functions at the time; initially, they have to be able to cover and maintain the active compound, namely, they must have the ability to be added to food without affecting appearance, flavor, texture, or the product's shelf life. Furthermore, the system must protect the active compound from chemical degradation during formulation, storage, transport, and usage. Also, release of the active compound must be controlled, and it has to respond to environmental stimuli as pH, temperature, or ionic strength for its release. Finally, it has to be formulated with food-grade ingredients (McClements et al., 2007; Djordjevic et al., 2008; Charoen et al., 2011).

Due to their nature, and as a consequence of several mechanisms such as coalescence and migration of liquids, emulsions are systems that tend to separate through time in their conforming phases (Rao et al., 2009; Peredo-Luna and Jiménez-Munguía, 2012). In order to overcome these drawbacks, and to fulfill encapsulation and release of the functional and nutraceutic ingredients, the food industry has increasingly been using nanoemulsions. Some of the advantages that nanoemulsions present over conventional emulsions are a higher stability of droplets against aggregation and gravitational separation as well as release of the encapsulated compound in the specific site. Moreover, due to its small droplet size, nanoemulsions exhibit a clear appearance and high solubility and stability, thus offering an excellent bioavailability and biological efficacy of the dispersed bioactive compounds (Abbas et al., 2013).

It is called a "nanoemulsion" to an emulsion, for which droplet size ranges between 20 and 500 nm (Bilbao-Sáinz et al., 2010). The application of nanoemulsions in the food science and industry field has been mainly focused as carrying systems of lipophilic bioactive compounds because of its minimal impact in the sensory characteristics of food as well as its high bioavailabilty (Choi et al., 2011; Donsì et al., 2012). Several studies have reported the addition of lipophilic bioactive compounds in food due to their properties such as flavoring and coloring agents, antioxidants, antimicrobials, assessing both natural and synthetic emulsifiers as well as different emulsion formation methods. The present review shows the state of the art of nanoemulsions formation as an encapsulation technology and its application in the food industry for the release of lipophilic compounds.

## 2 The Nanoscale and Its Applications in Food

Nanotechnology is concerned with the control or handling of materials, structures, devices, and systems that, due to their tiny size, have unique properties (Allhoff et al., 2010). Most of the applications of nanotechnology are focused in material engineering, informatics, and the medical field. However, researches have been growing in different fields such as agriculture and food. In the food industry, the application of nanotechnology has produced a noticeable change in production, process, storage, and development of innovative materials as well as in new food product development. With the application of nanotechnology in food, it is possible to develop new products with novel features such as texture, flavor, and stability during shelf life (Ezhilarasi et al., 2013; Soto-Chilaca and López-Malo, 2011).

Regarding the advances of nanotechnology applications in food packaging, there are two major areas studied: the inclusion of nanosensors that detect dangerous contaminants and the incorporation of nanoadditives, which are intended to be released in the final product in order to improve its quality. Additionally, in the last years, nanotechnology has been applied in the development of functional and nutraceutical foods and in the detection of pathogen microorganisms by using cubosomes, complex biopolymers, micelles, and nanoemulsions (Soto-Chilaca and López-Malo, 2011).

## 3 Nanoemulsions

### 3.1 Nanoemulsions Formation

A nanoemulsion is commonly formed by alipophilic phase dispersed in a continuous aqueous phase, in which each oil droplet is surrounded by a thin interfacial layer with the interaction of an emulsifier. Even though most of the authors support the idea that the only nanoemulsion that can be produced are the type of (o/w), Zuidam and Shimoni (2010) reported the production of w/o emulsions. In both cases, the size of the droplet in the emulsion has to be among the nanoscale (20–500 nm). Generally, due to its small size, nanoemulsions are highly stable and resistant to gravitational separation; furthermore, they show high resistance to aggregation because the range of attractive forces is lower as the size of the droplet decreases (Silva et al., 2011).

## 3.2 Nanoemulsions Stabilization Mechanisms

Depending on the application of the nanoemulsions, their stabilization must be improved by different methods.

### *3.2.1 Electrostatic Stabilization*

Electrostatic stabilization is related with the repulsive electrostatic forces effect that droplets show in the inner parts of the system. This effect is more noticeable when systems possess smaller sizes of the dispersed droplets. It is highly marked and partially responsible of the high stability of nanoemulsions (Garti and Lutz, 2004).

In nanoemulsions, when droplet coalescence occurs, droplets must be close enough until reaching contact with each other; this phenomenon is known as flocculation and, in order to avoid it, droplets need to stay separated owing to the repulsion between their charged surfaces. Some biopolymers with a specific surface activity such as proteins, polysaccharides, and their complexes, are able to provide electrostatic stability; their electrostatic interactions depend on their concentration, pH (isoelectric point), and the ionic strength of the solution (Dickinson, 1989, 1998).

When two particles or surfaces identically charged approach in a fluid medium, they develop a repulsive interaction that, depending on its size, the distance between them and the attractive forces generated, will keep them separated. These interactions represent an energy barrier that helps to reduce particles coalescence (Myers, 1996; Bergenstahl and Claesson, 1997).

For example, when proteins are in mediums with pH values distant from its isoelectric point, they are electrically charged and, therefore there is an electrostatic repulsion that prevents the particles to approach tightly with each other (Dickinson and McClements, 1996). However, this stabilization phenomenon is usually weak, but it promotes nanoemulsions stabilization, considering that it occurs simultaneously with steric stabilization and other mechanisms (Myers, 1996; Bergenstahl and Claesson, 1997).

An indicative parameter of the surface charge and, therefore of the particles electrostatic interactions, is zeta potential. According to some researchers (Aoki et al., 2005; Jiménez-Alvarado et al., 2009), zeta potential values between −30 and 30 mV, indicate a high stability of nanoemulsions because particles exhibit more distance between them and they are less susceptible to present coalescence.

### *3.2.2 Steric Stabilization*

Steric stabilization or steric impediment is a dominant factor when amphiphilic polymers are used as macromolecular

surfactants. Owing that these polymers form thick films, they help to improve the nanoemulsions stability and delay movement through interfaces (Garti and Lutz, 2004).

Among steric stabilization, three main mechanisms are distinguished; (1) exhaustion stabilization caused by nonadsorbent macromolecules that prevents collision between droplets and provides elasticity to the system, (2) electrostatic repulsion between two droplets with the same charge (Dickinson, 1998), and (3) stabilization as a result of hydrophobic interactions between adsorbed polymers (Garti and Lutz, 2004).

Amphiphilic polymers are adsorbed in the interface and they form semi-solid and thick films that promote stability and delay movement in the system; this is a very promising strategy because it requires less amounts of surfactant and they can be nonsynthetic macromolecules (Garti and Lutz, 2004).

For food applications, the most used biopolymers are proteins and polysaccharides. Researchers have successfully employed polymers such as bovine serum albumin, serum protein isolate, caseins, gelatin, maltodextrin, pectin, and complexes with hydrocolloids as xanthan gum, guar gum, arabic gum, and locust gum (Garti and Lutz, 2004; Dickinson, 2011).

Moreover, exhausting stabilization relies in the addition of agents that increase viscosity and gelling agents that reduce mobility of the entrapped ingredients. These ingredients are not considered as emulsifiers, but as stabilizers and they provide semisolid or gel-type features to nanoemulsions; particularly, gums can fulfill this task. However, these ingredients can affect some of the nanoemulsions properties, such as droplet size, consistency, and encapsulation efficiency. The thick interfacial layer and the viscous or gelled aqueous phase can exhibit some advantages when protection of sensitive compounds is required (Garti and Lutz, 2004).

### *3.2.3 Mechanical Stabilization*

Nanoemulsions shelf life can be improved by adding small solid surfactant particles to the system. The goal is to develop a mechanical barrier through the incorporation of small solid particles adsorbed in the interface (Garti and Lutz, 2004).

This stabilization method has been mostly applied in the pharmaceutical industry. Some experiments with microcrystalline cellulose, hydrophobically modified clay, and silica particles, have shown that the addition of solid particles improve the stability of nanoemulsions because they turn rigid the interface where they are adsorbed. Experiments that employed silica particles showed that particles concentration and its hydrophobic-hydrophilic nature, affect droplet size of the nanoemulsions (Garti and Lutz, 2004).

## 3.3 Factors Affecting Nanoemulsions Stability

### *3.3.1 Emulsifiers Used*

As well as in the formation of conventional emulsions, for the nanoemulsions it is necessary the action of an emulsifier to achieve stability of the emulsion for long-term storage. During the formation of the nanoemulsions, the emulsifier has to be able to quickly absorb around the dispersed phase, and at the same time assures not to break down when two droplets collapse, in order to avoid coalescence. It has to be predominantly soluble in the continuous phase and it has to be capable to emulsify the blend even in small concentrations (Cannon et al., 2008).

Emulsifiers can be classified in different ways, according to their number of hydrophilic regions; they are classified in monofunctional and multifunctional. Monofunctional emulsifiers possess only one hydrophilic region; meanwhile, multifunctional emulsifiers possess several hydrophilic groups and, in most cases, they also own more than one lipophilic group. According to their electric charge, they are classified in anionic, amphoteric, cationic, and nonionic. They can also be classified in polymeric and monomeric and, according to their origin, they can be classified natural and synthetic. These classifications are not exclusive. In the food industry, the most used emulsifiers are nonionic, anionic, or amphoteric. There is a wide variety of emulsifiers in the market; however, not all of them work appropriately in any food; according to their composition and state of dispersion, each system requires a specific emulsifier or a blend of emulsifiers that meets a specific hydrophilic-lipophilic balance (HLB) value.

HLB is a semi-empiric method that is widely used for the classification of emulsifiers. HLB value of an emulsifier indicates its solubility extent either in the aqueous or in the lipophilic phase. According to its chemical structure, each emulsifier has been assigned with a HLB value. Emulsifiers with HLB values ranging from 0 to 10 show high lipophilicity, while values from 10 to 20 show high hydrophilicity (Kralova and Sjöblom, 2009).

In the formation of nanoemulsions, both synthetic and natural emulsifiers have been studied in order to obtain stable emulsions, and they are not very different regarding the emulsifiers used in the formation of conventional emulsions. There are two main challenges to be achieved with the addition of emulsifiers in nanoemulsions;

1. the stabilization of the nanoemulsion without increasing the droplet size, since most emulsifiers are high-molecular weight polymers with big sizes;
2. stabilization under stress conditions.

In a broad investigation, Mao et al. (2009) assessed the emulsion formation capacity of some high molecular weight polymers to obtain β-carotene emulsions using high-pressure homogenization. The tested emulsifiers were Tween-20, decaglycerol monolaurate, octenyl succinate starch, whey protein isolate, and a mixture of Tween-20 and whey protein isolate. The results showed that each emulsifier tested offers different effects on the stabilization of nanoemulsions. Regarding Tween-20 and decaglycerol monolaurate, these reduced the interfacial tension and formed very small droplets, but they aggregated easily; on the other hand, when using octenyl succinate starch and whey protein isolate, the emulsions obtained resulted in bigger droplet size but showed higher stability due to their strong interfacial layers. Otherwise, the nanoemulsions stabilized with whey protein isolate provided the best protection to β-carotene, whereas those formed with octenyl succinate starch were rapidly degraded.

Moreover, Donsì et al. (2012) tested different natural emulsifiers and compared their effect with respect with some synthetic emulsifiers. The natural emulsifiers tested were modified starch, pea protein, sugar ester, and soy lecithin; meanwhile, the synthetic emulsifiers tested were sodium dodecyl sulfate (SDS) and Tween-80. The nanoemulsions were formed by applying high-pressure homogenization. The droplet size of the emulsions obtained oscillated between 90 and 190 nm and were as follows: 90 nm for sugar ester, 95 nm for Tween-80, 110 nm for SDS, 150 nm for modified starch, 180 nm for pea protein, and 190 nm for soy lecithin; according to the authors and as it was expected, those emulsions with the lowest droplet sizes, showed the highest stability.

The emulsifier that has been extensively used and tested for the formation of nanoemulsions in several researches is the whey protein isolate (WPI) (Li et al. 2011; Mao et al. 2009; Jafari et al. 2008, 2006). Lee et al. (2011) conducted research where they compared the stability, as well as the physical and chemical properties, of nanoemulsions and conventional emulsions using WPI as emulsifier. It was found that the nanoemulsions not only exhibited better stability under pH variations, salt addition, thermal processes, and freeze-thaw processes than conventional emulsions, but also showed better digestibility and higher oxidative stability. However, authors say that one of the disadvantages of the nanoemulsions they created is the low amount of oil contained, thus limiting their application in commercial products.

The use of modified starch as emulsifier in the formation of nanoemulsions was also reported by Liang et al. (2013), which was used for encapsulation of β-carotene. It was reported that after 30 days of storage under different conditions of temperature,

the most stable nanoemulsions where those stored under refrigeration (4°C), without showing creaming or phase separation. The low stability of nanoemulsions stored under high temperatures, was attributed to the loss of viscosity and the increase in the mobility of the system.

A broad study concerning the influence of the emulsifier type and concentration over the particle size of the nanoemulsions was driven by Yuan et al. (2008a). The nanoemulsions were produced by high-pressure homogenization. The emulsifiers used were different classes of polyoxythylene sorbitan esters of fatty acids (Tween-20, 40, 60, and 80) while the concentrations tested were 4, 6, 8, 10, and 12%. The smallest droplet size nanoemulsions were obtained with Tween-20 at all the tested concentrations and, as authors expected, the higher concentration of emulsifier used, the smallest droplet size of nanoemulsions was obtained. The small droplet size obtained when using Tween-20 was attributed to its hydrophilic-lipophilic balance (HLB) value (16.7), which was the highest among the emulsifiers tested.

### *3.3.2 Temperature*

Temperature is an important parameter in the formation of nanoemulsions. Among other several factors, temperature influences the type of interactions of the molecules in the system. Most of the experiments of emulsions formation are developed among 20–25°C, although some industrial processes are developed in different temperatures. Temperature has a direct impact on the solubility of all the compounds in the system (oils, proteins, polysaccharides, and emulsifiers) and their interactions, as well as in the viscosity of the nanoemulsion. The temperature at which the emulsion changes its dispersed phase to continuous, is called phase inversion temperature (PIT) and in order to avoid this phenomenon this value must be known before starting the preparation of the system; however, in some cases this phenomenon is induced and is considered as a low-energy formation process.

On the other hand, nanoemulsions with food components usually will require a thermic treatment that aids the antimicrobial stability. Some other treatments can include freeze-thaw processes. After completion, nanoemulsions are expected to resist certain temperature conditions predicted during storage (McClements et al., 2009).

Shinoda and Arai (1964) and Shinoda and Saito (1969) developed some experiments several years ago, and mentioned some interesting conclusions about temperature influence during emulsions formation that still apply in the nanoemulsions formation. They concluded that, (1) the more soluble is the nonionic

emulsifier with regard to the lipophilic phase and vice versa, the lower is the PIT; (2) when the PIT is increased, the clouding point increases; (3) the larger is the hydrophilic chain, the higher is the PIT and clouding point; (4) the droplet sizes in the emulsions change substantially when temperature and HLB value of emulsifiers change; (5) although the droplet size reached in a system is small, it is not stable to coalescence if it approaches PIT; (6) o/w emulsions can be obtained if PIT of the systems are 20–65°C larger than the storage temperatures; and finally (7) the emulsions stability can be improved if they are immediately cooled after being formed at temperatures close and below their PIT.

In addition to the previously cited, McClements et al. (2009) mentioned that in order to avoid lipids solidification during homogenization, it is important that the system temperature remain above the crystallization temperature of the containing lipids.

### *3.3.3 Z Potential, Droplet Size, pH, and Ionic Concentration*

As mentioned previously, systems with smaller droplet size show better stability. In the nanoemulsions stability, pH also plays an important role; this is because the variation of the electrostatic interactions changes and affect the biopolymers structure present in the system (McClements et al., 2009). The most common method to measure these interactions is through the zeta potential. A brief explanation of this concept and how it is related with pH and nanoemulsions stability is given ahead.

Nanoemulsions behave as any colloid, and its particles (dispersed phase) in suspension (continuous phase) are charged in a greater or lesser extent, what makes them repel one another avoiding coupling and agglomerations that could be present easily with fewer charges. These charges can oscillate from strong to weak; the stronger they are, the greater the repulsion will be and the more stable the nanoemulsion will be. If there is no repulsion, particles tend to couple with each other promoting agglomeration and phases separation.

Zeta potential is a measure of the electrical potential in the interface of suspended particles. Its units are millivolts (mV). As a rule, values from −30 to 30 mV suggest nonstability of the system, values below −30 and above 30 mV, suggest an increase in the stability, and it is greater as it increases and moves away zero.

The zeta potential measurement is carried out in an electrophoretic cell, with two electrodes connected to a power supply, thus creating an electrical field. Colloids migrate and depending on their movement and direction, zeta potential value is obtained. The zeta potential value is remarkably affected by pH and therefore in the nanoemulsion stability (Maldonado et al., 2011).

Another factor that directly affects zeta potential and nanoemulsions stability is the presence of ions in the aqueous phase of the system. Onsaard et al. (2005) conducted a research about properties and stability of nanoemulsions stabilized with coconut milk proteins. They analyzed, among other factors, the influence of pH, NaCl concentration in the aqueous phase and the temperature of the thermic treatment on the stability of emulsions. They found that zeta potential oscillates from 60 to −70 mV when it moves from pH 3 to 8, showing high stability when it reaches pH 7; particle size increases when pH is varied, showing maximum values in the isoelectric point (flocculation) and decreasing as it continues increasing. When NaCl concentration is increased, an increase of the particle size was detected. This statement was confirmed by Hsu and Nacu (2002) research of water in soy oil emulsions, stabilized by nonionic emulsifiers, the zeta potential presented a strong dependence of pH and these were greatly influenced by the $Na^{+1}$ and $K^{+1}$ ions concentration. The higher concentrations of these ions were able to separate the lipophilic phase of the system; meanwhile, divalent cations ($Ca^{2+}$ and $Mg^{2+}$) when present in high concentrations, led the system to charge zero, which is not caused with trivalent cations as $Al^{+3}$ and $Fe^{+3}$.

### *3.3.4 Viscosity*

According to Weiss and Mushiolik (2007), the emulsions stability increases as polarity decreases and viscosity of the lipophilic phase increases. Viscosity of oil has an important effect in the droplet sizes of an emulsion and thus in its stability. Likewise, fatty acids chain length has an effect of the systems stability; when the ratio of fatty acids with medium length chain (C8-C10) increases, viscosity decreases; in contrast, in the presence of more fatty acids with long length chain (C16-C20), viscosity increases.

In a study conducted by Bouchemal et al. (2004), authors agree that the average size of nanoemulsion droplets is very different according to the nature of the oil used in the system. In their research, they showed, as theoretically expected, that during the formation of nanoemulsions, hexyl laurate, with lower viscosity showed small droplet sizes than Myritol® 318 or Miglyol® 812. However, they revealed that α-tocopherol, the most viscous oil, gave smaller droplet size (171 ± 2 nm) in the nanoemulsions obtained, meanwhile hexyl laurate of low viscosity did not exhibit the smallest mean size in the final nanoemulsions.

## 3.4 Nanoemulsions Separation Phenomena

Rahn-Chique et al. (2012) mentioned that nanoemulsions are kinetically stabilized dispersions of o/w. During storage,

nanoemulsions are susceptible to nonstabilization and thus separation of their phases. The main mechanisms affecting nanoemulsions stability are Ostwald ripening, creaming phenomena, flocculation, and coalescence (Wang et al., 2009). These phenomena occur simultaneously and are not independent of each other. The small droplet size of nanoemulsions confers stability against creaming because Brownian motion and, consequently, the diffusion rates are sufficiently high.

### *3.4.1 Ostwald Ripening*

Ostwald ripening or molecular diffusion, which arises from emulsion polydispersity, is widely considered as the main mechanism for nanoemulsion nonstabilization (Wang et al., 2009). This separation phenomenon is a process in which very fine droplets of emulsion dissolved into the continuous phase, diffuse and redeposit upon larger droplets, thus increasing the average size of emulsion droplets. This problem should be tackled by using food-grade oils of fine-tuned hydrophobicity to achieve the desired size and stability of dispersed droplets. Hydrophobicity of oils can be enhanced by employing mixed oil systems in the nanoemulsions (Abbas et al., 2013). Ostwald ripening occurs due to the difference in the radius between droplets, and the driving force is the difference in chemical potential of the oil phase between different droplets. In the ripening process, larger droplets grow at the expense of smaller ones due to molecular diffusion through the continuous phase. According to the LSW (Lifshitz-Slezov-Wagner) equation, a linear relationship between the cube of the droplet radius or diameter and storage time should exist (Li and Chiang, 2012). Fig. 7.1 shows a typical representation of emulsion instability by Ostwald ripening with a good linearity between the cube of droplet diameter and storage time.

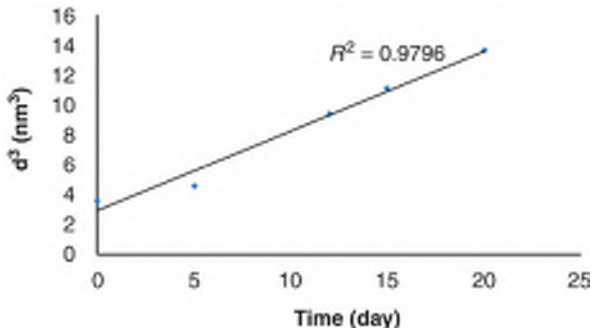


**Figure 7.1. Typical representation of nanoemulsion instability by Ostwald ripening: cube of the mean diameter as a function of time during storage.**

### 3.4.2 Coalescence and Creaming

Coalescence occurs when two adjacent droplets join into a bigger unique entity due to the disruption of the film in the interface that separates them. When this mechanism occurs there is no change in the interface of outer droplets (Appelqvist et al., 2007; Dickinson, 2011). As film becomes thinner, it breaks and this breakage depends on the relative hydrodynamic inside the film and other factor such as rheological properties of the continuous phase, the concentration of the dispersed phase and the effective stabilization of droplets and their capacity to keep distance between the droplets. Interfacial rupture depends on the mechanical properties of the film, and it is influenced by shear stress and temperature (Appelqvist et al., 2007).

After droplets coalesce in an irreversible way that creates bigger entities, gravity can lead to a phase separation producing the phenomenon known as creaming, which is the formation of a cream layer in the surface or sedimentation when the separated phase settles down (Appelqvist et al., 2007; Dickinson, 2011). This phenomenon occurs when repulsion between droplets is weak, interfacial tension and viscosity are low, too, and the interface film is not elastic enough (Friberg and Yang, 1996; Bergenstahl and Claesson, 1997).

### 3.4.3 Flocculation

Flocculation is the process by which nanoemulsion drops aggregate, without rupture of the stabilizing layer at the interface. It occurs when there is a net attractive force between droplets, which is large enough to overcome thermal agitation and cause persistent aggregation. The rate of flocculation depends on the product of a frequency factor (how often drops encounter each other) and a probability factor (how long they stay in contact). Therefore, the most obvious route to increasing stability toward flocculation is to reduce the rate and duration of droplets encounters. Nonionic surfactants normally have large hydrophilic groups, which spread out from the droplet surface presenting steric stabilizing barriers. However, some authors proposed that although nonionic surfactants suppress coalescence, they cannot prevent flocculation. Coadsorbed ionic surfactants may increase the emulsion droplet surface charge. The resulting electrostatic interactions are expected to stabilize the emulsions by reducing the rate of droplet encounters (Wang et al., 2009).

## 3.5 Formation Processes

Regarding the amount of energy used for the formation of nanoemulsions, because the mixture of a lipophilic and an aqueous

compound cannot be achieved naturally, the processes have been classified in two major groups: those that needs low amounts of energy and those using high amounts of energy (Ezhilarasi et al., 2013).

### *3.5.1 Low-Energy Processes*

The nanoemulsions formation by means of low-energy processes is based on the formation of droplets inside water-oil-emulsifier systems through the intentional modification of the environmental conditions or the composition of the system. Among the low-energy processes, the most common and most used processes are the spontaneous emulsification or phase inversion and the self-assembly process, as well as others less used such as membrane emulsification and solvent displacement (Abbas et al., 2013; Silva et al., 2011).

#### 3.5.1.1 Spontaneous Emulsification or Phase Inversion

The application of spontaneous emulsification is an efficient and less expensive alternative process compared with the high-energy processes. For the emulsion formation, this method has as advantage the chemical energy stored in the system, and it involves the phase inversion of the phase; this phenomenon refers to the process in which an o/w system becomes a w/o system and vice versa. A variant of this method is the catastrophic phase inversion (CPI) (Bilbao-Sáinz et al., 2010). According to Sajjadi et al. (2004), in the CPI method, the system starts as an abnormal emulsion, it means that it is an emulsion where the emulsifier has high affinity for the dispersed phase, and moreover, with the continuous stirring applied, the emulsions turns to be stable. The purpose of this method is to get the inversion of an emulsion, and it is induced by increasing its coalescence rate, hence, the balance between coalescence rate and droplet breakdown cannot be maintained. This phenomenon could be induced by modifying the variables, which increase the coalescence rate of the droplets, as with the addition of a known amount of the dispersed phase. The emulsion that starts for example, as a w/o emulsion, but as more droplets of water are added, they start increasing the size of the initial drops until they reach such a size that they become able to envelop the continuous phase (oil), therefore water becomes gradually the continuous phase of the emulsion inducing the phase inversion.

Among the research conducted using CPI for the formation of nanoemulsions, Bilbao-Sáinz et al. (2010) performed emulsions of Acetem/water/Tween-60, getting smaller droplet sizes and more stable emulsions than those made by stirring at a constant rate during 6 h. Ghosh et al. (2012), employed this technique during the formation of mustard essential oil nanoemulsions; they

establish that in order to get a successful nanoemulsion formation by means of CPI, it is necessary to control the oil-emulsifier ratio, as well as the addition rate of one of the phases to the existing system to accomplish the inversion.

#### 3.5.1.2 Self-Assembly

Choi et al. (2011) obtained nanoemulsions using the self-assembly method. This method consists in preparing a conventional emulsion with resulting droplet sizes among the nanoscale by applying stirring in the presence of a water-soluble emulsifier. In this technique, Tween-80 is commonly used as emulsifier. Up to this step, the system obtained is called a simple-layer nanoemulsion. Subsequently, a biopolymer is added to the nanoemulsion to wrap up the dispersed phase, getting a double-layer nanoemulsion. Finally, another biopolymer is added to the existing system, creating a triple-layer in the nanoemulsion, which final size ranges between 20 and 300 nm. In this study, it is used alginate and chitosan to encapsulate capsaicin, which was part of the lipophilic phase of the simple-layer nanoemulsion. Although it is reported that the size of the formed nanoemulsions by the self-assembly method ranges between 20 and 300 nm, among the results obtained in this study, authors reported that they obtained stable triple-layer nanoemulsions with droplet sizes equal or lower than 20 nm. Finally, based on the zeta potential values obtained, it was concluded that the addition of chitosan and alginate improved the stability of the nanoemulsions, suggesting to be a good option of material mixture for encapsulation and to give protection to bioactive compounds.

#### 3.5.1.3 Membrane Emulsification

The previous low-energy methods described, phase-inversion and self-assembly are the most commonly used, nevertheless there are other low-energy methods that are successfully utilized for the formation of nanoemulsions, such is the case of membrane emulsification. The membrane emulsification is a process that requires less amount of emulsifier than the high-energy processes and produces emulsions with narrower droplet size distributions. This method consists on forcing the dispersed phase to pass through a membrane in order to reduce its size.

The most important factors influencing membrane emulsification are divided into four main groups:

1. membrane parameters (mean pore size, number of active pores, porosity, wettability, permeability, and thickness),
2. phase parameters (interfacial tension, emulsifier type and concentration, viscosity and density of continuous and dispersed phases),

3. process parameters (wall shear stress, transmembrane pressure, temperature, membrane module configuration), and
4. emulsifying process (droplet size distribution, dispersed phase flux, and dispersed phase percentage) (Charcosset, 2009).

Van Der Graaf et al. (2005) mentioned that the main application of membrane emulsification in the food industry is in flavor delivery systems, both with simple and double emulsions. Nevertheless, Charcosset (2009) stated that the unique commercial product that employed this emulsification process up to 2009 was a very low fat spread, which have been reported to possess a very high stability that reaches up to 6 months without phase separation, showing creamy, soft, and tasty characteristics.

#### 3.5.1.4 Solvent Displacement

The solvent displacement technique consists in mixing a water-soluble organic solvent containing lipophilic functional compounds in an aqueous phase, with the presence of an emulsifier; the rapid diffusion of the organic solvent into the aqueous phase allows the formation of the nanoemulsions; as a final step, the solvent is evaporated (Mason et al., 2006).

A combination of the phase inversion and solvent displacement methods to formulate nanoemulsions was developed by Rao and McClements (2010). They tested a variation of the solvent displacement method; in an oil-water-surfactant model, they initially used a low phase inversion temperature emulsifier to form the emulsion. The emulsifier conferred a relative low stability to the system, therefore a second emulsifier with a much higher phase inversion temperature was added to the system; this last emulsifier was able to produce strong repulsive forces between the droplets, preventing droplet coalescence. At the end of the process this emulsifier displaced the first emulsifier from the droplet surface thus providing a higher stability to the nanoemulsion.

### *3.5.2 High-Energy Processes*

The formation of nanoemulsions by applying high-energy processes is characterized by submitting the system into high amounts of energy previously determined. The energy applied confers the system enough rearrangement initial predisposition to keep its stability, although some modifications in the formulation of the system is extremely important. These processes are based on the utilization of mechanical devices that produce the necessary disruptive forces to achieve the rupture of the macroscopic forces. Generally, these processes have shown more efficiency both in the time of formation of the emulsions and in the reduction of the droplet sizes of the dispersed phase, however, their industrial

application is still scarce. The most common high-energy processes are high-pressure process, ultrasound, and high velocity stirring (Abbas et al., 2013; Ezhilarasi et al., 2013; Silva et al., 2011).

#### 3.5.2.1 Ultrasound

The emulsification process was one of the first applications of ultrasound about 50 years ago. The ultrasound waves ranging from 20 to 100 kHz have the ability of producing physical and chemical modifications when they make contact with matter. When a plane surface vibrates with certain amplitude and frequency, longitudinal waves are generated and these are spread into the liquid or gaseous medium. These waves induce movements in the particles of the medium through several compressions and rarefactions under fluctuating pressure, leading to the phenomenon of acoustic cavitation. The formation of nanoemulsions is possible due to the phenomenon of cavitation (Jafari et al., 2006). Cavitation can be defined as the formation and collapse of steam cavities inside a liquid fluid; the collapse of these cavities causes powerful shock waves that irradiate along the fluid, thus breaking the dispersed liquid. The intense effect of the collapse of these created waves has as consequence the production of very small size droplets in the medium, allowing the formation of emulsions with a micro- and nanoscale size distribution as shown in Fig. 7.2 (Jafari et al., 2006; Abbas et al., 2013).

The most important parameters that have been evaluated, demonstrating a strong influence in the nanoemulsions formation by ultrasound-assisted emulsification are: frequency, ultrasonic power, and treatment time. With respect to the frequency, most authors establish that low frequencies for ultrasound processes ranging between 20 and 24 kHz are enough to produce nanoemulsions with very good long-term stability (Abbas et al., 2013). Regarding the treatment time, Leong et al. (2009) reported a decrease in the droplet size from 100 to 40 nm after increasing the treatment time from 5 to 40 min continuously applied in the process. Furthermore, Ghosh et al. (2013), formulated basil essential oil nanoemulsions testing different treatment times (5–15 min); the droplet sizes were reduced from 57.75 nm with 5 min of treatment to 41.15 nm with 15 min. These results confirm that increasing the treatment time, the more energy is available for the rupture of the fluids, and therefore the size reduction of the droplets.

In order to understand the influence of several process conditions of the ultrasonic homogenization for emulsification purpose and to optimize the process conditions, response surface methodology (RSM) has been used in some studies. Li and Chiang (2012) evaluated different parameters to prepare *d*-limonene

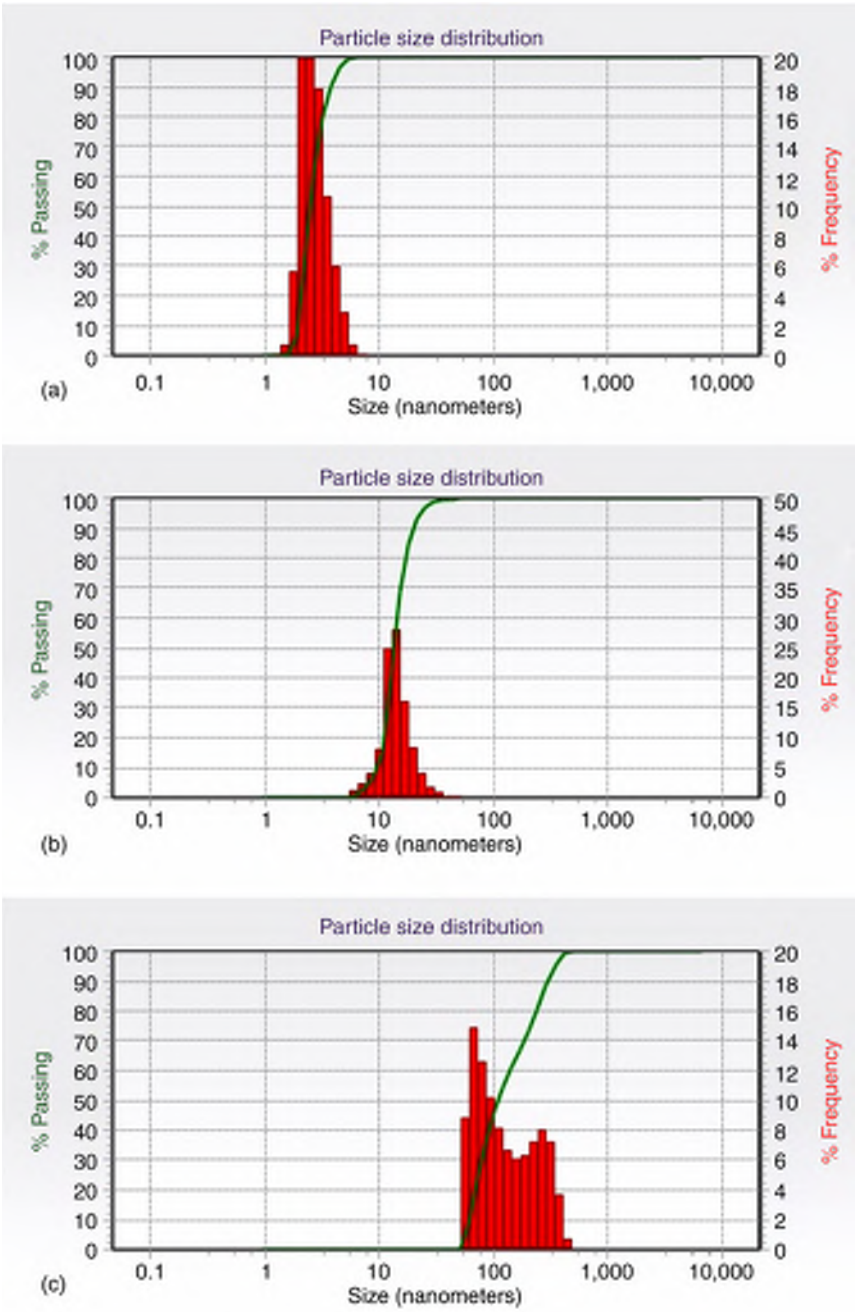


**Figure 7.2. Particle diameter distributions in a nanoemulsion influenced by sonication intensity (wave amplitude), (a) 100 μm, (b) 80 μm, (c) 60 μm.**

nanoemulsions: ultrasonic power, process time, and *d*-limonene to surfactant ratio. After performing 20 experiments designed by a central composite design (CCD), the authors concluded that an ultrasonic power of 18 W, applying 100–140 s of homogenization and with a *d*-limonene to surfactant ratio of 0.6–0.7, were necessary to obtain stable nanoemulsions, avoiding Ostwald ripening at room temperature. Similar process conditions were suggested by Salvia-Trujillo et al. (2013b) whom also investigated the influence of different processing parameters (amplitude and treatment time) for the ultrasonic emulsification of lemongrass essential oil. This study showed that in order to obtain small droplet size and stable nanoemulsions, increased sonication amplitude and time were needed during processing, specifically 100 μm for 180 s, respectively.

It has been suggested that the ultrasound power and time, which determines the total energy input to the system must be taken as control parameters of the process. Delmas et al. (2011) demonstrated that the emulsification efficiency only depends on the overall total energy applied to the system, because the saturated droplet size (minimum size attained during the ultrasonic process) is reached at whatever ultrasound power, when the process has been applied over a sufficient time. The droplet size evolution during the nanoemulsion formation while applying ultrasound can be described by an exponential function of the process time as well as the total sonication energy input (Fig. 7.3).

#### 3.5.2.2 High-Pressure Process

The most efficient method for the formation of nanoemulsions is the high-pressure homogenization (HPH), also known as micro

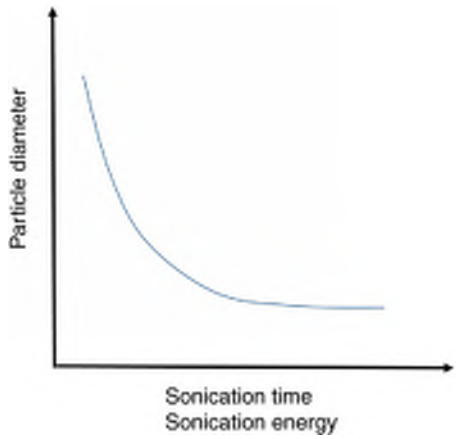


**Figure 7.3. Particle diameter of the dispersed phase in a nanoemulsion influenced by total sonication time or sonication energy input.**

fluidization (Donsì et al., 2010; Liang et al., 2012). The main parameters that have an important influence in the final droplet size of the nanoemulsions and therefore its further stability, are the level of pressure applied and the number of cycles or homogenization passes of the system. According to Donsì et al. (2010), due to the elevated levels of pressure applied to the fluid in HPH treatments, which are above 300 MPa in commercial systems, elevated tensions are exerted on the fluid when it is forced to pass through the small valve of the equipment, contributing to the reduction of the droplet size of the emulsion. This conclusion was arisen studying different HPH conditions to obtain emulsions in a nanoscale, testing pea protein as a novel emulsifier. The results demonstrated it was necessary to pass the fluid system up to 3 times through the homogenizer applying pressures of 200 to 300 MPa, in an aim to obtain nanoemulsions with droplet sizes below 100 nm. Another study supporting this conclusion was the one reported by Liang et al. (2012) when analyzing the stability of mint essential oil nanoemulsions. The tested pressures by these authors were 50, 100, or 150 MPa and 1, 3, 5, 7, 10, 15, or 20 passes. It was observed that with pressures of 100 and 150 MPa, the droplet diameters were similar and significantly smaller than those obtained applying a pressure of 50 MPa. Furthermore, it was observed that after 8 cycles, the droplet size of the nanoemulsions was remarkably reduced by applying the three pressures tested, however, after 10 cycles, the droplet size did not show a significant decrease in all the pressure levels tested. The emulsions with drop sizes smaller than 10 nm proved that HPH is an effective method to get droplet sizes among the nanoscale, conferring stability to the nanoemulsions during a storage period of 30 days, without showing phase separation or creaming.

However, an opposite behavior was reported by Jafari et al. (2006) when using HPH method for *d*-limonene emulsions formation. In this study, when the number of passes was increased, bigger droplet sizes were obtained in the nanoemulsions. The authors explained this result as an overprocessing phenomena, also associating it to a poor performance of the emulsifier, as well as an increase in the Brownian motion. This last mechanism of emulsion instability is defined as the random movement of particles suspended in a fluid (liquid or gas), induced by the rapid collision of its atoms and molecules, thus producing more coalescence between the suspended droplets. Similarly, Salvia-Trujillo et al. (2013a) evaluated the influence of HPH processing parameters (pressure and cycles) for nanoemulsions formation, containing lemongrass as the lipophilic phase. In this study, the authors obtained nanoemulsions with average droplet size of 7.35 ± 1.67 nm only after 3 cycles of HPH, working at a pressure of 150 MPa.

Moreover, the process temperature, as well as the formulation of the system, have also been identified as important parameters influencing the formation of nanoemulsions by HPH (Yuan et al., 2008a, b). A response surface methodology was performed by Yuan et al. (2008a) to predict and attain the optimal process conditions for β-carotene nanoemulsions applying HPH; the variables assessed were the β-carotene and emulsifier concentration and the homogenization temperature and pressure. The results showed that the β-carotene concentration, homogenization temperature and pressure had a significant effect on the stability of the nanoemulsions produced. In this study, the theoretical optimal process conditions of pressure and temperature to obtain stable nanoemulsions were 129 MPa and 47°C, when using a β-carotene concentration of 0.82% and an emulsifier concentration of 8.2%. Furthermore, Yuan et al. (2008b) reported that an increase in the homogenization temperature from 30 to 50°C leads to a reduction in the particle size of β-carotene nanoemulsions.

Moreover, Donsì et al. (2012) also suggested that the HPH equipment design may also affect the final droplet size of the nanoemulsions formation. Therefore, they tested different geometries and configurations of this chamber and compared it to the conventional equipments that did not have this chamber. However, only slight differences were encountered regarding size and shape of droplets of nanoemulsions obtained in the tested chambers.

## 3.6 Applications of Nanoemulsions as Additives in Food

The production of nanoemulsions is one of the most important applications of nanotechnology because they can be used as carrying or release systems of lipophilic compounds as nutraceutical ingredients, aroma, and flavor compounds as well as antioxidant and antimicrobial agents, among others. Due to their small particle size, one of the main advantages of nanoemulsions is their ability to improve the biodisponibility of the encapsulated compounds because these have a high surface-volume ratio in the system.

The application of nanoemulsions as food ingredients becomes an important item to control due to the diffusion and migration of the materials content through the food matrix (Dickinson, 2003). The current application of emulsions in food is mainly directed to develop food products that, in addition to showing functional benefits to human health, they could be able to offer good sensorial attributes to consumers (De Hoog, 2011).

Progress and innovation of the structure and content of the lipophilic phase has received major attention in the field of food emulsions. There is a growing interest in the use of oils with specific functional properties, such as polyunsaturated vegetable oils and essential oils or lipophilic compounds like antioxidants, phytosterols, antimicrobials, and vitamins (Muñoz et al., 2007; Charoen et al., 2011). In many cases, it is considered as an advantage to develop bioactive lipophilic compounds in an aqueous medium due to an increase in palatability (McClements et al., 2007). These lipids vary in their molecular properties (weight, structure, functional groups, polarity, and charge), which makes them different regarding their physicochemical and physiological properties (solubility, physical state, rheology, optical properties, chemical stability, surface activity, and bioactivity) (Charoen et al., 2011; Muñoz et al., 2007). Therefore, each system is developed in order to be compatible with a specific lipid. As an example, unsaturated fatty acids possess an important impact on human health because they fulfill numerous physiological functions, therefore their stability during storage has been studied in order to have better handling properties and dosification. Foods containing unsaturated fatty acids are prone to oxidation, thus nanoemulsions formulation becomes an effective option to preserve them (McClements et al., 2007; De Hoog, 2011).

### *3.6.1 Nanoemulsions as Carriers of Antimicrobial Agents*

Most of the researchers that utilize nanoemulsions as carriers of bioactive compounds with antimicrobial potential, report the use of essential oils in the lipophilic phase of these systems. Mustard essential oil is a lipophilic compound that has been subjected to several studies of encapsulation, through emulsification processes in aim to exploit its antimicrobial properties. The antimicrobial activity of the mustard essential oil encapsulated in nanoemulsions formed by the phase inversion method, in model systems against *Escherichia coli* was performed by Ghosh et al. (2012). When the nanoemulsion was directly applied to the inoculated bacteria, after 15 min of interaction, a reduction of 3 logarithmic cycles was observed. Since the release of the mustard essential oil contained in the emulsions was achieved gradually, after 60 min of interaction, the inactivation of the bacteria was complete.

Liang et al. (2012) evaluated the antimicrobial activity of mint essential oil nanoemulsion, against two Gram-positive bacteria, namely *Listeria monocytogenes* and *Staphylococcus aureus*. Before carrying out the experiments to test the antimicrobial activity, a compositional analysis of the pure essential oil and the oil content in the nanoemulsions were performed. It was demonstrated

that the compositions were similar without significant variations. The minimal inhibitory concentrations where the same for the nanoemulsions as well as the pure essential oil applied directly (0.5% v/v) for both tested bacteria; thus concluding that it is possible to use the nanoemulsions systems as an efficient encapsulation techique to protect essential oils, without affecting their antimicrobial activity.

The antimicrobial efficacy of thyme oil nanoemulsions, prepared by the HPH method, against *Zygosaccharomyces bailii*, an acid resistant spoilage yeast, was tested by Chang et al. (2012). In order to improve the stablity of the nanoemulsions produced, two lipophilic compounds (corn oil and a commercial triglyceride) were added to the oil phase. The nanoemulsions were directly added to a broth containing the yeast ($10^4$ CFU/mL). Authors concluded that although the corn oil and the commercial triglyceride used improved the nanoemulsion stability, these components-decreased the antimicrobial activity of thyme essential oil; therefore an equilibrium between the stabilizer and thyme essential oil must be found for the nanoemulsions. In the case of the nanoemulsions added with corn oil, the minimum amount of thyme oil required to inhibit *Z. bailii* after 5 days of storage was 375 μg/mL, containing 60 wt. % of corn oil in the lipophilic phase of the system. On the other hand, for the commercial triglyceride, with the same concentration (60 wt. %), the minimum concentration of thyme essential oil required to achieve the same inhibition of the yeast was 750 μg/mL.

Two antimicrobial compounds, an oily mixture of terpenes extracted from *Melaleuca alternifolia* and *d*-limonene were encapsulated, separately, by Donsì et al. (2011) using nanoemulsion technology, in order to assess different systems with controlled antimicrobial delivery. Nanoemulsions formulation was performed by HPH; sunflower oil or palm oil were used in the oleous phase and soy lecithin, Tween-20, glycerol monooleate and modified starch were used as emulsifying agents. The successful formulation of nanoemulsions of the terpenes mixture was achieved using soy lecithin, while *d*-limonene was succesfully encapsulated by using modified starch. The antimicrobial analysis showed that the nanoencapsulated terpenes lowered or equaled the minimal inhibitory concentrations and minimal antibactericidal concentrations of the values of the nonencapsulated mixture against *E. coli,* and *Saccharomyces cerevisiae*; however, nanoencapsulated *d*-limonene was only capable to reduce the minimal inhibitory concentration with respect to the nonencapsulated essential oil. Additionally, the encapsulated terpenes were added to orange and pear juices in order to test their antimicrobial activity against *Lactobacillus*

*delbrueckii*, resulting in the delay of the microbial growth or inactivation with concentrations of 0.1 and 0.5% w/v, respectively.

Eucalyptus oil nanoemulsions were formulated and characterized by Sugumar et al. (2013) in order to test their antimicrobial activity against food-borne pathogens. Nanoemulsions were obtained by ultrasound homogenization using Tween-80 as surfactant agent. Nanoemulsion bactericidal activity was assessed during a growth period of 24 h, control treaetments were eucalyptus oil and Tween-80 added separately. Authors found that bactericidal activity of eucalyptus oil was enhanced when added as part of the nanoemulsions, compared with the control treatments. Moreover, the nanoemulsions showed bactericidal activity against all bacteria tested (*Bacillus cereus, S. aureus,* and *E. coli*) after 24 h of direct exposure.

### *3.6.2 Nanoemulsions as Carriers of Antioxidant Agents*

Since some of the bioactive compounds demonstrating important antioxidant properties are mainly lipophilic, their application in food systems as o/w emulsions is a very good option, due to its low water-solubility. Besides, nanoemulsions have demonstrated to be an efficient encapsulating technique because these systems provide protection to the inner compounds, maintaining their functional properties.

Among the researches that have been performed to encapsulate antioxidant compounds through nanoemulsions, Donsì et al. (2011) utilized high-pressure homogenization and tested different emulsifiers in order to encapsulate peanut oil enriched with resveratrol and curcumin oleoresin. The final goal was to improve their dispersibility in aqueous systems and to protect the degradation of these two phytochemicals, as well as to keep or improve their antioxidant capacity. The results obtained showed that the stability of resveratrol was enhanced with encapsulation of these compounds (0.01% p/p) in nanoemulsions, reducing its degradation and transformation from cis- to transconfiguration. Additionally, when curcumin was encapsulated (0.01% p/p), the nanoemulsion promoted its dispersibility in water and avoided its recrystallization and settlement along storage.

### *3.6.3 Nanoemulsions as Carriers of Nutraceutical Ingredients*

Although it does not exist, a specific and global definition of nutraceutical ingredients, Wildman and Kelley (2007) define these products as any substance considered food or part of a food that provides medical and health benefits besides its nutritional contribution, including the prevention of and/or treatment of some

disease. Among these products, it can be mentioned the isolated ingredients, dietetic supplements, herbs and processed foods such as cereals, soups, and beverages.

In the field of encapsulation of bioactive compounds, the application of nanoemulsions has been reported for protection and carriage of different types of nutraceutical ingredients. Within this vast group of ingredients, carotenoids represent an extensive group of lipophilic organic pigments that are found in different fruits and vegetables. Due to the nutraceutical properties of carotenoids, Liang et al. (2013) tested the stability and bioavailability of β-carotene in nanoemulsions, using modified starch as emulsifier and HPH. After 30 days of storage at different conditions of light, oxygen concentration, and temperature, it was found that the retention of β-carotene in nanoemulsions was higher than 50% even at 25°C, both in presence of light and darkness; while when nitrogen was added to the headspace at stored at 4°C, retention was improved and increased. Therefore, the utilization of nanoemulsions as a protective technique of carotenoids was proved.

Qian et al. (2012) also prepared nanoemulsions containing β-carotene using HPH; β-lactoglobulin and Tween-20 were used as emulsifiers.In order to use them as nutraceutical ingredients, they subjected the nanoemulsions under environmental stresses such as pH, ionic strength, and temperature, which may be encountered in typical foods and beverages. Authors found that under acid conditions (pH 3), nanoemulsions tend to discoloration as a result of degradation, regardless the salt concentration (0–500 mM NaCl) used. Moreover, their results suggested that using β-lactoglobulin in the nanoemulsions formulation, the chemical stability of the system was increased, but if it was stored at temperatures greater than 37°C, the nanoemulsions tend to present droplet aggregation and a subsequent phase separation.

A similar study was carried out by Rao and McClements (2011). In this study, lemon oil was used in the lipid phase, an ingredient that, in addition to its nutraceutical demonstrated properties, it is commonly used as flavoring agent in foods, beverages, and fragrances. Nanoemulsions were formed using HPH and then subjected under stress conditions: under pH 6 to 7. The nanoemulsions showed relative stability, but exhibited droplet aggregation with the addition of NaCl (50 mM) at pH 7 after 1 month of storage. Similar to other studies, nanoemulsions behavior on storage at refrigeration (5°C) and room temperature (23°C) was stable, but exhibited coalescence above 40°C.

Emulsions and nanoemulsions successful formation and stability depend not only on stress conditions or external factors in

which the systems are subjected to, as the mentioned earlier, but also on specific factors and parameters during their formation as well as the method utilized. Saberi et al. (2013) encapsulated vitamin E in nanoemulsions formed by spontaneous emulsification; the main interest of this research was to develop vitamin E-based nutraceutical delivery systems that could be incorporated into transparent food, beverage, and pharmaceutical products. Authors found that the surfactant type had a remarkable influence in nanoemulsions droplet size. Among all the emulsifiers used (Tween-20, 40, 60, 80, and 85), Tween-80 produced nanoemulsions with the smallest droplet size. Furthermore, the droplet size of the vitamin E nanoemulsions was reduced by increasing the mixing temperature and stirring speed, achieving transparent nanoemulsions suitable to be included as nutraceuticals in different products.

With the purpose to evaluate the delivery properties of nanoemulsions, Yu and Huang (2012) encapsulated curcumin, a natural bioactive compound with many health promoting benefits. However, its low oral bioavailability limits its potential application in functional food. For the preparation of the nanoemulsions containing curcumin, a mixture of modified starch, whey protein concentrate, and Tween-20 was used as emulsifier and ultrasound was chosen as the homogenization method. Broadly, authors successfully developed curcumin-based nanoemulsions and accomplished a 9-fold improvement of curcumin bioavailability compared to the nonencapsulated one, thus confirming their hypothesis.

## 4 Conclusions

The use of high- and low-energy methods in the formation of emulsions can be applied to obtain small droplets size within the nanoscale, thus providing high stability during storage. The high-energy processes are the most effective in the formation of nanoemulsions, however industrial level scale-up have not been possible so far. On the other hand, despite low-energy processes are less efficient than high-energy processes, the nanoemulsions obtained by low-energy processes have also shown higher stability than microemulsions.

Besides, the application of nanoemulsions in food systems has resulted in the successful release and carriage of lipophilic compounds with functional and nutraceutical features. Therefore, it is concluded that the application of nanoemulsions is a good alternative for the addition of this class of ingredients into foods.

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# GELATION IN NANOEMULSION: STRUCTURE FORMATION AND RHEOLOGICAL BEHAVIOR

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## 1 Introduction

Many products in the food industry are made up of emulsions, for example, butter, margarine, salad dressing, sauces, and coffee creamer. Emulsions are colloidal dispersions of two immiscible liquids, in which one phase is dispersed as droplets and the other forms a continuous phase around them. By controlling droplet size, volume fraction, and their interdroplet interactions, the physicochemical properties of these foods can be significantly altered. Recently, it has been shown that textural and optical properties of emulsions can be significantly modified by reducing their droplet radii below 100 nm, that is, converting them into nanoemulsions (McClements and Rao, 2011). The extremely small droplet size of nanoemulsions make them kinetically more stable to droplet aggregation, gravitational separation, and coalescence compared to the conventional micron-scale emulsions (Huang et al., 2010). Nanoemulsions are also shown to increase the bioavailability of bioactive lipophilic substances due to their high surface-to-volume ratio (hence higher release of internal bioactive compounds in the gut), which makes them preferable over conventional emulsions in functional foods, nutraceuticals, and pharmacological applications (Silva et al., 2012; Maali and Mosavian, 2013). Their optical properties (significantly less turbid or translucent) also make them useful in many applications, for example, flavor delivery medium in clear beverages (Walker et al., 2014). However, almost all nanoemulsions used to date are liquids, which restrict their use in many soft materials, including gels, creams, and pastes. A viscoelastic nanoemulsion gel (or nanogel) with higher stability and novel structure can

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00008-8

have various applications in food, pharmaceuticals and cosmetics (Mason et al., 2006).

In conventional highly concentrated emulsions when droplet volume fraction ($\phi$) reaches a disordered close-packing limit, known as maximal random jamming ($\phi_{MRJ}$), the droplet surface starts to deform due to the pressure from the neighboring droplets and cannot flow past one another without the application of external force (Mason et al., 1997b). For disordered packing of monodisperse hard spheres $\phi_{MRJ} = 0.64$ (Berryman, 1983), while for polydisperse emulsions droplet packing and deformation can happen at a larger $\phi$ ($\phi_{MRJ\text{-polydisperse}} \geq 0.7$) as small droplets can fit into the interstices of larger packed droplets (Groot and Stoyanov, 2011). The energy needed to compress and deform the disorderly packed droplets against their interfacial tension leads to the elasticity of the structure (Wilking and Mason, 2007). The stability of these elastic emulsion gels comes from the presence of emulsifier layer at the oil–water interface and a thin film of water between the compressed droplets (Masalova and Malkin, 2007). Since nanodroplets are more stable to coalescence, gels made from nanoemulsions are expected to be more stable than the conventional highly concentrated emulsions. Moreover, nanoemulsions with extremely small droplet sizes have stronger elasticity compared to the conventional highly concentrated emulsions as the elastic storage modulus of the gels are proportional to the Laplace pressure (ratio of interfacial tension to droplet radius) of nondeformed droplets, and therefore inversely proportional to their radius (Fryd and Mason, 2012). Nanogels made from optically clear nanoemulsions also appeared as tranperant making them unique in properties. For example, Kawada et al. (2010) showed a photograph of a self-standing optically clear nanoemeulsion gel made from only 25 vol.% oil phase and anionic emulsifier (Fig. 8.1). This is a signifacnt advantage of nanoemulsion gels (nanogels), that they are able to reach jammed state at a much lower disperse phase volume fraction compared to conventional emulsion gels. For example, Weiss and McClements (2000) found that a liquid 25% n-octadecane emulsion stabilized by 50 mM sodium dodecyl sulfate (SDS) transformed into a viscoelastic gel when the droplet radius was reduced below 80 nm by multiple passes through a high-pressure homogenizer.

Nanogels can also be formed by inducing attractive interactions among the nanodroplets. Bibette et al. (1993) showed that the addition of an appropriate quantity of salt can transform a repulsive emulsion with charged droplets into an attractive elastic gel where the droplets are strongly aggregated in secondary minima of interdroplet pair potential. Such attractive gels were developed by Wilking et al. (2006) by adding ~700 mM NaCl to

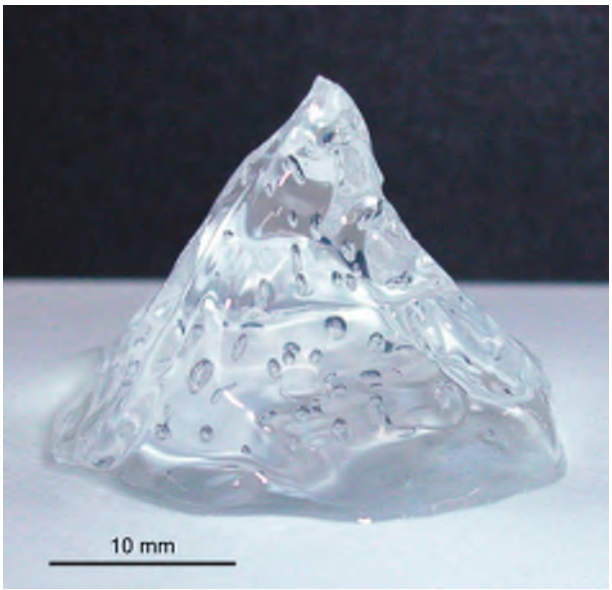


**Figure 8.1. Appearance of self-standing nanoemulsion gel made from 25 vol% oil + surfactant phase made by Kawada et al. (2010).** Reprinted with permission from Kawada et al., 2010. Langmuir, 26 (4), 2430–2437. Copyright 2010 American Chemical Society.

SDS-stabilized nanoemulsions with an average droplet radius ranging from 40 to 50 nm. Attractive interactions among the droplets can also be generated by depletion interactions where high concentrations of biopolymers or small nanoscale particles or emulsifier micelles in the continuous phase lead to an osmotic imbalance between the interdroplet region of two closely approaching droplets and the bulk continuous phase forcing them to aggregate (Berli et al., 2002; Mondain-Monval et al., 1996). The 3-dimensional network formed by the droplet aggregates immobilizes the continuous phase leading to the formation of emulsion gels (Datta et al., 2011; Berli et al., 2002).

As the gelation in nanoemulsions is obtained at much lower dispersed phase volume fraction compared to conventional emulsions, nanogels hold promise in wide applications. They also posses higher gel strength and are significantly more stable compared to their conventional counterpart. In this chapter after a brief introduction to nanoemulsions, rheology, and gelation in emulsions are reviewed, followed by a detailed discussion on how different factors influence nanocolloidal gelation, and current experimental techniques available to determine their nanostructure. The chapter ends by mentioning some of the potential applications of nanogels in food and related soft materials.

## 2 Nanoemulsions

Nanoemulsions are a new class of emulsions with droplet radius ranging from 10 to 100 nm (Tadros et al., 2004; Mason et al., 2007). However, this range of droplet size is arbitrary, and some authors consider the upper range to be 500 nm (Gutierrez et al., 2008). Nanoemulsions are kinetically very stable, sometimes over the years. Diluted nanoemulsions are less turbid or can even be transparent if their droplet size is much smaller than the visible light wavelengths (Qian and McClements, 2011). Nanoemulsions can be prepared by both high and low energy methods. In the former, the energy required for nanoemulsions is much higher compared to the conventional emulsions because of the creation of extremely small droplets with a very high interfacial area and the corresponding higher internal Laplace pressure of the droplets that must be overcome in order break up small droplets into nanoscale (Qian and McClements, 2011). Commonly used mechanical devices that generate intense disruptive forces to break the oil phase into nanodroplets are high-pressure homogenizers, microfluidizers, and ultrasonic transducers (Maali and Mosavian, 2013). In low-energy methods small oil droplets are spontaneously formed when the solution conditions (eg, dispersed phase concentration, emulsifier type and concentration) or environmental conditions (eg, temperature) are altered accordingly (Qian and McClements, 2011; Solans and Sole, 2012). However, often these low-energy approaches use a high amount of surfactant compared to high-energy methods, which is a drawback for its use in food emulsions.

### 2.1 Stability and Destabilization Mechanisms of Nanoemulsions

Nanoemulsions are kinetically more stable than conventional emulsions due to the small droplet size (Tadros et al., 2004). The destabilization mechanisms of nanoemulsions are similar to conventional emulsions and only differ by the magnitude. Extremely small droplet size in the nanoemulsions leads to more kinetic stability against particle aggregation and gravitational separation (Tadros et al., 2004; Rao and McClements, 2012). This can be explained using Stokes' law (Eq. 2.1.2), which states that the velocity of droplet movement is proportional to the square of droplet radius. Therefore, below a certain droplet size destabilization mechanism in nanoemulsions is mostly due to the Brownian motion whereas in case of normal emulsions it is gravitational separation (McClements and Rao, 2011). Brownian motion is the random

movement of particles suspended in a fluid (liquid or gas) (Kikoin and Maksimov, 1973). Flocculation and coalescence in nanoemulsions are less compared to conventional emulsions as the range of attractive interactions between the droplets decreases with the droplet size (Rao and McClements, 2012; Tadros et al., 2004). In nanoemulsions, the surfactant film thickness is significant relative to droplet radius, also making them more stable to coalescence (Fryd and Mason, 2012). Nevertheless, one of the most important destabilization mechanisms of nanoemulsion is Ostwald ripening. In Ostwald ripening mass transport of the molecules of dispersed phase droplets occurs from smaller droplets to larger droplets, increasing the size of larger droplets at the expense of smaller ones (Kabalnov, 2001; McClements, 2007). Ostwald ripening depends on the solubility and diffusion of the dispersed phase molecules in the aqueous phase (Weiss and McClements, 2000). As the nanoemulsion droplets are extremely small, their internal Laplace pressure and dispersed phase solubility are much higher compared to the conventional emulsions, making them unstable toward Ostwald ripening. It can, however, be inhibited by proper selection of dispersed phase that is more insoluble in the aqueous phase (eg, vegetable oil in water). Ripening inhibitors (eg, polyvinyl alcohol) can also be used which reduces the solubility of dispersed phase molecules in the continuous phase (Chang et al., 2012).

# 3 Rheology of Emulsions

## 3.1 Theory of Rheology

Rheology is the science that represents the study of deformation and flow of matter (ranging from liquids to soft solids) in response to mechanical stress (Barnes et al., 1989). Knowledge of rheological properties of the material is important in food industries as it helps food engineers in designing processing operations such as stirring, mixing, and pumping of food through heat exchangers, storage vessels, and packing into containers. Rheological properties such as creaminess, thickness, spreadability, and hardness also significantly influence sensory properties of foods (Fischer and Windhab, 2011; Rao, 2007).

Newton was the first to propose the theory of the flow of liquids and the conjecture of viscosity. According to him, viscosity, a measure of resistance to flow in liquid, is proportional to the velocity of a liquid (Barnes et al., 1989; Rao, 2005; McClements, 2005). Therefore, in an ideal liquid, the applied stress ($\tau$) is proportional to the change in displacement of the layers of the fluid per unit time, also

referred to as rate of strain ($\dot{\gamma}$) Eq. (8.1), where the proportionality constant $\eta$ is called the coefficient of viscosity.

$$\tau = \eta\dot{\gamma} \tag{8.1}$$

Water and pure vegetable oil are some of the liquids that obey the viscosity behavior according to Eq. (8.1) and are called Newtonian fluids. However, the majority of the liquids does not follow the Newtonian's law as their viscosity change with the rate of strain. These fluids are generally referred to as non-Newtonian fluids whose viscosity either decrease (pseudoplastic or shear-thinning) or increase (dilatant or shear thickening) with the rate of shear. For example, the viscosity of chocolate milk diminishes as shear rate increases (pseudoplastic behavior) and the viscosity of corn starch dispersion heated to a temperature of 70°C increases as shear rate increases (dilatant liquids) (McClements, 2005; Mewis, 2012). The viscosity of some materials also changes as a function of time at constant shear/strain rate. Such type of behavior is called thixotropy when viscosity decreases with time or rheopectic when viscosity increases with time (Meyers, 2009).

Many soft materials such as cream, toothpaste, yogurt, cheese, salad dressings, and pudding are not perfectly viscous, but possess some elastic solid-like properties and their flow behavior cannot be solely explained by Newtonian's law. It was proposed that these materials partially behave like elastic solids with some elements of flow properties associated with liquids and Hooke's law alone can not describe the response of such material (Barnes et al., 1989). Maxwell proposed a mathematical model to explain elastic properties of non-Newtonian fluids (Maxwell, 1866; Barnes et al., 1989). Such material that exhibits both elastic and viscous characteristics under stress are known as a viscoelastic material. In a viscous material, energy is lost upon application of stress. On the other hand, in pure elastic material, energy is not dissipated and stored when a load is applied. However, in a viscoelastic material, the viscous component results in partial loss of energy upon removal of applied load. This results in a hysteresis loop observed in a stress–strain curve after loading and during unloading passes, and the area of the loop is equal to the energy lost (Meyers, 2009). Unlike elastic solid material where deformation under stress is caused by atomic displacements on specific crystallographic planes, in viscoelastic material, a constant load results in the continuous displacement of atoms or molecules with time.

Characterization of material viscoelastic behavior is usually done by sinusoidally deforming the material and recording the resultant stress (Courtney, 1990). Let us consider a material is being subjected to an oscillatory strain with frequency $\omega$. In the

viscoelastic material, there is a phase lag $\delta$, between the applied stress ($\tau$) and the resulting strain. In the case of ideal elastic material, the phase shift $\delta = 0$, while for an ideal viscous material $\delta = 90$ degree (Meyers, 2009; McClements, 2005). The subsequent expression for stress and strain in sinusoidal deformation can be written as:

$$\tau = \tau_0 \sin \omega t \tag{8.2}$$

$$\dot{\gamma} = \gamma_0 \sin(\omega t + \delta) \tag{8.3}$$

where $\tau_0$ and $\gamma_0$ are yield stress and strain amplitudes, respectively. From these expressions, we can define two moduli that characterize the elastic and viscous behaviors of viscoelastic materials:

$$G' = \left(\frac{\gamma_0}{\tau_0}\right)\cos\delta \tag{8.4}$$

$$G'' = \left(\frac{\gamma_0}{\tau_0}\right)\sin\delta \tag{8.5}$$

where $G'$ is the storage modulus, which is the measure of stored energy or the elastic part and $G''$ is the loss modulus, which is the measure of lost energy in the form of heat or viscous component. Alternatively there is a complex modulus $G^*$, which represents both the $G'$ and $G''$ by the following equation in which $i$ is the complex number and is equal to $\sqrt{-1}$.

$$G^* = G' + iG'' \tag{8.6}$$

## 3.2 Rheology Measurement Techniques

Rheological behaviors of a wide range of materials, including dilute and very viscous liquids, solids, plastic, and viscoelastic material, can be characterized by a variety of instrumental methods. The different instruments vary depending on the type of measurement techniques they use, which include shear, compression, elongation, or any combination thereof. These methods provide information about the fundamental rheological constants of the material, such as $\eta$, $G'$, $G''$ (Steffe, 1992; Rao, 2005).

In simple capillary viscometer applied shear rate cannot be controlled and are suitable only for ideal liquids. However, many types of advanced mechanical viscometers and dynamic rheometers are available that can characterize fluids over a wide range of shear rates and are used to analyze both ideal and nonideal liquids (Morrison, 2001). In dynamic rheometers, shear is applied to

material held between different measurement cells (eg, concentric cylinders, cone and plate, parallel plates). In rotational rheometer deformation of the sample held between the two plates is obtained by rotating one plate relative to the other. This technique is used to determine transient and steady shear flow properties of materials, for example, typically shear viscosity, creep or stress relaxation. The viscoelastic properties of the material can be determined using oscillatory rheometry. The principle of the technique is that the material to be tested is held between the two plates of the rheometer and is subjected to a sinusoidal torque by oscillating one plate relative to the other (Findley, 1989). The response of the sample to sinusoidal displacement is used to record viscoelastic moduli ($G'$, $G''$, $G^*$) and phase angle ($\delta$).

## 3.3 Emulsion Rheology

Food emulsions exhibit a broad range of different rheological properties, ranging from low viscosity liquids (eg, milk, pourable salad dressings) to viscoelastic solids (shortening, margarine, butter, and other solid-like soft materials) (McClements, 2005; Rao, 2007). Rheological properties of emulsions provide information that helps to understand structural organization and interactions within emulsions. For example, viscosity versus shear rate data can provide an insight into the type and strength of interactions between the dispersed droplets in emulsions (Berli et al., 2002; Tadros, 1994). Shelf life of food emulsions also depends on its rheology (McClements, 2005). For example, continuous phase viscosity can influence the creaming or sedimentation of dispersed droplets leading to destabilization.

Emulsion rheology depends on many factors such as packing or volume fraction of the dispersed phase droplets, properties of the continuous phase, and the type, size and strength of interactions between the dispersed droplets (McClements, 2005; Mason et al., 1997b; Mewis, 2012; Datta et al., 2011). Depending on the dispersed phase volume fraction ($\phi$), rheological behavior of emulsions transform from liquids to viscoelastic solids (Fig. 8.2).

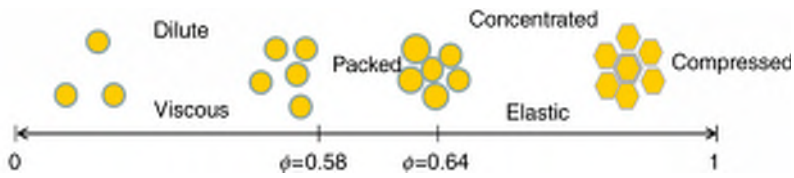


**Figure 8.2. Rheological behavior of emulsions containing various amount of compressible oil droplets ($\phi$).**

In dilute emulsions ($\phi < 0.05$) the droplets do not interact with each other as they are sufficiently far apart. Such emulsions exhibit relatively low viscosity, which is dominated by the influence of the continuous phase. As the system becomes more concentrated, ($0.05 < \phi < 0.49$), interactions between the droplets through collisions, hydrodynamic interactions become appreciably higher, hindering their movement within the continuous phase resulting in an increase in emulsion viscosity (Ochowiak et al., 2012; Sun and Gunasekaran, 2009). In further concentrated systems ($0.49 < \phi < 0.58$) viscoelastic behavior begins as a result of closer packing of droplets. For $0.58 < \phi < 0.64$, the movement of droplets become severely restricted as each droplet is caged between the neighbors and separated by a thin layer of continuous phase between them (Mason et al., 1995). These systems are known as colloidal glasses where the droplets can only oscillate but cannot move past each other smoothly. When $\phi$ is close to 0.64, a dramatic increase in viscosity and viscoelastic behavior of emulsions has been observed (Mason and Scheffold, 2014; Scheffold et al., 2013; McClements, 2005; Weiss and McClements, 2000). For monodispersed emulsions at $\phi = 0.64$ droplets surface touch with each other and when $\phi$ is higher than 0.64 droplets become compressed and deformed. This deformation leads to an increase in interfacial area, which results in energy stored in the droplets that manifest as elasticity ($G' > G''$) (Mason et al., 1995; Masalova and Malkin, 2007). This point, referred as random close packing (RCP), was later termed as maximal random jamming (MRJ) (Wilking and Mason, 2007; Truskett et al., 2000). At very high $\phi$, the droplets' surface become so deformed that they exist as hexagonal packing like foam (Fig. 8.2) (Meleson et al., 2004).

Droplet size is another major factor that influences emulsion rheological properties (McClements, 2005; Pal, 1996). As mentioned earlier in the case of emulsions with $\phi > 0.64$, elasticity results from the energy stored by deformation of droplets. The deformation is dependent on the Laplace pressure of droplets and hence elasticity is inversely related to the droplet size of emulsions. In electrostatically stabilized emulsions, a decrease in droplet size also results in an increase of $\phi_{eff}$ (see the section on nanocolloidal gelation), further enhancing the $G'$ of emulsions (Fryd and Mason, 2012).

In addition to aforementioned factors, properties of the continuous phase also have an influence on the emulsion rheology. Particularly in many food emulsions (eg, salad dressing, coffee creamer) where the droplet concentrations are well below the $\phi_{MRJ,}$ the continuous phase characteristics most effectively influence emulsion rheology (McClements, 2005). This is one of the reasons

why many additives such as thickening agents, gelling agents, polysaccharides, and proteins are often added to the aqueous phase of emulsions to modify their rheological properties (Pettitt et al., 1995; Pal et al., 1992). Apart from the aforementioned factors, crystalline properties and the structure of the dispersed and continuous phase also affect emulsion rheology (eg, ice cream, whipped cream, butter, and margarine) (Doublier, 1992; Fischer and Windhab, 2011).

## 4 Gelation in Emulsions

Gelation is the process of formation of gel from sol. According to the International Union of Pure and Applied Chemistry (IUPAC), a gel is defined as a "nonfluid colloidal or polymer network that is expanded throughout its whole volume by a fluid." A gel can be recognized as an intermediate state between liquid and solid, that is, they are liquid rich systems, yet possess solid-like property with no flow under gravity. One of the most important characteristic properties of the gel is its mechanical or rheological property. As gels are intermediate states between viscous liquid and elastic solid, their rheological characteristics vary between Newtonian viscosity and Hookean elastic properties (Katsuyoshi, 2009). In other words, they display viscoelastic behavior.

Structurally emulsion gels can be of two types: (1) dispersed particles filled biopolymer gels, and (2) particulate gels (Dickinson, 2013; Sala, 2007) (Fig. 8.3). In the former droplets are randomly dispersed in a biopolymer matrix that imparts gelation (Batchelor and Green, 1972; Dickinson, 2013). Here gelation is caused by the network of biopolymer or hydrocolloid molecules

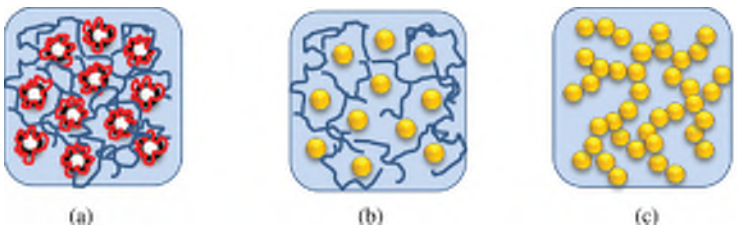


**Figure 8.3.** Schematic diagrams of (a) Active particle filled gel in which the network structure is caused by crosslinking the biopolymers/hydrocolloids in the continuous phase, and biopolymer/hydrocolloid stabilized droplets served as active fillers. (b) Inactive particle filled gel in which the network structure is caused by crosslinking the biopolymers/hydrocolloids in the continuous phase, and droplets served as inactive fillers. (c) Particulate gels in which network structure is caused by aggregation of attractive droplets in continuous phase.

in the continuous phase and the presence of droplets in the gelled matrix either strengthen (active filler) (Fig. 8.3a) or weaken (inactive filler) (Fig. 8.3b) the gel depending on their interfacial properties (Dickinson and Chen, 1999). Figs. 8.3a, b show the representative schematic diagrams of active and inactive emulsions filled gels. For example, it was observed that WPI stabilized oil droplets served as active fillers in gelatin gels as the WPI and gelatin interaction increased the gel strength of gels (Sala et al., 2007). Inactive filler gels can be observed in case of small-molecule emulsifier stabilized emulsions filled with a network of biopolymers in which the droplets may or may not react with the network. Sala et al. (2007) have found that Tween-20 stabilized dispersed oil droplets served as inactive fillers in gelatin gels and weakened the gel strength.

In particulate gels, dispersed particles/droplets form a 3-dimensional network which leads to emulsion gelation (Fig. 8.3c). The network is usually formed by secondary intermolecular forces (eg, hydrogen bonds, electrostatic, and hydrophobic interactions) driven aggregation of dispersed droplets. However, in case of some emulsions stabilized by proteins, covalent bonds also drive the droplets aggregation (Dickinson, 2013). For example, interaction among the interfacial biopolymers induced by enzymes and changes in the environmental conditions (temperature, pH, ionic strength, etc.) might also result in the formation of aggregated oil droplets, leading to gelation (Chen et al., 2001). In many practical applications, depending on the concentration and types of biopolymers, the structure of many emulsion gels lies between the particle filled gel and particulate gel (Dickinson, 2012). Particulate gels can also be classified into two types based on interdroplet interactions that lead to their gelation (Fryd and Mason, 2012). If attractive forces between the droplets lead to network they are called attractive gels. On the other hand, if the interaction between the droplets is repulsive, they are called repulsive gels. The Strength of both these gels depends on the nature and extent of interdroplet interactions and the dispersed phase volume fraction (Scheffold et al., 2014; Datta et al., 2011).

## 4.1 Gelation by Attractive Interactions

In the case of attractive emulsion gelation, aggregation is caused by the attraction between the emulsion droplets. If attractive interactions (van der Waal interactions, depletion forces, etc.) can be induced among the repulsive droplets (stabilized by ionic emulsifiers or thick layers of proteins), secondary attractive minima in their interdroplet pair potential can be developed which

would lead to droplet aggregation resulting in gelation (Fryd and Mason, 2012; Bibette et al., 1993). Attractive interactions induced gelation in nanoemulsions occurs by similar mechanisms as in the case of conventional emulsions. Attractive gelation in nanoemulsions has been observed by many researchers (Jorjadze et al., 2011; Datta et al., 2011). Interestingly, at the same dispersed volume fraction nanoemulsion gels have shown significantly higher gel strength compared to conventional emulsion gels (see in later sections for more details).

The kinetics of aggregation that leads to attractive gelation can be understood from the viewpoint of the underlying rate limiting processes. The aggregating particles form a complex pattern called fractals, which are self-similar at different length scales, that is, they appear the same when viewed over a range of scales (Sorensen, 2001). The advantage of using fractal dimension is that a single parameter can be used to describe an extremely complex structure (Bremer et al., 1993). The aggregation and fractal growth happen in 2 time scales: (1) diffusion time ($\tau_D$), which is the time needed for the particles or fractal clusters to come close together and aggregate through the process of diffusion through the continuous phase; (2) reaction time ($\tau_R$), which is the time needed for approaching particles to form bonds (Weitz et al., 1985; Jullien and Kolb, 1984). In this case, the bond formation can be instantaneous upon first contact or can be after many successive attempts (Matsushita, 1994; Weitz et al., 1985). If $\tau_R << \tau_D$, the aggregation process is called diffusion-limited aggregation (DLA) and if $\tau_D << \tau_R$, it is called reaction limited aggregation (RLA). In classic diffusion-limited aggregation (DLA), strong attraction among particles forces them to irreversibly stick to a growing cluster upon contact (Seager and Mason, 2007). In some cases, the aggregating clusters can themselves diffuse via diffusion-limited cluster aggregation (DLCA) to form a network of tenuous structures that kinetically traps large amounts of the continuous phase, thereby leading to gelation at a much lower $\phi$ (Wilking et al., 2006). In contrast, droplets in reaction-limited cluster aggregation (RLCA) exhibit weak attraction compared to the thermal energy of the system; hence they do not always aggregate upon contact and may pack more densely instead. Therefore, gelation requires a higher $\phi$ than DLCA (Wilking et al., 2006). Both DLCA and RLCA are purely kinetic phenomena commonly observed in colloidal hard sphere dispersions. However, deformable oil droplets of emulsions and nanoemulsions form shear-resistant aggregates through slippery DLCA (SDLCA), where rotational and translational diffusion of droplets over the surface of other droplets is possible due to the presence of a thin film of water between the deformed aggregated droplets

(Wilking et al., 2011; Seager and Mason, 2007). The fractal dimension for an open gel network formed by either DLCA or SDLCA is 1.7–1.9 (Seager and Mason, 2007), while for a more close-packed structure formed by RLCA it is 2.4–2.5 (Lu and Weitz, 2013b). Although fractal dimension is a useful tool to estimate the type of network, it cannot provide detailed structural information specifying the size of the nanodroplets and the characteristic length of the cluster that forms the basic unit of the entire 3D network (Wilking et al., 2006; Fryd and Mason, 2012).

The attractive interactions required for the above aggregation phenomena and cluster growth can be induced by two methods: depletion interaction or by charge screening due to the addition of ionic salt (Dickinson, 2013; Broide and Cohen, 1992; Tuinier et al., 2000).

### *4.1.1 Gelation by Depletion Attraction*

Depletion attraction between colloidal particles or emulsion droplets is observed when they are surrounded by smaller particles, emulsifier micelles or nonadsorbing polymer molecules (depletants) (Bibette et al., 1992; Tuinier et al., 2000). Due to the osmotic pressure difference between the surrounding continuous phase and the depleted interdroplet region, a net force is exerted on the droplets to aggregate, which is manifested as the attractive interaction, leading to droplet aggregation (Lekkerkerker and Tuinier, 2011). The origin of depletion interaction is entropic in nature. Depletants expelled from the interdroplet region have greater translational freedom due to increased available volume. This leads to a gain in translational entropy of depletants decreasing the free energy of the system, which causes a spontaneous colloidal flocculation (Butt et al., 2005; Sapir and Harries, 2015).

### *4.1.2 Gelation by Salt-Induced Attraction*

Gelation by attractive interactions can also be induced by the addition of ionic salt in the emulsions. In electrostatically stabilized emulsions the addition of an appropriate amount of ionic salt screens the charge on the droplets, thereby reducing the repulsive barrier between the droplets, leading to droplet aggregation in secondary minima of interdroplet pair potential (McClements, 2005; Datta et al., 2011; Fryd and Mason, 2012). However, salt added in excessive concentration can result in coalescence and the complete destabilization of the emulsions (McClements, 2005). Salt-induced attraction is also temperature-dependent. Raising the temperature above a critical value would increase the thermal energy of the system such that it become greater than the secondary

well depth leading to particles redispersion and gel breakdown (Derjaguin and Landau, 1993; Fryd and Mason, 2012). The addition of salt has also shown to induce gelation in protein-stabilized emulsions. Marangoni et al. (2000) and Maltais et al. (2005) have shown that the addition of $CaCl_2$ neutralizes the electrostatic repulsion between whey or soy protein particles and form salt bridges between them leading to gelation.

### 4.2 Gelation by Repulsive Interactions

Repulsive gelation in emulsions happens when volume fraction of ionic emulsifier-stabilized droplets with strong short-range repulsion increased to an extent that they become packed (for monodispersed systems at $\phi$ = 0.64), randomly jammed and the emulsion attains yield stress (Liu and Nagel, 2010). This transition from freely flowing to jammed state is termed as jamming transition (Hecke, 2010). The jamming transition has been a topic of interest since dense disordered sphere packaging was first proposed by Bernal and Mason (1960). The simulation studies on colloidal suspensions have shown that although jamming transition for monodisperse colloidal suspensions occur at $\phi$= 0.64 (maximal random jamming or $\phi_{MRJ}$), for polydisperse suspensions the transition need $\phi > 0.7$, depending on their polydispersity (Clusel et al., 2009; Groot and Stoyanov, 2011; Schaertl and Sillescu, 1994). This shows that the gelation and jamming transitions are often hard to define. The repulsive emulsion gels display elastic properties ($G' > G''$), thereby satisfying the rheological definition of gels without forming fractal clusters similar to their attractive counterparts.

The repulsive nanoemulsions display unique characteristics of jamming transition and gelation as compared to conventional emulsions due to significant effect of charge cloud on the overall nanodroplet size (Wilking and Mason, 2007). Because of this, repulsive gelation in nanoemulsions is observed at a much lower droplet volume fraction indicating significant advantage over conventional emulsions (more details in the next section).

## 5 Nanocolloidal Gelation

### 5.1 Repulsive and Attractive Gelation in Nanoemulsions

Repulsive gelation in nanoemulsions displays unique characteristics of jamming transition at a much lower droplet volume fraction compared to conventional emulsions. In conventional emulsions thickness of the electrical double layer formed by the

ionic emulsifier around the droplets has negligible influence on the total effective size and volume fraction of droplets. However, when the droplet size is decreased sufficiently (eg, less than100 nm radius), the thickness of the double layer becomes a similar order of magnitude of the nanodroplets, and the overall droplet size and the resulting effective dispersed phase volume fraction ($\phi_{eff}$) becomes significantly larger than the actual ($\phi_{core}$) (Qian and McClements, 2011). The resultant $\phi_{eff}$ and its dependence on the droplet size can be given by the following equation (Weiss and McClements, 2000; Wilking and Mason, 2007):

$$\phi_{eff} = \phi_{core}\left(1+\frac{\delta}{r}\right)^3 \tag{8.7}$$

where $\delta$ is the thickness of the electrical double layer and $r$ is the radius of the droplets. As the droplets size ($r$) decreases, the thickness of the interfacial layer ($\delta$) surrounding these droplets significantly contribute to the total volume fraction of the droplets ($\phi_{eff}$). Eq. (8.7) is illustrated in Fig. 8.4 where increase in $\phi_{eff}$ is plotted with reduction in droplet size at three different thicknesses of

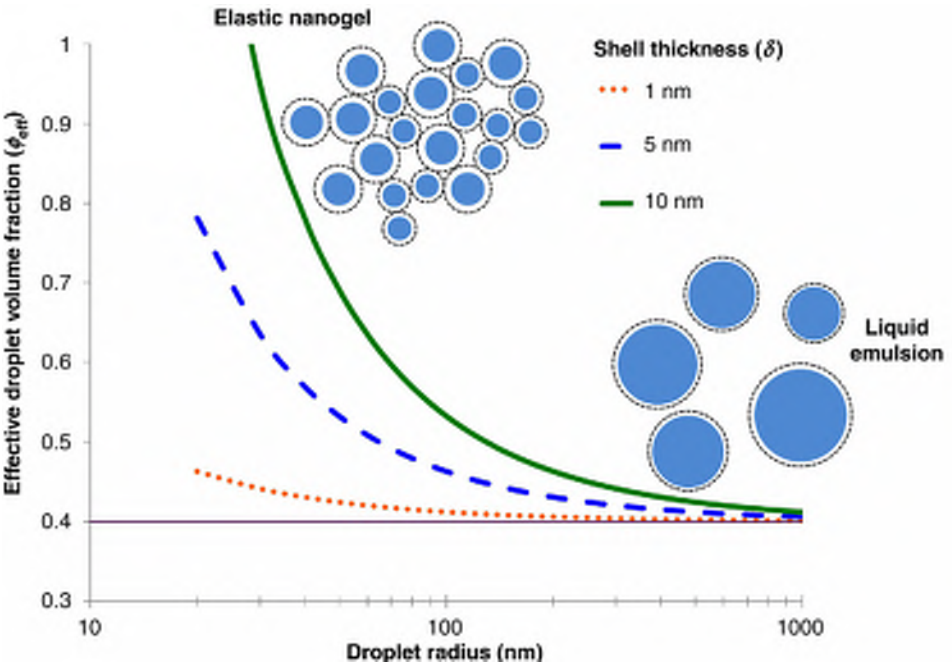


**Figure 8.4. Schematic representation of increase in $\phi_{eff}$ with the decrease in droplet size at different thicknesses of droplet interfacial shell layer according to Eq. 8.7.** Proposed microstructure of elastic nanogel and conventional liquid emulsion is also shown along with the $\phi_{eff}$ curve.

interfacial shell layer. When plotted for $\phi = 0.4$, the interfacial shell layer thickness ($\delta$) for conventional emulsions ($r \geq 250$ nm) has a negligible influence on $\phi_{eff}$. However, when $r$ is less than 100 nm, $\phi_{eff}$ rapidly increases and may even reach close packing ($\phi_{eff} > 0.7$), where random-jamming of nanodroplets transform liquid nanoemulsions into particulate gels (the so-called nanogel) at a much lower actual oil-phase volume fraction ($\phi = 0.4$). Experimentally, Wilking and Mason showed that an anionic emulsifier-stabilized 20% o/w nanoemulsion can be transformed into an elastic gel when the droplet size falls below 62 nm ($\delta \sim 3$ nm) (Wilking and Mason, 2007). In these nanoemulsions gels, the shell layer thickness is significantly influenced by the charge cloud (electric double layer) around the droplets. These nanoemulsion gels are referred to as repulsive nanogels as they have very strong short-range repulsive electrostatic interactions due to the charge on the droplets.

Attractive gelation in nanoemulsions has also been observed by many researchers (Jorjadze et al., 2011; Datta et al., 2011). Datta et al. showed that a silicon oil-in-formamide nanoemulsion sterically stabilized by Pluronic P105, a nonionic amphiphilic copolymer, transformed repulsive interaction at lower emulsifier concentration below critical micelle concentration (CMC) into an attractive one at higher concentration above CMC due to micelle induced depletion attraction. It was found that attractive interactions dramatically increased emulsion rheology and form soft gel even if the effective droplet fraction is below $\phi_{MRJ}$. The higher gel strength of attractive nanoemulsion gel compared to repulsive system has been attributed to the formation of droplet aggregates leading to a large-scale fractal cluster network encompassing the whole emulsion (Lu and Weitz, 2013a). Similar behavior in nanoemulsion gelation as a function of ionic emulsifier concentration has also been observed by Erramreddy and Ghosh (2014) where repulsively jammed nanogel transformed into attractive nanogel with higher gel strength by increasing emulsifier micelle concentration. In the following sections we have discussed how emulsifier type, concentration, nanodroplet size, and volume fraction influenced gelation in nanoemulsions.

## 5.2 Factors Affecting Nanocolloidal Gelation

### *5.2.1 Effect of Emulsifier Type and Micelle Concentration on Nanoemulsion Gelation*

Erramreddy and Ghosh (2014) prepared 40 wt.% canola o/w nanoemulsions with varying concentration of anionic SDS and nonionic Tween-20 using multiple passes through a high-pressure

homogenizer. The surface average droplet diameter of SDS nanoemulsions were ranged from 312 to 133 nm while for Tween-20 it was from 1212 to 120 nm with increasing emulsifier concentration. It was found that the nanoemulsion prepared with 0.5 CMC SDS immediately flowed under gravity while the flow of nanoemulsions slowed down beginning 1 CMC SDS. Consequently, nanoemulsions with 5, 10, and 15 CMC SDS did not flow at all during the span of observation (Fig. 8.5 inset). On the other hand, Tween-20 nanoemulsions flowed at all emulsifier concentrations (data not shown). Viscoelastic behavior of these nanoemulsions were determined using a AR G2 rheometer (TA Instrument, Montréal, QC, Canada) at room temperature by strain sweep measurement at 0.01–100 % strain and at a constant frequency of 1 Hz (6.28 rad/s) using a 40 mm diameter cross-hatched parallel plate geometry to avoid any wall slip effect (Fig. 8.5). For all nanoemulsions moduli were independent of strain below 2%, showing the existence of linear viscoelastic region (LVR). For these nanoemulsions, $G'$

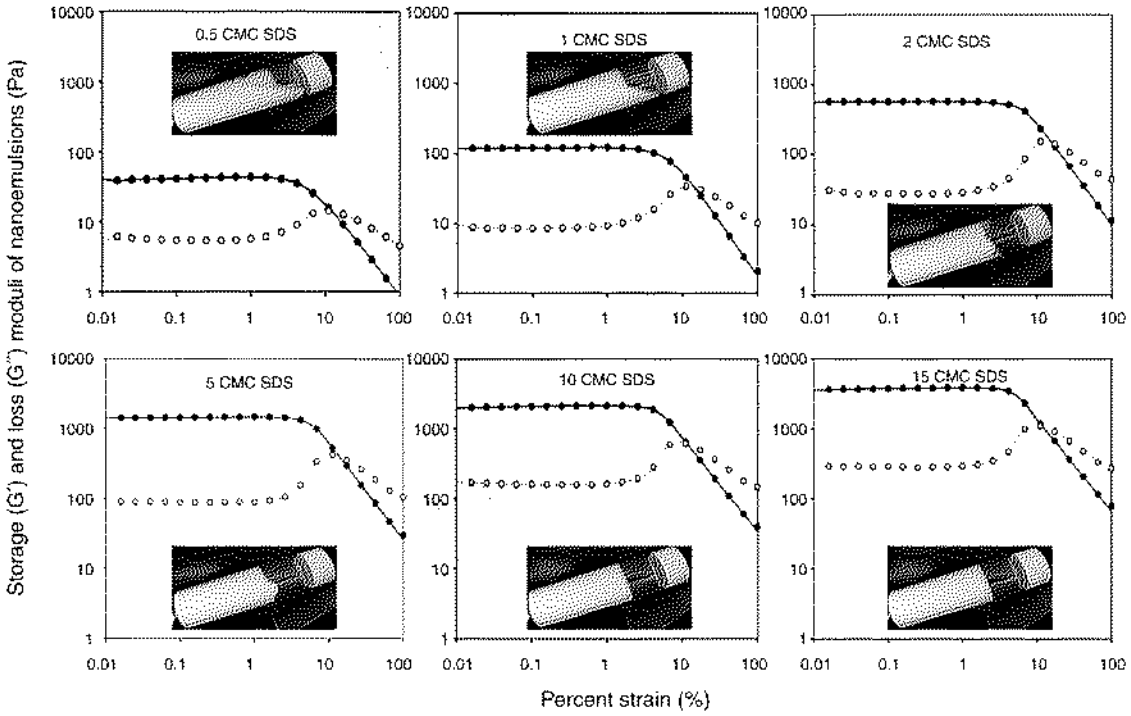


**Figure 8.5. Average strain dependent storage (G′) (●) and loss moduli (G″) (○) of different SDS nanoemulsions.** SDS concentration is expressed as multiples of its critical micelle concentration (CMC). Inset shows visual observation of nanoemulsions flow behavior under gravity. Adapted with permission from Erramreddy and Ghosh, 2014. Langmuir 30, 11062–11074. Copyright 2014 American Chemical Society.

was significantly greater than $G''$ within the LVR reflecting their dominant elastic nature. However, for 0.5 CMC and 1 CMC SDS nanoemulsions, despite the higher $G'$ than $G''$ and an LVR, they yielded under gravity in the visual observation experiment manifesting weak gelation. At a larger strain (beginning 5%), known as yield strain, the $G'$ values of these nanoemulsions showed a deviation from linearity and dropped gradually. At this time, the $G''$ values began to rise and formed a peak where it also crossed $G'$. The distinct peak in $G''$ was also observed by many researches during the viscoelastic studies of soft colloidal materials, and is considered to be due to the structural relaxation process leading to gel network breakdown and flow of materials (Datta et al., 2011; Koumakis and Petekidis, 2011). At higher strains compared to the peak value, $G''$ dominates over $G'$, indicating the liquid-like behavior of the nanoemulsions. In order to compare the elasticity of different nanoemulsions, the plateau storage and loss moduli at 0.1% strain is plotted as a function of emulsifier concentration in Fig. 8.6. It can be seen that $G'$ and $G''$ rapidly increased to 2 times SDS CMC followed by steady increased until 15 times CMC. The viscoelastic moduli for Tween-20 nanoemulsions at 0.1% strain is also plotted in Fig. 8.6. The $G'$ and $G''$ for Tween-20 nanoemulsions are significantly lower than that of SDS nanoemulsions. Also, within Tween-20 nanoemulsions the $G'$ is only marginally higher

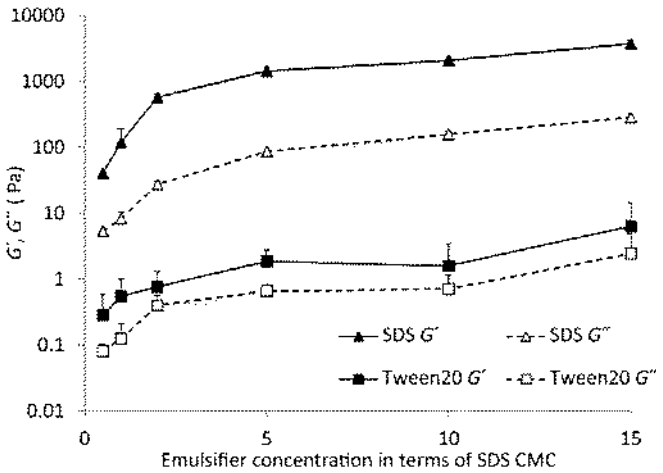


**Figure 8.6. Characterization of the viscoelastic behavior of SDS (triangle) and Tween-20 (square) nanoemulsions.** Values of storage ($G'$) (closed symbols) and loss ($G''$) (open symbols) moduli of nanoemulsions (at a frequency of 6.28 rad/s and 0.1% strain) were plotted from strain sweep measurements data in Fig. 8.5 against emulsifier concentration expressed as multiples of SDS CMC. Lines are used to guide the eye.

than $G''$, mimicking the characteristics of weak gel that yielded under gravity. It was proposed that the smaller droplet size of nanoemulsions along with increased influence of electrical double layer formed by the layer of charged SDS at the droplet surface increased the $\phi_{eff}$ beyond random jamming leading to repulsive gelation. Using the theory of Debye screening length ($\kappa^{-1}$) of ionic emulsifier and considering free sodium ion to act as counterions (with a dissociation factor of 0.1) the authros calculated the interfacial shell layer thickness ($\delta$) as 2.4 times of $\kappa^{-1}$ and the $\phi_{eff}$. It was found that the $\phi_{eff}$ increased to 0.71–0.75 for 0.5–2 times SDS CMC while for 5–15 times CMC it decreased to $\sim$ 0.5 due to presence of excess free counterion screening the $\kappa^{-1}$. For nonionic Tween-20 lack of charge on the droplets resulted in the absence of sufficient interfacial shell layer thickness leading to $\phi_{eff} \approx \phi_{core}$ and hence no repulsive gelation was observed. In contrast, $\phi_{eff}$ of 0.5, 1, and 2 CMC SDS nanoemulsions are well above the maximal random jamming limit ($\phi_{MRJ}$ = 0.64) for monodisperse emulsions leading to repulsize gelation.

Considerably higher gel strength was observed for 5, 10, and 15 CMC SDS nanoemulsions compared to 0.5, 1, and 2 CMC nanoemulsions in spite of their lower estimated values of $\phi_{eff}$, which was possible due to the appearance of attractive depletion interactions among the nanodroplets by excess SDS micelles in the continuous phase. Although the actual SDS micelle volume fraction was quite low at this concentration, the presence of a charge cloud around them increased the effective micelle volume fraction to a great extent. The interfacial electrical double layer around the droplets also contributed towards increased excluded volume in the interdroplet region, which further increased depletion interaction. The long-range attractive interaction (despite short-range repulsion due to interfacial SDS) formed a tenuous network of aggregated droplets, which prevented the flow of water and increased the gel strength of the nanoemulsions. In contrast, Tween-20 micelles and the droplets stabilized with it did not have enough charge leading to a lower excluded volume and depletion attraction among the droplets (Mondain-Monval et al., 1996).

### *5.2.2 Effect of Nanodroplet Size and Volume Fraction on Nanoemulsion Gelation*

In order to understand how nanodroplet size influences both repulsive and attractive gelation, we have selected the strongest nanogels in both regimes (made with 2 and 15 times SDS CMC, respectively) and their droplet size was varied by collecting samples during multiple passes of high-pressure homogenization (Erramreddy and Ghosh, 2015). The droplet size and viscoelastic

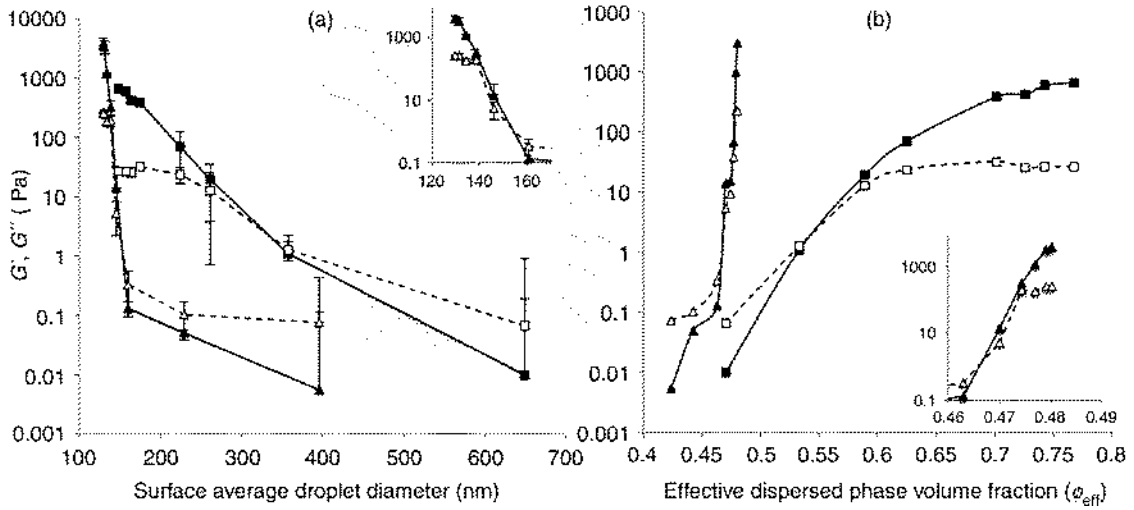


**Figure 8.7.** Viscoelastic behavior nanoemulsions stabilized with 2 times SDS CMC (square symbols) and 15 time SDS CMC (triangle symbols) as a function of (a) surface average ($d_{32}$) droplet diameter and (b) effective dispersed phase volume fraction. The average storage ($G'$) (closed symbols) and loss ($G''$) (open symbols) moduli were obtained using strain sweep experiments at 0.1% strain and 6.28 rad/s frequency. The inset curves show zoomed view for 15 CMC nanoemulsions.

behavior of each of this nanoemulsions were determined and the plateau storage and loss moduli were plotted in Fig. 8.7a. In the case of 2 CMC nanoemulsions, $G'' > G'$ above 300 nm droplet size. But with a decrease in droplet size the elastic nature superseded the viscous nature, and below 300 nm droplet diameter $G'$ became higher than $G''$. Similarly for 15 CMC nanoemulsions, $G''$ was initially higher than $G'$ at larger droplet sizes, however, below 150 nm (after around fourth pass) $G'$ dominated over $G''$ (inset of Fig. 8.7a). It can also be seen that elasticity of 2 CMC nanoemulsion gradually increased with reduction in droplet size, however, for 15 CMC nanoemulsions, elastic behavior rapidly increased once the droplet size was below a critical level of 160 nm. In order to understand why the increase in gel strength took two very different approaches, we have calculated the effective droplet volume fractions for both nanoemulsions and the strength of the attractive interaction for 15 CMC nanoemulsions (Erramreddy and Ghosh, 2015). In order to understand how $\phi_{eff}$ influences $G'$ and $G''$, viscoelastic moduli of the nanoemulsions from all replicates were replotted against $\phi_{eff}$ in Fig. 8.7b. For 2 CMC nanoemulsions $\phi_{eff}$ increased from 0.46 to 0.78, whereas for 15 CMC nanoemulsions the maximum was 0.47. The lower $\phi_{eff}$ for 15 CMC nanoemulsions is due to the higher number of counterions influencing the Debye screening length ($\delta$). In the case of 2 CMC SDS, at higher droplet size (>250 nm),

the emulsions existed as fluids where $G' < G''$. As the droplet size decreased further below 200 nm, $\phi_{eff}$ of these emulsions got closer to the glass transition limit ($\phi_{eff}$ is close to 0.6) at which $G' > G''$ and the liquid like nanoemulsions are transformed into weak gels. With further decrease in droplet size below 180 nm ($\phi_{eff}$ is close to 0.7), the glassy nanoemulsion entered jamming transition where the droplets are jammed into cage formed by surrounding droplets. At this point, the $G' >> G''$ and the liquid like emulsions are transformed into strong nanogels. Therefore, it can be said that the gelation in 2 CMC nanoemulsion is controlled by $\phi_{eff}$ that resulted in the fluid to glassy to jamming transition.

In contrary, for attractive nanoemulsions decrease in droplet size manifested a different mechanism of gelation. Erramreddy and Ghosh (2015) showed that the strength of the attractive interaction ($W_d/k_BT$) decreased with a drop in droplet size, as more emulsifier molecules got adsorbed on the newly created interfacial area leaving less micelles in the continuous phase for depletion attraction. It was proposed that despite higher depletion interaction energy, the fluid-like behavior of large droplet size nanoemulsions was due to structural forces generated by the presence of higher micelle concentrations (depletion stabilization) (Basheva et al., 2007). With a decrease in droplet size and a corresponding drop in micelle concentration, the interdroplet interaction transformed from structural force into depletion attraction (Petsev et al., 1995) leading to gelation by the fractal network in nanodroplets. It was proposed that the rapid increase in $G'$ cannot alone be explained by depletion interaction, and the decrease in droplet size and corresponding increase in their number might have an integral impact on the fractal nature of the aggregates thus influencing gelation behavior of the attractive nanoemulsions.

Recently we have also tested at what minimum droplet volume fraction nanoemulsions would transformed into nanogels. For this we have prepared nanoemulsions with oil volume fraction ($\phi$) starting from 0.3 to 0.6. The emulsifier to oil ratio was kept constant throughout based on 2 times SDS CMC for $\phi = 0.4$. The effective oil phase volume fraction (actual plus the thickness of the electrical double layer), given by $\phi_{eff}$, calculated using the theory described earlier and plotted as a function of surface-average droplet diameter of nanoemulsion samples collected after each pass during high-pressure homogenization (Fig. 8.8). It can be seen that the decrease in droplet size with degree of homogenization at a constant $\phi$ propelled an increase in $\phi_{eff}$ for all emulsions. In fact, $\phi_{eff}$ increased to as much as 0.8 even at $\phi$ as low as 0.3 due to a reduction in droplet sizes to nanoscale and formation of charge cloud of comparable size around the droplets.

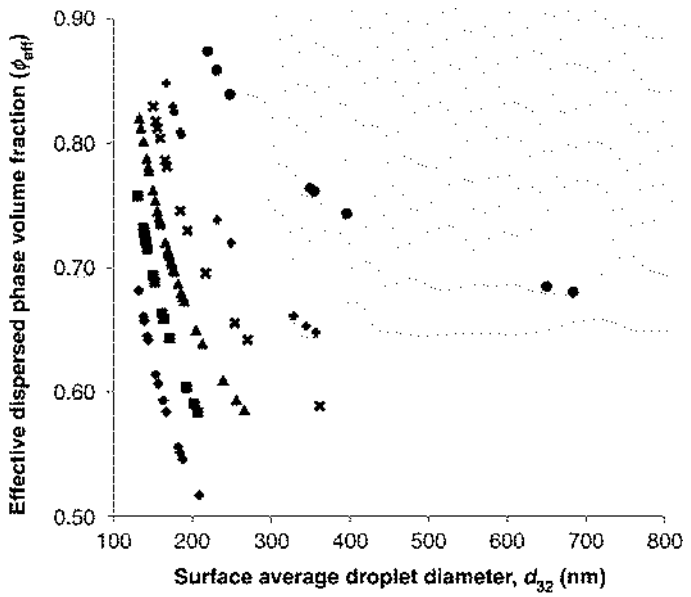


**Figure 8.8. Calculated values of $\phi_{eff}$ as a function of average droplet diameter ($d_{32}$) at different dispersed phase volume fractions: 0.3(◆), 0.35(■), 0.4(▲), 0.45 (x), 0.5(+), 0.6(●).**

To understand how such a large increase in $\phi_{eff}$ would influence the elastic properties of the nanoemulsions, the plateau storage moduli ($G'_p$) of all individual nanoemulsions at 0.1% strain (within the LVR region) obtained from strain sweep measurements were plotted in Fig. 8.9a as a function of $\phi_{eff}$. It can be seen that the initial rapid increase in $G'_p$ values slow down as $\phi_{eff}$ approach 0.7. Similar behavior of initial rapid increase (at $\phi_{eff}$ = 0.64) followed by a slow increase of $G'_p$ (at $\phi_{eff}$ = 0.7) was also observed by many researchers (Wilking and Mason, 2007; Scheffold et al., 2014; Graves et al., 2008). We already know that close packing of droplets resulted in a rapid increase in $G'$ as $\phi_{eff}$ approached jamming transition volume fraction ($\phi_j$). As our aim was to calculate the critical volume fraction for this transition, we used the semi-empirical model for elasticity of monodisperse emulsions developed by Mason et al. (1995):

$$\frac{G'_p}{\gamma/R} = K\varphi(\varphi - \varphi_j) \tag{8.8}$$

where $G'_p$, the plateau elastic modulus of jammed emulsions is scaled by Laplace pressure and $K$ is a constant that combines the dimensionless shear coupling parameter and the

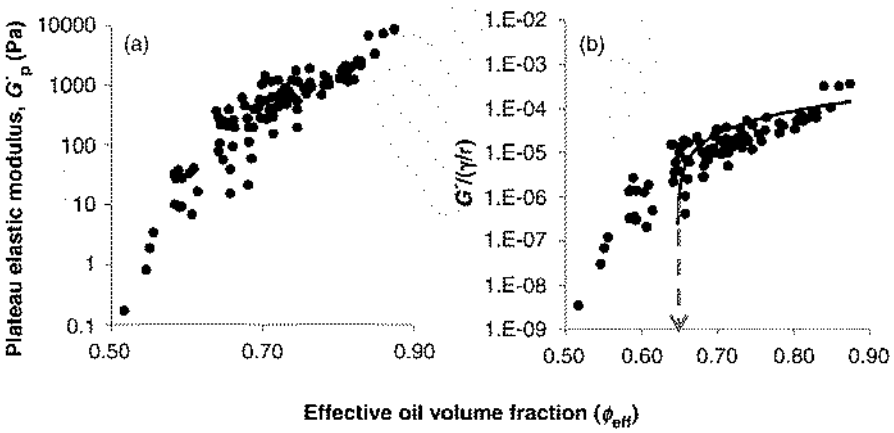


**Figure 8.9.** (a) Plateau storage moduli ($G'_p$) of all individual nanoemulsions at 0.1% strain obtained from strain sweep measurements as a function of $\phi_{eff}$. (b) $G'_p$ from (a) scaled with Laplace pressure as a function of $\phi_{eff}$ at different dispersed phase oil concentrations. The data was modeled with Eq. (8.8) with $r^2$ value of 0.47. The arrow indicates jamming transition volume fraction ($\phi_j$ = 0.66) prediction by the model.

interfacial-entropic coupling parameter (Mason and Scheffold, 2014). Similar scaling expression for elastic modulus of jammed emulsions has also been used to fit experimental results before (Princen and Kiss, 1986). The earlier model was applied to the data in Fig. 8.9a with $\phi_j$ used as a fitting parameter (Fig. 8.9b). The fit of the model to our data gave $r^2$ values of 0.47. Such a low number means the empirical model's fit to these polydisperse nanoemulsions was not great. Nevertheless, the model's prediction of $\phi_j$ of 0.66 means this is the minimum effective nanodroplet volume fraction required for jamming transition and corresponding repulsive nanogel formation in this particular system.

The jammed state model presented here assumes that elasticity vanishes below $\phi_j$, and it predicts a continuous drop in $G'$ at $\phi_j$, which can be seen in Fig. 8.9b. However, in actual case the droplets remained in a crowded state and formed cages around each other without the need to deform as they do not touch. This is termed as colloidal glassy state where the droplet movement is characterized by Brownian motion and each droplet can explore the free volume around it leading to an increase in total entropy of the system. Any applied strain in this region would change the available free volume thereby increasing the energy of the system, which is stored as elastic modulus (Mason and Scheffold, 2014; Ikeda et al., 2012). The volume fraction ($\phi_g$) for this transition generally occurs at $\phi$ = 0.58 for monodispersed emulsions (Berthier et al., 2011; Clusel et al., 2009; Lu and Weitz, 2013a). Recently,

Mason and Scheffold (2014) showed that for conventional emulsions the entropic contribution towards $G'$ is substantially below the jamming state when scaled by Laplace pressure, while for nanoemulsions entropic contribution become significant towards overall magnitude of $G'$ under jammed state. Below the glass transition the crowding effect of droplets subsides and this results in liquid like response where $G'' > G'$.

# 6 Characterization of Nanostructure of Nanogels

The appearance, texture, and stability of the nanogels strongly depend on the characteristics of the individual nanodroplets (size, number, charge), their interdroplet interactions, and the mode of aggregation (Lu et al., 2008; Lu and Weitz, 2013b). Two different techniques, scattering and imaging, are commonly used to characterize the nanostructure of nanogels. Of the former, small-angle light scattering (SALS), X-ray scattering (SAXS), and neutron scattering (SANS) are commonly used to study wave number-dependent intensity profile of nanoemulsions that can be modeled with appropriate theory in order to calculate aggregation number, interdroplet interaction and separation distance between the droplets and clusters (Fryd and Mason, 2012). However, as scattering techniques do not provide real-space images of nanogels; transmission and scanning electron microscopy techniques are also necessary to visualize the dense packing of nanodroplets (Fryd and Mason, 2012).

## 6.1 Scattering Technique for Nanogel Characterization

Various scattering techniques have been used to investigate nanocolloidal gelation (Corredig and Alexander, 2008; Hyland et al., 2013). Liu et al. (2007) used diffusive wave spectroscopy to investigate the structure of flocculated oil droplets in the presence of biopolymers in the continuous phase. Scattering techniques are also used to identify the evolution of the cluster network during the process of gelation. Wu et al. (2005, 2013) studied in situ gelation kinetics by mixing flurorinated polymer nanoparticles (with refractive index close to water) with NaCl solution and transferring them into the measuring cell of a scattering instrument. As gelation proceeds the evolution of structure factor was collected as a function of time (Fig. 8.10). The light scattering structure factor curve consisted of two regions, the first at higher

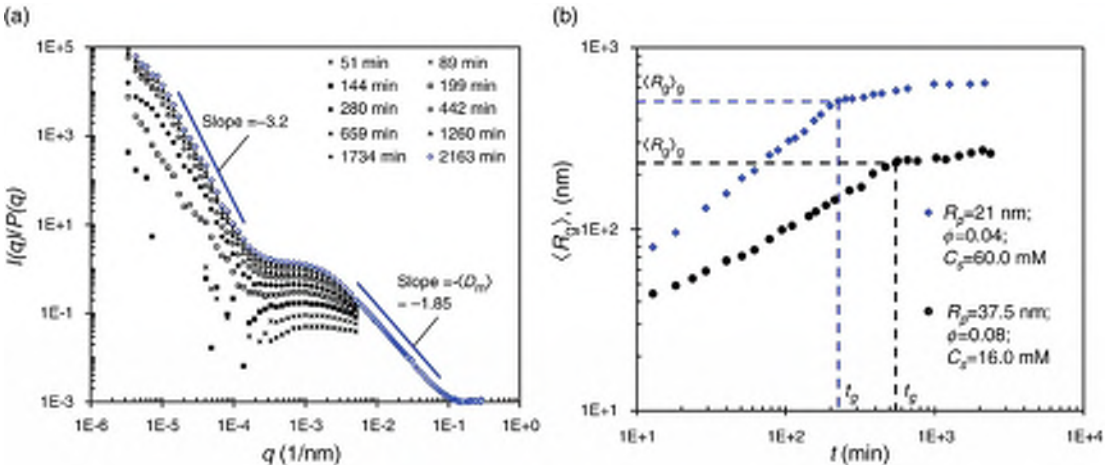


**Figure 8.10. Kinetics of salt-induced nanocolloidal gelation in fluorinated polymer nanoparticles tracked using SALS, WALS, and SANS.** (a) Time evolution of SLAS structure factor for nanoparticles with radius 21 nm, volume fraction 0.04 and NaCl concentration of 60 mM. The final structure factor at 2163 min was obtained by combining SALS, WALS, and SANS. (b) Scaling of gelation point with time ($t_g$) using the rate of change in average radius of gyration ($R_g$) of the fractal clusters for nanocolloidal gels with radius 21 nm and 37.5 nm, calculated from the structure factor curves. Reproduced from Wu et al., 2013, with permission from the Royal Society of Chemistry

wave numbers ($q > 1 \times 10^{-3}$ nm$^{-1}$) corresponds to growth of clusters due to nanoparticle aggregation, which started flatter and moved upward toward higher structure factor as clusters grow with time. In order to obtain structure factor at even higher wave numbers that are not possible by SALS, the authors used wide angle light scattering (WALS) and SANS and the data was overlapped with the SALS by vertical shifting (the final data at 2163 min on Fig. 8.10a). The SANS data at high wave number showed a linear behavior in log-log scale, the slope of which can give the fractal dimension of the clusters constituting the gel. In the second regime, at lower wave numbers ($q < 1 \times 10^{-4}$ nm$^{-1}$), structure factor increased linearly with decrease in wave number and corresponds to the length scale larger than that of the clusters and considered as fractal structure of the super-aggregates formed from smaller clusters of lower fractal dimensions. This structure appeared only after a gel was formed, hence only the scattered data was obtained at initial time scale. The two linear regions of the structure factor curves joined by a bended region, which can be modeled with Guinier plot to give estimation of radios of gyration ($R_g$) of the clusters (Fig. 8.10b). The change in $R_g$ showed a transition from faster to a slower growth rate indicating gelation. The authors attributed the slow increase in $R_g$ even after gelation to the progressive attachment of smaller free clusters to the gel network holding

the nanoparticles. From Fig. 8.10b it can also be seen that gelation happened earlier for the smaller nanoparticles, whose the critical $R_g$ for gelation $\langle R_g \rangle_g$ is higher than the larger nanoparticles. Nevertheless, when the $\langle R_g \rangle_g$ values for different gels with varying size and salt concentrations were scaled with nanoparticle size and plotted against dimensionless aggregation time all values below gelation point collapsed on a single master curve indicating same aggregation kinetics of gelation were followed irrespective of nanoparticle size, volume fraction and salt concentration. At the gelation point, the $\langle R_g \rangle_g$ values decreased with increase in particle volume fraction, while the effective volume fractions of the clusters required for gelation remain constant independent of actual particle volume fraction, indicating a critical requirement of same crowding condition in order to form gel. Finally, the Wu et al. (2013) also showed that the effective cluster volume fraction required for gelation increased with decrease in particle size. This work shows how scattering data from a nanoparticle gel can be utilized to obtain various information on gelation kinetics and the structure of the gel at various length scales.

Research work by Mason et al. (Mason et al., 1997b; Graves et al., 2005; Mason et al., 1997a, 2006; Wilking et al., 2006, 2011) have significantly impacted our understanding on the use of SANS to probe the structure and gel breakdown of concentrated nanoemulsions. In order to understand droplet deformation in nanoemulsions, the authors measured SANS intensity as a function of wave number for silicone o/w nanoemulsions stabilized with SDS with a range of $\phi$ from 0.005 to 0.72 and whose droplet size distribution was narrowed down by ultracentrifugation, during which water was replaced by $D_2O$ for enhanced neutron scattering and quick data collection (Mason et al., 2006). When the low wave number intensity of the nanoemulsions were plotted as a function of droplet volume fraction and modeled with disordered monodisperse Percus-Yevick hard spheres, good prediction was obtained until $\phi = 0.545$, above which scattering intensity remained higher than the model prediction, indicating droplet deformation. Wilking et al. (2006) used time-resolved SANS to study cluster formation during the process of droplet aggregation and gelation in attractive silicone oil nanoemulsions stabilized by SDS and prepared by adding salt and quenching temperature, and proposed that the dense clusters formed with tetrahedra of four nanodroplets as the primary rigid building blocks, aggregate to form rigid fractal gel.

Quick data collection capabilities of SANS made it possible to track in-situ structural changes in soft materials coupled with rheology in the length scale of ~1 nm to 600 nm. Eberle and Porcar (2012) reviewed application of rheo-SANS techniques in various

colloidal soft matters. Wilking et al. (2011) used rheo-SANS to determine structure evolution in nanoemulsions during shearing in a Coquette cell of a rheometer in order to understand the mechanisms of gel breakdown. Similar SDS-stabilized silicone oil nanoemulsions with $D_2O$, as used by Mason and coworkers in their other works, was also used with the addition of salt to induce attractive gelation. The samples were preheated to 50°C to disintegrate the droplets, poured into a concentric cylinder Couette cell of a rheometer and cooled down to 23°C to reform the gel. The cell was centrally located in a neutron beam path such that the beam is perpendicular to the rotational axis of rheometer. Using simultaneous rheology and SANS measurements the authors were able to identify three distinct regimes of viscosity change and structural evolution under steady shear flow. Strong shear thinning behavior at low and high strain rate was separated by an intermediate region where viscosity was weakly depended on shear rate. In the low shear regime the gels showed strong scattering at low wave number, which decreased with an increase in shear rate indicating gel network breakdown into clusters. In the second regime at intermediate shear rate, the low wave number scattering intensity again increased with shear rate where the bonds between the droplet clusters are continuously being broken and reformed and the clusters tumbled in the shear field. In the strong shear thinning, high shear rate regime, low wave number scattering intensity significantly dropped indicating complete disruption of dense clusters into individual droplets (Wilking et al., 2011).

## 6.2 Imaging of Nanoemulsions and Nanogels

Visualization of the dense packing of nanodroplets in nanogels is necessary in order to complete our understanding of their nanostructure. However, only high-resolution electron microscopy techniques are suitable for determining nanostructure of nanogels as the small nanodroplets are not clearly visible in optical or confocal microscope. Even then a suitable instant freezing technique is necessary in order to preserve the nanodroplet network structure. Difficulty in imaging such soft material might be the reason for lack of available literature demonstrating nanostructure of nanogels. Kawada et al. (2010) were the first to show the freeze fracture transmission electron micrograph (FF-TEM) of nanoemulsion gel (Fig. 8.11). The crystal-like hexagonal lattice packing of the repulsive nanodroplets can be seen from the micrograph where the size of the droplets can be estimated as 20–40 nm, while the interdroplet distance was 50–60 nm leading to a large increase in effective droplet volume fraction and jamming.

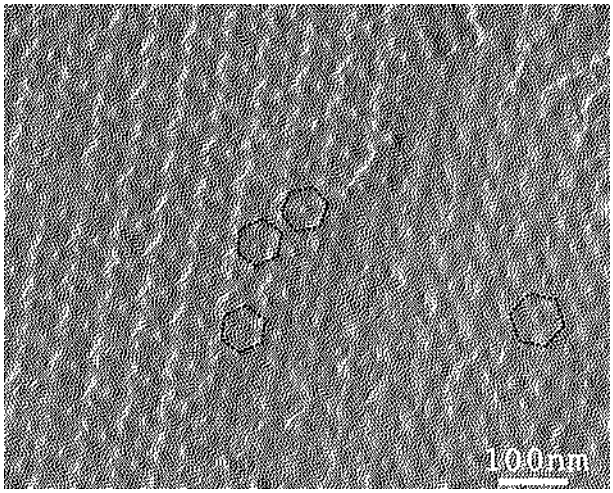


**Figure 8.11. Freeze-fracture transmission electron microscopy (FF-TEM) image of hexagonal close packing of nanodroplets in a repulsive nanoemulsion gel of prepared by Kawada et al., 2010** . Reprinted with permission from Kawada et al., 2010. Langmuir 26 (4), 2430–2437. Copyright 2010 American Chemical Society

Fryd and Mason (2012) showed nanostructure of repulsively jammed SDS-stabilized silicone oil nanoemulsions using negative staining TEM, which is probably one of the best known images of nanogels reported so far. Recently, we were able to obtain freeze-fracture cryo-scanning electron microscopy (SEM) images of attractive nanogel-stabilzied with 10 times SDS CMC (Fig. 8.12). It can be seen that the nanogel was made by aggregating clusters of nanodroplets, where the clusters were themselves made by strong aggregation among the nanodroplets. Further advancement in sample treatment is necessary in order to obtain better images of nanogels. Also, technology for in situ visualization of nanostructure during gelation and shear-induced gel breakdown would further enhance our knowledge on nanoemulsion gelation and their application in food, pharmaceuticals and cosmetic products.

## 7 Potential Application of Nanogels in Food and Related Soft Materials

Gels are widely used in our day-to-day lives, from toothpaste used in the morning and margarine applied on a piece of toasted bread to foam mattresses used for comfortable sleep in the night. They are widely used in pharmaceutical, biomedical, cosmetic, and food applications. In pharmaceuticals, many formulation include gels for sustained drug release (Djabourov and Simon, 2013;

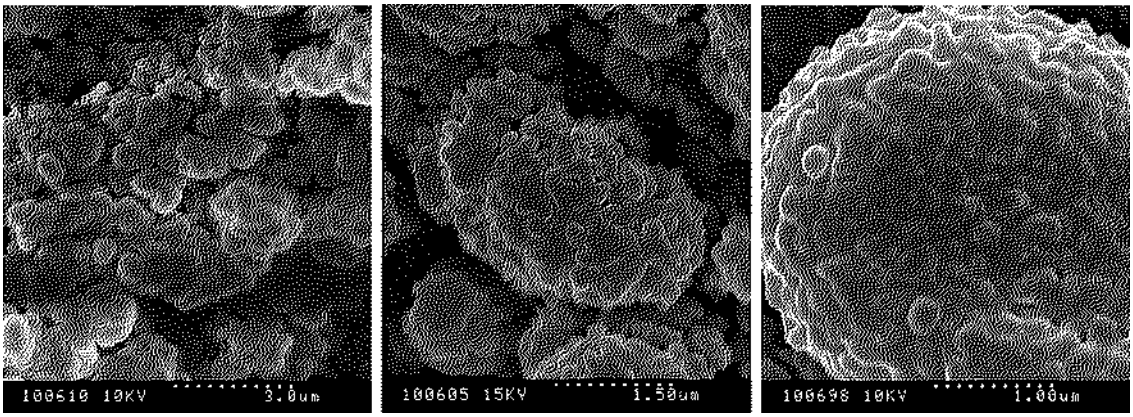


**Figure 8.12. Freeze-fracture cryo-scanning electron microscopy image of attractive nanogel made with 10 time SDS CMC.** From left to right progressive higher magnification shows cluster aggregation, nanostructure of a cluster and individual nanodroplets making a cluster, respectively.

Garcia-Gonzalez et al., 2011). They are also essential components of many cosmetics. Gels and gelling agents are used for obtaining desired textural and sensory properties of food products like jams, custards, yogurts, ice-creams, creams, and confectioneries (Banerjee and Bhattacharya, 2012). Besides that they are also used for a number of other functions like stabilizing foams and emulsions, controlling syneresis, and water retention (Florian et al., 2005). In order to form gels in foods, gelling agents are added. Most of the gelling agents used in foods are food hydrocolloids that constitute polysaccharides and biopolymers like pectins, gums, starches, proteins, and so on (Richardson et al., 1998). On the other hand, gels formed by the bottom-up approach, that is, by controlling interdroplet interactions does not require any additional gelling agents and if necessary their state of gelation can be switched.

Studying the gelation of emulsions and nanoemulsions is important as a lot of colloidal suspensions in different applications exist in a gelled or jammed disordered state (Hecke, 2010). For example, structure and flow behavior of toothpaste, shaving foam, cosmetic creams, and topical medicine gels depend on interparticle interactions and gelation. Emulsion gels used in food applications include yogurt, ice cream, butter, margarine, salad dressings, cheeses, and so on (Dickinson, 2012). As gelation in nanoemulsions can be observed at much lower dispersed phase volume fractions compared to conventional emulsions, nanogels can help to reduce the fat content in the emulsion gels while maintaining the structure (Datta et al., 2011; Mason et al., 2007; Erramreddy and Ghosh, 2014). Besides close-packed association of nanodroplets, nanogels are more stable against coalescence

compared to conventional emulsion droplets due to their higher Laplace pressure and formation of thick interfacial shell around the droplets (Fryd and Mason, 2012). Nanodroplets are also known to improve the bioavailability of internal bioactive components, which can further enhance nanogels' application (Huang et al., 2010; McClements et al., 2007). However, more research is necessary in order to better understand the long-term stability of the nanogels, and their potential application in food and related soft materials.

## 8 Conclusions

Traditionally, structure formation in lipid-rich foods (eg, spreads, chocolate) come from the microscopic fat crystal network made of saturated and trans fats, which holds liquid oil immobile, providing shape and form to the product. However, the epidemic of obesity and cardiovascular disease forced us to limit saturated and trans fat in our diet. In this chapter, a novel approach to provide structure to lipid-rich foods by developing nanogels from low oil containing liquid nanoemulsions was proposed. It was observed that liquid nanoemulsions transformed into viscoelastic gels at a specific concentration range of anaionic SDS emulsifier while no gelation was observed for nonionic Tween-20. The apparent viscosity, yield stress, and storage moduli ($G'$) of the nanogels increased with SDS concentration. At low SDS concentration (0.5–2 CMC) the repulsive charge cloud acted as interfacial shell layer on the nanodroplets and significantly increased the effective oil phase volume fraction ($\phi_{\text{eff}}$) beyond random jamming leading to elastic behavior. Nanoemulsions prepared with nonionic Tween-20 showed weak elastic behavior and flowed under gravity, as the lack of charge cloud did not influence $\phi_{\text{eff}}$. With increase in SDS concentration to 5–15 CMC, excess SDS moleculs formed micelles in the continuous phase, which created depletion attractions among the nanodroplets and led to their aggregation resulting in attractive nanogels with high gel strength ($G' >> G''$). Moreover, elasticity of repulsive nanoemulsion gradually increased with reduction in droplet size, while for attractive nanoemulsions, elastic behavior rapidly increased once the droplet size was below a critical level. A semiemperical model proposed by Mason et al. (2014) predicted a value of 0.66 as the critical volume fraction required for jamming transition in repulsive nanogel, although the need for a better model, which takes into account attractive interactions and polydispersity of the nanogel could not be ignored. Of the many techniques used to characterize the nanostructure of the nanogel, small-angle light scattering (SALS), X-ray scattering (SAXS) and neutron scattering

(SANS) are most commonly used. These techniques used a wave number-dependent intensity profile of nanoemulsions that can be modeled with appropriate theory in order to calculate aggregation number, interdroplet interaction and separation distance between the droplets and clusters of a nanogel. However, as scattering techniques do not provide real-space images of nanogels; transmission and scanning electron microscopy techniques are also necessary to visualize the dense packing of nanodroplets. Nevertheless, difficulty in imaging such soft material might have prevented widely available literature demonstrating such data.

In the future, there is a need to develop nanogels using food-grade emulsifiers. However, in order to achieve this, the emulsifiers should be able to achieve extremely smaller droplet size, have a substantial interfacial shell layer thickness and at the same time be able to provide long-term stability to the interface. Therefore, finding appropriate food-grade emulsifiers and suitable emulsification methods is necessary for applications of these nanogels in food and more research is needed in order to fully understand their potential as a viable system with long-term stability and delivery of bioactive materials.

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# 9

# NANOEMULSION-BASED DELIVERY SYSTEMS: PREPARATION AND APPLICATION IN THE FOOD INDUSTRY

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## 1 Introduction

Emulsion-based delivery systems are increasingly being used in food and pharmaceutical industries to encapsulate, protect, and deliver bioactive components (McClements, 2012; Dizaj, 2013; Atashafrooz et al., 2014). These emulsions can be categorized into three groups based on the emulsion droplet size: macroemulsions (100 nm–100 μm), nanoemulsions (10–100 nm), and microemulsions (2–100 nm) (Mason et al., 2006b). Comparison of the thermodynamic stability of colloidal dispersions containing oil, water, and emulsifier has shown that macroemulsions and nanoemulsions are thermodynamically unstable while microemulsions are stable systems (Mason et al., 2006b). Studies have shown that the surface-to-mass ratio of emulsions, nanoemulsions, and microemulsions are 0.07–70, 70–330, and 330–1300 $m^2/g$, respectively (Tadros et al., 2004). The optical properties are expressed for typical oil and water systems, where there is a significant refractive index contrast for macroemulsions, nanoemulsions, and microemulsions that are turbid/opaque, clear/turbid, and clear/turbid, respectively (McClements and Rao, 2011; Tadros et al., 2004).

"Microemulsions" and "nanoemulsions" are two common kinds of colloidal delivery systems with appropriately small particles to attain optical transparency (Kabalnov and Wennerström, 1996).

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00009-X

In scientific literature, sometimes it is confusing to use the term microemulsion or nanoemulsion and due to similarities of these systems various definitions for nanoemulsions have been proposed in the literature (McClements, 2012). However, some researchers apply the word nanoemulsions only for emulsions with droplet size in the nanometer range obtained by high shear methods. They believe that the emulsions prepared by the methods such as self-emulsifying technique or phase inversion methods should not be considered as nanoemulsions, although possessing an extremely small droplet size (Kabalnov and Wennerström, 1996; McClements, 2012; McClements and Rao, 2011). Their argument is due to the influence of the preparation method on droplet size, stability, and other properties of developed emulsion (Aboofazeli, 2010). However, in enormous studies the emulsions prepared from these methods are also named nanoemulsion.

The reduced particle size of both nanoemulsion and microemulsion systems presents a number of potential benefits for certain applications; for example, enhanced long-term stability, high optical clearness as well as increased bioavailability (McClements, 2012). One of the main differences between these two systems is that nanoemulsions can be formed at moderate surfactant concentration while formation of microemulsion needs high concentration of surfactants (Izquierdo et al., 2002). In nanoemulsion systems, "Brownian motion" can prevent the droplets from creaming or sedimentation and therefore coalesce (Aboofazeli, 2010). Furthermore, there are differences between the production methods, the stabilization methods, and the techniques used to design the functional attributes of various emulsion types (Atashafrooz et al., 2014; Dizaj, 2013; Azeem et al., 2009; Chime et al., 2014).

Nanoemulsions have numerous applications such as personal care, cosmetics, health care, food and agrochemicals (Maali and Mosavian, 2013). Interest has also been raised on utilization of nanoemulsions in the food industry due to the physicochemical properties and biological performance related to alterations in particle size (Guttoff et al., 2015). Food nanoemulsions suggest some advantages over conventional delivery systems in the case of nutraceuticals/ingredients. These advantages include protection of the bioactive and enhancement of its solubility, stability, and bioavailability (Aboofazeli, 2010). Various bioactive agents including nutraceuticals and pharmaceuticals that proposed for the oral ingestion are highly hydrophobic compounds with low solubility in water and therefore poor bioavailability (Li et al., 2012). Delivery systems based on nanoemulsions are predominantly

suitable carriers for encapsulating, protecting, and delivering the poorly water-soluble nutraceuticals and drugs for both food and pharmaceutical applications.

Particle size reduction in a nanoemulsion system has a number of results that may be beneficial for certain food applications. These systems show great stability to droplet aggregation and gravitational separation. Furthermore, higher optical clarity and an increased oral bioavailability can be achieved through them (McClements, 2012; McClements and Rao, 2011). Nanoemulsions may be mainly useful for encapsulating lipophilic bioactive components (such as oil-soluble vitamins or nutraceuticals) within aqueous-based food products that should be optically clear, such as fortified waters, soft drinks, or juices (Guttoff et al., 2015). Literature review showed that the nanoemulsions have been studied extensively as carriers for the bioactive components. In addition, these nanostructures have been investigated for delivery of different drugs as well as treatment of the diseases. The application of nanoemulsions in cosmetics, as a mucosal vaccine, and in cell culture technology is also reported in the literature (McClements, 2012; McClements and Rao, 2011).

In this chapter, we focus on fundamental principles of emulsification process; the role of surfactants; the types of nanoemulsions and their production methods as well as the application of nanoemulsions in the food industry.

## 2 Nanotechnology in the Food Industry

Nanotechnology is the science of manufacture, manipulation, and characterization of the structures, devices, or materials that have at least one nanoscaled dimension (Duncan, 2011). Considering the fact that the surface/volume ratio of the nanoparticles increases by decreasing the size, nanoparticles have revealed different properties comparing to the larger particles of the same material (Adibkia, 2014; Adibkia et al., 2011b). Nanotechnology has provided some strategies to improve the material properties in order to increase their activities (Dingman and Rehs, 2008; Adibkia et al., 2011a).

For the first time, the application of nanotechnology in food industry was used by the US Department of Agriculture Food (Dingman and Rehs, 2008; Fao and Foods, 2004). It was applied to enhance the quality and safety of food packaging, to increase nutritional value, to preserve the aroma and taste of the food and to reduce the costs in the food industry. Nanotechnology has shown potential applications in a wide range of food industry

from modification of natural protein, carbohydrate, and fat molecules to attain extra or improved functionalities in order to use nanoparticulated materials in food packaging or as food ingredients (Pavelková, 2012; Bakoš, 2008). Modification of the polymers and carbohydrates structure as well as the assembly of natural biopolymeric food components is currently under research in order to use as nanostabilizers for bioactive components. Engineering in the areas related to protein-carbohydrate coupled with enzymatic functionalization can also be applied to prepare the nanostructures with new functionality in food industry (Ireland, 2008). Different nanotechnology-based techniques may be used to manufacture the beneficial foods. These techniques can also play an important role in elimination of pathogenic bacteria from the food. Nanotechnology also shows potential benefits to produce new food packaging with less weight and better resistance (Dingman and Rehs, 2008).

In the agri-food sector novel and fantastic applications of nanotechnology can play great roles. This section can include nanosensors, tracking devices, targeted delivery of food components, food safety area, development of new product and smart food processing/packaging (Pavelková, 2012; Bakoš, 2008). Nanotechnology also can be employed to a range of storage conditions of product. For example, some products need to be stored at low temperatures and then heated for consumption. The nanoparticle stability within the food as well as changing the properties of the biomolecules in the food can affect this process. Alteration the stability and texture of food products is also another particular area of interest for application of nanotechnology in food industry (Ireland, 2008). It can be detailed that the short term applications of nanotechnology can be in food including packaging, while the long-term applications appears to be focused on controlled release of encapsulated food ingredients or nutrients. Manufacturing the engineered organic forms of natural food components using nanotechnology is probably the main area of interest in the food industry (Ireland, 2008). Fig. 9.1 shows the application of nanotechnology in food industry schematically.

## 3 Nanoemulsion

Emulsion is a system that contains two immiscible phases; one is internal phase of dispersed phase (as droplets) and the other is the external phase (continuous phase) (Maali and Mosavian, 2013; Becher, 1965). Generally, there are two types of emulsions; oil-in-water (o/w) or direct emulsion and water-in-oil (w/o) or inverse emulsion (Mason et al., 2006a). Emulsions can also be categorized

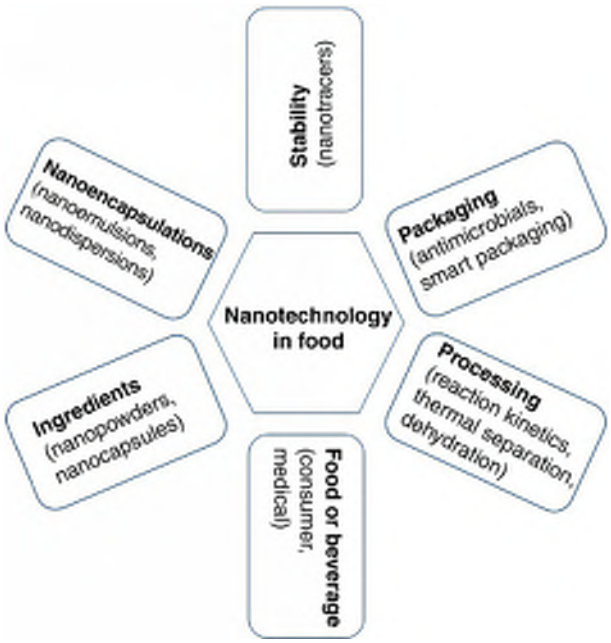


**Figure 9.1. Application of nanotechnology in food industry.**

into three groups based on the emulsion droplet size: emulsions (100 nm–100 μm), nanoemulsions (10–100 nm), and microemulsions (2–100 nm) (Mason et al., 2006b).

Nanoemulsions as isotropic and stable emulsions are non-equilibrium systems with a natural tendency to separate into the constituent phases while they possess a relatively high kinetic stability for a long time. Unlike microemulsions, nanoemulsions cannot be formed spontaneously and an energy input is needed (Aboofazeli, 2010). Nanoemulsions can also be categorized into two main groups depend on droplet size, that is, transparent (50–200 nm) and milky (up to 500 nm) (Izquierdo et al., 2002). In another classification system, nanoemulsions can grouped into three groups depending on the constituent materials; the first group is o/w, the second one is w/o, and the last group is named bicontinuous (rich in water or rich in oil) (Atashafrooz et al., 2014; Dizaj, 2013). Indeed bicontinuous phase is formed when the amount of water and oil are similar in the system (Devarajan and Jain, 2015). Fig. 9.2 shows these three groups schematically.

Due to the small size of droplets, nanoemulsions often have a long-term physical stability and considering a range of various conditions, the physical properties of nanoemulsions are superior to the properties of other conventional emulsions (Liu et al., 2006). To stabilize the system, a thin layer made of phospholipids,

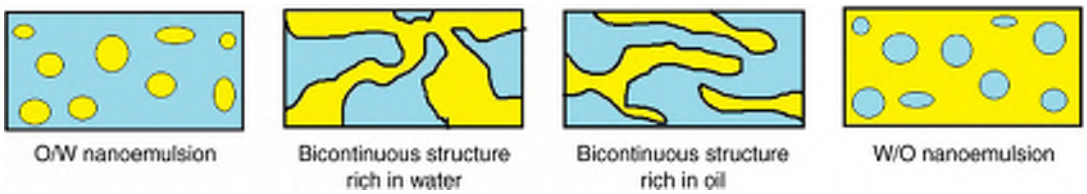


**Figure 9.2. Types of nanoemulsions depending on the constituent materials; o/w, w/o and bicontinuous structures.**

surfactants, or proteins can be introduced to emulsion systems (Sanguansri and Augustin, 2006). Due to some great characteristics, nanoemulsions are attractive systems in food and pharmaceutical industries. Thanks to the very small droplet sizes that reduces the gravity force and Brownian motion, no creaming or sedimentation occurs on storage of nanoemulsion. Furthermore, no separation happens in nanoemulsion because the small droplet size avoids any flocculation. Comparing with microemulsions, preparation of nanoemulsion needs lower concentrations of surfactant (5–10%) (Tadros et al., 2004; Bouchemal et al., 2004). Nanoemulsion can also be diluted with water without any changing of the droplet size and size distribution.

Nanoemulsion reveals unique properties such as high solubilization capacity, high kinetic stability, and optical transparency (Saberi et al., 2014). They can be applied for delivery of active compounds to the different targets in the body or to stabilize biologically active ingredients. Nanoemulsions are applied to deliver of low-soluble food materials such as oil-soluble vitamins (Fathi et al., 2012). These systems can be used to increase the solubility and absorption of bioactive food materials in the body. Furthermore, they can be used to extend the shelf life of products due to enhanced stability and increased viscosity at lower concentrations of oil phase. Studies has also revealed that stabilized o/w or w/o nanoemulsions can be used for controlled release of nutraceutical and other bioactive components in food (Weiss et al., 2008). This technology can be combined with other advanced technologies to develop novel microencapsulated products that allow controlled release of food bioactive materials in the gastrointestinal tract.

## 4 Essential Component in Preparation of Stabilized Nanoemulsions

The preliminary stage of nanoemulsion preparation is the selection of the suitable oil phase on which other components depend on (Silva et al., 2012). Various nonpolar components

including triacylglycerols, free fatty acids, essential oils, mineral oils, waxes, oil soluble vitamins, and many lipophilic nutraceuticals can be used to form nanoemulsions (He et al., 2011). Triacylglycerol oils such as corn, soybean, sunflower, safflower, olive, flaxseed, algae, or fish oils due to their low cost as well as nutritional attributes have the most attention in the food industry (McClements and Rao, 2011).

The choice of emulsifier/surfactant is the second step and plays a main role in nanoemulsion preparation (He et al., 2011). An emulsifier is a surface-active molecule with the ability to adsorb on droplet surfaces and to facilitate droplet disruption as well as to protect droplets from aggregation (McClements and Rao, 2011). Most food-grade ionic surfactants are negatively charged, however, lauricarginate as positively charged surfactant is also available for certain applications (Silva et al., 2012). Nonionic surfactants have also been broadly used to prepare nanoemulsions due to their low toxicity and lack of irritability (McClements and Rao, 2011). In the case of the stability of improved nanoemulsions the type of emulsifier/surfactant is critical. It has been reported that formation and stability of nanoemulsions can be enhanced via combinations of emulsifiers rather than using a single emulsifier (McClements and Rao, 2011; Tadros et al., 2004). Nanoemulsions stabilized by natural emulsifiers such as food proteins can prevent toxicological concerns for long-term utilization. An o/w nanoemulsion system with good biocompatibility that has stabilized using food proteins (as emulsifier) presents better and more rapid absorption of lipophilic compounds (He et al., 2011). He et al. investigated the potential of food proteins including soybean protein isolate, whey protein isolate, β-lactoglobulin as safer stabilizers for nanoemulsions (preparing by HPH method) to deliver different hydrophobic compounds. They tested the toxicity of the prepared nanoemulsions in Caco-2 cells using the 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium-bromide viability assay as well as in vivo evaluation of absorption in rats. Based on their results, food protein stabilizers with small particle size and good size distribution exhibits more stable and more biocompatible nanoemulsions compared with nanoemulsions formed by traditional emulsifiers. Among them, β-lactoglobulin showed a better emulsifying capacity and biocompatibility than the other two food proteins (He et al., 2011).

Hydrophilic lipophilic balance (HLB) value is an important factor in the selection of surfactant. Hydrophilic surfactants and cosurfactants prefer the interface and need low energy to form the nanoemulsions, thus they can improve the stability

(Kommuru et al., 2001). Selection of the right mixture with suitable proportions of different surfactants with low and high HLB results in formation of stable nanoemulsions (Azeem et al., 2009).

Many of the preparation techniques are codependent of solvent type (Adibkia et al., 2014). Food grade solvents are generally recognized as safe solvents to use in preparation of nanoemulsions. Some of these utilized solvents are not food grade and if they are, they are not well accepted by the consumers. This issue needs to be improved and solvents like n-hexane and others may be replaced by safer solvents such as sunflower oil and MCTs (Silva et al., 2012). Typically water is used as the aqueous phase to prepare a nanoemulsion, however, the aqueous phase may also contain other polar components such as simple alcohols and polyols, carbohydrates, proteins, minerals, acids, and bases (McClements and Rao, 2011). Controlling the aqueous phase composition to optimize the nanoemulsion formation is a proper approach to improve the stability of nanoemulsions (McClements and Rao, 2011).

Cosurfactants, surface active amphiphilic molecules, can be used as another component of nanoemulsion formulation. They are not good emulsion stabilizers by themselves due to the small size of their polar head groups. Cosurfactants can facilitate nanoemulsion formation via a number of mechanisms; they can fluidize the interface, optimize the disperse-to-continuous phase viscosity ratio, act as spacers to reduce the electrical repulsion between the head groups of ionic surfactants and also induce the suitable interfacial curving (Gradzielski, 1998).

Nanoemulsions can be applied to encapsulate a wide range of lipophilic compounds, including lipids, flavors, antimicrobials, antioxidants, and drugs (Chen et al., 2006; Shefer and Shefer, 2003; Ubbink and Krüger, 2006), however, four categories of lipophilic functional compounds (including fatty acids, carotenoids, antioxidants, and phytosterols) more often need to be incorporated in nanoemulsion systems (McClements et al., 2007).

## 5 Nanoemulsions Preparation Methods

The main components of an emulsion are oil, water, surfactant, and energy. From the thermodynamically point of view, the total free energy (ΔG) needed for formation of an emulsion can be gained using the following equation (Maali and Mosavian, 2013):

$$\Delta G = \Delta A\gamma + T\Delta S$$

Where $\Delta A$ is the amount of increase in interfacial area, $\gamma$ is the interfacial tension, T is the temperature of procedure, and $\Delta S$ is

the amount of change in entropy. Since the value of entropy of dispersion $T\Delta S$, which is positive, is greater than absolute value of consumed energy ($|\Delta A\gamma|$), the value of $\Delta G$ would be positive. Because of the positive amount of $\Delta G$, energy is required for nanoemulsion formation. In contrast, to form macroemulsions, high speed stirrers is adequate (Maali and Mosavian, 2013).

A number of techniques have been utilized in the preparation of nanoemulsion such as phase inversion method, sonication technique, higher pressure homogenization (HPH) procedure, micro fluidization, and spontaneous emulsification (Saberi et al., 2014). The required energy to produce a nanoemulsion can be gained from mechanical methods and devices or from the chemical potential of its components. Principally, preparation of nanoemulsions can be classified in two groups: high energy emulsification methods (dispersion methods) and low energy emulsification methods (condensation) (Solans et al., 2005).

The methods in the first category use high mechanical forces to disrupt droplets into smaller sizes and include the use of mechanical devices (Solè et al., 2006). These methods can be categorized in three groups of HPH (Solans et al., 2005), microfluidization (Jafari et al., 2007), and ultrasonication (Solans et al., 2005). As a disadvantage, this category needs high energy for preparing of nanoemulsions and therefore is not favorable for much industrial utilization (Tadros et al., 2004). In these methods the formation of nanoemulsion droplets depends on some formulation parameters such as the amount of energy, the type and the amount of surfactant as well as the nature of the components (Anton et al., 2008). HPH is the most common method of prepare emulsions with small droplet sizes in the food industry (Schubert et al., 2003; Sekhon, 2010). Generally a coarse emulsion is prepared using a high shear mixer and is then fed into the HPH. Microfluidizers have conventionally been used in the pharmaceutical, food, and beverage industries to produce emulsion-based products. A microfluidizer technique also involves using high pressures to force a premix emulsion via a fine orifice to disrupt the droplet. In ultrasonic homogenizers high-intensity ultrasonic waves are used to produce the intense disruptive forces in order to break up the droplets into very small sizes (Kentish et al., 2008; Leong et al., 2009).

In contrast, low-energy methods only need mild mixing and include the physicochemical properties of the surfactants or cosurfactants (Fernandez et al., 2004; Liew et al., 2010). These methods are based on the spontaneous formation of emulsion under determined conditions that are outcomes of change in interfacial properties (Anton and Vandamme, 2009). These changes can be

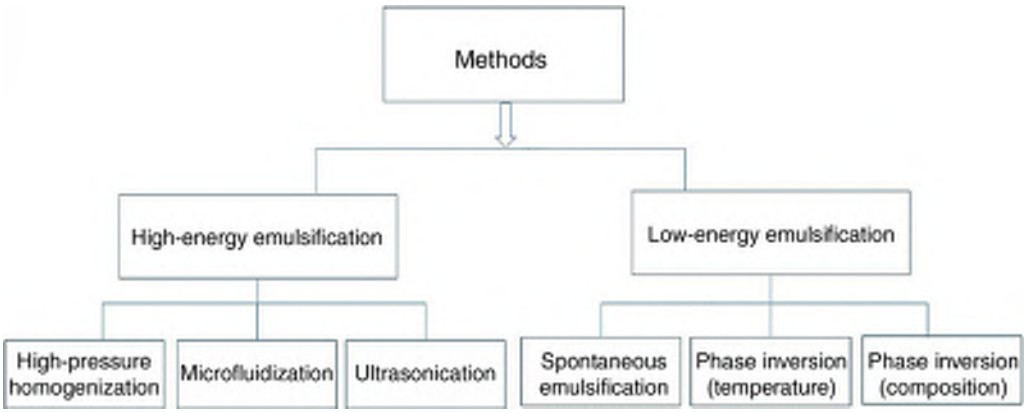


**Figure 9.3. Nanoemulsions preparation method.**

obtained both when the composition is fixed and the temperature is altered (known as phase inversion temperature (PIT)) or when the temperature is kept constant and the composition is changed (known as phase inversion composition (PIC)) (Liew et al., 2010). Low-energy methods are categorized in three general groups: PIT (Rao and McClements, 2011), PIC (Fernandez et al., 2004), and spontaneous emulsification (Bouchemal et al., 2004). An important benefit of low-energy methods is that no expensive preparing equipment such as a high-pressure homogenizer or sonicator is required. Fig. 9.3 shows nanoemulsions preparation methods.

In spontaneous emulsification process an emulsion is spontaneously formed when two liquids are mixed together (Miller, 1988; Anton and Vandamme, 2009). There are some proposed mechanisms for spontaneous emulsification process (Horn and Rieger, 2001). For example, in the connection boundary of the two phases, some of the component with tendency to both phases may move from its location in one phase into the other phase. When this component moves it can cause an increase in oil-water interfacial area and interfacial turbulence, then spontaneously formation of droplets occurs (McClements and Rao, 2011).

The certain mixtures of oil, water, and nonionic surfactant can form nanoemulsions using the PIT method by varying the temperature-time profile. This method involves controlled transformation of an emulsion from one type to another (w/o to o/w or vice versa) via an intermediate crystalline phase or a bicontinuous phase (McClements and Rao, 2011). PIC method as another phase inversion method is slightly similar to the PIT method, but the optimum curvature of the surfactant is changed by altering the formulation (composition) of the system, rather than the temperature (Anton and Vandamme, 2009). An example for PIT

method is adding salt to an o/w emulsion stabilized by an ionic surfactant to phase invert to a w/o emulsion (McClements and Rao, 2011).

In recent years, low-energy methods have grown great attention because of their soft and nondestructive nature as well as the high safety of encapsulated molecules in these systems. Furthermore, these methods are energy-saving and therefore more applicable for large-scale production (Koroleva and Yurtov, 2012). Among these methods, the spontaneous emulsification method as simple and low-cost technique has great potential for forming nanoemulsion-based delivery systems for foods. As a major advantage, this method needs no sophisticated or expensive equipment to form the nanoemulsions. However, a major disadvantage of this method is a relatively high surfactant concentration is need to form nanoemulsion (Gulotta et al., 2014).

The high- and low-energy methods that mentioned previously are mainly applied for production of o/w nanoemulsions (Tadros et al., 2004). Preparation of w/o (reverse) nanoemulsions is more difficult due to their high viscosity. The high- and low-energy combination method is proposed to prepare of reverse nanoemulsions. Low-energy methods lead to reverse nanoemulsions with smaller droplet sizes; however, the fraction of the internal phase in these emulsions is rather low. In the other hand, high-energy methods cause to an increase in the fraction of the internal phase that leads to rapid increase in the droplet size due to an increase in the viscosity of the emulsion. None of the modern methods of formation of nanoemulsions gives reverse nanoemulsions with a high content of the internal phase and small particle size. Therefore, a combined method of preparation is needed to prepare reverse phase nanoemulsions. In this process, at first reverse macroemulsions with an average droplet size is prepared by high-energy stirring and then using low-energy emulsification a large number of water droplets are formed (Koroleva and Yurtov, 2012).

## 6 Characterization of Nanoemulsions

Understanding the formation and characteristic properties of nanoemulsions for developing nanoemulsion-based food products in the food industry is important. Nanoemulsions can be physically and chemically characterized related to appearance, pH, viscosity, the compatibility of components, isotropicity of the formulation, content uniformity, density, conductivity, surface tension, size, and zeta potential of the dispersed phase (Chime et al., 2014).

Dynamic light scattering (DLS) [also named as photon correlation spectroscopy (PCS)] can be used to analyze the nanoemulsion droplet size, polydispersity, and zeta potential. The morphology of nanoemulsions can be studied by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). SEM technique provides a 3-dimensional image of the globules (Aulton and Wells, 2002). Zeta potential of nanomaterials gives information on their surface charge. Zeta potential value between −10 and +10 mV are considered nearly neutral, while zeta potential values greater than +30 mV or less than −30 mV are considered strongly cationic and strongly anionic, respectively (Clogston and Patri, 2011). Zeta potential value is significant parameter for predicting the stability of the nanomaterials and their interaction with the biological environment such as cellular uptake by macrophages (Vogelman et al., 1988). For colloidal dispersions, zeta potential can be related to the stability that indicates the degree of repulsion between adjacent similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability, that is, the solution or dispersion will resist to aggregation (Silva et al., 2012).

Differential scanning calorimetry (DSC) can be used to provide information about the interactions of different components. Polarized light microscopy that can distinguish between isotropic and anisotropic materials is employed to confirm isotropicity of nanoemulsion formulation (Chiesa et al., 2008). The structural features of nanoemulsions can be studied using self-diffusion nuclear magnetic resonance (SD NMR) and small angle X-ray scattering (SAXS) techniques. Viscosity measurements determines the existence of reverse micelles and conductivity measurements can provide information about continuous phase of nanoemulsion that is whether oil- or water-continuous. Furthermore, it is a means for monitoring phase inversion phenomena (Chiesa et al., 2008).

The charge of droplets in o/w nanoemulsions is another property that needs to be measured. Droplets are usually negatively charged this is due to the presence of free fatty acids. Combination of a cationic lipid, such as oleylamine (1–3%) will also produce cationic nanoemulsions (Mandal and Bera, 2012; Aulton and Wells, 2002). Nanoemulsion droplet polarity is an important factor in determination of emulsification efficiency. Polarity denotes the affinity of the cargo compound for oil or water and the type of formed forces and the release of the cargo into the aqueous phase is supported by the polarity (Chiesa et al., 2008). Dielectric measurement is another assay related to emulsions that can be a powerful means of probing both the structural and dynamic features of a nanoemulsion system. The important parameters that

can show an impact on the polarity of the oil droplets are included HLB, molecular weight of the hydrophilic portion, chain length, degree of unsaturation of fatty acids and concentration of the emulsifier (Mandal and Bera, 2012; Aulton and Wells, 2002).

Viscosity of a nanoemulsion is a function of its components including the surfactant, water and oil and their concentrations and can be carried out using a viscometer. For an emulsion system, increasing the water content drops the viscosity and decreasing the amount of surfactant or cosurfactant increases interfacial tension between water and oil resulting in increased viscosity (Chime et al., 2014). Electric conductivity is a useful and very simple method in characterization of type of microemulsions or nanoemulsions that they can be in o/w, w/o or bicontinuous types (Atashafrooz et al., 2014; Dizaj, 2013). The conductivity is initially low in o/w but increases with increase in aqueous phase. A bicontinuous phase has maximum amount of electric conductivity and for w/o emulsion the conductivity decreases with increasing of aqueous content. We used this method to detect the phase boundary of microemusion area for vitamin A palmitate and Cucurbita pepo oil microemulsion systems (Atashafrooz et al., 2014; Dizaj, 2013).

In most cases detection of nanoemulsions in food matrixes is not possible. Hence, separation techniques such as size-exclusion chromatography or ion exchange chromatography are necessary to isolate the nanoemulsions from food prior to their characterization (Silva et al., 2012). These methods are the most proper types of liquid chromatography for the separation of nanoemulsions from the food matrix. Field flow fractionation is a method for the separation of analytes including macromolecules (such as proteins) and other particles such as whole cells. As a main advantage, very broad ranges of molecular sizes can be separated, in a one single run (Luykx et al., 2008). The separation of particles due to their Stokes radius can also be performed by FFF (Dulog and Schauer, 1996). Characterization method for determining the properties of nonoemulsions are summarized in Table 9.1.

Plotting of phase diagrams is the best way to study all types of formulations that are prepared from the mixing of surfactants, water and oil. Such a system also shows the whole probabilities of mixing ratios in a more systematic way (Hadzir et al., 2013). The ternary phase diagram can be used for the detecting of nanoemulsion phase behavior. Indeed, nanoemulsion formation is a function of its composition. Then, the existence of nanoemulsion formation zone can be illustrated using the ternary or pseudo-ternary (more than three component) phase diagrams (Azeem et al., 2009). For oral administration usually formulations with the lowest surfactant concentration is selected from the nanoemulsion

## Table 9.1 Characterization Method for Determining the Properties of Nonoemulsions

| Properties | Characterization Technique(s) |
|---|---|
| Droplet size, polydispersity, and zeta potential | DLS (PCS) |
| Polarityand type of nanoemulsion | Polarized microscopy |
| Interactions of different components | DSC |
| Structural features | SD NMR, SAXS |
| Morphology | SEM, TEM |
| Viscosity | Viscometer |
| Electric conductivity, type of nanoemulsion | Conductometer |
| Separation of analytes | SEC, IEC, FFF |

*DLS*, Dynamic light scattering; *PCS*, photon correlation spectroscopy; *DSC*, differential scanning calorimetry; *SD NMR*, self-diffusion nuclear magnetic resonance; *SAXS*, small angle X-ray scattering; *SEM*, scanning electron microscopy; *TEM*, transmission electron microscopy; *SEC*, size-exclusion chromatography; *IEC*, ion exchange chromatography; *FFF*, field flow fractionation.

formation zone. High surfactant concentration decreases the thermodynamic activity of the cargo in the carrier because the affinity of the cargo to the carrier becomes greater (Beg et al., 2012). Therefore, formulations should be optimized using phase diagrams.

Nanoemulsions are metastable systems with tendency to breakdown over time via a variety of different physicochemical mechanisms. The stability of nanoemulsions can be influenced by the different stresses such as chilling, thermal processing, mechanical agitation, freezing, and dehydration during the production, transportation, storing, and application (Azeem et al., 2009). During a research, stress testing is required in order to exclude the possibility of metastable nanoemulsion formulations. For this propose, some representative formulations should be taken from the nanoemulsion region of the phase diagram and tested by the thermodynamic stability tests such as heating–cooling cycle, freeze–thaw cycle, and centrifugation. Phase separation, turbidity, creaming, or cracking should not be observed in the selected formulations (Beg et al., 2012; Azeem et al., 2009).

It is essential to recognize the requirements to develop a stable emulsion by the use of food grade components such as emulsifiers and stabilizers for food supplements and nutraceuticals. Developing the multilayer emulsions provides different functionalities by using different combinations of surfactants and biopolymers (Sharma et al., 2010).

## 7 Increased Bioavailability and Controlled Delivery via Nanoemulsions

Bioavailability can be defined as the fraction of a component that ultimately ends up in the systemic circulation. The overall bioavailability of a lipophilic compound with poor water-solubility depends on a number of factors:

$$F = F_B \times F_A \times F_M$$

Where $F$ is overall bioavailability and $F_B$ is the fraction of lipophilic component released from the carrier into the lumen of the gastrointestinal tract to become bioavailable. $F_A$ signifies the fraction of the released component that is absorbed across the intestinal epithelia and $F_M$ represents the fraction of the absorbed component that reaches the systemic circulation without being metabolized (Versantvoort and Rompelberg, 2004; Acosta, 2009; McClements and Rao, 2011).

Nanoemulsions are particularly proper for delivering lipophilic compounds. These propose can be carried within the oil region and protected by the emulsifier interfacial membrane. There are a number of studies about the enhanced bioavailability of highly lipophilic functional components due to encapsulation within lipid droplets of nanoemulsions (Acosta, 2009; McClements and Rao, 2011). This improved bioavailability has a number of probable reasons; the small droplets of nanoemulsions are the first reason which presents a large surface area that they may be digested more rapidly by digestive enzymes in the body so that their contents are released and absorbed more easily and efficiently. Indeed the water solubility of highly lipophilic components enhances as the droplet size decreases that may increase absorption (Yu and Huang, 2012). Another reason is that the nanosized droplets of a nanoemulsion system can penetrate into the mucous layer of the small intestine; therefore it may increase their residence time near to their absorption site (Acosta, 2009; McClements and Rao, 2011). Very small droplets of nanoemulsions may be transported across the epithelium cell layer by either paracellular or transcellular mechanisms. It has been reported that by incorporating curcumin within nanoemulsions the oral availability and therefore anti-inflammation activity of curcumin of it can be increased (Wang et al., 2008). Yu and Huang (2012) reported that the oral bioavailability of curcumin in the nanoemulsion formulation increased by ninefold compared with unformulated curcumin.

It has also been reported that these formulations may also be used for oral delivery of poorly soluble nutraceuticals with high

loading capacity (Koroleva and Yurtov, 2012). It has been reported that the penetration rate of the cargos using nanoemulsions is much higher than other vehicles such as macroemulsions, micellar solutions, gels, and suspension (Koroleva and Yurtov, 2012). Nanoemulsion systems can effectively deliver nutraceuticals and food ingredients to specific sites in human body thus it may improve their efficacy (Vishwanathan et al., 2009). Recently some multilayer o/w emulsions have been developed using the layer-by-layer technique (Güzey and McClements, 2006). This method involves direct adsorption of an oppositely charged polyelectrolyte (such as biopolymers) layer on an initial layer of ionic emulsifiers (Fig. 9.4). In 2007, Djordjevic et al., reported that multilayer o/w emulsions could improve the stability of encapsulated limonene against oxidation (McClements and Rao, 2011; Djordjevic et al., 2007). The two coating layers of multilayer emulsions present strong electrostatic and steric repulsive forces that stabilize the emulsion over a wide range of situations (Moreau et al., 2003; Guzey et al., 2004).

Multiple layers strategy can keep the emulsions stable, and controls the release of cargo within the body (Gu et al., 2005).

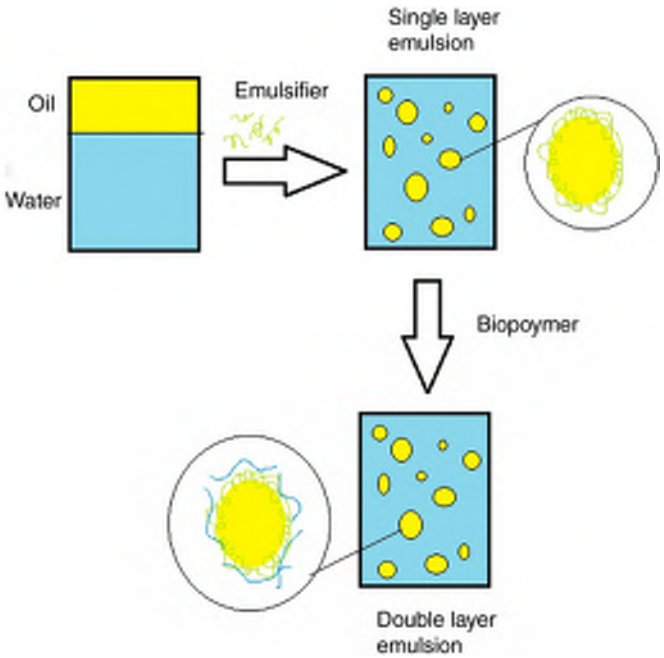


**Figure 9.4. Multiple layers nanoemulsion approach; the method includes direct adsorption of an oppositely charged polyelectrolyte (such as biopolymers) layer on an initial layer of ionic emulsifiers.**

Multilayer o/w emulsions have shown a great advantage over other delivery systems because of a complex interfacial system with simultaneously controlling the release of encapsulated bioactive cargos (Benjamin et al., 2012). These systems can retain their cargos via a variety of environments, and be tailored to release the encapsulated compound at the right point. The release of encapsulated active ingredient from emulsion droplets may be either triggered by changing the emulsion layer composition or by altering physical properties such as permeability, thickness, and charge. Specific environmental stresses may also be used to this purpose (Benjamin et al., 2012). Gu et al. reported that by varying the pH, the secondary biopolymer layer of multilayer emulsion separated due to weaker electrostatic attraction. This system can be used as a multi delivery system to deliver different types of compounds simultaneously. So far most studies around multilayer emulsions were related to improve the physical stability of such systems, rather than examining their abilities to use as delivery tool for different cargos (Gu et al., 2005).

## 8 Delivery of Bioactive Food Materials by Nanoemulsions

The beneficial aspects of nanotechnology in food sciences is in its initial stage, however, the food industry is slowly embracing it (Saberi et al., 2014). Foods are naturally rich in phytochemicals derived from plants, fruits, and vegetables. Recently, bioactive phytochemicals have gained so much attention because of their health benefits in the prevention and treatment of different diseases. Incorporation of materials such as vitamins, antimicrobials, plant sterols, lycopene, Co$Q_{10}$, and protein-based bioactives into both o/w or w/o nanoemulsions has been successfully carried out using this technology (Saberi et al., 2014). Typically, lipophilic bioactives, as functional ingredients, from natural origins including antioxidants, phytosterols, antimicrobial, ω-3 fatty acids, flavors, and other components are broadly used in food industry. As a main problem, most of these ingredients have very low solubility in water and also are unstable at a particular environmental stimulus including light, oxygen, and temperature during manufacture, transportation, storing, and application (Dizaj, 2013; Guttoff et al., 2015). From this point of view, there are some strategies to improve the low stability and bioavailability of the low-soluble bioactive materials (Atashafrooz et al., 2014; Acosta, 2009). The most important bioactives that has been studied as nanoemulsion delivery system are briefly discussed in the next sections.

## 8.1 Oil-Soluble Vitamins

As an obvious point, vitamins are essential for human body. For example, vitamin A can act as antioxidant, and it is also needed for general cellular growth and bone cell repair, and for healthy teeth (Mora et al., 2008). Vitamin D is important material for absorption of calcium, for maintaining bone health, and also it is involved in our bodies' immune function as well as preventing inflammation (Mora et al., 2008). Vitamin E is another oil-soluble vitamin with ability to support immune function and can work with vitamin A to maintain healthy cholesterol levels. It can also act as an important antioxidant in human body (Sesso et al., 2008). Nanoemulsion-based delivery systems has shown increasing application to encapsulate lipophilic bioactive components (Gulotta et al., 2014). An o/w nanoemulsion is a suitable system for encapsulation of lipophilic nutraceuticals such as oil soluble vitamins this is because of their ability to form stable and transparent systems with enhanced oral bioavailability (Guttoff et al., 2015). These products may vary in their pH, ionic composition, ingredient interactions during the preparation procedures or storage conditions. Therefore, studding the stability of vitamins and their delivery systems under the conditions that they can be applied in commercial products is necessary. In a research by Guttoff et al., nanoemulsion-based delivery systems were produced by spontaneous emulsification method for vitamin D. They evaluated the influence of system composition and preparation conditions on the particle size and stability of vitamin D nanoemulsion. According to authors the smaller oil droplets was formed when an oil/surfactant mixture is titrated into an aqueous solution. Their results showed that nanoemulsions with small droplet size formed using Tween 80 and the thermal stability of the nanoemulsions improved by adding sodium dodecyl sulfate (SDS) as cosurfactant (Guttoff et al., 2015).

The type of nanoemulsion oil carrier has a significant impact on vitamin bioaccessibility. Based on reports, nanoemulsions prepared using long chain triglycerides (such as corn or fish oil) had shown effective impact on increasing vitamins bioaccessibility. Ozturk et al. tested the influence of carrier oil type on the bioaccessibility of vitamin D3 encapsulated within o/w nanoemulsions prepared using quillajasaponin (a natural surfactant). Their results within simulated gastrointestinal tract model, mouth, stomach, and small intestine showed that the rate of free fatty acid release decreased in the following order: MCT> corn oil≈fish oil> orange oil> mineral oil. So therefore, the bioaccessibility of vitamin D3 decreased in the following order: corn oil≈fish oil> orange oil> mineral oil> MCT (Ozturk et al., 2015).

It has been reported that nanoemulsions are thermodynamically unstable systems, which break down over time. The effect of cosurfactants on the stability of vitamin nanoemulsions has been studied by researchers. Saberi et al. studied the influence of posthomogenization of cosurfactant addition on the thermal and storage stability of vitamin E acetate nanoemulsion. They evaluated the impact of cosurfactant addition (Tween-20, SDS, lauricarginate) on the stability of vitamin E nanoemulsion system. According to their results, such additions caused little change in droplet charge for nonionic Tween-20 whereas addition of anionic SDS or cationic lauricarginate cosurfactants caused the droplets to be more negatively or positively charged, respectively. Based on their results, Tween-20 showed little influence on the cloud point of vitamin E nanoemulsions and storage stability. Lauricarginate or SDS decreased the storage stability of the nanoemulsions at elevated temperatures due to the influence of these ionic surfactants on droplet growth through Ostwald ripening or coalescence mechanisms (Saberi et al., 2014).

## 8.2 Phytosterols

Phytosterols and their esters are cholesterol-lowering agents. Natural phytosterols have a low solubility in both water and fat that causes their poor absorption (Panpipat et al., 2012). Phytosterols act as inhibitors of cholesterol absorption by interfering with cholesterol synthesis and enhancing cholesterol excretion and are include brassicasterol, campesterol, stigmasterol, β-sisterol, fucosterol, δ-avennasterol, and α-spinnasterol. They are not synthesized in human body and therefore incorporation of phytosterols into foods is required (Schneider et al., 2009; Cantrill and Kawamura, 2008). Such incorporation is difficult due to the high melting point of them and their affinity in forming insoluble crystals. In aqueous-based foods, they need to be either suspended or emulsified due to stated disadvantages (Schneider et al., 2009; Panpipat et al., 2012).

To improve the intestinal absorption and bioavailability of phytosterols, conversion of phytosterols into enzymeliable lipophilic derivatives, such as fatty acid esters was one of the possible strategies. Panpipat et al. tested the improvement of productive yield related to a series of β-sitosteryl fatty acid esters in order to investigate the properties of nanoemulsion of those compounds. They prepared nanoemulsions of a series of β-sitosteryl fatty acid esters using sonication method. A nanoemulsion system can enhance the solubility and therefore bioavailability and absorption of these compounds because of the reduced droplet size of nanoemulsion system (Panpipat et al., 2012).

## 8.3 Coenzyme $Q_{10}$

Coenzyme $Q_{10}$ ($CoQ_{10}$), also known as ubiquinone, acts as an antioxidant. It is important for the cell respiration, electron transferring, and then energy production within cells (Åberg et al., 1992). Due to these main functions, it finds great application in different commercial areas such as drugs, food supplements, and cosmetics. The absorption and bioavailability of this lipophilic vitamin-like nutrientis poor from food and supplements due to insolubility in water. Some approaches including emulsification, complexation, and using liposomal systems can be improved the solubility, absorption, and therefore the bioavailability of $CoQ_{10}$ (Bhandari et al., 2007; Thanatuksorn et al., 2009).

Recent researches demonstrate that $CoQ_{10}$ nanoemulsions can improve the bioavailability of the substance after oral application (Pandey et al., 2005). According to researches, daily intake of 300 mg of $CoQ_{10}$ results in only a serum concentration of 1.8 μg/ml after 16 months while the same daily dosage of $CoQ_{10}$ nanoemulsions enhanced serum concentration up to 5.2 μg/ml after only 6 weeks (Shults et al., 2002; Zuelli et al., 2006). They added that these nanoemulsions can also improve dermal bioavailability compared to conventional formulations (Zuelli et al., 2006).

Zhou et al. formulated $CoQ_{10}$ in a lipid-free nanoemulsions system to increase its solubility and oral bioavailability using the hot HPH method. According to their results, after oral administration in rats, nanoemulsion systems meaningfully enhanced $CoQ_{10}$ bioavailability as compared to a $CoQ_{10}$ powder suspension (Zhou et al., 2014).

## 8.4 Omega-3 Polyunsaturated Fatty Acids

Health benefits of omega-3 polyunsaturated fatty acids (ω-3 PUFA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been broadly attributed owing to their benefits for reduction the risks of cardiovascular disease, mental disorders as well as diseases related to immune response disorders (Jensen, 2006; Hibbeln et al., 2006). These are essential parts of the diet since human cannot synthesis these fatty acids. The main problem is that ω-3 PUFA are very oxidatively unstable, especially in the case of DHA (Frankel, 2014). This problem can be solved by encapsulation by both the wall material and the emulsifiers coating the oil.

Gulotta et al. used a spontaneous emulsification method to prepare nanoemulsions from food-grade polyunsaturated ω-3 fatty acid delivery systems suitable for utilization in transparent foods and beverages. Their method included injecting an oily

phase (consisting of a hydrophilic surfactant, a carrier oil, and a lipophilic bioactive) into an aqueous phase (consisting of water and cosolvent) with continuous stirring. Based on their results, the size of the droplets in the prepared nanoemulsions can be controlled by manipulating the surfactant-to-oil ratio, oil composition, and aqueous phase composition (glycerol, ethanol, propylene glycol, and water). Results showed that the nanoemulsions prepared using the lemon oil were more suitable because they had relatively small droplet diameters at relatively high fish oil contents. Furthermore, lemon oil can mask any flavors related to fish oil. The authors stated that the existence of the glycerol molecules in the aqueous phase of nanoemulsion changed the nature of the colloidal structures formed at the boundary of the oil/surfactant and aqueous phases when the two phases were brought into contact. Consequently, the spontaneous formation of ultrafine droplets at the boundary may have been simplified in this way (Gulotta et al., 2014).

## 8.5 Carotenoids

Carotenoids are a class of naturally occurring pigments synthesized by photosynthetic plants, algae, bacteria, and some fungi, and contribute to the yellow and red colors of many foods (Qian, 2013). Carotenoids are in two main groups including oxygen containing (are known as xanthophylls) and without oxygen (are known as carotenes). Their main biological activities are the scavengers of active oxygen species and antioxidative activity. It has been reported that their antioxidative ability can decrease the risk of some disease such as cancer, heart disease, and macular degeneration (Stringham and Hammond, 2005). Despite great potential, these compounds have some limitations for food utilizations. The first limitation is the lipophilicity, which has low bioavailability of them. High melting point makes them to be in crystalline form at food storing and body temperatures. The distribution of carotenoids in the cellular system can also be influenced by their structures (Qian, 2013).

Qian et al. tested the nanoemulsion of β-carotene via mixed micelles using Tween-80 as emulsifier and long chain triglycerides (LCT) as carrier. Due to the large sizes of the mixed micelles, a high bioavailability (about 66%) was obtained for their nanoemulsion system. They also investigated the bioavailability of β-carotene in orange oil nanoemulsion and medium chain triglycerides (MCT). Due to the absence or small formation of mixed micelles to solubilize the β-carotene the bioavailability of β-carotene was negligible in that system (Qian, 2013).

Lycopene, a fat soluble acyclic open-chain unsaturated carotenoid, exists in red tomatoes, water melon, and their processed products with several health benefits. Undesirable oxidation as well as low solubility of this compound in water restricts its health benefits and also affects the sensory quality of food products containing lycopene. Health benefits of lycopene in food formulations can be enhanced by preventing its degradation by incorporating it into an o/w nanoemulsion. Indeed, adding oil absorption is improved when oil is added to the diet, causing much of the ingested lycopene to pass through the body. To stabilize lycopene and other carotenoids in the final food products during the preparation and storage, the development of suitable methods such as nanoemulsions is important. Isomerisation of lycopene under the influence of excess heat and light can lead to better bioavailability and stability of it due to the presence of lipids (Schieber and Carle, 2005). To enhance the bioavailability of lycopene in tomato products, oil-based food systems have been studied (Xia et al., 2006).

Kim et al. prepared lycopene nanoemulsions using an emulsification technique. Their results based on response surface methodology predicted an optimum nanoemulsion formulation containing specified amounts of lycopene extract and emulsifier as well as homogenization cycles with the smallest droplet size, maximum emulsion stability, and suitable emulsification efficiency (Kim et al., 2014).

## 8.6 Astaxanthin

Astaxanthin is a fat-soluble xanthophyll with strong antioxidant properties, which shows low oral bioavailability due to its lipophilicity. Astaxanthin contains 13 conjugated double bonds and its two functional groups, ketonic and hydroxilic groups. Some commercial products of astaxanthin as antiinflammatory and anticancer as well as immunostimulants foods, are available in the market (Higuera-Ciapara et al., 2004). The use of nanoemulsion systems is one of the efficient methods to improve the bioavailability of lipophilic entities such as astaxanthin (Affandi et al., 2011). Preparation of the o/w nanoemulsions containing astaxanthin is one of the main methods to improve its absorption and stability.

The small droplets of nanoemulsions are the first reason that presents a large surface area and then may increases the absorption of water insoluble compounds. Kim et al. prepared o/w nanoemulsions of astaxanthin by HPH method in the mean diameter ranged from 160 to 190 nm. According to their results, the nanoemulsions prepared with glyceryl citrate/lactate/linoleate/oleate showed smaller particle sizes compared to the emulsion prepared

with hydrogenated lecithin. Results showed that the nanoemulsion was not mainly affected during storage (under light and thermal condition) for 1 month. They stated that because of a zeta potential of less than −41 mV, the nanoemulsion has been stable (Kim et al., 2012).

## 8.7 Antimicrobials

A number of reports had shown that nanoemulsion can be an effective means of encapsulating and delivering antimicrobial agents (Teixeira et al., 2007; Hamouda et al., 1999). This type of nanoemulsion has mainly been developed for the decontamination of food packaging equipment (Sekhon, 2010). These results can lead to the design and application of nanoemulsions as antimicrobial delivery systems in the food industries (Hamouda et al., 2001).

Spore-forming microorganisms become a serious food safety risk due to their probable proliferation (Ultee et al., 1999). A strategy to reduce the proliferation of microorganisms is the use of antifungal and antibacterial agents (Conner, 1993; Ultee et al., 1998). Preservation technologies possess ever-increasing important in modern food industries. Mild preservation processes are often combined to gain safe products with improved quality. Essential oils can be used as antimicrobial compounds due to their safe nature when comparing to synthetic additives. However, their lipophilic nature is the main limitation to use in food products that they need to overcome using encapsulation in a proper carrier (Donsì et al., 2014). Solid food preservation with essential oils needs to utilize appropriate carriers. For this purpose, nanoemulsions can be used because they are able not only to promote dispersion (in the aqueous part of foods), but also to improve mass transfer within the food matrix (Donsì et al., 2014).

Carvacrol is the major component of the essential oil fraction of oregano and thyme (Lagouri et al., 1993) that exerts a distinct antimicrobial action among the various group of chemical components in essential oils. Carvacrol is added to different food products such as baked goods (15.75 ppm), nonalcoholic beverages (28.54 ppm/0.18 mM), and chewing gum (8.42 ppm) (Burdock, 2009). Donsi et al. studied the fundamental aspects of the use of nanoemulsion delivery systems for essential oils. They investigated the impact of nanoemulsion characteristics on the mass transfer in solid food products as well as their antimicrobial effects (Donsì et al., 2014). Furthermore, the impact of nanoemulsion characteristics on the rate of infusion of carvacrol in food matrices was studied using image analysis of micrographs

of histological sections of zucchini cylinders upon infusion with different emulsions stained with fluorescent dyes as well as using microbiological assays in zucchini and cooked meat sausages. Their results showed that nanoemulsions meaningfully enhanced effective diffusivity that lead to more efficient antimicrobial action of carvacrol (Donsì et al., 2014).

Donsì et al. investigated the preparation of nanoemulsion delivery systems by HPH. They studied the antimicrobial activity of some essential oil components in the nanoemulsion systems against *Escherichia coli*, *Lactobacillus delbrueckii*, and *Saccharomyces cerevisiae*. Their emulsion systems were including limonene, carvacrol, and cinnamaldehyde encapsulated in the sunflower oil droplets. The prepared system stabilized by different emulsifiers included lecithin, pea proteins, sugar ester, and a combination of Tween-20 and glycerol monooleate. Their results revealed that obtained antimicrobial activity was mainly affected by the nanoemulsion system. According to authors, the effect of the nanoemulsion system, as delivery systems, on the antimicrobial activity was because of the concentration of essential oil components in the aqueous phase. In fact, the interaction of these active components with the cell membrane of microorganisms is influenced by the dissolution of them in the aqueous phase (Donsi et al., 2012).

Cheng et al. presented a method based on spontaneous emulsification for production of antimicrobial nanoemulsions using essential oils. According to authors, oil phase composition (carvacrol to MCT mass ratio) showed a main impact on initial droplet diameter and the smallest droplets being formed at 2.5 wt% carvacrol and 7.5 wt% MCT. Their results showed that the carvacrol concentration had main effect on the stability of the nanoemulsions and the droplet growth during storage. They proposed that the concentration of carrier oil must be well-ordered to gain proper physical stability alongside with good antimicrobial efficacy. The stability of the nanoemulsions decreased as the carvacrol concentration in the oil phase increased. The carvacrol concentration showed converse effect on the antimicrobial efficacy of the nanoemulsions so that increased as the carvacrol concentration increased. Tween-80 gave the smallest droplets from a group of surfactants including Tween-20, 40, 60, 80, and 85. As the total surfactant concentration was increased from 5 to 20 wt%, the droplet size also decreased from 5000 to 25 nm (Chang et al., 2013).

In another research by Chang et al., they prepared o/w nanoemulsions for thyme as potential antimicrobial delivery systems and examined physically stable thyme oil nanoemulsions for their antimicrobial activities against an acid-resistant spoilage yeast,

*Zygosaccharomyces bailii* (ZB). Due to the relatively high water solubility of thyme oil, their prepared nanoemulsions were highly unstable to droplet growth and phase separation that was related to Ostwald ripening. They could inhibit Ostwald ripening by mixing thyme oil with a water-insoluble ripening inhibitor including 60 wt% corn oil or 50 wt% MCT in the lipid phase before homogenization. Finally using ripening inhibitor yielded nanoemulsions with proper physical stability. However, increasing the ripening inhibitor concentrations in the lipid phase reduced the antimicrobial efficacy of the nanoemulsions. Ripening inhibitor types also effected on antimicrobial activity of nanoemulsions and at the similar concentration in the lipid phase, MCT decreased the antimicrobial efficacy of thyme oil more than corn oil (Chang et al., 2012). The most important bioactives that has been studied as nanoemulsion delivery system are summarized in Table 9.2.

## 9 Challenges of Nanoemulsion

Despite the great potentials of nanotechnology, we also need to point out what can be seen as the negative side of this technology. The application of nanoemulsions in food industries have some challenges that need to be resolved; from the production process (especially their cost) to the characterization of both the prepared nanoemulsions and the food product in terms of product safety and acceptance (Siegrist et al., 2007).

Like other nanoparticles, nanoemulsions may show cellular toxicity effects. However, there is little experimental evidence on the toxicity effects of food-grade nanoemulsions. This challenge is serious for nanoparticles because their size allows them to easily penetrate cell membranes and biological barriers, including, perhaps, the blood–brain barrier (Yang et al., 2010). Some safety concerns associated with the utilization of very small lipid droplets in foods have been reported so far. For example, systems like nanoemulsions may change the extent or route of absorption of lipophilic components. Therefore, the bioavailability or probable toxicity of a lipophilic material encapsulated within nanoemulsion system containing lipid droplets may be significantly different from when it is dispersed within a bulk lipid phase. Currently, there is no standardized protocol to assess the potential toxicity of nanoemulsions proposed for applications in food industries (Maynard et al., 2006).

Generally, nanotechnology needs to become easier to use in order to convert to an efficient standard methodology. Another important challenge of nanoemulsions is the cost for development

# Table 9.2 The Most Important Bioactives That Has Been Studied as Nanoemulsion Delivery System

| Compound Name | Benefits as Food | Limitations for Use in Food Systems or Products | Usefulness of Nanoemulsion |
|---|---|---|---|
| Oil soluble vitamins | – As antioxidant, needed for general cellular growth and bone cell repair, and for healthy teeth (vitamin A) (Mora et al., 2008).<br>– As important material for absorption of calcium, needed for maintaining bone health, involved in our bodies' immune function, and can help to prevent inflammation (vitamin D) (Mora et al., 2008).<br>– As an important antioxidant, supports immune function and works with vitamin A to maintain healthy cholesterol levels (vitamin E) (Sesso et al., 2008). | – Low solubility in water.<br>– Variable and unstable in during the preparation procedures or storage conditions (low stability) (Gulotta et al., 2014). | – The improvement of the stability, absorption and bioavailability.<br>– To form transparent systems Dizaj (2013), Guttoff et al. (2015), Ozturk et al. (2015). |
| Phytosterols | – Cholesterol lowering agents in human by interfering with cholesterol synthesis and enhancing cholesterol excretion (Cantrill and Kawamura, 2008). | – Low solubility in both water and fat causing a poor absorption of them (Cantrill and Kawamura, 2008; Schneider et al., 2009). | – Enhanced absorption and bioavailability Li et al. (2012) |
| Coenzyme $Q_{10}$ | – For cell respiration<br>– Electron transfer<br>– For energy production within cells<br>– Act as antioxidant. Shults et al. (2002) | – Poor absorption and bioavailability due to insolubility in water (Shults et al., 2002; Thanatuksorn et al., 2009; Zuelli et al., 2006). | – The improvement of the solubility, absorption and bioavailability. Shults et al. (2002), Thanatuksorn et al. (2009), Zuelli et al. (2006) |

# Table 9.2 The Most Important Bioactives That Has Been Studied as Nanoemulsion Delivery System (*cont.*)

| Compound Name | Benefits as Food | Limitations for Use in Food Systems or Products | Usefulness of Nanoemulsion |
|---|---|---|---|
| Omega-3 Polyunsaturated Fatty Acids | – Able to reduce the risks of cardiovascular disease, diseases affected by immune response disorders and mental disorders. Jensen (2006), Hibbeln et al. (2006) | – Very oxidatively unstable.<br>– Low solubility in both water and fat causing a poor absorption of them. Frankel (2014) | – The improvement of the stability, absorption and bioavailability. Gulotta et al. (2014) |
| Astaxanthin | – As anticancer.<br>– Antiinflammatory<br>– Immunostimulant Higuera-Ciapara et al. (2004) | – Low solubility in both water and fat causing a poor absorption of them. Affandi et al. (2011) | – The improvement of the stability and bioavailability. Kim et al. (2012) |
| Carotenoids | – Antioxidative activity.<br>– Decrease the risks of some disease such as cancer, heart disease and macular degeneration. Stringham and Hammond (2005) | – Low bioavailability of them.<br>– High melting points (make them to be in crystalline form at food storage and body temperatures).<br>– The distribution of carotenoids in the cellular system is also influenced by their structures. Qian (2013) | – The improvement of the stability, absorption and bioavailability. Xia et al. (2006), Kim et al. (2014) |
| Antimicrobials (Essential oils) | – For the decontamination of food packaging equipmentand for application to various food surfaces.<br>– To reduce the proliferation of microorganisms as the antifungal and antibacterial agents Teixeira et al. (2007), Hamouda et al. (1999) | – Their lipophilic nature is the main limitations to use in food products Donsì et al. (2014) | – The improvement of the stability, absorption and bioavailability.<br>– Nanoemulsions lead to more efficient antimicrobial action Chang et al. (2012) |

and scaling-up of high-energy methods that are still very expensive due the pressures needed to process high volumes. Developing the simpler methods of nanoemulsion production or further technical improvements in the conventional methods can be useful to reduce such costs. Among the nanoemulsion preparation methods, the spontaneous emulsification method as simple and low-cost technique has great potential for forming nanoemulsion-based delivery systems for foods. As a major advantage, this method needs no sophisticated or expensive equipment to form the nanoemulsions. However, a major disadvantage of this method is that a relatively high surfactant concentration is needed to form nanoemulsion (Gulotta et al., 2014).

The other limitation about the development of nanoemulsions is their stability. According to reports these systems can remain stable even for years, however, due to the small droplet size the Oswald ripening could damage nanoemulsions. Some main reasons including creaming (Stokes, 1851), flocculation (Denkov et al., 1995), coalescence(Boschkova et al., 2002), and Ostwald ripening cause to the instability of nanoemulsion (Lifshitz and Slyozov, 1961). Ostwald ripening is the main reason for nanoemulsion instability because due to minimizing the particle size of nanoemulsion and using nonionic surfactants. Because of Ostwald ripening, two droplets diffuse and become one large droplet and therefore after the storage for a long time period, droplets size distribution shifted to large sizes and the nanoemulsion became unclear. It is also known that Ostwald ripening is a problem during the delivery of formulations. Then, in most cases it is necessary to prepare nanoemulsions shortly before their use (Sharma et al., 2010). Tadros et al. (2004) reported that the addition of polymeric surfactants on the interface, which increase the elasticity of droplets and further reduce the effect of Ostwald ripening.

In reverse nanoemulsions the Ostwald ripening can be prevented by adding an electrolyte to the internal phase. Indeed, diffusion of water from smaller to larger droplets of the internal phase cause to an increase in the electrolyte concentration in the smaller droplets and then the osmotic pressure would be increased. This process can prevent further water transfer and therefore Ostwald ripening (Koroleva and Yurtov, 2012).

## 10 Conclusions

Food nanoemulsion formulations can present some advantages over conventional delivery systems in the case of nutraceuticals or food ingredients. Delivery of bioactive components using nanoemulsion-based systems provides a maximum

stability, high protection, and also permits an efficient release of encapsulated components into the body. Reviewed literature showed that nanoemulsions can be useful for delivery and control the release of nutraceuticals or other bioactive components. Combination of nanoemulsion methods with progressive processing technologies can develop novel encapsulated products with ability to release food bioactive components in the gastrointestinal tract by a controlled release profile. These products can be included in different designs with diversity in ingredients in a wide range of materials. The small size droplet of nanoemulsion makes its appearance transparent and its stability improved by Brownian motion that plays a main role to avoid creaming. The small particles of a nanoemulsion system can lead to enhancement of delivery characteristics; improve biodistribution and pharmacokinetics of food ingredients. Nanoemulsion requires little energy input and possesses a long shelf life due to thermodynamic stability. Nanoemulsion delivery system improves the solubility and therefore the bioavailability of food ingredients including antimicrobials, antioxidants, vitamins, minerals, and the other food compounds. However, in thermodynamic point of view, it has been shown that food emulsions are unstable and they ultimately break down because of the increasing of interfacial area after emulsification. Thermodynamically instability leads to the physical instability of an emulsion system. The processes such as flocculation, creaming, coalescence, phase inversion, and Ostwald ripening are the different outcomes of the physical instability. However, nanoemulsion-based delivery systems are predominantly suitable technology for the production of encapsulating systems for functional compounds as it avoids their degradation and improve their bioavailability. Since requirements for safer food products and the need for delivery systems with the ability to encapsulate, protect, and release functional compounds, investigators are recently concentrating their efforts in nanobased technologies in order to resolve the issues related to food and nutrition.

## 11 Future Outlook

Due to outstanding properties, nanoemulsions can apply in the future in order to develop food products especially low-fat ones. Despite of all advances in the research areas, no marketable products are in the form of food nanoemulsions so far. With increasing researches and also development of related knowledge, nanotechnology can significantly contribute to food industries for certain applications including production of transparent foods

and beverages with increased bioavailability and improved physical stability. The attention of research and development efforts on nanoemulsions has increased because of their potential advantages over conventional emulsions. There is some outlook in the application of nanoemulsions in food systems in terms of the production process as well as the characterization of both resulting nanoemulsions and food systems. For example, economically suitable procedures must be identified for scaling-up and to fabricate food-grade nanoemulsions. Discovering and developing the simpler methods of nanoemulsion production or further technical improvements in the conventional methods can be useful for nanoemulsions scaling-up. In addition, appropriate food-grade ingredients must be identified and utilized for preparing food nanoemulsions. A number of the utilized components in production of nanoemulsions are synthetic surfactants, synthetic polymers, synthetic oils, and organic solvents, for which they are not suitable for food applications. Production of nanoemulsions from acceptable commonly used food-grade components such as flavor oils, proteins, and polysaccharides must be developed in order to gain efficient healthy and safe food products with improved bioavailability and stability in the near future.

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# 10

# BIOPOLYMERS-EMBEDDED NANOEMULSIONS AND OTHER NANOTECHNOLOGICAL APPROACHES FOR SAFETY, QUALITY, AND STORABILITY ENHANCEMENT OF FOOD PRODUCTS: ACTIVE EDIBLE COATINGS AND FILMS

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## 1 Introduction

Edible films (EFs) and edible coatings (ECs) based on natural ingredients provide one of the most promising and currently investigated approaches for food products' quality and shelf-life enhancement (Valencia-Chamorro et al., 2011). EFs can be used as covers, wraps, packages, or separation layers for food products. ECs form the film directly onto a food surface and provide a part of the final product (Bourtoom, 2008).

EFs and ECs can inhibit food senescence processes, protect it from mechanical and microbial damages, decrease escape of volatile sensitive food components, and minimize undesired modifications. They can also improve food appearance and contribute to the environment (Dhall, 2013). Due to their biodegradable nature, EFs and ECs provide a viable alternative to contamination caused by synthetic packaging and coatings. Rising public requirements

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00010-6

for healthier food that does not contain synthetic additives, together with awareness regarding environmental issues have promoted a substantial interest in EFs and ECs and encouraged advanced developments in this research field (Bourtoom, 2008; Valencia-Chamorro et al. 2011; Umaraw and Verma 2015). An example of such a development is the utilization of EFs and ECs as a matrix for the delivery of active agents (natural antimicrobials, nutraceuticals, antibrowning agents, natural flavor, and aroma compounds). Such active EFs and ECs may effectively extend food shelf life and at the same time improve its quality and safety (Debeaufort et al., 1998; Dhall, 2013).

In recent years, novel nanotechnology techniques have begun being exploited in the EFs and ECs research field. Nanotechnology especially is utilized for the incorporation of active compounds into the EFs and ECs matrices. In this chapter, we provide a brief description of the content, properties, and applications of EFs and ECs, and review nanotechnology application in this field. The chapter covers the utilization of nanoemulsions, nanoparticles, nanofibers, and layer-by-layer as techniques to form active EFs and ECs. Active packages that are based on nonedible materials are not covered in this chapter.

## 2 Materials Used to Form Edible Films and Edible Coatings Matrices

EFs' and ECs' most prominent features are often determined by a formulation's main component (its matrix). EFs and ECs matrix-forming materials can be classified into three groups: (1) lipids, (2) proteins, and (3) polysaccharides. In addition, the film or coating matrix can be a composite, based on a combination of several materials.

### 2.1 Lipids

Lipids were the first materials that composed the earliest EFs and ECs. In the 12th century, waxes were applied on citrus fruit and later on in the 16th century lipids were used to cover the surface of fresh meat in a process called "larding." Waxes, paraffin, acetoglycerides, organic fatty acids, and resins are among the materials classified as lipids that form EFs or ECs (Valencia-Chamorro et al., 2011). These materials' hydrophobic nature results in good moisture barrier properties, which are important for preventing physiological deterioration of food products (Umaraw and Verma, 2015). In addition, many lipid materials may impart

an attractive gloss once applied to food products. However, lipids have poor mechanical and optical properties and lipids-based films are therefore usually nontransparent, relatively thick, and brittle (Bourtoom, 2008). An additional limitation of lipids-based coatings is their poor adhesion to food products with hydrophilic surfaces (Dhall, 2013).

## 2.2 Proteins

Various proteins can be used as EFs and ECs matrix materials. Among the reported proteins are gelatin, casein, whey protein, corn zein, wheat gluten, soy protein, mung bean protein, peanut protein, and more (Arvanitoyannis and Gorris, 1999). Proteins can interact in aqueous solutions and create ionic bonds, hydrogen bonds, or hydrophobic interactions. They are not always water soluble, as this depends on their constituting functional groups. If the selected proteins tend to form ionic or hydrogen bonds, the formed EFs and ECs will usually display good gas permeability and mechanical properties, but a low moisture barrier (Umaraw and Verma, 2015). Proteins with poor water solubility are usually less permeable to gases and vapors. Since proteins offer a high variety of properties in terms of film forming ability and physical and mechanical features, they can fit specific needs for different food products (Arvanitoyannis and Gorris, 1999). However, proteins are considered as materials that have a relatively high probability of causing allergenic reactions, thus limiting their use (Bourtoom, 2008).

## 2.3 Polysaccharides

Polysaccharides are extensively investigated biopolymers in the field of EFs and ECs (Debeaufort et al., 1998). They are highly available and often of relatively low cost. Most of the polysaccharides dissolve in water and form strong hydrogen bonds that impart good mechanical properties on the formed EFs and ECs (Valencia-Chamorro et al., 2011). A hydrophilic nature of polysaccharides make them permeable to moisture and reduce their ability to prevent water loss from food. A notable advantage of polysaccharides is their defined chemical structure that allows controlling EFs' and ECs' features. Unlike lipids, polysaccharides usually have a very low impact (or none at all) on food appearance or flavor. Unlike proteins, polysaccharides usually do not arouse allergic reactions (Bourtoom, 2008; Umaraw and Verma, 2015). The most investigated polysaccharides in the EFs and ECs field are cellulose derivatives, chitosan, alginate, and other algae derived materials such as starch, pectin, and gums (Bourtoom, 2008).

### 2.4 Composites

Composite EFs or ECs contain materials from two different classification groups, such as lipids and proteins, lipids and polysaccharides, or polysaccharides and proteins. Formation of EFs and ECs based on a combination of two or more components can provide an improved efficacy in comparison to a single-type based formulation. With this approach, the weakness of one substance may be compensated by the addition of another (Kester and Fennema, 1986). For example, in order to overcome the poor mechanical strength of lipid substances, they can be combined with water soluble proteins or polysaccharides (hydrocolloids), which are known for their excellent mechanical properties. At the same time, the high moisture permeability which characterizes hydrocolloids can be reduced by lipids (Dhall, 2013).

## 3 Applications

In light of the wide variety of EFs and ECs present nowadays, it is possible to find the most suitable and effective formulations for almost every type of food product (Debeaufort et al., 1998). So far, EFs and ECs have been applied on meat, poultry, seafood, grains, nuts, confectioneries, fruits, and vegetables (whole or fresh-cut). The following sections present a few examples for such applications.

### 3.1 Meat, Poultry, and Seafood

Application of EFs and ECs on meat, poultry, or seafood products, whether fresh or frozen, benefits numerous advantages in terms of product quality and safety and also allows prolonging the products' shelf life. EFs or ECs reduce moisture loss and therefore inhibit texture degradation, avoid unattractive dripping of product juices, and reduce economic losses (Umaraw and Verma, 2015). EFs and ECs may also reduce biochemical product degradation since they inhibit aggravating proteolytic enzymes, protect products' lipids and proteins from oxidation, delay products' rancidity, and prevent undesired color changes (Gennadios et al., 1997). Active antimicrobial EFs and ECs also contribute to products' microbial safety and decrease their microbial spoilage (Khan et al., 2013).

### 3.2 Grains and Nuts

Grains and nuts are defined as products with low moisture content (around 10%). The reasons to use ECs on low moisture

content products are as follows; ECs help control an undesirable "mass" transfer between the products and atmosphere. Moisture transfer from the atmosphere leads to water absorption that affects products' sensorial quality and promotes their spoilage (Mendoza et al., 2010). Mass transfer from the products causes surface dehydration, aroma and flavor loss and fat migration (Umaraw and Verma, 2015). By adding antioxidants to ECs, it is possible to minimize lipid oxidation in the products (ECA et al., 2014). Grains and nuts are considered as fragile foods and can easily break by pressure and vibration, and therefore ECs can also provide protection by layering products from physical and mechanical damages (Kester and Fennema, 1986). In addition, ECs can impart brightness, provide a homogeneous stable color and keep the product surface nonsticky (Dhall, 2013).

## 3.3 Confectioneries

Application of ECs on the surfaces of candies and confectioneries can help to reduce unwanted phenomena attributed to these products such as stickiness, agglomeration, moisture absorption, and oil migration in the case of fat containing confectioneries (Debeaufort et al., 1998). Hydrocolloids-based EFs can delay chocolate whitening or blooming due to lower lipid permeability (Nelson and Fennema, 1991).

## 3.4 Fruits and Vegetables

High losses of fresh fruit and vegetables occur during the products' storage period. They are mainly caused due to moisture loss, shrinkage, microbial and biochemical damage, mechanical injuries, and senescence processes (Dhall, 2013). Maintaining the quality of fruits and vegetables during their storage period is probably more complicated and challenging compared to other food sectors due to the fact that fresh produce continue breathing even after their harvest (Valencia-Chamorro et al., 2011). Therefore, when applying an EC on fresh fruit or vegetables, several considerations should be taken into account. The main consideration is attributed to the EFs' or ECs' gas permeability features. EFs and ECs may reduce the income of oxygen during storage periods. A lack of oxygen leads to a disruption in ethylene production, resulting in an inhibition in ripening processes. This means the produce remains firm, fresh, and attractive for longer periods (Park, 1999). On the other hand, application of EFs and ECs with very low gas permeability can adversely modify fruit' or vegetables' internal atmospheres and encourage development of anaerobic conditions

that lead to alcoholic fermentation and off-flavors buildup (Mishra et al., 2010). Consequently, it is very important to choose the most appropriate coating for the specific produce that ensures a gentle balance between inhibition of senescence processes and prevention of off-flavors development (Debeaufort et al., 1998). Additional requirements that should be satisfied upon application of ECs and EFs on fresh produce include perfect adhesion, effective moisture barriers, a homogeneous cover, and pleasant sensorial effects (Lin and Zhao, 2007). So far, ECs have been extensively applied on citrus fruit, apple, pear, cherry, banana, guava, mango, lychee, date, coconut, peach, grape, melon, carrot, cucumber, root crop, pumpkin, sweet corn, eggplant, pepper, tomato, asparagus, celery, radish, potato, turnip, and more (Baldwin 1994; Debeaufort et al., 1998).

ECs are also being utilized on minimally processed produce such as fresh-cut fruit and vegetables. In order to maintain their quality during storage, one should face the unique challenges attributed to fresh-cut produce such as browning, texture breakdown, rapid off-flavor development, and enhanced proneness to microbial spoilage (Rojas-Graü et al., 2009a). Another major challenge of fresh-cut produce is their hydrophilic surfaces that cause adhesion complications (Vargas et al., 2008). Despite all these difficulties, ECs are much desired in the field of fresh-cut fruit and vegetables. Fresh-cut fruit and vegetables suffer from extremely short storage periods and ECs can significantly prolong their shelf life. ECs were reported to profit fresh-cut apple, melon, banana, pear, pineapple, mango, strawberries, raspberries, water chestnut, papaya, carrots, broccoli, and more (Galgano et al., 2015; Rojas-Graü et al., 2009a; Vargas et al., 2008).

## 4 Edible Films and Edible Coatings Parameters that Effect Food Quality

Analyzing the quality attributes of coated products is usually performed to evaluate the ECs' and EFs' impact on them. This impact is usually derived from the formulation content and microstructure, which dictate physicochemical and biological properties of the film or coating. Food features and especially food surface properties are also of high importance (Vargas et al., 2008).

### 4.1 Moisture Loss

Moisture loss from food products causes an unattractive texture and appearance (Dhall, 2013). In addition, moisture loss may

lead to economic losses because food is often sold by weight. These problems constituted the original reasons for developing coating (and later on, edible coatings) for food products (Arvanitoyannis and Gorris, 1999). Application of ECs enables to retard moisture loss. Lipids-based EFs and ECs retard moisture loss due to their hydrophobic properties that result in a high moisture barrier. Hydrocolloids-based EFs and ECs usually have relatively low moisture barriers. They can, however, delay moisture loss from food products by sacrificing their own moisture first (Bai and Plotto, 2011). Water vapor permeability (WVP) is measured to define EFs' and ECs' moisture barrier features. There is a method based on gravimetric techniques, which enables to measure WVP of edible films (Baldwin, 1994; Vargas et al., 2008). However, this methodology is not compatible to measure WVP of edible coatings since food products have irregular shapes that complicate the accurate measurement of their surface area. It is therefore always recommended to measure the direct weight loss from coated foods (Vargas et al., 2008).

## 4.2 Mechanical Properties

The terms "texture" or "mechanical properties" for food products refer to firmness, crispness, juiciness, and toughness (Baldwin, 1994). Food texture can usually be evaluated by the consumers and therefore often serves as a product quality indicator (Dhall, 2013). Accurate texture measurements can be obtained using apparatus such as a texture analyzer, a universal tensile testing machine, or a dynamical mechanical thermal analyzer, which provide data on the tensile strength, elongation, deformability, and elastic modulus of the food product (Vargas et al., 2008). Generally, poor mechanical properties are resulted by moisture loss, microbial damage, and in the case of fresh produce, also overripening. Application of ECs can reduce occurrence of these damages and therefore, maintain the foods' texture (Dhall, 2013).

## 4.3 Gas Permeability

EFs' and ECs' gas permeability (to $O_2$ and $CO_2$) is an attribute that is mostly important for application on fresh fruit and vegetables (Bai and Plotto, 2011). As was discussed previously, EFs or ECs have to balance between the purpose of inhibiting overripening and senescence processes of the fresh produce by slowing gas exchange, and allowing gas exchange to avoid development of adverse anaerobic conditions, which lead to alcoholic fermentation and off-flavors (Lin and Zhao, 2007; Mishra et al., 2010; Park, 1999). The main parameters that affect EFs' and ECs' gas permeability are the formulation components and thickness.

### 4.4 Adhesion

EFs and ECs are designed to serve as a protective layer on the food surface, hence they should provide good adhesion to the food surface. This is especially true for ECs (Falguera et al., 2011). The parameters that are responsible for good adhesion involve matrix components that dictate viscosity, density, and surface tension of the formulation, and the food's surface tension and roughness (Lin and Zhao, 2007). For instance, hydrophilic ECs show poor adhesion to naturally waxed fruits because of their surfaces' hydrophobic character. However, addition of surfactants helps to reduce the coating formulation's surface tension and enhance the adhesion of hydrophilic formulations to hydrophobic food surfaces (Vargas et al., 2008).

### 4.5 Appearance

The term "general appearance" encompasses color, glossiness, and absence of defects in shape or skin. Food products' general appearance is the most important feature that will eventually determine whether they will be purchased (Falguera et al., 2011). Products are susceptible to several negative effects during their storage periods, which may hinder their appearance. Oxidation reactions and enzymatic browning cause undesired color changes. Moisture loss might lead to inconsistent unattractive shrinkage and reduction of gloss. Pests, microbial damage, and mechanical damages may cause skin blemishes (Lin and Zhao, 2007). Applications of EFs or ECs can inhibit these deleterious effects by serving as a protective layer. This is especially the case when EFs or ECs carry active agents like antimicrobial substances or antioxidants. Additionally, some materials such as chitosan, gelatin, and lipid waxes can enhance the food products' gloss levels and increase their visual attractiveness in the eyes of the consumers (Bai and Plotto, 2011).

## 5 Incorporation of Active Agents into Edible Films and Edible Coatings Matrices

One of the most innovative functions of EFs and ECs utilizes their potential to serve as a matrix for agents that have beneficial activity (Dhall, 2013). For this reason, they are called "active edible films or coatings" (Debeaufort et al., 1998). The most common active agents that are currently incorporated into EFs and ECs are antimicrobials and antioxidants. There are, however, many types of active compounds such as texture enhancers,

aroma, and flavor compounds and nutraceuticals (Umaraw and Verma, 2015). It is important to note that all additives incorporated into EFs and ECs should be edible and generally recognized as safe (Falguera et al., 2011). Examples of active compounds and their incorporation into EFs and ECs are described in the next sections.

## 5.1 Antimicrobials

The first active EFs and ECs were developed for the delivery of antimicrobial substances. Currently, antimicrobials also represent the most popular class of compounds that are incorporated into active EFs and ECs. These antimicrobial agents include organic acids, polypeptides (lysozyme, peroxidase, lactoferrin, nisin) and essential oils (oregano, lemongrass, cinnamon, tea tree, etc.). Organic acids such as acetic, benzoic, citric, fumaric, lactic, malic, propionic, sorbic, succinic, and tartaric acid are more active in their neutral (protonated) form and can penetrate the microorganism cytoplasm (Dhall, 2013). Essential oils originated from plants are a prominent example of natural antimicrobial substances. Numerous studies have reported good antimicrobial effects of essential oils against different types of microorganisms including human pathogens (Rojas-Graü et al., 2009a). However, it is important to remember that along with a beneficial antimicrobial activity, essential oils may also affect foods' organoleptic features (Franssen and Krochta, 2000).

Incorporation of antimicrobials into EFs and ECs helps to increase food products safety and prolong their shelf life (Valencia-Chamorro et al., 2011). However, the question arises: Why are antimicrobial additives not implemented directly onto the food surface? When antimicrobial substances are applied directly onto the food surface they can easily diffuse or react with food components. This results in loss of their bioactivity or even formation of new and not necessarily safe compounds. Incorporation into EFs or ECs carrying matrices effectively helps to control and maintain antimicrobials' efficacy (Rojas-Graü et al., 2009a).

## 5.2 Antioxidants

Antioxidants can increase food safety and quality by binding free radicals and protect the products from damaging oxidative reactions. These reactions, such as enzymatic oxidation, may cause undesired color changes, altered flavor and odors (oxidative rancidity), as well as softening and even nutritional loss (Villa-Rodriguez et al., 2015). Many types of antioxidants were examined as active agents in EFs and ECs (ECA et al., 2014). A growing

demand for natural components in foods has induced research on natural-origin antioxidants such as plant extracts, essential oils, α-tocopherol, ascorbic, and citric acid, individually or combined (Rojas-Graü et al., 2009a).

## 5.3 Nutraceuticals

Nutraceuticals are compounds derived from food sources that provide health benefits (Cencic and Chingwaru, 2010). Incorporation of nutraceuticals can also compensate the nutritional losses caused to foods during their processing (Quirós-Sauceda et al., 2014). However, direct incorporation of nutraceuticals can be problematic because they are often sensitive to oxidation processes and undesired side reactions with food components. EFs and ECs may serve as both a delivery and protective matrix for these sensitive compounds (Dhall, 2013). Currently, only few studies have reported the integration of nutraceuticals in EFs and ECs in order to enhance nutritional quality (Han et al., 2004; Mei and Zhao, 2003; Park and Zhao, 2004; Tapia et al., 2008).

## 5.4 Texture Enhancers

Deterioration of mechanical properties is a common phenomenon that occurs during processing and storage due to moisture loss and enzymatic activity (Dhall, 2013). The dramatic softening can be prevented by application of ECs with good moisture barrier features and by treatment with texture enhancers such as calcium salts (Rojas-Graü et al., 2009a). Coatings or films with a good moisture barrier can physically prevent the escape of water, while calcium ions (or other divalent cations) interact with carboxylate containing polymers to form a cross-linked network that increases firmness. For instance, calcium ions interact with carboxylate containing pectic enzymes in fruits and vegetables, thus delaying senescence and controlling physiological disorders (Poovaiah, 1986).

## 5.5 Flavor and Aroma Compounds

During prolonged storage periods, flavor and aroma components found in foods may be altered or escape because of their volatile nature or degradation (Belitz et al., 2009). Since flavor is a key contributor for food acceptance, its maintenance is essential for later consumption (Quirós-Sauceda et al., 2014). Essential oils are typical example for natural flavoring agents and include a wide diversity of aroma components (Burt, 2004). Usually, natural

aroma compounds are sensitive, volatile, and expensive. In addition, incorporation of flavoring agents into solid foods is a technically difficult task. EFs and ECs allow a delicate and effective addition of natural flavor compounds into solid food products (Bai and Plotto, 2011).

# 6 Nanotechnologies in Edible Films and Coatings

Nanotechnology is a multidisciplinary technological and scientific field that undergoes rapid development. Size reduction to the nanometer scale ($10^{-9}$ m) may fundamentally modify compounds' physical, chemical, and biological properties resulting in new application potential (Duncan, 2011). The potential benefits of nanomaterials in the food industry are tremendous and cover many aspects such as food safety, nanosensors, nutrients delivery systems, bioavailability, new materials for pathogen detection and packaging materials (Duran and Marcato, 2013). In the following sections we present various nanotech approaches in the field of EFs and ECs.

## 6.1 Nanoemulsions

Emulsion is defined as a system containing two immiscible liquids in which one is dispersed homogenously in the other, in the form of globules (Fig. 10.1).

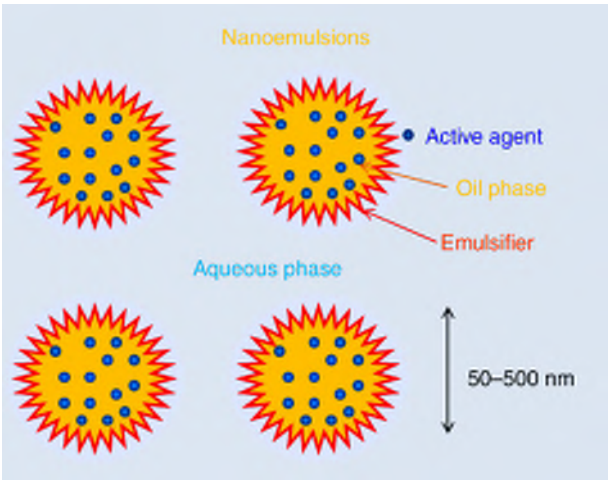


**Figure 10.1. Schematic representation of oil/water nanoemulsion.**

The preparation method of conventional emulsions involves addition of surfactants and applying mechanical homogenizing (Duran and Marcato, 2013). The size of the droplets and their distribution are two of the most significant parameters on the emulsion nature in terms of stability, rheology, appearance, color, and texture. Therefore, fluctuation in these parameters may lead to sizable alterations in emulsion features. In this sense, nanoemulsions and conventional coarse emulsions (both based on the same contents) can have completely different features as well as applications (Duncan, 2011).

Nanoemulsions are a class of emulsions with droplet sizes that typically range between 50 and 500 nm, although there is no clear agreement in the scientific community on this point (Mason et al., 2006). In order to reach this size scale, powerful dispersing equipment such as ultrasonicators, micorfluidizers, and high-pressure homogenizers are usually utilized (Gutiérrez et al., 2008). Due to a higher ratio of droplet surface per mass unit, nanoemulsions have high delivery/encapsulation ability. In addition, activity and bioavailability of an active agent are increased upon nanoemulsification (Chaudhry et al., 2008).

Nanoemulsions are utilized in the food sector, mainly as a method to disperse lipophilic active ingredients in an aqueous media (Mason et al., 2006). They present unique physicochemical and functional characteristics like supreme kinetic stability and clear or translucent appearance that make them compatible as delivery systems mainly in drinks and beverages (Duncan, 2011). However, engagement of nanoemulsions for delivery of active agents to solid foods is more challenging (Duran and Marcato, 2013). For this purpose, EFs and ECs based on nanoemulsion containing solutions can be utilized (Mason et al., 2006).

Essential oils are the most common active agents encapsulated into nanoemulsified EFs and ECs (Gutiérrez et al., 2008). They have proven to be effective natural antimicrobial agents and gained particular interest due to their ecofriendly properties, relative safety and acceptance by consumers (Burt, 2004). EFs and ECs with nanoemulsified essential oils are mainly applied on fruits and vegetables in order to inhibit or prevent microbial growth (Donsì et al., 2015; Kim et al., 2013; Salvia-Trujillo et al., 2015; Sessa et al., 2015; Severino et al., 2014, 2015). For example, Sessa et al. (2015) encapsulated nanoemulsified lemon, mandarin, oregano, and clove essential oils to modified chitosan ECs and examined their antimicrobial effect. The combined use of nanoemulsified lemon essential oil with a modified chitosan showed a remarkable in vitro antimicrobial activity. It was therefore further

examined on leaf vegetables, such as rucola, resulting in shelf-life extension from 3 to 7 days. Salvia-Trujillo et al. (2015) studied the effect of alginate ECs with several concentrations of nanoemulsified lemongrass essential oil (LEO) on the bacterial growth on fresh-cut Fuji apples. Comparison of alginate coatings with coarse-emulsified and nanoemulsified LEO showed that coatings with nanoemulsified LEO exhibited a faster and greater inactivation of *Escherichia coli* (E. Coli).

Incorporation of essential oils to EFs and ECs in a nanoemulsion form can also reduce their sensory impact. In addition, the nanoemulsion method of encapsulation enables to minimize the essential oil concentration required for antimicrobial activity by increasing its accessibility (Duran and Marcato, 2013). Kim et al. (2013) have developed a carnauba wax EC for plums incorporated with nanoemulsified LEO. The effect of the coating on *Salmonella typhimurium* and *E. coli* growth inhibition was examined alongside the impact on the plums' other quality attributes during storage at 4 and 25°C. The results showed a reduction in bacterial growth in the nanoemulsion-based formulation coated plums. The flavor, physical, and biochemical parameters were not impaired by the nanoemulsified LEO coatings.

Nanoemulsified ECs can also be combined with other methods. For example, two studies performed by Ferrari group have examined the antimicrobial effect of ECs based on modified chitosan and nanoemulsified mandarin essential oil against *Listeria innocua* on green beans (Donsì et al., 2015; Severino et al., 2014). In both studies the applications of nanoemulsified coatings were combined with nonthermal antimicrobial treatments. In the first study three treatments were utilized; γ-irradiation, UV-C, and ozonized water (Severino et al., 2014). The combination of active ECs with γ-irradiation or UV-C treatments resulted in a constant synergetic antimicrobial effect compared to application of only a single method. However, the combined treatment with ozonized water was less effective against bacteria compared with treatment of active ECs alone. The second study utilized two nonthermal treatments, high hydrostatic pressure (HHP), and pulsed light (PL) (Donsì et al., 2015). The obtained results showed beneficial antibacterial effect of active EC and HHP treatment against *L. innocua* during all storage periods. In contrast, the combined treatment with PL was only effective at the first day of storage, while at the rest of the storage period, treatment of active EC alone was more effective. Thus, not every treatment can enhance the antimicrobial activity of the nanoemulsified active coating; moreover, some treatments may even have detrimental effects. The same group has also studied antibacterial activity of modified chitosan EC with

nanoemulsified carvacrol essential oil against *E. coli* O157:H7 and *S. typhimurium* on green beans (Severino et al., 2015). The effect of γ-irradiation and modified atmosphere packaging (MAP) treatments against the two bacteria was examined when combined and separate. It was found that a combination of γ-irradiation treatment, EC and MAP was extremely effective against tested bacteria in terms of both controlling the growth and reducing inoculated population.

Otoni et al. (2014b) have utilized EFs based on methylcellulose and nanoemulsions of clove bud or oregano essential oils as shelf-life extenders for sliced bread. It was reported that both essential oils acted as plasticizers and also provided the EFs with antimicrobial activity against yeasts and molds. It was found that the films that contained nanoemulsions demonstrated better mechanical and antimicrobial properties than the films that contained regular coarse emulsions of essential oil.

Acevedo-Fani et al. (2015) have evaluated the effect of nanoemulsified alginate films containing thyme, lemongrass, and sage essential oils on the physical, mechanical, and antimicrobial properties). It was found that the type of added essential oil has an impact on the average droplet size of the nanoemulsion and therefore also on the barrier, color, and mechanical properties of the films. Incorporation of sage essential oil exhibited the smallest droplet size, probably due to better affinity to the alginate phase, resulting in more flexible and transparent films with better water vapor resistance. The antimicrobial activity of the film was better influenced by the essential oil nature than by its size.

Another type of natural active agent that can be encapsulated into EFs or ECs via the nanoemulsion technique is the isolated active component in essential oils, like cinnamaldehyde in cinnamon essential oil or citral in citrus-origin essential oils. To the best of our knowledge, there are only two publications in this subject, both of them examine the antimicrobial effect of nanoemulsified cinnamaldehyde incorporated into EFs for packaging purposes. In the first study, performed by Shaaban et al. (2014), the EF was based on pectin, while in the second study performed by de Moura group the EF was based on pectin and papaya puree (Otoni et al., 2014a). The results obtained from both studies presented uniform films with good mechanical and antimicrobial properties. Due to reduction in droplet size in cinnamaldehyde containing emulsions, higher surface area was obtained (Otoni et al., 2014a; Shaaban et al., 2014).

It is important to note that although nanoemulsions benefit a relatively good stability, the small droplet size makes them prone to break up by the Ostwald ripening mechanism (Gutiérrez

et al., 2008). Therefore, in order to maximize nanoemulsion-based EFs' and ECs' effectiveness, it is recommended to apply them quickly after the preparation (Sanguansri and Augustin, 2006; Weiss et al., 2006).

## 6.2 Layer-by-Layer Edible Films and Coatings

The Layer-by-Layer (LbL) technique is an approach that originated from materials science and is based on the alternate deposition of various polymers to produce multilayers with controlled properties (Decher, 1997). Due to a nanometer scale buildup, the approach facilitates a delicate and precise tuning of the materials' features. The LbL approach is extensively explored and applied in various fields including electronic, optics, and medicine (Weiss et al., 2006). It is especially valuable in the field of drug release systems due to its potential abilities to control and manipulate material properties and incorporate a wide range of functional biomolecules without substantial loss of their biological functions (Hammond, 2012).

The LbL approach has entered the field of food nanotechnology relatively recently (Weiss et al., 2006). Since then, multilayered EFs and ECs have demonstrated their abilities to enhance food safety and quality (Dhall, 2013). The majority of multilayered EFs and ECs developed for food products are applied on fruit and vegetables, whole or fresh-cut (Vargas et al., 2008). Frequently such LbL EFs and ECs consist of oppositely charged polyelectrolytes and are applied by utilizing the electrostatic deposition method (Fig. 10.2).

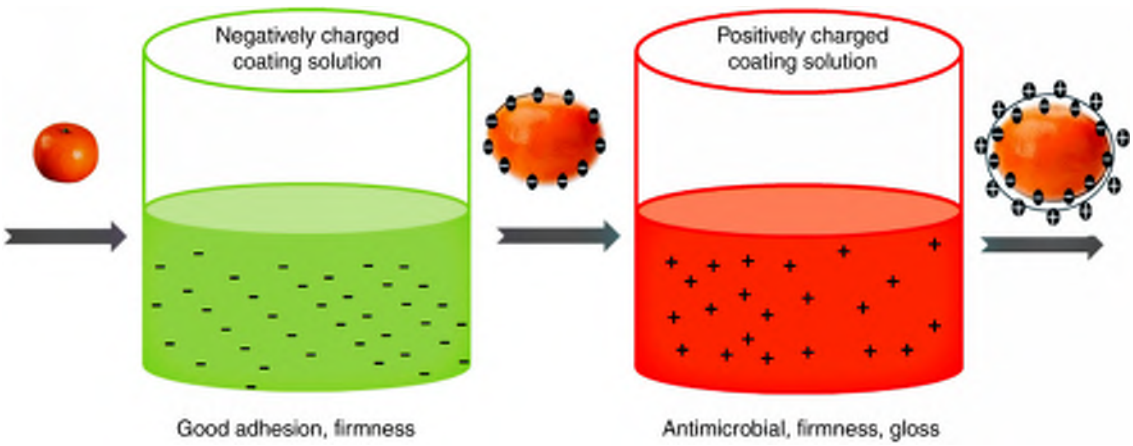


**Figure 10.2. Schematic representation of edible coating applied by utilizing the LbL electrostatic deposition method.**

### 6.2.1 Polysaccharides-Based LbL

Alginate, cellulose derivatives, pectin, chitosan, and carrageenan represent the most utilized polysaccharides in the field of LbL EFs and ECs. An exactly defined chemical structure of a polysaccharide's repetitive monomer unit allows the prediction and as a consequence, the design of EFs' and ECs' properties.

Poverenov's group has published three papers on polysaccharide-based LbL edible coatings (Arnon et al., 2014, 2015; Poverenov et al., 2014a). Two of these studies examined carboxymethylcellulose (CMC)/chitosan bilayered ECs on citrus fruit and the third study examined an alginate/chitosan EC on fresh-cut melons. Chitosan, a positively charged polysaccharide, served as the external layer in all three studies due to its eminent antimicrobial activity, while the negatively charged CMC or alginate constituted the inner layer due to their satisfying adhesive properties. These formulations allowed for the bilayer coatings to gain the individual advantages from both comprising polymers.

In the first study Arnon et al. (2015) performed a series of systematic experiments with the aim of finding polysaccharide-based ECs that convey enhanced quality, improved storage duration, and had an attractive appearance of citrus fruit. Such polysaccharides-based coatings may provide a natural alternative to synthetic waxes that are currently utilized on citrus fruit (Bai and Plotto, 2011). After screening a series of different polysaccharides on mandarin fruit, CMC in a fixed concentration of 1.5% w/v was chosen as an inner coating layer due to its best adhesion properties in addition to good gas and water vapor permeability. Chitosan was applied as an external active layer. Chitosan was found to cause an improvement in fruit texture and gloss. Glycerol and fatty acids were added to the CMC coating formulation instead of the second chitosan layer, in order to compare their effect with the LbL approach. However, these auxiliary additives did not result in a desired improvement of coating properties. Moreover, in several cases they caused deterioration in the coating's properties. Various chitosan concentrations were examined in order to formulate the optimal LbL coating. The obtained results showed that the higher the chitosan concentration used, the more gloss was seen in coated fruits, as well as an improvement in firmness and a ripening rate inhibition (Fig. 10.3). On the other hand it should be noted that excessive chitosan content causes an increase in ethanol concentrations. An accumulation of high ethanol concentration is not desired, since it may cause off-flavors. This is especially important for mandarins, which are the most off-flavor sensitive among all the citrus fruit.

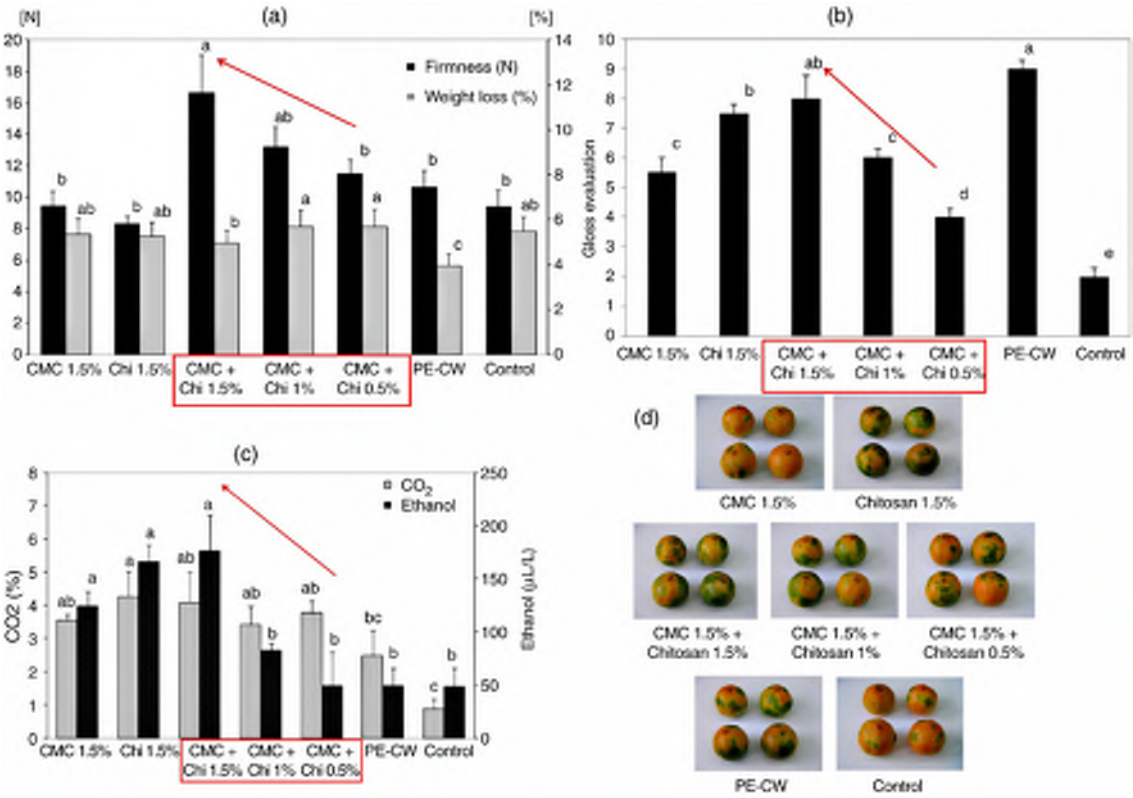


**Figure 10.3.** The effect of chitosan concentration in the LbL EC on (a) firmness/weight loss, (b) gloss, (c) $CO_2$/EtOH accumulation, and (d) progress of ripening in mandarin fruit. This was compared to the effect of single layered coatings, synthetic polymer-based commercial wax (PE-CW), and uncoated fruit (control). Measurements were taken after 10 days at 20°C and represent means of replications ± SE. Different letters indicate significantly different values (at $p \leq 0.05$) according to a Tukey-Kramer HSD test (Arnon et al., 2015).

Bilayered coating formulations of 1.5% (w/v) CMC and 1% (w/v) chitosan were selected as the optimal coating content. In the second study Arnon et al. (2014) have examined the effects of this coating formulation on the postharvest quality of oranges, grapefruit and two types of mandarins at simulated storage conditions. The coatings demonstrated good performance and showed that can serve as a considerable alternative to the currently used synthetic waxes.

The third study published by Poverenov's group involved the implementation of a bilayered polysaccharide-based coating composed of polyanionic alginate and polycationic chitosan on fresh-cut melons (Poverenov et al., 2014a). The application of ECs to minimally processed fruits faces some technical problems. These are related to the difficult adhesion of materials to the cut fruit hydrophilic surface. In addition, fresh-cut fruits are

usually very prone to microbial spoilage and texture deterioration. The alginate/chitosan LbL coating was found to possess beneficial properties from both ingredients. The bilayer combination allowed for a good adhesion to the melon matrix by the inner alginate layer, along with good antimicrobial activity provided by the outer chitosan layer (Fig. 10.4).

This antimicrobial protection allows reducing bacteria, yeast, and fungi counts. Both layers contributed to the melons' texture enhancement; alginate due to a presence of $Ca^{+2}$ ions in its formulation and chitosan by avoiding microbial damages that affect fruit entirety. The coating slowed down tissue texture degradation, so that after 14 days of storage, only LbL samples maintained an appreciable firmness. This is unlike uncoated melons or fresh-cut melons coated with a single layer of alginate or chitosan. Surprisingly, unlike the previously described studies on citrus fruits coated by a bilayered coating, the bilayered coating exhibited enhanced gas exchange properties that exceeded those of both monolayer

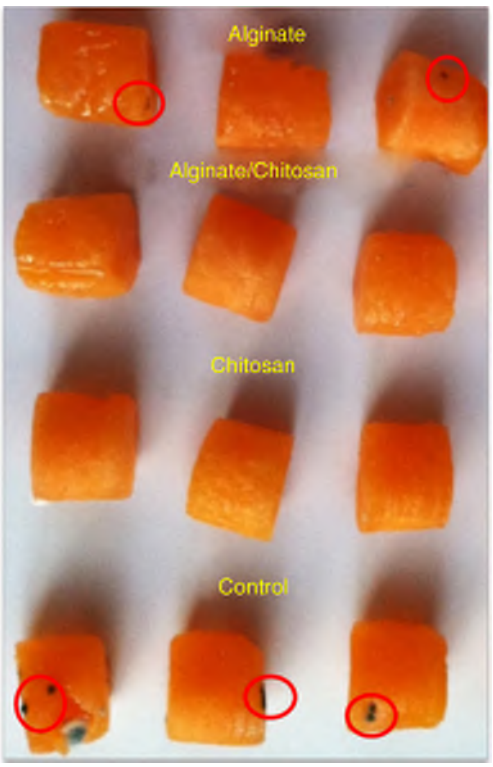


**Figure 10.4. Effect of edible coating on the microbial quality of melons after 10 days of storage at 6°C.**

coatings and even of the noncoated control. As a result, the bilayered coating prevented an increase in headspace $CO_2$ and ethanol concentrations, which are synonymous to hypoxic stress and off-flavor development as observed in other samples, especially in alginate-coated melons.

Polysaccharide-based EFs and ECs prepared by the LbL electrostatic deposition approach can consist of more than two layers, as was demonstrated by two papers published by Vincente's group (Medeiros et al., 2012a; Souza et al., 2015). In the first study, nanomultilayer EFs were prepared, consisting of five alternating layers of polyanionic pectin and polyactionic chitosan (Medeiros et al., 2012a). This formulation was first characterized in terms of water vapor and oxygen and $CO_2$ permeability. Further on, the nanomultilayer system was applied on whole "Tommy Atkins" mangoes and the layers' adsorption was confirmed by changes in the coated fruits' skin's contact angle. After 45 days in storage, coated mangoes presented a better external appearance and a less dehydrated surface. No fungal growth was observed, with a much more preserved flesh in comparison to the uncoated fruit. These findings suggest that a combination of chitosan's antimicrobial and gas barrier properties, along with pectin's low oxygen permeability, were possibly efficient in the reduction of gas flow and on the extension of the mangoes' shelf life. In the second study, a nanomultilayer EC consisting of five layers of polyanionic alginate and polycationic chitosan were prepared and applied on fresh-cut mangoes (Souza et al., 2015). The fruit quality was evaluated after 14 days of storage at 8°C. The LbL coated fresh-cut mangoes exhibited a high value of titratable acidity and lower values of soluble solids, mass loss, pH and browning rate. In addition, the LbL alginate/chitosan EC improved the fruits' microbial quality during storage and extended their shelf life up to 8 days in 8°C when compared to uncoated fruit (<2 days).

### *6.2.2 Proteins-Based LbL*

Rudra et al. (2006) have reported LbL edible films based on polypeptides, incorporated with an antimicrobial protein. In this case, the active protein was egg white lysozyme (HEWL), which is widely employed as a human food preservative, and carries a positive charge at an acidic pH. The nanofilms were made of poly(L-glutamic acid) (PLGA), which has a high negative charge in the mildly acidic pH range. The results obtained showed that PLGA/HEWL nanofilms inhibited the model microbe *Micrococcus luteus* growth in the surrounding liquid medium following the release of HEWL molecules over time. In addition, it was

found that the amount of HEWL released depends on the number of its layers and therefore on its total amount in the film (Rudra et al., 2006).

### 6.2.3 Composite Polysaccharides/Proteins-Based LbL

Medeiros et al. (2012b) have reported EFs and ECs comprising of a LbL formulation based on a combination of polysaccharides and proteins. The aim of this study was to construct and characterize a nanomultilayer coating system composed of five layers of κ-carrageenan and lysozyme and the evaluation of its effect on the shelf life of fresh-cut and whole "Rocha" pears. Construction of the nanolayered film was assembled at first on aminolyzed/charged polyethylene terephthalate (PET) pieces, which acted as a support, by alternate five-layer depositions. This procedure was performed to allow the characterization of the nanomultilayer system in terms of WVP and oxygen permeability. Indeed, the obtained values of WVP and oxygen permeability were much lower than the values reported in the literature for carrageenan and lysozyme. Further on, the nanomultilayer system was applied on whole and fresh-cut "Rocha" pears, resulting in a lower mass loss, a lower TSS, and a higher TA when compared with uncoated fruit. Moreover, application of a nanomultilayer coating prevented the enzymatic browning and dark color on fresh-cut "Rocha" pears compared with uncoated fruit. These findings suggest that the nanolayered coating of κ-carrageenan and lysozyme assembled on the pears' surface had a significant positive effect on the quality and shelf-life extension of the fruit.

Previous reports have stated that mixing chitosan with other hydrocolloids, in particular with proteins, may result in a significant improvement in its physical properties and adhesion (Abugoch et al., 2011; Martinez-Camacho et al., 2010; Rivero et al., 2009). Poverenov et al. (2014b) also conducted a study about the integration between proteins and polysaccharides in EFs and ECs using two different approaches: blending and LbL electrostatic deposition. The mechanical, physical, and optical properties of chitosan- and gelatin-based films prepared by blending and LbL method were examined and also compared with a single component films. In addition, edible coatings based on these formulations were applied on fresh-cut melon. The effect of two formulations on microbial and physiological food quality was studied in comparison with the noncoated control. It was found that EFs prepared by the LbL approach have advanced mechanical properties in comparison to the blended films. It was also found that films based on two components possess higher permeability than the films based on

single-component. ECs based on a combined formulation were applied on fresh-cut melons. The LbL and blended formulations showed significant antimicrobial activity.

Both combined coatings did not obstruct fruit gas exchange or caused accumulation of off-flavor volatiles. However, the LbL formulation was superior in the preservation of fruit textures and also slightly reduced fruit weight loss, while the blended formulation did not improve these parameters.

### *6.2.4 Adding Lipids to LbL Formulations*

EFs and ECs formulations that are based on hydrocolloids (proteins and polysaccharides) exhibit good mechanical properties. However, they are usually characterized by a poor water vapor barrier (Bourtoom, 2008). Adding lipids as a layer to the LbL film/coating formulations may enhance water barrier efficiency by 10–1000 times (Debeaufort and Martin-Polo, 1993; Kester and Fennema, 1989). Debeaufort et al. (2000) have studied a lipid layer containing LbL edible films. The bilayered films composed of a methylcellulose layer coated with triglycerides or alkanes were prepared and examined in terms of solid fat content, thickness, melting point, water vapor transmission rate (WVTR), and mechanical properties. The results showed that the films' mechanical properties were barely influenced by thickness of the lipid layer. Mechanical resistance was mainly attributed to the methylcellulose matrix. However, the WVTR decreased notably when film thickness increased, but only up to a certain value. Above this value, WVTR values were independent of the measured thickness.

Velickova et al. published two papers on the combination of bees-wax and chitosan. In the first paper, a combined three layered beeswax-chitosan-beeswax coating was applied on fresh strawberries (Velickova et al., 2013). Improvement of the original chitosan films' WVP barrier was examined and the quality of coated strawberries at ambient temperature of 20°C was evaluated. The performance of the multilayered coating was compared to composite coatings based on a chitosan and beeswax emulsion and to coatings based on chitosan alone. The obtained results showed that addition of beeswax as a separate layer or as a component in the composite coating reduced weight loss and respiration rate, inhibited fungal growth and maintained fruit firmness and color as well as titratable acidity, soluble solids, and pH. However, the composite chitosan and beeswax coating was transparent and had higher overall acceptance. The three-layer-coatings were mat, slightly white and were less accepted by panelists, even though

they maintained the strawberries' best quality during storage period. In the second paper, chitosan-based EFs were prepared and subjected to cross-linking reactions using sodium tripolyphosphate and/or to beeswax coating (Velickova et al., 2015). In addition, chitosan-beeswax emulsion-based films were produced. The goal of these modifications was to decrease chitosan films' water affinity. The cross-linking with tripolyphosphate decreased both the water vapor permeability and the water absorption capacity. However, there was an increase in the films' stiffness, revealed by the increased Young's modulus. The multilayered wax-chitosan-wax films exhibited a similar improvement of the barrier properties to water vapor, with the advantage of maintaining the mechanical properties of the original chitosan films. However, these wax-coated films showed a higher water absorption capacity, which is believed to be a consequence of water entry into small pores between the film and the wax layers. Regarding the film samples subjected to cross-linking and further coating with beeswax, a similar behavior as the uncoated cross-linked films was observed. The emulsion-based composite films were characterized by a substantial decrease of the water vapor permeability, along with a decrease in their stiffness.

Meyers reported that addition of a lipid layer to a preexisting hydrocolloid-based layer, thus forming a bilayer EF, improved adhesiveness when applied on chewing gum sheets. The bilayer formulation also reduced moisture loss and extended their shelf life. These successful traits led to the formulation eventually being utilized and patented by the JR Wrighley Company (Meyers 1994).

### *6.2.5 Adding Active Agents to LbL Formulations*

The LbL films/coatings have already been reported as an effective drug delivery biomedical system that allows controlling the release of functional biomolecules without substantial loss of their bioactivity through manipulating film/coating's properties (Jiang and Li, 2009; Wang et al., 2007; Zhong et al., 2007). Vincente's group have developed nanolayered active κ-carrageenan and chitosan EFs that could act as a support system for the incorporation of bioactive compounds (Pinheiro et al., 2012a). The potential of a κ-carrageenan/chitosan nanolayered coating to incorporate methylene blue (MB), a monovalent dye cation was demonstrated (Pinheiro et al., 2012b). MB is commonly utilized as a model for loading and releasing experiments of small molecules due to its clear absorption peak in the visible range. Loading MB dye molecules allows the investigation of electrostatic interactions between oppositely charged polyelectrolytes in multilayer

films/coatings (Tedeschi et al., 2000). In Vicente's group study, MB was incorporated at different positions of the nanolayered coating and its loading and release behavior was evaluated. UV–VIS spectroscopy and quartz crystal microbalance analysis showed that the amount of MB loaded increased with the distance from the first layer, suggesting that the MB was able to diffuse into the κ-carrageenan/chitosan nanolayered coating. In addition, for most of the tested conditions, the MB release from the κ-carrageenan/chitosan nanolayered coatings was successfully described by the linear superimposition model, which allowed concluding that MB transport is driven by both concentration gradients and the polymer relaxation of the nanolayers. However, different results were observed depending on the position of MB incorporated on the nanolayered coating or the pH and temperature of the medium. This work clarified the release mechanisms of active agent in nanolayered EFs systems. Such findings are of high importance for the application of active multilayer films and coatings in food products, as a strategy for shelf-life extension (Pinheiro et al., 2012b).

Another approach for encapsulating active agents in LbL EFs and ECs involves the formation of an inclusion complex (IC) with the active agent (guest molecule) using cyclodextrins. An IC enhances the solubility of an active agent, controls its volatility and protects its bioactivity. In case of active agents with dominant flavors such as essential oils, an IC may mask undesired organoleptic effects (Del Valle, 2004). Castell-Perez's group have published four papers concerning the encapsulation of *trans*-cinnamaldehyde in a β-cyclodextrin IC to polysaccharide-based LbL ECs (Brasil et al., 2012; Mantilla et al., 2013; Martiñon et al., 2014; Sipahi et al., 2013). The coatings' effect on fresh-cut fruit was examined after a storage period of 15 days at 4°C. Two of these studies were engaged with LbL coatings based on alginate and pectin. In the first study the coating was applied on fresh-cut pineapple and in the second it was applied on fresh-cut watermelon. Microbial analyses of both studies led to the conclusion that a LbL coating containing β-cyclodextrin-*trans*-cinnamaldehyde IC is effective against microbial growth. On the other hand, physiological and sensorial features were less affected by the encapsulation of an IC (Mantilla et al., 2013; Sipahi et al., 2013). Another study engaged with a chitosan and pectin LbL edible coating for fresh-cut cantaloupe. The obtained results showed that the microbiological quality of LbL-coated fresh-cut cantaloupes was enhanced due to the antimicrobial IC. However, above a certain concentration of IC, a negative effect on the sensorial properties was observed (Martiñon et al., 2014).

Another Castell-Perez group's study reports the encapsulation of *trans*-cinnamaldehyde to a LbL coating based on chitosan and pectin, by utilizing a β-cyclodextrin IC (Brasil et al., 2012). In this study three different packaging types were examined: polypropylene tray and lid (Ziploc®), Saran® wraps and plain cheesecloth. The objective of this study was to evaluate the packaging effect on the efficacy of the LbL coating applied on fresh-cut papaya during 15 day storage at 4°C. The obtained results showed improvement in the microbial and physiochemical qualities of fresh-cut papaya. The coating extended the shelf life of the fruit up to 15 days at 4°C while uncoated fruits did not reach as long (<7 days). The Ziploc® packaging enhanced the quality of LbL coated fruit compared with unpacked LbL coated fruit (cheesecloth).

The triangle antimicrobial effect of designated packaging and chitosan/pectin LbL coatings encapsulated with a β-cyclodextrin and *trans*-cinnamaldehyde IC was further studied (Moreira et al., 2014). The antimicrobial preservation system was applied on fresh-cut melons and their quality was evaluated after 15 day storage at 4°C. The conclusions drawn from this study again demonstrate the potential of the antimicrobial multilayered coating and the Ziploc® packaging to serve as a commercial preservation method for fresh-cut fruit. LbL ECs have proven their preservation potential and quality enhancement, mainly when applied on whole or fresh-cut fruit and vegetables.

However, LbL coatings also showed a stabilizing potential in other food sectors. For example, Klinkesorn et al. (2005) have utilized the LbL electrostatic deposition technique to improve liquid and dry tuna oil-in-water emulsions' stability. This technique enhanced the emulsion ability to protect ω-3 fatty acids, found in fish oil, against oxidative stress, thus maintaining the beneficial health properties of the tuna oil and allowing its incorporation in food.

## 6.3 Nanoparticles

Incorporation of nanometric-sized particles was found to enhance physical, optical, and mechanical properties of EFs and ECs (Acevedo-Fani et al., 2015). Nanoparticles (NPs) incorporated into EFs and ECs can also serve as delivery systems for active agents (Rojas-Graü et al., 2009b). This has led to the development of a variety of NPs reinforced EFs and ECs, also termed as "nanocomposites" (Duncan, 2011). NPs can often be produced from food-grade biopolymers such as polysaccharides and proteins or natural bioactive compounds such as curcumin and solid lipids.

NPs preparation methods compatible with food products include salting out, spontaneous emulsification/diffusion, solvent evaporation and nanoprecipitation (Weiss et al., 2006).

### 6.3.1 Biopolymeric Nanoparticles

Biopolymeric NPs are prepared from biocompatible and biodegradable polymers in the 10–1000 nm size range. The active agent is entrapped by the polymer NP matrix. The majority of biopolymeric NPs in EFs and ECs are based on polysaccharides such as chitosan and cellulose derivatives (Duncan, 2011). Such particles can be formed by promoting self-association/aggregation of single biopolymer or by inducing phase separation in mixed biopolymer systems (Weiss et al., 2006).

De Moura's group published numerous papers about incorporating chitosan NPs in polysaccharides-based EFs. Chitosan is also well known for its antimicrobial activity against a wide variety of microorganisms including fungi, algae, and bacteria (Elsabee and Abdou, 2013). The broad antimicrobial properties of chitosan NPs have also been reported and attributed to their high surface area and charge density (Qi et al., 2004). de Moura et al. (2008) have studied the effect of chitosan NPs' addition to the mechanical, and water vapor and oxygen permeability properties of HPMC films. SEM images revealed that chitosan NPs tend to occupy the pores' empty spaces in the HPMC matrix, inducing the pores to collapse and thereby improving film tensile and barrier properties. In an additional study, chitosan's ability to form inter- and intramolecular cross-linkages with tripolyphosphate (TPP), a multivalent food grade polyanion with good gelation ability, was investigated (de Moura et al., 2009). The obtained results showed that chitosan-TPP NPs improved HPMC films' barrier and mechanical properties. SEM images revealed that HPMC films became more compact and dense when chitosan NPs were added. NPs also improved HPMC's thermostability, an important applicative parameter. They have also increased HPMC film's thermal degradation temperature from 232 to 271°C. In an additional study, de Moura et al. (2011) have investigated the effect of chitosan NPs on CMC-based edible films' properties. In this study, the morphology of chitosan NPs was tested by transmission electron microscopy (TEM), revealing the NPs size in the range of 80–110 nm. Addition of chitosan NPs led to homogeneous and manageable CMC films. Films containing 110 nm NPs exhibited the most suitable properties for the packaging industry (lowest water solubility and better mechanical properties). For instance, only 110 nm NPs successfully improved films' elongation percentage (%E). The films'

thermal degradation temperature increased from 240 to 266°C. Two other publications by the de Moura group have reported the incorporation of chitosan NPs into EFs containing fruit puree (Lorevice et al., 2012; Martelli et al., 2013). In the first paper, the EFs were based on guava puree and HPMC. The thermal degradation and glass transition values remained practically unchanged due to the presence of chitosan NPs, whereas films' mechanical and water vapor barrier properties were significantly enhanced (Lorevice et al., 2012). In the second paper, the edible films were based on banana puree mixed with pectin in order to compensate the lack of endogenic pectin in bananas. Glycerol was added to the film formulation to improve its toughness and processability. A small amount of chitosan NPs was found to be sufficient as a reinforcement material, enhancing the overall mechanical properties and reducing significantly the films' water vapor permeability (Martelli et al., 2013).

Edible coatings based on biopolymeric nanoparticles were also studied to enhance quality of fruit and vegetables. de Moura group compared the effects of chitosan-TPP based NPs coating with the effects of conventional chitosan coating on the microbiological quality and additional quality attributes of fresh-cut "Gala" apple slices during 10 days of storage at 5°C. The obtained results revealed a better antimicrobial efficiency from coatings with NPs when compared to a conventional chitosan coating and uncoated fruit (Pilon et al., 2014).

Antoniou et al. (2015) have conducted a direct comparison of the antimicrobial activity, thermomechanical, physicochemical, morphological, and barrier properties of bulk chitosan and chitosan NPs within glycerol plasticized Tara gum (TG) films. Microbial analyses have revealed that chitosan NPs were less effective against *E. coli* and *S. aureus* when compared with bulk chitosan. On the other hand, the presence of chitosan NPs significantly improved the TG films' tensile strength without reducing their elongation. The improvement in mechanical properties was attributed to the reduction of free volume in the polymer matrix by the films' more compact structure with chitosan NPs compared to those containing only bulk chitosan.

Bao et al. (2009) have incorporated chitosan-TPP nanoparticles containing tea polyphenol in fish skin gelatin edible films. The objectives of this study were to investigate the antioxidant effects of these films against the oxidation of fish oil, as well as the release of the tea polyphenol. The addition of tea polyphenol-loaded chitosan NPs has greatly decreased the tensile strength and slightly decreased the elongation at break and the oxygen permeability of gelatin edible films, while at the same time increasing its water

vapor permeability and barrier to UV light. The release of tea polyphenol from the NPs was achieved according to the increased radical-scavenging activity of the films during their storage period and oxidation of fish oil was effectively retarded over the whole incubation period.

Poor mechanical properties and excessive water vapor permeability are two main challenges when developing edible films and coating (Bourtoom, 2008). Bilbao-Sainz et al. (2010) have attempted to improve the water barrier properties by forming lipid coated microcrystallaine cellulose (LC-MCC) nanoparticles and incorporating them in an HPMC matrix. Incorporation of MCC nanoparticles in HPMC film systems exhibited water barrier properties that improved with MCC increasing size and quantity. Lipid coatings of MCC resulted in further improvement of the water vapor barrier properties.

### *6.3.2 Natural Bioactive Compounds-Based Nanoparticles*

Nanoparticles can be made from various bioactive compounds without involving polymers. Rapid expansion of subcritical solutions into liquid solvents (RESOLV), which has been developed based on supercritical fluid technology, is a versatile technique for producing stable suspension of well-dispersed and uniform NPs (Sane and Limtrakul, 2009; Sane and Thies, 2005). In this process, a subcritical solution containing a solute and a solvent is directly expanded across a nozzle into a liquid receiving solution. This method is capable of producing NPs from a variety of compounds (Sane and Limtrakul, 2011). Sonkaew et al. (2012) have utilized this technique for the production of curcumin NPs (Ccm-NPs) and/or ascorbyl dipalmitate NPs (DPA-NPs) and incorporated them in cellulose-based edible films. Ccm-NPs' and ADP-NPs' antioxidant activities were investigated using four assays, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ABTS radical cation decolorization, β-carotene bleaching, and ferric reducing antioxidant power. These assays proved that Ccm-NPs and ADP-NPs had higher antioxidant activities to inhibit the oxidation of β-carotene, scavenge DPPH, and ABTS free radicals, and reduce the ferric complex to the ferrous form than those of regular Ccm and ADP. This study has also demonstrated that antioxidant components of Ccm and ADP can be nanoparticulated and incorporated into cellulose-based biopolymers while retaining their antioxidant activities. Cellulose-based edible films can act as reservoirs and release Ccm and ADP to maintain an inhibitory effect at the food surface. This demonstrates a promising potential of Ccm-NPs and ADP-NPs for application in antioxidant packaging films and coatings.

### 6.3.3 Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are lipid colloidal submicronic systems that have been developed to encapsulate and deliver lipophilic functional components. SLNs are systems with a high level of technological potential in different areas, including the food industry. SLNs are typically prepared using the hot homogenization process where a lipid and an aqueous surfactant solution are homogenized at a temperature above the lipid's melting point to produce an oil-water nanoemulsion. This hot nanoemulsion is then cooled to room temperature leading to the formation of solid particles (Quintanar-Guerrero et al., 1996; Vitorino et al., 2011). Zambrano-Zaragoza et al. (2013) have published a paper about utilizing nanocomposite edible coating made of xanthan gum and Candeuba® wax SLNs for extending guavas shelf life. SLNs were prepared using the hot high shear stirring method (Solans et al., 2005) with a concentrations range of 60–80 g/L and were compared with the control and xanthan gum. The film-forming dispersion was applied by dipping the guavas and storing them under refrigeration at 10°C and 85% RH for 30 days in order to assess the effect of maturation on the changes in weight loss, chemical quality, texture, and color. The best results were obtained with SLN concentrations of 60 and 65 g/L because at these concentrations guavas showed the lowest range of weight loss and preserved the best quality compared to the fruits processed at concentrations above 70 g/L. In addition, high contents of SLN cause physiological damages and also delay the fruit maturation.

## 6.4 Nanocellulose in Edible Films and Coatings

Cellulose is the most prevalent material in the food technology research utilized as a basis for nanostructures, such as nanofibers and nanocrystals (Kriegel et al., 2009). Edible polymers usually demonstrate poor mechanical properties in comparison to synthetic polymers. Hydrocolloid biopolymers often have insufficient water vapor barrier properties as well. Nanocellulose additives can improve these features. Therefore, nanocellulose containing EFs and ECs provide an extensive and rapidly developing research area. Cellulose nanofibers (CNF) (Kriegel et al., 2009; Wang and Sain, 2007; Weiss et al., 2006) also called microfibrillated cellulose (MCF) and nanocrystaline cellulose (NCC) (Azizi Samir et al., 2005) also referred to as nanowhiskers, were reported by a number of groups to improve mechanical and also WVP properties of edible films and coatings (Andrade et al., 2014, 2015; Azeredo et al., 2009, 2010, 2012; da Silva et al., 2012; Fakhouri et al., 2014; Oun and Rhim, 2015; Pereda et al. 2011, 2014).

## 7 Conclusions and Future Applications

Utilizing advanced nanotechnology approaches is the relatively new field of research of active edible films and coatings. It is attractive from both a fundamental scientific and applied point of view. Nanoemulsions allow the delivery of bioactive agents and significantly increase bioavailability and stability of these compounds. Delivery of active agents provides food products with better physiological properties (freshness, firmness, appropriate gas exchange, and wettability), microbiological protection and can even improve the products' nutritional values and organoleptic properties. Nanoparticels can provide both enhancement of mechanical and physical properties of the film and coatings and also serve as encapsulating systems for the delivery of active components. Nanocellulose crystals and fibers can effectively improve mechanical and physical properties of edible films and coatings. LbL methods allow the build up of active films and coatings with precise nanolevel molecular tuning of their properties. Thus, the nanotechnology methods allow a rational design of active edible films and coatings and may have clear benefits with regard to food products' quality and safety.

## Acknowledgments

We would like to gratefully acknowledge the Chief Scientist of the Israeli Ministry of Agriculture and Rural Development and European Union Seventh Framework Programme (Project QUAFETY) for financial support. We would like to thank Roi Rutenberg for his great help in writing of this chapter. We also thank our collaborators Dr Ron Porat, Dr Victor Rodov, and Prof Eli Fallik who worked with us on active edible coating projects.

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11

# NANOEMULSIONS AS POTENTIAL DELIVERY SYSTEMS FOR BIOACTIVE COMPOUNDS IN FOOD SYSTEMS: PREPARATION, CHARACTERIZATION, AND APPLICATIONS IN FOOD INDUSTRY

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## 1 Introduction

Bioactive compounds including carotenoids, essential oils, antioxidants, or flavors are widely incorporated into food products in order to enhance their sensory properties or to develop their nutritional and health properties. However, due to their low solubility in aqueous phases, their higher instability in food products during processing and preparation as well as their low bioavailability, the use of these active substances is sometimes limited. Encapsulation of bioactive compounds has been proposed as potential approaches to stabilize such bioactives and to control their release and increase their bioavailability. Microencapsulation of bioactive compounds is defined as a process by which particles or droplets are surrounded by a coating or embedded in a homogeneous or heterogeneous matrix giving small capsules with active properties (Fig. 11.1).

The development of microencapsulated products started in 1950s in the research into pressure-sensitive coatings for the manufacture of carbonless copying paper (Green and Scheicher, 1955).

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00011-8

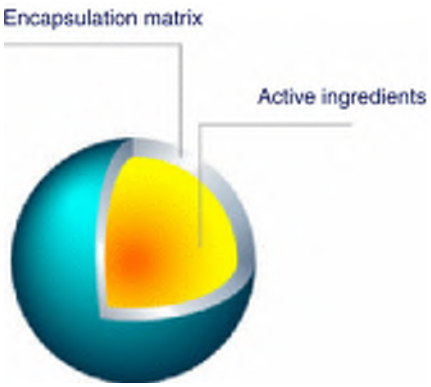


**Figure 11.1. Schematic presentation of encapsulated bioactive compounds (active ingredients) through microparticles.** www.polaris.fr/microencapsulation

Then, this technology has been well developed in the pharmaceutical, cosmetic, chemical, and food industries (Augustin et al., 2001). Microencapsulation is able to provide an efficient barrier between the bioactive molecules, called also core materials or active ingredients or encapsulated agents, and the external environment. Encapsulation process is defined as the technique by which liquid droplets, solid particles, or gas compounds are entrapped into thin films called coating materials or encapsulation matrices. The core material, which is the bioactive compound to be coated or encapsulated, can be liquid, solid, or gas in nature. Moreover, it can be composed of one or several ingredients. However, coating material can be single or double-layered and it should be able to form cohesive film with the core material. On the other hand, the properties of strength, stability, impermeability, thickness, and flexibility of the coating film should be well studied in order to ensure the successful encapsulation process of the bioactive compound.

In the food industry, several bioactive compounds have been protected by microencapsulation (Table 11.1) such as aroma compounds and flavors, vitamins, fats and oils, colorants, enzymes and microorganisms, agents with undesirable flavors or odors (Gibbs et al., 1999; Dziezak, 1988). Moreover, typically the coating material used are natural biopolymers (Table 11.1) such as gums, lipids, carbohydrates.

Generally, microparticles can be classified according to their shape into two groups: microcapsules and microspheres (Fig. 11.2). Microcapsules are particles consisting of an inner central core containing the bioactive substance, covered with a polymer layer that constitutes the capsule membrane. However,

## Table 11.1 Essential Techniques Used to Encapsulate Bioactive Compounds for Food Industry Applications

| Techniques | Encapsulated Bioactives | Encapsulation Matrices | References |
|---|---|---|---|
| Spray drying | Fish oil, flavors, lycopene, β-carotene | Starch, chitosan, modified starch, proteins, natural gums | Jafari et al. (2008); Risch and Reineccius (1988); Reineccius (1989) |
| Coacervation | Polyunsaturated fatty acids, essential oils, flavors, enzymes | Gums, proteins, starch, maltodextrin, xanthan gum, pectin | Porzio and Madsen (1997); Tamjidi et al. (2012); Gouin (2004); Mendanha et al. (2009) |
| Extrusion | Flavor, carotenoids, probiotic bacteria, oils | Sodium alginate, maltodextrin, modified starch, pectin | Risch (2009); Wang et al. (2013); Rijo et al. (2014) |
| Fluid bed coating | Probiotic bacteria, vitamins, lactic acid carotenoids | Cellulose, maltodextrin gums, proteins | Strasser et al. (2007); Knezevic et al. (1998) |
| Nanoemulsions | β-carotene, aroma, curcumin, oils | Chitosan, gums, carbohydrates | Jafari et al. (2006); Yuan et al. (2008); Tan and Nakajima (2005) |

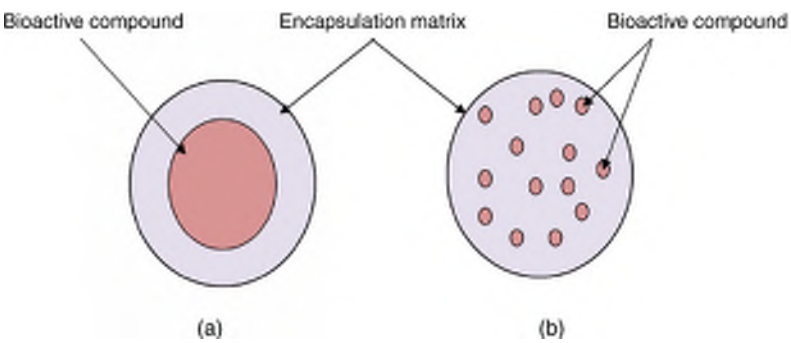


**Figure 11.2. Structural models for the incorporation of bioactive compounds into microparticles (a) microcapsule and (b) microsphere.**

microspheres are matrix systems in which the active molecule is uniformly dispersed in a polymer network (Vilstrup, 2001).

Moreover, the encapsulation process gives in general small particles with diameters ranging between nanometers and a few micrometers. Then, we can distinguish the macroparticles (>5 μm), microparticles (0.2–5 μm), and nanoparticles (<0.2 μm).

Nanoparticles can be produced by several nanoencapsulation processes. The term nanoencapsulation describes the encapsulation of bioactive compounds on the nanometer scale with films, layers, or simply nanodispersions.

## 2 Nanotechnology in Food Industry

Nanotechnology can be defined as the engineering of very small systems and structures. It involves research, technology, and control of structures with sizes ranging from 1 to 100 nm (Quintanilla-Carvajal et al., 2010). Recently, nanotechnology has been developed and used in several sectors and activities. The food industry becomes one of the major sectors where the application of nanotechnology is more and more expanded (Neethirajan and Jayas, 2010; Rizvi et al., 2010; Sanguansri and Augustin, 2006). It offers the potential to significantly improve the solubility and bioavailability of various functional ingredients included in the formulation of food products. Moreover, the applications of nanotechnology in the food industry include smart packaging and interactive foods. This means that consumers will be able to modify food depending on their own nutritional needs or tastes and other sensory attributes. Furthermore, the application of nanotechnology in food industry may allow the modification of the stability during shelf life, thermal stability of food products, and oral bioavailability and solubility of functional compounds (McClements et al., 2009; Huang et al., 2010). Moreover, nanocapsules containing bioactive compounds such as flavor, antioxidant, and vitamins would be released only when triggered by the consumer.

One of the most promising applications of nanotechnology as delivery systems for bioactive compounds in food industry includes their nanoencapsulation through nanoemulsions.

## 3 Nanoemulsions as Delivery Systems for Bioactive Compounds

The protection of bioactive compounds of food grade properties has been investigated for a long time using microencapsulation systems. Recently, research proved that delivery of bioactive agents inside and outside the body, their release as well as their stability and bioavailability are directly affected by particle size (Tiwari and Amiji, 2006). Then, advancements in nanotechnology have been investigated to ameliorate all these properties of encapsulated bioactive compounds. Indeed, nanosized particles provide more surface area, increase the solubility and the bioavailability of the nanoencapsulated agents, and control their release in the body. Reactive or sensitive bioactive molecules could be turned into stable agents through encapsulation in nanocarrier systems such as nanoemulsions. In general, three steps are involved in the encapsulation of bioactive compounds: preparation

of the wall material; incorporation of the bioactive agents' subject of the encapsulation; and the encapsulation techniques including chemical or physical techniques. In this chapter, we focus on the nanoemulsion technology and its application for the nanoencapsulation of food bioactive compounds.

## 3.1 Emulsions: Background

Conventional emulsions are formed by mixing two immiscible liquids namely water and oil stabilized by an emulsifying agent also called surfactant (Tadros and Vincent, 1983). Due to the differences in attractive interactions between the molecules of the two immiscible phases, an interfacial tension exists between them (Myers, 1999). To maintain a homogenous and stable system, the surface tension between the two phases should be significantly decreased by adding the emulsifier or the surfactant. These are amphiphilic surface active molecules, which are soluble in one of the liquid phases. The nature of emulsion, that is, oil in water (o/w) or water in oil (w/o), is mainly governed by the affinity of the surfactant toward the water or oil phase. When an emulsion is formed, surface area expansion is created between the two phases. Generally, emulsifiers or surfactants are used to stabilize emulsions by producing small droplet sizes. Indeed, they can reduce the interfacial tension between the two immiscible phases, which reduces the energy necessary to disrupt the droplets. On the other hand, emulsifiers can also stabilize emulsions by forming a thin layer around the dispersed droplets, which leads to the prevention of coalescence and the decrease of emulsions stability (McClements, 1999). Therefore, the excess surface free energy is dependent on the droplet size and the interfacial tension. Moreover, the time required for the emulsifying agent to adsorb at the interface between oil and water is also important because the emulsifier must adsorb quickly at the droplets in order to give time to the interfacial tension to be reduced, which facilitates the droplet disruption.

The process used to make an emulsion by mixing two immiscible phases and the emulsifying agent is known as emulsification or homogenization. In the food industry sector, for example, high-speed mixers are probably the most used homogenizers. Moreover, ultrasonic homogenizers are recently largely used.

According to the size of their dispersed droplets, we can distinguish three types of emulsions: conventional emulsions, also known as macroemulsions with droplets $>500$ nm; miniemulsions, with droplets between 50 and 500 nm and microemulsions, with droplets $< 50$ nm. Both macroemulsions and miniemulsions are thermodynamically unstable.

In recent years, stable emulsions have rapidly emerged as one of the most promising and attractive methods used as a potential delivery system for bioactive compounds in food systems. Indeed, the high hydrophobicity of some bioactive substances makes them insoluble in aqueous systems, which reduce their bioavailability in the body. Then, to improve the solubility in aqueous systems of many bioactive compounds, to increase their bioavailability during gastrointestinal passage, and to increase their stability in food systems and during storage, these bioactive molecules could be incorporated in the fine droplets of o/w or w/o emulsions. Stable encapsulated bioactive molecules could be produced by forming stable emulsions. This can be achieved through the addition of emulsifiers and stabilizers as well as by producing emulsions with submicron droplets (Mao et al., 2009). Nanoemulsions are kinetically stable emulsions that are obtained when the size of an emulsion globule reaches approximately 50–200 nm. The small droplet size can resist the physical destabilization caused by flocculation, coalescence, and gravitational separation. These properties make nanoemulsions potential delivery systems for bioactive molecules (Shafiq et al., 2007).

## 3.2 Nanoemulsions

### *3.2.1 Definition*

Nanoemulsions are optically clear dispersions consisting of two immiscible liquids, with one liquid phase being dispersed as nanometric droplets into another continuous liquid phase and stabilized through an interfacial film of surfactant molecules or emulsifiers. They appear visibly different from conventional emulsions because their droplets are much smaller than optical wavelengths of the visible spectrum (Van de Hulst, 1981). We can distinguish two types of nanoemulsions, which are most likely to be formed depending on the affinity of the emulsifier toward the aqueous or oil phase: o/w nanoemulsions and w/o nanoemulsions. In particular, o/w nanoemulsions, which are of prevalent interest for food delivery systems, are composed of oil droplets dispersed in an aqueous medium and stabilized by a food-grade surfactant or biopolymeric layer (Mason et al., 2006b). They are systems with a typical particle size range of 50–200 nm (Chen et al., 2011). The small droplet size of nanoemulsions can affect properties such as particle stability, rheology, appearance, texture, and shelf life (Becher, 2001). Different from microemulsions, which are thermodynamically stable and form spontaneously, nanoemulsions are kinetically stable and need high-energy input to be produced. The small droplet size, characterizing nanoemulsions,

can resist the physical destabilization caused by gravitational separation, flocculation, and/or coalescence (McClements, 2005). It also avoids the creaming process because the droplet's Brownian motion is enough to overcome the gravitational separation force (McClements, 2005; Thadros et al., 2004). In addition, nanoemulsions show a lower tendency to droplet aggregation than conventional emulsions as the strength of the net attractive forces acting between droplets usually decreases with decreasing droplet sizes (McClements, 2005). Moreover, nanoemulsions stability can be affected by coalescence phenomena due to Ostwald ripening, where the larger droplets grow at the expense of the smaller ones because of molecular diffusion of oil between droplets through the continuous phase. In recent years, nanoencapsulation of bioactive compounds in o/w nanoemulsions becomes an effective approach for incorporating bioactive molecules in foods matrices known as functional foods.

Depending on the formulation used, in terms of ingredients and preparation procedures, the nanoencapsulated bioactive molecules could have different localizations within the o/w nanoemulsions droplets (Fig. 11.3). The bioactive compound can be entrapped in the inner oil phase or can diffuse into the outer emulsifier film. It is so important to study the localization of the bioactive molecule as it influences the stability, the release, and the bioavailability of the nanoencapsulated agent. For example, the entrapment of the bioactive agent in the emulsifier film is likely to reduce the possibility of a controlled release process. However, the migration of the encapsulated agent from the inner oil phase to the emulsifier film can increase its stability against chemical degradation (Baspinar et al., 2010).

### *3.2.2 Nanoemulsions of Bioactive Compounds*

#### 3.2.2.1 Challenges of Bioactive Compounds

The term bioactive is an alternative term for biologically active. A bioactive compound is a substance that has a biological activity. A bioactive compound is a substance having biological activity

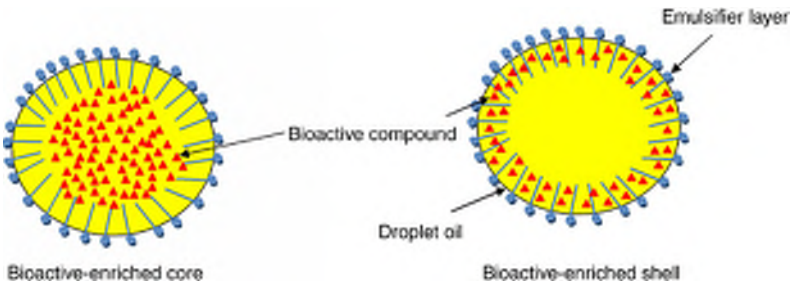


**Figure 11.3. Localization of encapsulated bioactive compound in o/w nanoemulsion droplet.**

affecting directly a living organism. The effect of bioactive compounds could be positive or negative depending on the substance, the dose and its bioavailability (Cammack, 2006). However, in general, it is claimed that bioactive compounds consumed at sufficient levels provide health benefits as prevention or treatment of several diseases (Chu et al., 2007). Recently, the increase in dietary-related diseases as cardiovascular diseases, obesity, diabetes, and cancer, have made the development of functional foods a priority in the food industry sector for the prevention of several diseases. Many researchers proved that the health benefits may be derived from the incorporation of bioactive compounds in food products also known as functional foods. Indeed, incorporating bioactive compounds in food products is able to enhance the nutritional function of foods and contribute health benefit to brain, heart, and immune system and reduce the risk of chronic diseases. For example, antioxidants are known by their main antioxidant activity, they protect human cells and help reduce the risk of chronic and cardiovascular diseases. Moreover, they are known as anticancer agents (Krishnaiah et al., 2010). Another example of bioactive compounds is the omega-3, 6, and 9 fatty acids. They are polyunsaturated fatty acids known by their immune function, heart disease prevention, and cancer prevention.

However, many challenges and difficulties are associated with the inclusion of many bioactive compounds in food systems. The main factors, reducing the biological and functional properties of bioactive agents in food matrices, are their low solubility in aqueous systems and their instability and high degradation rate by environmental conditions as pH, oxygen, and temperature affecting their bioavailability and release processes. The degradation of bioactive molecules during formulation, processing, and storage affects their biological activity and functionality as well as their antioxidant activity. Furthermore, some health functional compounds are hardly absorbed; however, some other molecules react with other components, which reduces their absorption. In addition, some other bioactive substances are known by their undesirable odor and flavor limiting their incorporation in food products such as fish oils rich in polyunsaturated fatty acids.

Then, when developing functional foods, it becomes necessary to encapsulate bioactive compounds using edible delivery systems to efficiently protect and ameliorate their bioavailability, their solubility, and their release. Nanoemulsions are one of the most interesting fields of application as they can act as carriers or delivery systems for bioactive compounds as flavors, antioxidants, and antimicrobial agents (McClements et al., 2007; Weiss et al., 2008; Wissing et al., 2004).

#### 3.2.2.2 Nanoemulsions as Delivery Systems for Bioactive Compounds

The pharmaceutical industry is the dominant field where most applications of nanoemulsions are proposed and where research has been conducted to use nanoemulsions as delivery systems for poorly soluble drugs and to improve their bioavailability (Shah et al., 2010; Shafiq et al., 2007). Recently, nanoencapsulation of bioactive compounds in o/w nanoemulsions has been found to be an effective approach for incorporating highly instable or poorly water-soluble compounds in foods. Indeed, encapsulation of bioactive compounds using nanoemulsions in the food industry represents an efficient method to increase the physical stability of the bioactive molecules, to protect them from the interactions with the environment, to decrease their volatility and toxicity, to enhance their bioactivity and to modulate their release (Salvia-Trujillo et al., 2013).

Several lipophilic bioactive substances of food grade with health interest have been encapsulated in nanoemulsions (Table 11.2). Omega-3 fatty acids, β-carotene, tocopherols, curcumin, essential oils are the mainly lipophilic bioactive compounds incorporated to fortify food products. The low bioavailability, poor water solubility, oxidation, release, and sensorial detection of these substances in food products are the main challenges that can be solved by their incorporation in nanoemulsions.

## Table 11.2 Examples of Nanoemulsion-Based Delivery Systems

| Techniques | Emulsifiers | Oil Phases | Bioactives | References |
|---|---|---|---|---|
| High-pressure homogenization | Tween-20, 40, 60, and 80<br>Sodium caseinate | Medium chain triglycerides (MCT)<br>Stearin-rich milk fat | β-carotene, α-tocopherol, curcumin | Yuan et al. (2008); Wang et al. (2008) |
| Ultrasound | Lecithin, Tween-80, sodium dodecyl sulfate | MCT, sunflower oil, soybean oil | d-limonene | de Araújo et al. (2007); Jafari et al. (2007) |
| Microfluidization | Modified starch, sodium caseinate, whey protein isolate | — | d-limonene, fish oil | Jafari et al. (2007); Jafari et al. (2006) |
| Solvent displacement technique | Tween-80–Span20 | — | α-tocopherol | Kong et al. (2011) |
| Emulsification–evaporation method | Tween-20 | — | Lycopene, β-carotene | Ha et al. (2015); Tan and Nakajima (2005) |

## 3.3 Nanoemulsions Production

O/w nanoemulsions are prepared using oils, emulsifiers, and aqueous phase. Emulsifiers are used to stabilize nanoemulsions by reducing the interfacial tension between the two immiscible phases. An effective emulsifier should have three characteristics: (1) rapidly adsorb to the oil/water interface of newly formed droplets during emulsification; (2) reduce the interfacial tension; and (3) form an interfacial membrane to stabilize the nanoemulsion by steric or electrostatic interactions between droplets. A large number of food grade biopolymers exhibit such properties and they can be used as emulsifiers to stabilize nanoemulsions for food industry applications (Table 11.2).

Moreover, as delivery systems for bioactive compounds in food industry, oils used in o/w nanoemulsions formulation should be of food grade properties; moreover, the solubility of the bioactive compounds in the oil phase should be an essential criterion for the selection of this phase. Indeed, the ability of nanoemulsion to maintain encapsulated agent in solubilized form is influenced by the solubility of this agent in the oil phase. Another important parameter influenced by the solubility of the active compound is the controlled release of this agent in the formulation and during the consumption of the fortified food product with nanoemulsions encapsulating bioactive substances. Table 11.2, summarizes some lipid phases and emulsifiers frequently used for the formulation of nanoemulsions encapsulating bioactive molecules and designed for the food industry.

However, as delivery systems in food products, the most important parameter for the selection of all these nanoemulsions components is that they should be of food grade depending on the requirement and falling under the “generally recognized as safe” (GRAS) category.

As with conventional emulsions, o/w nanoemulsions are usually prepared by homogenizing oil phase in an aqueous phase together in the presence of water-soluble emulsifier. The lipid or oil phase of o/w nanoemulsions acts as a carrier of lipophilic active substances. Then, this bioactive compound is dispersed in the oil phase prior to homogenization or formulation of the nanoemulsions. However, the role of the emulsifier is to stabilize and prevent the breakdown of the nanoemulsion structure once is formed increasing the stability of the encapsulated bioactive. Then, the formulation and preparation of these nonequilibrated systems require the use of an energy source for the homogenization (Anton and Vandamme, 2009).

In general, two essential methods have been introduced to produce nanoemulsions: high-energy and low-energy methods.

High-energy methods include microfluidization, high-pressure homogenization and ultrasonication (Tadros et al., 2004; Anton et al., 2007; Jafari et al., 2008). However, low-energy methods include phase inversion temperature (PIT) (Fernandez et al., 2004; Shinoda and Saito, 1968), phase inversion composition also called emulsion inversion point (EIP) (Pey et al., 2006; Porras et al., 2008) and solvent displacement technique (Solans et al., 2005; Porras et al., 2008). The furnished energy will be able to deform the interface between the immiscible phases and the droplets will be subsequently broken up or disrupted into smaller ones.

### *3.3.1 High-Energy Methods*

These are high-energy techniques using mechanical devices to create intensely disruptive forces, which break up the dispersed droplets to form nanosized droplets. This include high-pressure homogenization, microfluidization, and ultrasonication (Mason et al., 2006a; Graves et al., 2005; Jafari et al., 2007; Anton et al., 2008).

High-pressure homogenization is the most used method to produce fine emulsions or nanoemulsions in the food industry. To produce a fine nanoemulsions using high-pressure homogenization methods, a coarse emulsion is usually produced using a high-speed mixer, which is then fed into the input of a high-pressure valve homogenizer at a pressure in the range of 500–5000 psi (Fig. 11.4). The emulsification process using high-pressure homogenization can be represented by two stages. First, the disruption of the droplets, which increases the surface area of

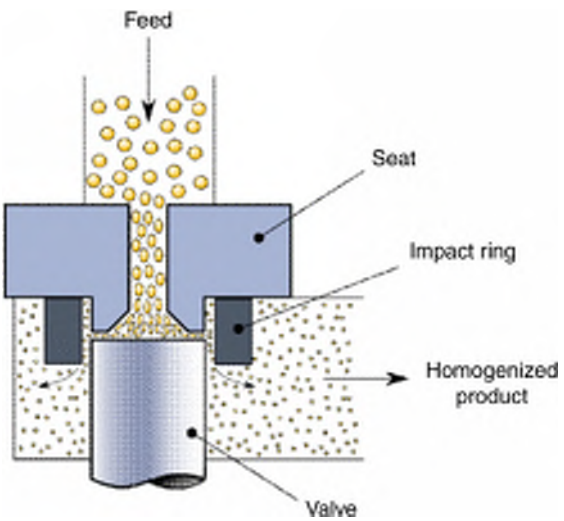


**Figure 11.4. Schematic representation of high-pressure homogenizer.**

the emulsion, then the stabilization of the droplets by means of the adsorption of the emulsifier at the newly formed interfaces (Hakansson et al., 2009a,b).

Nanoemulsion droplet size as small as 100nm can be produced using this method if there is sufficient emulsifier present to completely cover the oil-water interface and the adsorption kinetic is high to prevent droplet coalescence (McClements, 2005). Moreover, homogenization of the nanoemulsion can be repeated many times to ensure the production of extremely small particle sizes (Mason et al., 2006b).

For the microfluidization process, a coarse emulsion is pumped under a very high pressure (up to 40,000 psi) through a patented interaction chamber with microchannels of fixed geometry. The coarse emulsion feed through the microchannels into a collision chamber to form a fine nanoemulsion (Fig. 11.5).

For these two methods, the operating pressure and the number of passing cycles of the coarse emulsion through the homogenizer or the microfluidizer have a significant effect on the particle size of the formed nanoemulsions.

The use of these two methods to produce nanoemulsions or to produce nanoemulsions of bioactive compounds has been studied in several works (Lee et al., 2014; Tan and Nakajima, 2005; Qian and McClements, 2010; Yuan et al., 2008). Tan and Nakajima (2005) have successfully encapsulated β-carotene in nanoemulsions prepared by high-pressure homogenization and they produced a fine nanoemulsion of β-carotene with droplet size ranging from 60 to 140 nm. Jafari et al. (2007) produced d-limonene nanoemulsions

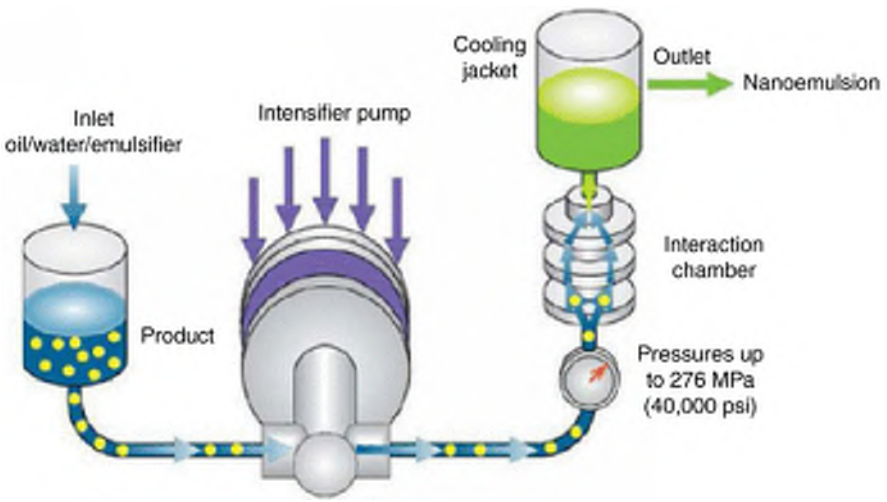


**Figure 11.5. Schematic representation of microfluidizer processor.**

with droplet size of 150–700nm using microfluidizer at pressure ranging from 35 to 105MPa. Microfluidizer was also successfully used by Mason et al. (2006b) to produce silicon oil nanoemulsions stabilized by SDS with very fine droplets diameter of 50nm.

Ultrasound is also another mechanical method used to produce fine emulsions in nanoscale droplet size (Galindo-Alvarez et al., 2011; Bhanvase et al., 2012). It consists on the formation and collapse of vapor cavities in a flowing liquid. First, the application of acoustic field produces interfacial waves resulting in the dispersion of the dispersed phase in the continuous phase to form droplets. Then, the application of ultrasound causes acoustic cavitations leading to the formation and subsequent collapse of microbubbles by the pressure flocculation of a simple sound wave. Then, a highly localized turbulence can be created, which break up primary droplets into nanoscale size (Li and Fogler, 1978; Paulusse and Sijbesma, 2006). By varying the time and the ultrasound energy input, we can produce nanoemulsions with very fine droplets sizes.

The use of ultrasound to produce stable nanoemulsions has been well documented (Jafari et al., 2007; Carneiro et al., 2013; Kentish et al., 2008; Jadhav et al., 2015; Abbas et al., 2014; Tan et al., 2013). Efficiency of nanoemulsification by ultrasound (considering droplet size and required time to prepare fine nanoemulsion), depends both on the emulsion composition (lipid and aqueous phases, emulsifier) and the power of the device (Leong et al., 2009). However, in spite of high potential of ultrasound for nanoemulsion formulation, the use of this method in the food industry is not practical and high-pressure homogenization or microfluidization are often preferred (Samer and Schork, 1999).

### *3.3.2 Low-Energy Methods*

Nanoemulsions could be produced using nonmechanical techniques. These methods are based on physicochemical behavior of the system by altering the spontaneous curvature of the surfactants (Carneiro et al., 2013). The most used methods are Phase Inversion Temperature (PIT), Phase Inversion Composition (PIC), and solvent displacement technique. PIT and PIC methods are based on the phase transitions taking place during the emulsification process. They result from changes in the spontaneous curvature or solubility of the emulsifier or the surfactant that can be achieved: (1) at constant composition by changing the spontaneous curvature or solubility of the emulsifier with temperature (PIT); or (2) at constant temperature by varying the composition of the system (PIC). For the PIT method, the emulsification is based on the changes of the solubility of nonionic surfactants

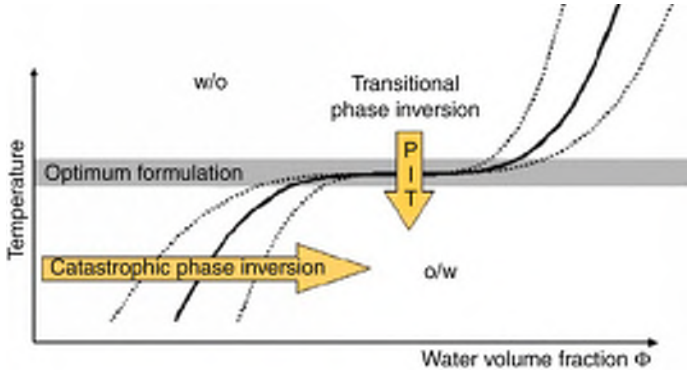


**Figure 11.6. Schematic illustration of both catastrophic and transitional phase inversion for the preparation of finely dispersed o/w emulsions.** With permission from Fernandez et al., 2004. Colloids Surf. A Physicochem. Eng. Aspects 251, 53–58.

or emulsifiers with temperature. The surfactant is hydrophilic at low temperatures but becomes lipophilic with increasing temperature forcing a transition from o/w emulsion at low temperature to a w/o emulsion at high temperatures (Fig. 11.6). During cooling, the system crosses a point of zero spontaneous curvature and minimal surface tension, leading the formation of very fine dispersed droplets (Morales et al., 2003; Fernandez et al., 2004; McClements, 2010).

For phase inversion composition (PIC), the temperature is maintained constant; however, the emulsion composition is changed. This method consists of a progressive dilution of the system by adding water or oil in order to create stable nanoemulsions (Sadtler et al., 2010).

Finally, solvent displacement technique is based on mixing a water-soluble solvent-containing oil phase, in which the lipophilic bioactive compound is dispersed in an aqueous phase containing an emulsifier. The rapid diffusion of the organic solvent in the aqueous phase leads to the formation of nanoemulsions. The organic solvent can be removed by evaporation under reduced pressure (Yin et al., 2009). However, due to the use of organic solvents, application of nonmechanical methods is limited in the food industry.

## 3.4 Characterization of Nanoemulsions

After preparation, identification and characterization of nanoemulsions are essential stages to understand the stability of these delivery systems leading to the stability of the encapsulated compound, their potential toxicity as well as to study the biological

properties of the encapsulated agents (their bioavailability, their controlled release, their biological activities, etc.).

### 3.4.1 Physical Properties of Nanoemulsions

Nanoemulsions are characterized by numerous interesting physical properties that are different from those of larger microscale emulsions. In this section, we focus on the most important properties that distinguish nanoemulsions from the other conventional emulsions as droplet properties (size, polydispersity, and zeta potential), morphology, and stability of nanoemulsions.

### 3.4.2 Droplet Size, Polydispersity Index, and Zeta Potential

These parameters are related to the stability, appearance, and rheology of the prepared nanoemulsions. Dynamic light scattering (DLS), also called photon correlation spectroscopy (PCS), is used to analyze the fluctuations in the intensity of scattering by droplets or particles due to Brownian motion (Ruth et al., 1995). The Brownian motion measurements are related to the particle size of nanoemulsions through Stokes-Einstein theory. It is a rapid technique usually used for the determination of the size distribution profile of particles dispersed in suspensions or in solutions (Silva et al., 2011; Preetz et al., 2010). However, sometimes, using DLS technique, a large error can occur for inhomogeneous or polydisperse nanoemulsions with a wide particle size distribution. In this case, some supplemental tools can be used to characterize size and size distribution of nanoemulsion droplets. Electron microscopy and cryogenic transmission electron (Cryo-SEM) are commonly used to confirm droplet size and size distribution in this case of nanoemulsions.

DLS can also measure the polydispersity index and the zeta potential of nanoemulsions. Polydispersity index reflects the broadness of the size distribution derived from the cumulative analysis of DLS. This is an important parameter that indicates the homogeneity of the nanoemulsions (Li et al., 2011). Zeta potential reflects the electrokinetic potential in colloidal systems (Mills et al., 1993). Zeta potential value can be related to the stability of the nanoemulsions indicating the degree of repulsion between similarly charged particles. In the case of nanoemulsions, small droplets in the nanoscale range will confer higher stability to the dispersion, which will resist more to the aggregation. In contrast, low zeta potential, attractions between droplets exceed repulsions and the dispersion can then flocculate. In general, zeta potential from 0 to 30mV indicates instability; however, zeta potential higher than 30mV indicates stability (ASTM, 1985). Recent research indicated

that, for nanoemulsions, highly charged droplets surfaces are stable and the nanoemulsions will resist to droplets aggregation (Preetz et al., 2010; Gao et al., 2011).

### *3.4.3 Morphology of Nanoemulsions*

The morphology of nanoemulsions can be determined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM examination gives a 3-dimensional (3-D) image of the droplets. Using this technique, a good analysis of surface morphology of nanoemulsions droplets can be obtained. SEM is the most used technique in research areas because of its greater resolution, ease of sample observation, higher magnification, and larger depth of field (Luykx et al., 2008). However, this technique requires high vacuum and high sample conductivity that make it an expensive technique. TEM can give higher resolution images of the dispersed phase in the order or 0.2 nm (Luykx et al., 2008). For this technique, the studied samples should be very thin and be able to resist the high vacuum present inside the apparatus (Wang, 2000). In general, TEM resolution is about an order of magnitude higher than SEM resolution. Nevertheless, SEM image relies on surface processes rather than TEM, which makes SEM techniques able to image bulky samples, so it can produce images with good representation of the 3-D structure of the examined sample (Luykx et al., 2008).

## 4 Nanoemulsions Stability: Encapsulated Bioactive Compounds Protection

The protection of bioactive compounds encapsulated into nanoemulsions and incorporated into food matrices, against degradation or interactions with food ingredients, occurs through formulation of stable delivery systems. Indeed, an efficient nanodelivery system, such as nanoemulsions, must be stable in the food matrices during food preparation and over long periods of time. Such stability is able to ensure an efficient controlled release of the active substances when the fortified food matrices are consumed.

However, although nanoemulsions are kinetically stable, stability of the encapsulated compounds is one of the major problems associated with the development of nanoemulsions during storage or during incorporation in food products. Several parameters associated with the prepared nanoemulsions should be determined to ensure the stability of the encapsulated bioactive substances. For example, droplet size, viscosity, turbidity,

refractive index, the bioactive content should be determined during storage. Any significant changes of these parameters reflect nanoemulsion instability indicating the possible degradation of the encapsulated agent. Moreover, some analytical techniques, such as chromatography techniques, can be used to analyze and identify the encapsulated substance in the formulated nanoemulsions or to identify this bioactive compound in the fortified food products. These techniques will be able to identify the amounts and the biological activities of the encapsulated agents when they are incorporated to fortify foods.

Then, to ensure the stability of the encapsulated bioactive substance, the stability mechanism of nanoemulsions used as delivery system should be optimized.

In general, nanoemulsions are kinetically stable systems. However, different processes of physical and chemical instability can occur resulting in the alteration of these delivery systems, which can affect the biological properties of the encapsulated bioactive substances. Chemical instability, in general, results in the alteration of the encapsulated agent, such as oxidation or hydrolysis. However, the physical destabilization of nanoemulsions is generally related to the spontaneous trend toward a minimal interfacial area between continuous and dispersed phases. Three essential mechanisms can be the origin of the destabilization of nanoemulsions (Fig. 11.7): Creaming, the flocculation followed by coalescence and the Ostwald ripening (McClements, 2005; Anton et al., 2008).

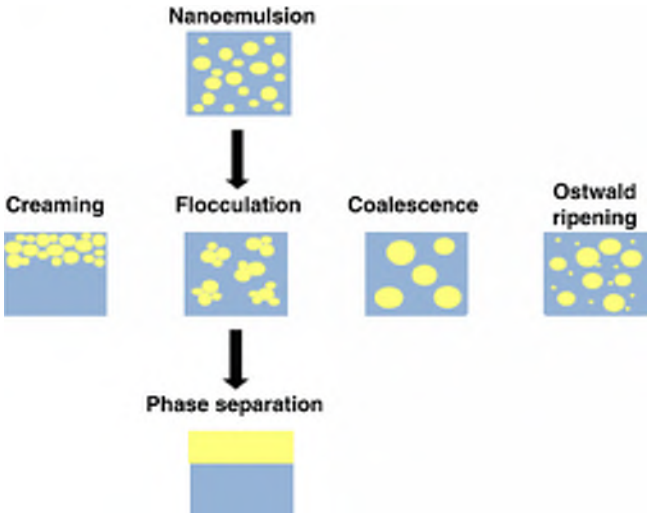


**Figure 11.7. Schematic representation of the physical destabilization of nanoemulsions.**

In general, creaming is the mechanism by which the dispersed droplets of prepared emulsions move due to the action of gravity forces on the density differences between the two immiscible phases (Tadros and Vincent, 1983). It is a reversible process with a minimal energy input. However, nanoemulsions are in general highly stable to gravitational separation thanks to small droplet sizes as the creaming rate depends on the square of the droplet diameter, emphasizing the need for efficient homogenization (McClements, 2011).

Flocculation consists on the reversible aggregation of the droplets into clusters (Binks, 1998). It occurs when there is a weak, net attraction between droplets. Moreover, flocculation results from sedimentation aggregation or from Brownian motion aggregation of the droplets. Some authors considered that nanoemulsions can resist against flocculation (Anton et al., 2008), due to the high curvature reducing the region of contact of two droplets, which reduces the probability of two droplets to aggregate.

The coalescence fact is an irreversible destabilization mechanism characterized by the rupture of the interfacial film between the dispersed droplets and each individual droplet merges with other droplets forming a bigger one with lower interfacial area (Walstra, 1993). It consists on a first approach of droplet oils to close proximity; then the film surrounding the droplets and separating them must drain and allow the droplets' contents to flow together (McClements, 2005). In this case, the measurement of the zeta potential of nanoemulsions is an important parameter because we can have an idea about the charged droplets. In fact, coalescence and film drainage could be significantly inhibited through adequate repulsive forces between droplets. On the other hand, the mechanical properties of the film are able sometimes to limit the coalescence especially when the emulsifier chains are long enough or if the emulsifier concentration is high enough to produce a strong interfacial film.

Finally, Ostwald ripening is a mechanism by which the larger droplets grow at the expense of the smallest ones because of molecular diffusion of oil between droplets through the continuous phase. Being its rate dictated by the solubility of the oil in the continuous phase, Ostwald ripening rate can be reduced by selecting oil with low aqueous phase solubility. For example, in the case of essential oils nanoemulsions, which are relatively soluble in the continuous phases, Ostwald ripening can be reduced by adding second oil with lower water solubility. In this case, the second oil will change the partitioning of the essential oil between lipid and aqueous phase (Sagalowicz et al., 2006).

## 5 Improved Bioavailability of Nanoencapsulated Bioactive Compounds

In order to exert the nutritional and health benefits, the bioactive compound should withstand food processing, be released from the food matrix during the postingestion step and be accessible in the gastrointestinal tract and reach the target tissue of action. All of this process can be summarized in the bioavailability of the bioactive compound incorporated in a food product or functional food (Espìn et al., 2007; Manach et al., 2005).

The term bioavailability designs the portion of a particular nutrient that is digested, absorbed, and metabolized through normal pathways. There are diverse factors that limit the bioavailability of bioactive compounds through the gastrointestinal tract during the consumption of functional food or food products fortified with active substances. The essential factors are pH and enzymatic activities in the stomach of the intestinal tract.

Several bioactive compounds of interesting biological and nutritional properties are poorly absorbed and quickly metabolized such as carotenes, curcumin, essential oils, omega-3 fatty acids. The major challenge of the use of these active agents to fortify food products is their low solubility and bioavailability in aqueous phases preventing these molecules from reaching the target organs in active form. This problem is the major challenge limiting the use of these active compounds to fortify food products in order to produce functional foods. Moreover, the bioavailability of these bioactive substances depends on the complexity of food formulations and their preparation processes as well as their transition during digestion (Acosta, 2009). In general, food, after consumption, undergoes a gastrointestinal digestion process that is able to affect the native bioactive substances properties present in the functional food product. Nanoemulsions are delivery systems that can contribute to the increase the bioavailability of bioactive compounds and they can improve their solubility and stability. These systems are able to improve the uptake of the encapsulated bioactives in the gastrointestinal tract and enhance their transport to the target sites.

Then, to understand the effect of these delivery systems on the bioavailability of bioactive compounds incorporated into a food matrix, we have to understand first the effect of each stage of food digestion on the stability and the bioavailability of the active compound.

The term bioavailability is defined as the proportion of a nutrient or a bioactive compound used for normal physiological functions (Fairweather-Tait, 1993). The bioavailability includes two

important stages: bioaccessibility and bioactivity. Bioacessibility can be defined as the fraction of the active compound that can be released from the food matrix in the gastrointestinal tract and available for intestinal absorption. Especially, bioaccessibility includes the known events taking place during food digestion from the first stage, which is the food mastication to the transformation into potentially active substances, absorption, and presystemic metabolism. Then, the first step of the bioaccessibility process of a functional food product is the mastication in the mouth, which initiates the process of digestion. At this stage, the food product is masticated and mixed with saliva and broken down in small pieces. Then, in the stomach, and throughout the remainder of the gastrointestinal lumen, the food product is subject to a complex series of physicochemical changes induced by the highly acidic environment as well as enzyme hydrolysis (Gropper and Smith, 2009). Then, in the case of a functional food matrix, bioactive substances will never resist at acidic pH or enzymatic activities in the stomach. Therefore, when we manipulate with an encapsulated bioactive compound through a delivery system such as o/w nanoemulsions, this means that these active and instable molecules are surrounded or coated into a lipid droplet, which is surrounded by a multilayer emulsifier or an emulsifier film. This coating film is a physical barrier between the encapsulated agent and the acidic aqueous phase where the digestive enzymes are located.

Then, these nanosized delivery systems are able to facilitate entering the bioactive agents through a biological barrier limiting the effect of the metabolic modifications that lead to the instability, alteration, and low absorption (Xie et al., 2011).

After that, the digested functional food will leave the stomach to enter in the small intestine. At this stage, digested food product is broken down by bile, pancreatic, and other enzymes. The lipid fraction of the food product as well as hydrophobic compounds will be emulsified by bile and transported to the entrocyte cells where they will be absorbed. In general, the absorption of bioactive compounds in the intestine is achieved through active and passive transport.

However, the second stage related to the bioavailability of the bioactive compound is its bioactivity. It includes events linked to the transport of the bioactive compound to reach the target tissue, its interactions with biomolecules, its metabolism, and the generation of biomarkers and the physiologic responses (Fernández-García et al., 2009).

The particle size of the delivery system is an important parameter that can affect the absorption of the bioactive compounds through the intestinal walls. Indeed, a nanoscale delivery system is able to increase the absorption of the nanoencapsulated agent,

by improving the passive transport thanks to the nanosized particles (Hussain et al., 2001). Several works studied the effect of the nanoencapsulation of bioactive compounds on their bioavailability and absorption. The main observations proved that there was a clear correlation between the particle size of the encapsulated agent and its bioavailability as well as its absorption through the intestinal walls. In general, active compounds with particle size below 500nm showed higher absorption and bioavailability (Acosta, 2009). Indeed, due to their small size, nanoemulsions are characterized by a large surface area allowing a rapid penetration of the active compound through the intestinal walls.

For example, curcumin is well known for its interesting biological and pharmacological properties (Menon and Sudheer, 2007; Zhou et al., 2011). However, it is known by its poor solubility in aqueous phases limiting its bioavailability (Lin and Lin-Shiau, 2009; De et al., 2009). Curcumin was encapsulated through poly-lactic-co-glycolic acid nanoparticles and experiments made with the incorporation of these nanoparticles in rats showed that their bioavailability was improved 5.6-fold higher compared with free curcumin (European Food Safety Authority, 2011). Another example of the effect of the droplet size on the bioavailability of the encapsulated compound is the polyunsaturated fatty acids. Indeed, several studies showed that the incorporation of fish oil containing high amount of polyunsaturated fatty acids (PUFAs) in microparticles, liposomes, gel emulsions, and plant spores showed an increase in their bioavailability when decreasing the particle sizes (Haug et al., 2011; Wakil et al., 2010).

Salvia-Trujillo et al. (2013) studied the effect of the particle size on lipid digestion and β-carotene bioaccessibility using as delivery systems corn o/w emulsions differing by their initial droplet diameters. The authors proved in their study that the bioaccessibility of the encapsulated β-carotene increased with decreasing the droplets diameter. The authors explained these results by the fact that emulsions with the largest droplets size were highly susceptible to coalescence and disruption within different regions of the gastrointestinal tract, while those with smaller droplets showed more stability against flocculation.

## 6 Controlled Release of Nanoencapsulated Bioactive Compounds

Another important parameter that can be controlled by the encapsulation of bioactive compounds in nanoscale delivery systems is their release to their target site, at specific rate and at specific time (Pothakamury and Barbosa-Canovas, 1995). Releasing

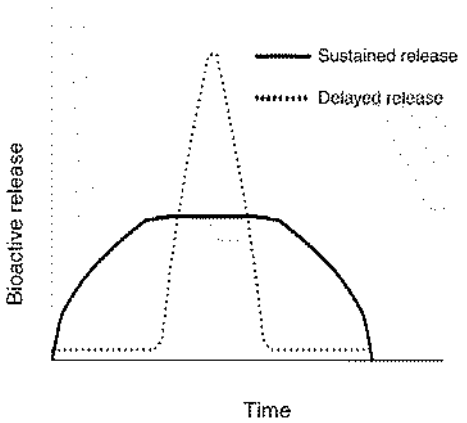


**Figure 11.8. Mechanisms of release of bioactive compounds.** With permission from Fathi et al., 2012. Trends Food Sci. Technol., 23, 13–27.

mechanisms can be controlled by external conditions as pH, enzymes, temperature, and moisture (Desai and Park, 2005). Moreover, the properties of the nanoparticles can also affect the release of the nanoencapsulated bioactive compound.

Nanoscale delivery systems are able to provide a large surface area for interaction with the encapsulated agent. In general, two methods of controlled release can occur during delivery of encapsulated bioactive compounds (Fig. 11.8): delayed and sustained release (Fathi et al., 2012; Lakkis, 2007). Delayed release is a mechanism by which the release of the active compound is delayed from a finite lag time up to a point where its release is favored. However, for sustained release, the concentration of the encapsulated active agent is maintained constant over a prolonged release time (Fathi and Mohebbi, 2010). Delayed release is used for achieving the protection of active agents during gastric digestion and the release in the gastrointestinal tract.

Controlled release of encapsulated agents can be influenced by various factors including environment conditions, form, and shape of the nanoparticles, the particle sizes, the bioactive solubility and diffusivity as well as the porosity of the wall material used for the encapsulation (Zhang et al., 2003; Briones and Sato, 2010). Indeed, nanometric-size delivery systems offer considerable advantages not only for increasing the stability and solubility of bioactive compounds in food products but also for controlling their release improving their bioaccessibility and absorption into the body.

Another important parameter that can affect the release and the bioavailability of the encapsulated agent is its localization

within the nanoemulsion droplets. This parameter has been proved by Baspinar et al. (2010) who studied the encapsulation of prednicarbate through nanoemulsions prepared by high-pressure homogenization and stabilized by polysorbate and purified egg lecithin as emulsifiers. The authors observed that the higher stability of the encapsulated agent was obtained with low homogenization pressure and higher temperature but with higher numbers of homogenization cycles. They attributed this interesting result to an effective immigration of the encapsulated agent from the inner oil to the stable emulsifier outer layer.

# 7 Applications of Nanoemulsions as Delivery Systems in Food Industry

In recent years, nanotechnology has been developed to involve several industrial sectors. Food industry is one of the recent promoting area that can promote from the advantages of the nanotechnology (Neethirajan and Jayas 2011).

Nanoemulsions could be used as potential delivery systems of bioactive compounds. The produced nanosized particles could be incorporated into food matrices in order to produce functional food products of interesting biological and nutritional properties. Several bioactive compounds with high biological activities could be of interest in the food industry such as carotenoids, vitamins, flavoring agents, antioxidants, essential oils, antimicrobial agents.

We give three examples of bioactive compounds, encapsulated through nanoemulsions, which are essential oils, polyunsaturated fatty acids, and β-carotene. These bioactive agents are known by their high biological activities, their health benefits, and their nutritional properties. However, the major challenges limiting the incorporation of these active substances into food matrices is their low solubility in aqueous phases, their low bioavailability, and their instability during the different food preparation stages as well as through the gastrointestinal tract during the consumption of the fortified food.

## 7.1 Nanoemulsions of Essential Oils

Essential oils are volatile natural, aromatic oily liquids that can be obtained from several parts of plants especially from the aerial ones. They are complex mixtures of volatile compounds such as terpenoids, aliphatic compounds, and phenol-derived aromatic components. Essential oils are used for their interesting biological

properties including bactericidal, fungicidal, antioxidant, and antimicrobial properties (Abdelouaheb and Amadou, 2012; Tetsuo et al., 2000; Riccardo et al., 2013). The use of natural essential oils for their biological properties in food products is more and more developed (Burt, 2004). They can be also used in food packaging especially in the formulation of active edible coatings for food products (Salvia-Trujillo et al., 2015).

Several recent researches focused on the encapsulation of essential oils in order to protect and improve their biological properties using several methods. The most used methods for essential oils encapsulation are spray drying (De Souza et al., 2013; Carneiro et al., 2013; Charve and Reineccius, 2009) and complex coacervation (Dima et al., 2014). The encapsulation of essential oils in nanoscale delivery systems is another interesting area to protect and increase their solubility and bioavailability. Encapsulation of essential oils in nanoemulsion systems would be an alternative to increase their solubility and limit their oxidation process (Chang et al., 2012; Salvia-Trujillo et al., 2013; Donsì et al., 2011). Such nanoscale delivery systems have been well studied by many authors in the past few year and they have been used as delivery systems to improve the biological functionalities and properties of essential oils (Ziani et al., 2011; Salvia-Trujillo et al., 2015; Donsì et al., 2011; Liang et al., 2013).

Salvia-Trujillo et al. (2015) proved that o/w nanoemulsions produced by microfluidization could be a potential technology for the formulation of lemongrass nanoemulsions with droplet size of 135 nm. However, they observed that the conditions of this technology should be optimized in order to limit the degradation of the antimicrobial properties of the essential oil.

Donsì et al. (2012) studied the encapsulation of carvacrol, limonene, and cinnamaldehyde in the sunflower oil droplets of nanoemulsions prepared by high-pressure homogenizations and stabilized by lecithin, pea proteins, sugar ester, and a mixture of Tween-20 and glycerol monooleate. The authors studied the antimicrobial activity of the encapsulated essential oils against *Escherichia coli*, *Lactobacillus delbrueckii*, and *Sccharomyces cerevisiae*. They observed that the effect of the delivery systems on the antimicrobial activity was correlated to the concentration of the essential oil in the aqueous phase in equilibrium with the nanoemulsion droplets. These results proved that the efficiency of the biological activity of the essential oil as an antimicrobial agent is directly associated to their dissolution in the aqueous phase. Such results proved that nanoemulsions are a good candidate for delivery of poorly water-soluble substances as essential oils, which are able to enhance their absorption in the gastrointestinal tract.

Li and Chiang (2012) studied the encapsulation of d-limonene in nanoemulsions systems prepared by ultrasonic emulsification method. The authors used a mixture of sorbitane trioleate and polyoxyethylene (20) oleyl ether as surfactants. They used response surface methodology for the optimization of the formulation of stable nanoemulsions. The authors obtained stable d-limonene nanoemulsions with particle size below 100 nm. Moreover, they showed that nanoemulsions of d-limonene stored at room temperatures were stable against Ostwald ripening for 8 weeks. Furthermore, the authors proved that the particle size of the delivery system for has a significant effect on the stability of d-limonene. Indeed, they compared the stability of d-limonene encapsulated into conventional emulsions with large droplet sizes to that of d-limonene encapsulated into nanoemulsions with droplet sizes below 100 nm. They proved that decreasing the droplet sizes of the delivery system was able to decrease the degradation and the oxidation of encapsulated d-limonene. Such important results proved that nanoemulsions could be an effective solution for the oxidation and low bioavailability problems for d-limonene suggesting that these delivery systems could be successfully applied for food industry to encapsulate lipophilic agents.

Moreover, nanoemulsions could be successfully used as delivery systems of essential oils with antimicrobial activity incorporated into edible coatings to control the postharvest quality of fruits and vegetables during their storage. Recently, nanoemulsions containing lemongrass essential oil as an antimicrobial agent were used as edible coatings to control the safety and quality parameters of fresh-cut fuji apples during storage. The efficiency of these nanoemulsions edible coatings was compared to that of an edible coating obtained from conventional emulsions (Salvia-Trujillo et al., 2015). The results of this study showed that the microbial quality of coated fruits with antimicrobial nanoemulsions edible coating was maintained during storage compared to antimicrobial conventional emulsions edible coating. Indeed, *E. coli* was greater inactivated during storage using nanoemulsions as edible coating.

## 7.2 Nanoemulsions of β-carotene

Carotenoids pigments are a diverse group of lipophilic compounds contributing to the yellow to red color of fruits and vegetables. The most common carotenoids types are β-carotene, lycopene, and lutein. β-carotene (pro-vitamin A) is a hydrocarbon containing 11 conjugated double bonds. It is also an effective

antioxidant and it plays an important biological role in the body (Naves and Moreno, 1998). Consumed in sufficient levels, β-carotene can reduce the risk of many chronic diseases such as cancer, cardiovascular diseases, and cataracts (Failla et al., 2007). Fortifying food products with β-carotene is recently the most rational way of ensuring a daily intake of this active substance. While due to its lipophilic nature as well as to its low stability against high temperature, oxygen, and light; the use of beta-carotene as an active agent to fortify food matrices is so limited. Moreover, the absorption of β-carotene through the gastrointestinal tract is often insufficient for numerous reasons. First, β-carotene is a lipophilic compound, so it is characterized by a very low water-solubility, which limits its bioavailability and absorption (Boon et al., 2010). Second, β-carotene is sensitive to light, oxygen, and heat limiting its application in the food industry (Rodriguez-Huezo et al., 2004). Encapsulation of beta-carotene has been found to offer possible solutions to enhance stability, water solubility, and to improve bioavailability of such hydrophobic biomolecule (Rascon et al., 2011; Sutter et al., 2007). Then, as β-carotene is highly soluble in lipid phases, o/w nanoemulsions may be suitable delivery systems for this bioactive substance. In general, β-carotene o/w nanoemulsions are prepared in few stages. First, β-carotene is dispersed in the oil phase followed by emulsification in aqueous phase containing the emulsifier (Zuidam and Nedovic, 2010). In this form, the release and bioavailability of β-carotene can significantly increase.

In recent years, many researchers have been interested in the use of nanoemulsions as delivery systems for β-carotene especially for food applications. Nanoemulsions of β-carotene have been formulated by different methods and in each study the effect of the used formulation technique on the stability of the nanoemulsions as well as on the β-carotene stability have been well established. For example, Silva et al. (2011) studied the stability of β-carotene nanoemulsions prepared by high-energy emulsification-evaporation technique. The objective of the authors was to look for a novel emulsification technique that can substitute the high-energy methods usually used as high-pressure homogenization. According to this study, β-carotene nanoemulsions showed a good physical stability in terms of particle size. However, the major problem showed using this technique of emulsification-evaporation was the chemical instability of the encapsulated β-carotene during storage. Indeed, nanoemulsions showed a significant decrease in term of β-carotene retention during storage. Another problem that can limit the use of this technique in the food industry is the use of organic solvent as hexane during the nanoemulsions preparation.

However, Yuan et al. (2008) and Tan and Nakajima (2005) successfully prepared stable β-carotene nanoemulsions using high pressure homogenization technique.

The bioavailability of β-carotene has often been found to be limited depending on the complementary components present in the food (Ribeiro et al., 2008).

Moreover, several studies showed that nanoemulsions are able to improve the solubility of β-carotene in water (Horn and Rieger, 2001) and increase its bioavailability during gastrointestinal tract (Yuan et al., 2008; Horn and Rieger, 2001).

Qian et al. (2012) prepared nanoemulsions of β-carotene using different oil phases as carriers, which are long chain triglycerides (LTC), medium chain triglycerides (MCT), and orange oil. The authors observed that the bioaccessibility of β-carotene in orange oil nanoemulsions was negligible and they associated this result to the lack of mixed micelles formed to solubilize β-carotene. However, the higher bioaccessibility of β-carotene was observed in LCT nanoemulsions compared to MCT nanoemulsions. The authors attributed these results to the fact that the mixed micelles formed by a long chain have larger hydrophobic cores than those formed by medium a chain. These results are so important for the choice of an appropriate oil phase for the preparation of β-carotene nanoemulsions. Indeed, as β-carotene is a lipophilic compound, the carrier phase, which is the oil fraction of the nanoemulsions, will have the key role in the release, bioaccessibility, and absorption of the encapsulated β-carotene in the gastrointestinal tract.

In another study, Liang et al. (2013) studied the encapsulation of β-carotene in o/w nanoemulsions stabilized with modified starches and prepared by high-pressure homogenization technique. The authors were interested in the study of the efficiency of nanoemulsions as delivery systems to improve the stability and the bioaccessibility of nanoencapsulated β-carotene. Then, an in vitro digestion study showed that the bioaccessibility of the encapsulated β-carotene in nanoemulsions systems increased from 3.1 to 35.6%. Moreover, the authors observed that modified starch with high dispersed molecular density was able to increase the retention of β-carotene, however, its bioaccessibility decreased significantly. The authors related these results to the nature of the formed interfacial layer around the oil droplets protecting the β-carotene. Indeed, it seems that modified starch with higher molecular weight produced a thick and dense interfacial layer around the oil droplets, which, in spite of its effect on the physical stability of the nanoemulsions, it decreased the bioaccessibility of the encapsulated β-carotene.

## 7.3 Polyunsaturated Fatty Acids (PUFAs) Nanoemulsions

PUFAs, in particular omega-3 PUFAs, play an important role in maintaining health as the prevention of cardiovascular diseases, the improvement of brain function, and the reduction of cholesterol (Kargar et al., 2011; Walker et al., 2013). Fish oils as well as some seeds and nuts are naturally rich in omega-3 PUFAs. Consumption of adequate levels of rich oils in omega-3 PUFAs has been shown to provide several health benefits associated with cancer, cardiovascular diseases, and inflammation (Kris-Etherton et al., 2009). However, omega-3 PUFAs are fatty acids with three or more double bonds that lead to higher oxidative degradation rate, structural changes, isomerization, and polymerization processes. Therefore, adding such bioactives to fortify food products presents many challenges, mainly the degradation of omega-3 PUFAs and the development of undesirable flavors and odors. Encapsulation is an alternative to limit these nutritional and sensorial problems of these bioactive oils. Several techniques including spray drying, complex coacervation, freeze drying, fluidized bed coating, and polymer gelation have been used for the encapsulation of oils rich on omega-3 PUFAs (Klinkesorn and Sophanodora, 2005, 2006; Klaypradit and Huang 2008; Drush and Berg, 2008; Tamjidi et al., 2012). The formation of o/w emulsions to encapsulate oils rich on omega-3 PUFAs could be a suitable method to protect the biological and functional properties of these lipophilic compounds. Furthermore, nanoscale delivery systems could be the most appropriate solution to protect these active substances against oxidation and to increase their bioavailability as well as to decrease their undesirable odor and flavors.

Esquerdo et al. (2015) studied the encapsulation of rich fish oil on omega-3 PUFAs in o/w nanoemulsions stabilized by chitosan as wall material. Moreover, the authors used different levels of chitosan in order to study the effect of the concentration of the wall material on the encapsulation efficiency as well as on the stability of the fish oil against oxidation. The main results of this study showed that using a low chitosan concentration provided nanoparticles of fish oil with smallest sizes (332 nm). These stable nanoemulsions were then subjected to freeze drying in order to increase the stability of nanoparticles of PUFAs. Fish oils particles with an irregular porous microstructure were obtained with high potential to decrease the primary oxidation rate of unsaturated fatty acids.

Walker et al. (2015) studied the effect of the surfactant concentration and particle size on the oxidative stability of fish oil

nanoemulsions prepared by spontaneous emulsification. The stability of these nanoemulsions was compared to that of nanoemulsions prepared by high-energy method (microfluidization). The main results of this study showed that the particle size was not a major factor that impacts the stability of the encapsulated fish oil against oxidation. The same results were observed for the effect of the emulsifier concentration on the stability of the encapsulated oil against oxidation. Moreover, the authors proved that low-energy method could be a successful technique for the spontaneous formulation of fish oil nanoparticles with high stability against oxidation.

In conclusion, practically, all original and review papers on nanoemulsions of bioactive compounds are limited on the study of the process of nanoencapsulation through nanoemulsions, the effect of several homogenization techniques, emulsifiers as well as the nature of oil phases on the stability of the formulated nanoemulsions and on the retention and the in vitro bioavailability of the encapsulated bioactives. While, in spite of all these very interesting studies, reports on direct applications of these nanoemulsions as potential delivery systems in food products are not as numerous as expected. Indeed, only general suggestions about the potential applications of nanoemulsions as delivery systems were reviewed in several papers (Wang et al., 2007; Yuan et al., 2008). Moreover, detailed review on the effective emulsifiers and about the potential emulsification techniques has been presented in several papers and review (Samer and Schork, 1999; Anton et al., 2008; Tan and Nakajima, 2005; Jafari et al., 2006; Lee and McClements, 2010; Mason et al., 2006b; Porras et al., 2008). However, no direct or specific applications in food matrices are indicated.

## 8 Major Challenges of Nanoemulsions Delivery Systems

Nanotechnology becomes an emerging technology that holds the potential to develop the food industry (Luykx et al., 2008). The application of nanotechnology as delivery systems to the food industry may allow modifying many sensorial, nutritional, and functional properties of food products. Acting as delivery systems, nanotechnology can also improve the stability, the bioavailability, as well as the functional properties of bioactive compounds (McClements et al., 2009). Especially, nanoemulsions have been more and more developed and investigated to be potential delivery systems for lipophilic bioactive compounds in food products.

However, although nanoemulsions provide great advantages as delivery systems in food industry, they suffer from major challenges and limitations including:

- *One problem associated with the stability of nanoemulsions*. It is generally admitted and proved that nanoemulsions are kinetically stable and they could remain stable even for years. However, it has been proved that there have been two major problems related to the stability of these systems. First, it has been reported that nanoemulsions have low stability in acidic conditions, which can affect their stability in the gastrointestinal tract characterized by their acidic conditions (Klinkesorn and McClements, 2009). Second, nanosized droplets characterizing nanoemulsions prevent the coalescence, flocculation, and sedimentation processes occurring to destabilize these systems. However, it has been reported that these small droplet sizes may allow the destabilization of nanoemulsions within the Ostwald ripening process, which is responsible of the deterioration of nanoemulsions limiting their applications.
- *Another problem is associated with the formulations mechanisms of nanoemulsions*. In fact, production of nanoemulsions requires significant energy input especially using high-energy processes. The formulation of such nanosized delivery systems could be an expensive process due to size reduction of droplets, which requires special instruments involving large amounts of financial support. On the other hand, low-energy methods are not, all the time, for food industry scale manufacture because these methods require the use of high concentrations of emulsifiers and sometimes we need to use some chemical solvents that are not allowed in the food industry.
- *Several researches focused on the effect of nanoemulsions*. They focused on the effect on the stability and bioavailability of bioactive compounds, but there is a lack of studying the incorporation of bioactives encapsulated into nanoemulsions in food matrices and understanding their stability through these food products as well as the study of their bioavailability after the consumption of such products.

## 9 Future Industrial Perspectives

Nanoemulsions are now an interesting emerging technology proposed for applications in several sectors such as for drugs delivery in the pharmaceutical sector or for active substances for cosmetic products. And recently, several researches proved that nanoemulsions could be potential carriers for bioactive compounds for food industry applications. These delivery systems are

able to increase the solubility of lipophilic compounds in aqueous phases. They can also increase the bioavailability of encapsulated substances during the gastrointestinal tract. Future perspectives of nanoemulsions are very promising for different applications in food products; especially their incorporation in the formulation of functional foods. An example of future application of nanoemulsions as delivery platform is the encapsulation of probiotics through nanoemulsions. Indeed, such bioactives are usually encapsulated through liposomes. Moreover, it will be so interesting to develop the scientific researches in vivo after the consumption of functional foods fortified with encapsulated bioactives through nanoemulsions.

## 10 Conclusions

Nanoemulsions offer numerous interesting advantages for the delivery of bioactive compounds and they are receiving increasing attention to improve the delivery of bioactive food ingredients. This promised technology has been well studied and developed to overcome the poor bioavailability, solubility in aqueous phases, and stability of lipophilic bioactive compounds. Especially, the advantages of nanosized droplets, characterizing nanoemulsions, were proved through numerous studies mainly their role in increasing the stability as well as the bioavailability of encapsulated bioactives through the gastrointestinal tracts. However, some scientific and industrial luck about the direct incorporation of these delivery systems to develop functional foods and their effects on the in vivo bioavailability of these bioactives still the major limiting factors.

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12

# PRODUCTION, STABILITY AND APPLICATION OF MICRO- AND NANOEMULSION IN FOOD PRODUCTION AND THE FOOD PROCESSING INDUSTRY

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## 1 Introduction

Emulsion is a mixture that consists of at least one immiscible liquid dispersed in another in the form of droplets (Cindio et al., 1991). Emulsion science is a multidisciplinary subject combining chemistry, physics, and engineering (McClements, 2005; Serdaroğlu et al., 2015). It has widespread application in pharmaceutical, cosmetics, and the food industry. The purpose of emulsion in the food industry is to develop and improve food quality attributes along with facilities the processing and production techniques by benefiting from emulsion principle. Emulsions involve partly or wholly in the structure on many natural or processed foods such as milk, cream, and soft drinks. However, there are also some food products, which have been emulsified during the production such as powdered soups, sauces, and coffee creamers (McClements, 2005, 2012). In food processing industry emulsions are used to control physicochemical and sensory properties of food products (eg, texture, appearance, mouthfeel, flavor, and shelf life).

The quality attributes of emulsion-based food products depends on the type of emulsion, emulsification process, and nature

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00012-X

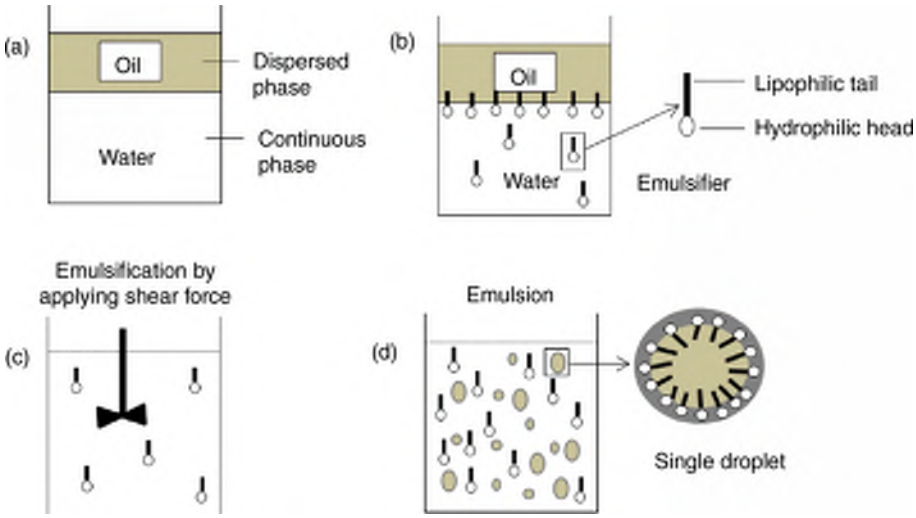


**Figure 12.1. Formation of oil-in-water (o/w) emulsions.** (a) Two separate phases, oil and water; (b) a surfactant soluble in water phase and adsorb on the interface; (c) shear force is applied to form oil droplets; and (d) emulsion is formed.

of emulsifier. The few common terms that are related to emulsion science are given later (Fig. 12.1).

*Emulsifier*: It is a substance that stabilizes the emulsion. The emulsifier positions in emulsion at the interface between oil and water. An emulsifier comprises a water-loving hydrophilic head and an oil-loving hydrophobic tail. The hydrophilic head involves the aqueous phase and the hydrophobic tail to the oil phase. In terms of the ability of emulsion formation, emulsifiers are efficient to interact with other food ingredients. The few common emulsifier are carbohydrate materials (acacia, tragacanth, agar, pectin), protein substances (gelatin, egg yolk, caesin), high molecular weight alcohols (stearyl, cetyl alcohol, glyceryl monostearate).

*Emulsification*: The process of making emulsion where the breakdown of large fat globules into uniform small droplets occurs by means of mechanical or chemical force.

*Dispersed phase and continuous phase*: The droplet phase is termed as the dispersed phase and the surrounding phase is known as continuous phase (Boom, 2008). In the oil in water (o/w) emulsion, oil droplets are dispersed phase and water is continuous phase.

*Texture modifier*: These are the substances which usually are used in food products to improve or modify desired texture. In emulsion texture modifiers help to increase the stability of emulsion by thickening or gelling the continuous phase, which retard

or prevent droplet movement. In the food industry, biopolymers such as proteins or polysaccharides are the mostly used texture modifiers.

*Weighting agent*: To reduce the gravitational separation such as creaming or sedimentation of emulsion weighting agent is added during emulsification. The main function of weighting agents is to match the density of dispersed particles to that of surrounding continuous phase.

In the recent time, food manufacturers are showing more interest in the use of micro- and nanoemulsion in food processing for their unique properties and applicability over conventional emulsion. It offers a number of advantages as (1) because of a very small particle size it scatters light weakly and looks transparent or less turbid. Hence, it is very suitable to convey oil soluble food compound (eg, flavor, vitamins, and other functional ingredients) in transparent or less turbid food products; (2) more stable against gravitational separation as the droplet size is in nano scale and obtain necessary Brownian motion to overcome this types of break down; (3) the small droplets also prevent their surface fluctuations and coalescence. Thus all ingredients disperse uniformly in the food; (4) nanosize emulsion particle increase the bioavailability of nutraceuticals and functional food elements; (5) nanoemulsion provides more efficient encapsulation to bioactive and volatile food compounds than other types of emulsion; (6) nanoemulsions modify the food structure very internally and improve functional and sensory properties such as mouthfeel, retain the color and flavor for a longer time in food products, reduce losses of active ingredients during food processing (Tadros et al., 2004; McClements, 2005, 2012; Chakraborty et al., 2009; McClements and Li, 2010). This chapter discusses current approaches of nanoemulsion formation, emulsion droplet properties, physicochemical properties of nanoemulsions, emulsion stability, approaches use for observing the properties of micro- and nanoemulsion, possible risk associated with nanoemulsion and potential application in food production and food processing industry.

## 2 Classification of Emulsions

In general, an emulsion contains two immiscible liquids; one of the liquid dispersed as small droplets in other liquids (McClements, 2005, 2010). The droplet size of emulsion has great influence on stability, applicability, optical properties, rheology, and quality of emulsion. According to the droplet size range emulsions can be classified into three groups; that are, macroemulsion or conventional emulsion, nanoemulsion, and microemulsion or

**Table 12.1 Comparison of Thermodynamic and Physicochemical Properties of Different Types Emulsion Prepared from Oil, Water, and Emulsifier (Solans et al., 2005; Flanagan and Singh, 2006; McClements and Rao, 2011; Zhang, 2011; Serdaroğlu et al., 2015)**

| Type of Emulsion | Droplet's Radius Range | Thermodynamic Stability | Surface-to-Mass Ratio ($m^2/g$) | Optical Property |
|---|---|---|---|---|
| Macroemulsion | 0.1–100 μm | Instable | 0.07–70 | Opaque/turbite |
| Nanoemulsion | 20–200 nm | Instable | 0–330 | Lucent/turbite |
| Microemulsion | 5–100 nm | Stabile | 30–1300 | Clear |

miniemulsion. Macroemulsion contains the droplet size between 0.1 and 100 μm. This type of emulsion is thermodynamically unstable and optically turbid or opaque due to containing droplets that have similar dimensions to the wavelength of light ($d \approx \lambda$) and strongly scatter light (provided the refractive index contrast is not close to zero). Nanoemulsions contain nanosized droplets ranging from 20 to 200 nm. These types of emulsions are thermodynamically more stable compared to the conventional emulsion. It is also optically more clear than macroemulsion because the droplet size is smaller than light wavelength ($d \ll \lambda$). According to Tadros et al. (2004), the smaller droplet size makes it more stable against gravitational separation and aggregation. Microemulsions possess additional thermodynamical stability and optical clarity with nanoemulsion (Table 12.1).

Based on characteristics of dispersed phase, the emulsions are categorized as (1) oil-in-water emulsions (o/w). In this type of emulsion, oil droplets disperse into water (Fig. 12.2). Oil exists as a disperse phase and water as the dispersion medium (continuous phase) in the emulsion system. For example milk and ice cream. (2) Water-in-oil emulsion (w/o): this type of emulsion is totally opposite to the o/w-type emulsion. In w/o emulsion water forms the dispersed phase and the oil acts as the dispersion medium. Butter, margarine, and cold cream are typical example of these kinds of emulsion.

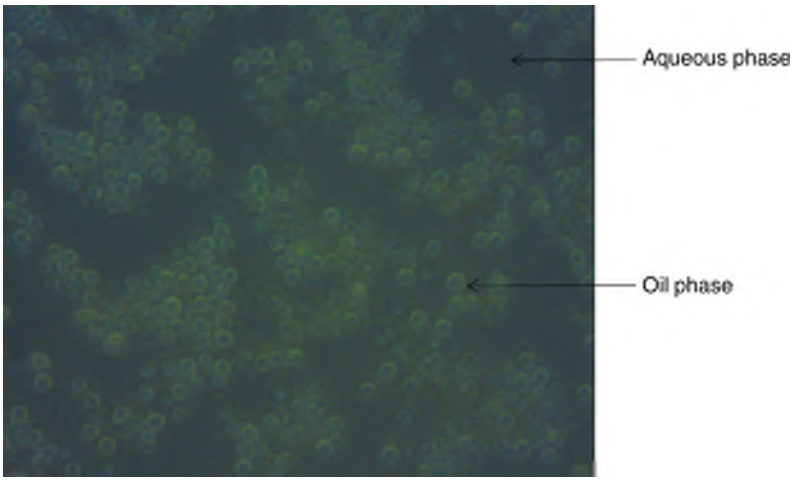


**Figure 12.2. o/w emulsion from orange oil, water, and gum arabic.**

Emulsions are also classified as multiple layer emulsion or double emulsion. In the double emulsion, droplets of one dispersed liquid (emulsion, microemulsion, liposome, etc.) are further dispersed in another liquid (water or oil), generating double layered liquid droplets (Guzey and McClements, 2006; Garti and Aserin, 1996). Double emulsions are emulsions within emulsions of two major types of water-in-oil-in-water (w/o/w) and oil-in-water-in-oil (o/w/o) (Dickinson, 2011). These structures consist of two layers of emulsifiers, the inner phase covered by emulsifiers (stabilizers), further dispersed and covered by another layer of emulsifiers in an outer phase (Garti and Aserin, 1996). Among the different types of emulsions o/w nanoemulsions are widely used in food processing industry currently (McClements, 2010).

## 3 Nanoemulsion Formation

In order to the prepare emulsion, oil, water, surfactant, and energy are needed (Tadros et al., 2004). Oil and water acts as disperse or continuous phase according to the nature of emulsion. Surfactant works at the interface between oil and water to stabilize the emulsion system. The energy is required to make homogenized droplets. Emulsifiers/stabilizers are dissolved in the water or oil depending on solubility of emulsifier and on the type of emulsion expected (Adheeb Usaid et al., 2014). The emulsifier dissolution is required before it goes through emulsification process. There are a number of techniques available to produce nanoemulsion, which are categorized as (1) high energy/intensity approach, (2) low

energy/intensity approach (Tadros et al., 2004; Acosta, 2009; Leong et al., 2009; McClements, 2010; Qian and McClements, 2011; Adheeb Usaid et al., 2014). High-energy approaches use intense disruptive forces, produced by mechanical devices, to break the oil droplets such as high-pressure valve homogenizers, microfluidizers, and sonication methods (Gutierrez et al., 2008; Leong et al., 2009; Velikov and Pelan, 2008; Wooster et al., 2008; McClements, 2010). In contrast, low-energy approaches are based on spontaneous formation of tiny oil droplets within mixed oil-water-surfactant systems, when solution or environmental conditions are transformed, for example, phase inversion and solvent mixing methods (Anton et al., 2008; Bouchemal et al., 2004; Chu et al., 2007; Freitas et al., 2005; Tadros et al., 2004; Yin et al., 2008). The selection of specific emulsification approach and minimum size of droplets produced by that method depends on many factors, such as, type and nature of emulsifier, ratio of emulsifier in the emulsion system, compositions of the oil phase and viscosity of the phases.

## 3.1 High-Intensity Approach

At the surface, two immiscible fluids present an interfacial tension, which is known as interfacial energy. This is generated due to the difference in cohesion between molecules of the two phases. High-intensity force is used to produce tiny oil droplets, which increase total interface. It requires energy to create interface between two phases and the amount of energy is proportional to the amount of interface produced. The amount of (Gibbs) energy required to generate 1 $m^2$ of interfacial zone is termed as the interfacial tension or interfacial energy (N/m or J/$m^2$).

$$\Delta G = \int_0^A \sigma \, dA \tag{12.1}$$

Here, $\Delta G$ the Gibbs energy (J) needed to create total interfacial area $A$ ($m^2$) and $\sigma$ is the interfacial tension (N/m or J/$m^2$). The interfacial tension is always positive, with the exception of microemulsions. This shows that $\Delta G$ is (almost) always positive: making small droplets cost energy (Boom, 2008). Since smaller droplets become, the larger is the total interfacial zone.

In high-intensity approaches, mechanical devices generate extremely intense disruptive forces, which must exceed the restorative forces holding the droplets into spherical shapes. In the droplet form, one immiscible fluid in another, the interface is curved. The interfacial tension exerts a force perpendicular to the interface, directed to the concave side of the interface. The size of the force is proportional to the interfacial zone, and hence, it is defined

as a pressure, which is termed as the Laplace pressure. This pressure is proportional to the curvature of the interface (Boom, 2008). The Laplace Pressure can be calculated by the equation:

$$\Delta P = \frac{\gamma}{2r} \tag{12.2}$$

Where, $\Delta P$ represents the difference in pressure between inside and outside of the droplet, which increases with the decreasing of droplet radius ($r$) and increasing interfacial tension ($\gamma$). The smallest size of the droplets formed using a high-intensity approach relies on the type of homogenizer, operating conditions of homogenizer (eg, energy intensity, time, and temperature), composition of the sample (eg, oil type, emulsifier type, relative concentrations), and the physicochemical properties of the component phases (eg, interfacial tension and viscosity) (Kentish et al., 2006; Wooster et al., 2008; McClements, 2010).

### *3.1.1 High-Pressure Homogenizer*

High-pressure homogenizer is the most common equipment that is widely used to produce fine emulsion. Currently it is a very popular method of creating nanoemulsion for the food industry (Schubert and Engel, 2004; Piorkowski and McClements, 2014). In this technique, oil, water, and surfactant mixture is exposed to very high pressure and is pumped through a resistive valve. This device is very effective in reducing the size of oil droplets through high shear force and preparing fine emulsion from the separate oil and aqueous phase. However, it becomes more effective when it goes through the two-step process. At first oil, water and surfactant mix together by using high speed mixture or blender to prepare coarse emulsion (Troncoso et al., 2012; Piorkowski and McClements, 2014; Tabibiazar et al., 2015). After that, the coarse emulsion is pumped into a homogenizer chamber on its backstroke and consequently forces it through a narrow valve at the chamber end on its forward stroke. During passing through the valve the course emulsion goes through a combination of intense disruptive forces that result in the breakdown of large droplets into several smaller droplets (Fig. 12.3). Different valve designs and at different pressures homogenizers show various efficiencies. The droplet size formed from the high-pressure homogenizer reduces with increased number of passes through the homogenizer and pressure intensity. It relies on the viscosity ratio of the two phases (typically oil and water) being homogenized as well. Small droplets can only usually be formed when the disperse-to-continuous phase viscosity ratio decreases within a certain

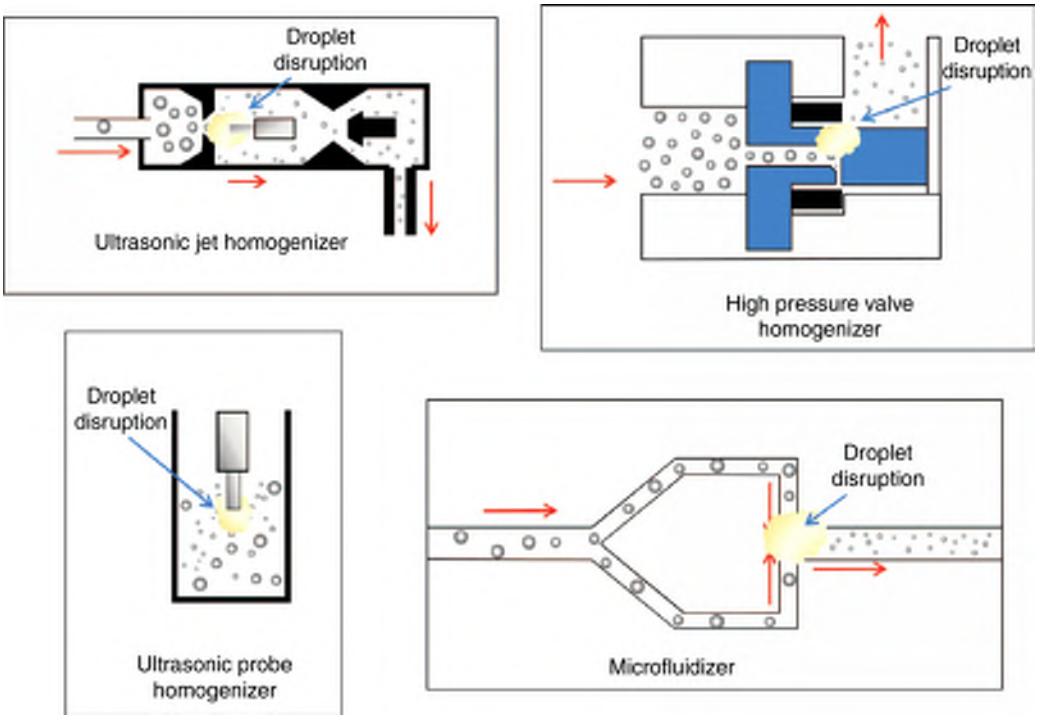


**Figure 12.3. Schematic representation of mechanical devices that can be used to produce beverages emulsions using the high-energy approach: high pressure valve homogenizer, microfluidizer, and continuous ultrasonic homogenizer** (McClements and Rao, 2011).

range (Tadros et al., 2004; Walstra, 1993, 2003; Piorkowski and McClements, 2014). McClements (2005) reported there is a linear relationship between the logarithm of the homogenization pressure ($P$) and the logarithm of the droplet diameter ($d$): $\log d \propto \log P$, with the constant of proportionality depending on homogenizer type. However, temperature of emulsion increases with rising homogenization pressure and number of passes. Increased temperature has adverse effects on the stability of emulsion. Therefore, necessary cooling systems need to be installed during producing emulsion with very small droplet size ($r < 100$ nm). During producing very fine emulsion droplet size as small as 100 nm or below, the important point is to add adequate surfactant to fully cover the o/w interface generated and the adsorption kinetics is high enough to avoid droplet coalescence (Jafari et al., 2008; Quintanilla-Carvajal et al., 2010). To produce nanoemulsion by using high-pressure homogenizers, certain factors are very crucial such as high emulsifier levels, low interfacial tensions, and appropriate viscosity ratios (Piorkowski and McClements, 2014).

### 3.1.2 Ultrasounic Homogenization

Sonication methods need high-intensity ultrasonic waves (frequency > 20 kHz) to produce emulsions containing very fine droplets (Abismaïl et al., 1999; Jafari et al., 2007; Kentish et al., 2008; Leong et al., 2009). In ultrasonic method, emulsion droplets are produced by cavitation, when two immiscible liquids come to high-frequency sound waves under a surfactant. It results in intense shock waves in the surrounding liquid and the development of emulsion droplets happened due to the development of liquid jets at high speed (Silva et al., 2012). In the flowing liquid, formation and collapse of vapor cavities is the main phenomenon of this method (Fig. 12.3). There are two set of mechanisms involved with emulsification by using ultrasonic method. First, dispersion of the oil phase (in the form of droplets) in the continuous phase happened by the action of acoustic field produced by interfacial waves. Second, the formation and following collapse of microbubbles by the pressure fluctuations of the simple sound wave, which produces extreme levels of highly localized turbulence (Gadhave, 2014). However, the turbulent microimplosions break up primary droplets into submicron size. Even though the ultrasonic technique can offer high shear stress owing to acoustic cavitation, the final size of the nanoemulsion droplet is reliant on the dual effect of shear rate and emulsion rheology (Mason et al., 2006). In general, batch and continuous ultrasonic homogenizers are used for emulsions (Leong et al., 2009). Therefore, continuous ultrasonic homogenizers are the most popular for the huge scale production of fine emulsions. The nature and quantity of emulsifier, viscosity of the disperse, and continuous phases has a major influence on emulsification/homogenization efficiency (Jafari et al., 2006; Kentish et al., 2006; Leong et al., 2009; Maa and Hsu, 1999). Ultrasonic homogenizers are mainly applied for low-viscosity fluids, but are less appropriate for high viscous fluids (Piorkowski and McClements, 2014). Ultrasonic homogenization need to install a cooling system with the sonication chamber because of disruption of air bubbles releasing the heat energy, which increases the temperature of emulsion (Abbas et al., 2013).

### 3.1.3 Microfluidizers

Microfluidizers are similar in design to some extent with high-pressure homogenizer because they pump the coarse emulsion premix and pass through high pressure to produce fine droplets (Schultz et al., 2004; Jafari et al., 2006, 2007; Kentish et al., 2006). However, the channels through which the coarse emulsion is prepared to flow within the equipment is different (Piorkowski

and McClements, 2014). In high-pressure homogenizer the point where the fine emulsion droplet produces is the pressure valve; on the other hand, in microfluidizers fine emulsion is produced just after passing through the narrow orifice. The microfluidizer divides coarse emulsion flow into streams and the two streams flow through two channels under high pressure, finally streams impinge on each other at high velocity in an interaction chamber (Fig. 12.3). The intense disruptive forces produced in the interaction chamber lead to the break down of large droplets into very fine emulsion droplets. A number of researchers have observed the potential application of microfluidizers for the production of food-grade nanoemulsions (Abismaïl et al., 1999; Jafari et al., 2006; Leong et al., 2009; Henry et al., 2010; Klein et al., 2010). It is found that the droplet size of prepared emulsion tends to reduction with increased homogenization pressure, number of passes, emulsifier concentration, and decreased dispersed-to-continuous phase viscosity ratio (Wooster et al., 2008). There is a logarithomic linear relationship between homogenization pressure and droplet diameter. The logarithm of the mean droplet diameter reduced linearly as the logarithm of the homogenization pressure increased: $\log d \propto \log P$. Here $d$ is the mean droplet diameter and $P$ is the homogenization pressure (McClements, 2011; Qian and McClements, 2011). Furthermore, the viscosity of organic and aqueous medium and ionic strength of surfactant affect the size of droplets in nanoemulsion.

## 3.2 Low-Intensity Approaches

In low-energy approaches, the formation of emulsion depends on the spontaneous formation of tiny oil droplets within oil–water–emulsifier mixtures, when either their composition or the environmental conditions are changed (Anton and Vandamme, 2009; Anton et al., 2008; Bouchemal et al., 2004; Yin et al., 2009; Piorkowski and McClements, 2014). A number of methods are available based on low-energy technique, including spontaneous emulsification, phase inversion methods, and membrane emulsification (Anton and Vandamme, 2009; Anton et al., 2008; Fernandez et al., 2004; Maestro et al., 2008; Piorkowski and McClements, 2014; Gadhave, 2014). Some of these low-energy techniques are currently used in food processing for the formation of o/w nanoemulsion, especially in the beverage production (Piorkowski and McClements, 2014). To produce small droplet size, low-energy methods are often more efficient than high-energy approaches, while low-energy methods have few limitations on certain types of oil and emulsifiers. For example, in most of the low-energy approaches, proteins or polysaccharides are not

suitable to use as emulsifiers to form very fine emulsions. Instead, it requires high concentrations of synthetic surfactants to produce stable emulsion, which limits their application in some food products (Piorkowski and McClements, 2014).

### *3.2.1 Phase Inversion Methods*

Phase inversion methods include phase inversion temperature (PIT) and emulsion inversion point (EIP) inverse the phase from a w/o to o/w form in order to formulate fine emulsion (Fernandez et al., 2004; Thakur et al., 2008). Transitional phase inversion involves the PIT method; whereas, catastrophic phase inversion involve in the EIP method (Piorkowski and McClements, 2014). The variation of surfactant properties by adjusting a formulation variable, such as temperature, pH, or ionic strength results in transitional phase inversion. On the other hand, when the ratio of the oil-to-water phases is changed, the surfactant properties keep constant, and then a catastrophic phase inversion occurs.

#### 3.2.1.1 Phase Inversion Temperature Method

The PIT method basically depends on the changes in the optimum curvature and relative solubility of nonionic surfactants with varying temperature (Anton et al., 2007; Anton and Vandamme, 2009; Gutierrez et al., 2008). Nanoemulsion can be instinctively produced using the pin version temperature method by changing the temperature-time profile of appropriate mixtures of surfactant, oil, and water. It includes the controlled alteration of an emulsion from one type to another (eg, w/o to o/w or vice versa) through a transitional state (McClements, 2010; Piorkowski and McClements, 2014). The variations in physicochemical properties of surfactant with temperature are the key driving force for this kind of phase inversion.

The implementation of PIT method is comparatively direct than other methods. For example, when a mixture of surfactant, oil, and water (SOW) is primarily heated up to a temperature nearby or to some extent above the PIT, it causes the development of a microemulsion or liquid crystalline phase. The SOW system is then cooled down to a temperature well under the PIT with constant stirring, which causes the spontaneous development of an o/w emulsion or nanoemulsion (Anton and Vandamme, 2009). Rao and McClements (2010) reported production of nanoemulsion from a mixture of nonionic surfactant (13% Tween-80), a flavor oil (10 wt% lemon oil), and water (77%). Initially the mixture was opaque. Upon heating reaching up to PIT, the system becomes transparent, and then becomes opaque, when heated up beyond

the PIT due to formation of a w/o emulsion. Upon cooling, the system transforms from turbid to transparent representing the nanoemulsion formation ($d = 45$ nm).

One of the major drawbacks of the PTI-produced emulsion is frequently high prone to droplet coalescence during the storage at temperatures approaching the PIT. This might be a problematic issue in food and beverage applications, which need several kind of thermal actions (eg, pasteurization, sterilization, or cooking) or that are stored at high temperatures (eg, in warm or hot climates). To overcome this problem Rao and McClements (2010) reported an approach to produce the emulsion using a nonionic surfactant with a relatively low PIT, and then diluting the developing emulsions in a solution comprising another surfactant with a high PIT.

#### 3.2.1.2 Emulsion Iinversion Point Method

EIP method that alters one type of emulsion to another (eg, w/o to o/w or vice versa) is through a catastrophic phase inversion (CPI) (Fernandez et al., 2004; Thakur et al., 2008). The implementation of EIP method is very simple, which involves titrating increasing amounts of an aqueous phase with continuous stirring into an organic phase to induce a catastrophic phase inversion from a w/o to an o/w system (Piorkowski and McClements, 2014). The dimension of produced emulsion droplets depends on the stirring speed, the rate of addition of aqueous phase, concentration of surfactant (Thakur et al., 2008). In this method the organic phase is to prepare first by adding hydrophilic surfactant with oil with constant stirring. During addition of water to w/o emulsion, two-step changes often occurs at first the emulsion converted into a multiple emulsion o/w/o then turned into o/w emulsion (Jahanzad et al., 2009; Sajjadi, 2006). The small molecule surfactants are often used in catastrophic phase inversion emulsification, which are able to stabilize both w/o emulsions (at least over the short term) and o/w emulsions (long term), usually. Emulsion inversion point techniques can be utilized to form submicron-sized emulsion. In recent times, it is found that the CPI method can be utilized to create nanoemulsions ($r < 100$ nm) from food-grade ingredients (Bilbao-Sáinz et al., 2010; Ostertag et al., 2012).

### *3.2.2 Spontaneous Emulsification*

Spontaneous emulsification has been reported as spontaneous formation of emulsion or nanoemulsions, when two immiscible liquids (usually an organic phase and an aqueous phase) are mixed together at a certain temperature (Miller, 1988; Pouton and Porter, 2006; Anton and Vandamme, 2009). Practically, the work

procedure of this method can vary in various ways. The real application of this technique can differ in a number of ways: the compositions of the organic and aqueous phases; the environmental conditions (eg, temperature, pH, and ionic strength); and the mixing conditions (eg, stirring speed, rate of addition, and order of addition). For instance, two phases can be carried out together in a number of ways: an organic phase comprising of nonpolar oil and a hydrophilic surfactant and/or water-miscible organic solvent may be titrated into the aqueous phase (Anton and Vandamme, 2009), or alternatively the aqueous phase may be titrated into an organic phase containing nonpolar oil, water-miscible organic solvent, and surfactant (Sonneville-Aubrun et al., 2009). The hydrophilic surfactant and/or solvent transfers from the oil phase to the aqueous phase, when two phases come into contact at the phase boundary in order to produce tiny oil droplets (Horn and Rieger, 2001). The droplet size can be modified by changing the composition of phases and mixing environment and conditions. Spontaneous emulsification technique is lately compared with high-intensity methods; where oil, water, and surfactant are mixed together and then pass through mechanical equipment and emulsion produce by means of intense shear force. On the other hand, in spontaneous method oil phase, aqueous phase and suitable emulsifier mix together slowly, then emulsion is produced automatically (Yang et al., 2012). Recent studies using the same composition microfluidization method produce emulsion with droplet size 110nm, whereas the spontaneous method produced emulsion droplet size 140nm (Piorkowski and McClements, 2014).

### *3.2.3 Membrane Emulsification*

Membrane emulsification has received increasing attention in recent time (Joscelyne and Trägårdh, 2000; Charcosset, 2004). In the membrane emulsification method, formation of emulsion occurs by a drop-by-drop mechanism through a microporous membrane (Charcosset, 2004; Piacentini et al., 2014). The dispersed phase is forced through the pores of a microporous membrane, while the continuous phase flows along the membrane surface. Droplet detachment of the membrane surface depends on four main forces (Schröder et al., 1998): shear (induced by continuous phase movement, or membrane movement), interfacial tension between two emulsified fluids, inertia/pressure from the flow through the membrane, and buoyancy (Fig. 12.4). The dispersion phase in the form of droplets can be as a pure liquid or an emulsion. It works as an alternative of shear-force emulsification method. This method offers uniformity of droplet size and control over droplet dimension compared to other methods (eg, high pressure

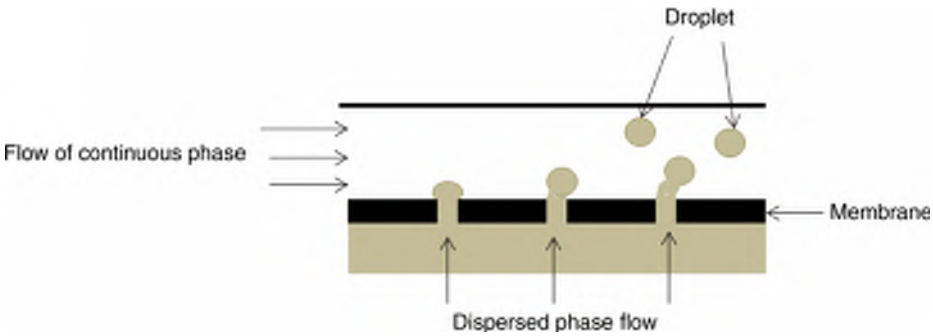


**Figure 12.4. Membrane emulsification.**

homogenization, ultrasonication). This method is very suitable to produce specific-sized droplets. However, this technique has constraint, such as the low flux of the dispersed phase through the membrane, this being an issue during scale-up (Sanguansri and Augustin, 2006).

# 4 Stability of Nanoemulsion

Stability of emulsion is very important in terms of application and storage. Emulsion is an equilibrium system, but thermodynamically unfavorable systems, which tend to break down over time due to a number of physicochemical mechanisms, containing gravitational separation, flocculation, coalescence, and Ostwald ripening (Dickinson, 1992; Friberg et al., 2004; McClements, 2005). Structural organization of different components varies in the system in case of all these types of instability mechanisms. However, alterations in the chemical configuration of active components can lead to variations in physical constancy, and vice versa (Piorkowski and McClements, 2014). In case of gravitational separation and droplet aggregation, nanoemulsions exhibit greater physicochemical stability compared to conventional emulsions, with the same composition. Therefore, in case of chemical degradation and Ostwald ripening, nanoemulsions show least stability than the conventional emulsions owing to their small particle size.

## 4.1 Gravitational Separation

Separation of two phases based on relative density occurs in gravitational separation. It is the most common form of emulsion instability. It could allow the form of either creaming or sedimentation based on the relative densities of the dispersed and

continuous phases. Creaming is the upward movement of droplets owing to the circumstance that it has a lower density than that of the surrounding liquid, whereas sedimentation is the downward movement of droplets owing to the reason that it has a higher density than that of the surrounding liquid (McClements, 2010, 2012; Piorkowski and McClements, 2014). Food-grade creaming is more often for o/w emulsion and sedimentation is more often in w/o emulsion because the density of edible oil is normally lower than water. Nevertheless, oil droplets in emulsion might be encircled by comparatively heavy and dense surfactant coatings, which might affect their tendency to either cream or sediment.

The velocity of an oil droplet's movement to upward direction in an emulsion owing to gravitational separation is presented by Stokes' law:

$$\upsilon = -\frac{2gr_{\text{particle}}^2(\rho_{\text{particle}} - \rho_0)}{9\eta_0} \tag{12.3}$$

Where, $\upsilon$ = creaming velocity, $r_{\text{particle}}$ = droplet radius, $\rho_{\text{particle}}$ = the droplet density, $\rho_0$ = aqueous phase density, $\eta_0$ = aqueous phase viscosity, and $g$ = gravitational acceleration. From this equation it is cleared that the decrease of droplet creaming happens because of the decrease of droplet size, which leads to the decrease of density difference or increase of the aqueous phase viscosity. The droplet movements either upward or downward due to gravitational force depending on the droplets relative density to continuous phase. Therefore, droplets would gather at either the top or the bottom of an emulsion. In fact, Brownian motion causes droplet movement with the thermal energy of the system. It also offers the droplets in a random distribution all over the entire dimensions of the emulsion, rather than their gathering at either the top or bottom. Gravitational forces have a tendency to direct the droplet movement in emulsions having comparatively large droplets ($r > 100$ nm), whereas Brownian motion forces be likely to direct droplet movement in emulsions having smaller droplets (McClements, 2011). Therefore, emulsion stability against gravitational separation (creaming or sedimentation) decrease with decreasing droplet size because of increasing Brownian motion.

Now it is clear that the droplet dimension and density difference are the main governing factors for action of gravitational force. Emulsifier coating contributes a considerable portion of the total volume of the droplets and leads to change in density of the oil phase. In that case overall particle radius becomes:

$$r_{\text{p}} = r_{\text{c}} + \delta \tag{12.4}$$

Where, $\delta$ is the thickness of the emulsifier coating on droplet, $r_c$ is the radius of original droplet and $r_p$ is the particle radius. The overall density of a particle comprising of an oil droplet surrounded by an emulsifier layer is calculated as follows:

$$\rho_{particle} = \Phi_s \rho_s + (1 - \Phi_s)\rho_c \tag{12.5}$$

Where, $\rho_{particle}$ is the overall particle density, $\phi_s$ is the volume fraction of the shell, $\rho_s$ and $\rho_c$ is the density of droplet shell and core materials, respectively. Usually the density of shell material is higher than the core material. Therefore, the surfactant coating not only increases the droplet radius but also it increases the density of droplets.

The previous discussion indicates that there are a number of techniques that could be utilized to avoid gravitational separation. First, the density difference between two phases is the main key factor that is responsible for this type of breakdown of emulsion. So, the prevention of gravitational separation can be performed by the density matching of the dispersed (oil) and continuous (aqueous) phases. Additionally, oil phase is lighter than the aqueous phase and it tends to move upward and form cream. This problem can be overcome by the addition of a necessary weighting agent to the oil phase, or by maintaining the thickness and density of the emulsifier layer. Second, according to Stoke's law, creaming velocity and droplet size squared are correlated with a proportional relationship. However, the prevention of gravitational separation can be done by decreasing the droplet size. Additionally, if the droplets are sufficiently small and suitable for Brownian motion, then the emulsion system remains constant to creaming or sedimentation by the action of Brownian movement of droplets. Third, there is an inversely proportional relationship between droplet movements and viscosity of continuous phase. Therefore, the inhibition of gravitational separation can occur by rising the viscosity of the aqueous phase, such as by the addition of thickening or gelling agents (Piorkowski and McClements, 2014).

## 4.2 Droplet Aggregation

Droplet aggregation leads to gravitational separation and changing its appearance (cloudiness or homogeneity). Emulsion with small droplet size (micro- and nanoemulsion) normally have greater stability to droplet aggregation (flocculation and coalescence) then conventional emulsions due to the effect of the small particle size on the colloidal interactions (Tadros et al., 2004). When two drops collide, it can flocculate, if the intermolecular

repulsive forces are adequately strong enough to keep the droplets detached at a small equilibrium distance, or it may coalescence, if the interfacial membrane ruptures. The drop-drop interaction depends on size and interfacial properties of droplets. In food-grade emulsion the interaction between droplets can be influenced by several factors (eg, emulsifier type, charge density, pH, and salt concentration); therefore, it is more practical to consider steric interactions induced by an adsorbed interfacial membrane (Tadros et al., 2004; Sagalowicz and Leser, 2010). The approximate colloidal interactions between two droplets can be defined by the sum of the van der Waals ($w_{VDV}$), electrostatic ($w_E$), steric ($w_S$), and hydrophobic ($w_H$) interactions (McClements, 2005; Anton and Vandamme, 2009).

$$w(h) = w_{VDV}(h) + w_E(h) + w_s(h) + w_H(h) \tag{12.6}$$

The van der Waals and hydrophobic interactions are attractive, while the steric and electrostatic interactions are generally repulsive. The steric interaction is a strong small range repulsive interaction, while the magnitude and range of the electrostatic repulsion rely on the electrical charge on the droplets and the ionic composition of the aqueous phase. The magnitude of both the attractive and repulsive colloidal interactions generally tends to rise with increase droplet size (Anton and Vandamme, 2009).

On the other hand, coalescence indicates the thinning and/or disruption process of the liquid film between the droplets with the consequence of fusion of two or more droplets into larger ones (Tadros et al., 2004; McClements, 2010; Hu et al., 2015). Subsequently, larger droplets formed and then move from the continuous phase. Finally, the two distinct phases separate completely (Fig. 12.5). Movement of large oil droplet happened based on Stokes' Law equation.

## 4.3 Ostwald Ripening

Ostwald ripening is the main mechanism of instability of nanoemulsion. It involves the mean size of the droplets in an emulsion increases over time owing to the diffusion of oil molecules from small to large droplets (Kabalnov, 2001). The physical basis of Ostwald ripening is due to the Laplace's Law effect on the solubility of the dispersed phase in the continuous phase (Izquierdo et al., 2002; Meinders and Vliet, 2004). The solubility of the dispersed phase in the continuous phase just at the boundary of the drop of radius ($r$) is predicted by the Kelvin equation (Smet et al., 1999; Meinders et al., 2001; McClements, 2005).

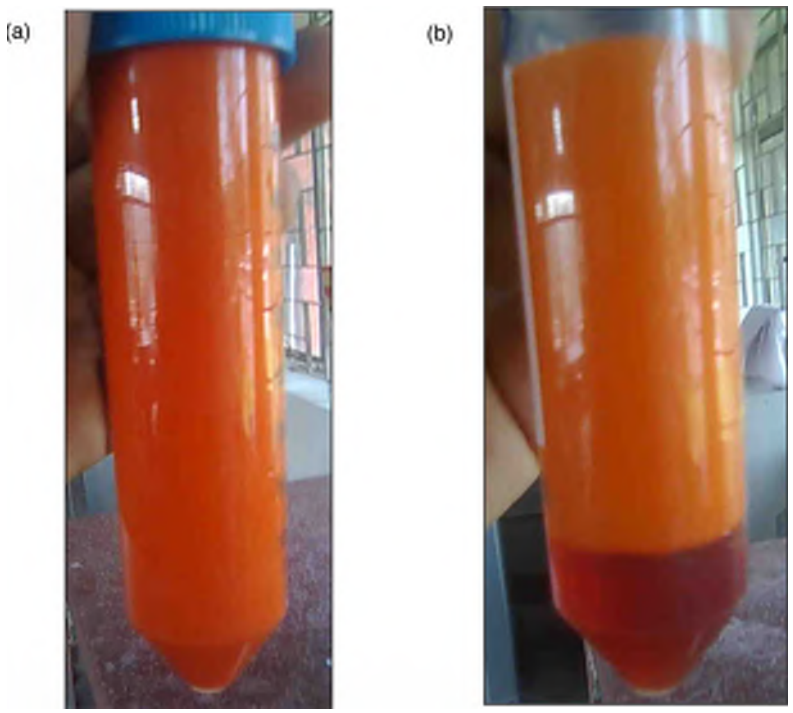


**Figure 12.5.** (a) Orange flavor emulsion, (b) phase separation in orange flavor emulsion.

$$S(r) = S_{\infty} \exp\left(\frac{2\gamma V_{\mathrm{m}}}{r\mathrm{R}T}\right) \tag{12.7}$$

Where, $S(r)$ is the solubility of the dispersed phase in the continuous phase just at the boundary of the droplet of radius ($r$); $S_{\infty}$ is the solubility of the dispersed phase in the bulk of continuous phase $V_{\mathrm{m}}$ is the molar volume of the dispersed phase; $\gamma$ is interfacial tension; R is gas constant; and $T$ is absolute temperature. The equation shows that solubility of emulsion droplet content increases with reducing droplet size, which refers to higher concentration of solubilized oil molecules in the aqueous phase surrounding a small droplet than surrounding a larger one. Therefore, diffusion of solubilized oil (dispersed phase) molecules occurs from the smaller droplets to around the larger droplets due to the concentration gradient (McClements, 2011). After achieving the steady state, the rate of Ostwald ripening is mentioned by Kabalnov and Shchukin (1992):

$$r^3 - r_0^3 = \omega t = \frac{4}{9} \propto S_{\infty} Dt \tag{12.8}$$

Where, $r$ is the number of mean droplet radius at time ($t$); $r_0$ is the initial number mean droplet radius; $D$ is the translational diffusion coefficient of the oil through the aqueous phase; $\alpha = 2\gamma V_m/RT$; and $\omega$ is the Ostwald ripening rate.

Ostwald ripening is the major challenge in producing stable nanoemulsions for practical applications. There are a number of affecting factors involved in Ostwald ripening in emulsions, such as solute solubility, droplet size, droplet composition, interfacial diffusion, interfacial tension (Kabalnov and Shchukin, 1992; Weers, 1998). Obviously decreasing oil solubility in the aqueous phase is the most effective method to retard or prevent Ostwald ripening in o/w nanoemulsions. It could be obtained by increasing the droplet size fairly big enough and by confirming a narrow droplet size distribution in emulsion. The greater solubility rate of dispersed phase in the continuous phase, exhibits quicker Ostwald ripening rate. Therefore, Ostwald ripening becomes slow, when o/w emulsion having lipid, which is fairly water soluble (eg, triacylglycerols).

## 4.4 Chemical and Biochemical Stability

Along with physical instability, there are number of chemical or biochemical reactions, which might have negative effects on the quality of food emulsions. As, for instance, biopolymer hydrolysis, lipid oxidation, flavor or pigment degradation could happen (Fennema, 1996). The most common type of quality degradation of food nanoemulsion is lipid oxidation, which leads to the change of unwanted "off-flavors" (rancidity) and potentially toxic reaction products. Furthermore, it might lead to the physical instability of some emulsions (Coupland and McClements, 1996; McClements and Decker, 2000). There are number of reaction products formed during lipid oxidation; these may act as surface active agents. Hence, they enable interaction with the interfacial membrane surrounding the droplets in such a way as to lead to droplet coalescence (McClements, 2005). In addition, micro- and nanoemulsions are transparent. Hence, visible light and UV can enter into them easily that may stimulate any light-sensitive reactions. Also, there is quality degradation of food-grade emulsion by the action of biochemical reactions. For example, the food emulsion properties can be changed considerably when the adsorbed proteins are cleaved by enzyme hydrolysis. Therefore, additional steps are required to increase the chemical stability of labile components encapsulated within nanoemulsions by the addition of antioxidants or chelating agents (McClements and Decker, 2000; Mao et al., 2009).

# 5 Nanoemulsion Droplet Properties

In the emulsion system, emulsion droplet characteristics (such as composition, concentration, size range, physical state, electrical charge, and interfacial properties) control the major physicochemical properties and quality of emulsion (McClements, 2005). To get the desired types of emulsion, it is very important to control the droplet properties. The droplet properties of emulsion varied by number of factors; for example, materials used for the formation of emulsion, production method, and other factors related to the emulsion production. In this section, we discuss the food-grade nanoemulsion droplet properties.

## 5.1 Droplet Composition

Emulsion droplet composition mainly depends on the types of ingredients used in the formation of emulsion. In the production of food-grade emulsion careful selection of ingredients is required. The selection of ingredients is also limited by the ingredients physicochemical properties (such as polarity, water-solubility, density, viscosity, refractive index, physical state, and melting point) and production methods (see production approaches Section 3). In o/w type emulsion; oil droplets are coated with a thin layer of adsorbed emulsifier molecules, for instance, surfactants, phospholipids, proteins, or polysaccharides (McClements, 2005). The main components of the droplets are lipophilic core material that might consist of one or more nonpolar components, containing triacylglycerols, diacylglycerols, monoacylglycerols, flavor oils, essential oils, mineral oils, fat substitutes, waxes, weighting agents, oil-soluble vitamins, and nutraceuticals (such as carotenoids, phytosterols, curcumin, and coenzyme Q). The coating layer surrounding the core content might also consist of one or more materials, including surfactants, phospholipids, proteins, polysaccharides, and minerals (McClements, 2011; Piorkowski and McClements, 2014). In the conventional emulsion the droplet layer thickness ($\delta_s$), is much smaller thickness compare to the radius ($r$) of the core content ($\delta_s \ll r$), and therefore the droplet composition is mainly dominant by the lipophilic core content. On the other hand, nanoemulsion thickness of emulsifier layer is very close to the lipophilic core ($\delta_s \approx r$) and therefore the coating layer has significant influence on the overall droplet composition (Tadros et al., 2004; Mason et al., 2006; McClements, 2010). This influence can be defined by the following equation:

$$\phi_s = \frac{(r+\delta_s)^3 - r^3}{(r+\delta_s)^3} \tag{12.9}$$

Here, $\phi$ ($=V_s/V_{effective}$) is the volume of the droplet coating layer by the effective volume of the overall droplet (oil droplet + emulsifier shell), $r$ is the radius of the inner oil droplets (core material) and $\delta_s$ is the thickness of the droplet coating wall. Therefore, it is obvious that the composition of the droplets in conventional emulsion may be quite different from those in micro- and nanoemulsion emulsion made from the same ingredients.

## 5.2 Droplet Concentration

The concentration of droplets in an emulsion may be defined as the number, mass, or volume of droplets per unit volume or mass of emulsion (McClements, 2005). For instance, the disperse phase volume fraction ($\phi = V_D/V_E$), which is the volume of emulsion droplets ($V_D$) divided by the total volume of emulsion ($V_E$). The droplet concentration in an emulsion can be altered by varying the proportion of dispersed phase and continuous phase. The droplet concentration in a prepared emulsion with specific droplet concentration can be changed by either diluted (eg, by adding more continuous phase) or concentrated (eg, by gravitational separation, filtration, centrifugation or evaporation) (McClements, 2010). Like droplet composition droplet concentration in micro- and nanoemulsion is quite different from the conventional emulsion. The effective volume fraction ($\phi_{effective}$) of the coated droplets is greater than the volume fraction ($\phi$) of the uncoated droplets:

$$\phi_{effective} = \phi\left(1+\frac{\delta_s}{r}\right)^3 \tag{12.10}$$

In conventional o/w-type emulsion, the thickness of emulsifier layer ($\delta_s$) around the droplet is normally smaller than the radius ($r$) of oil droplet ($\delta_s \ll r$), and hence the effective droplet concentration is similar to the oil droplet concentration ($\phi_{effective} \approx \phi$). On the other hand, in micro- and nanoemulsion the droplet coating layer thickness is nearly similar to the droplet radius ($\delta_s \approx r$), hence the effective droplet concentration in emulsion is higher compared to the oil concentration (McClements, 2010, 2011). The effective thickness of coating material depends on the ionic strength of the surrounding aqueous phase. One considerable point is the effective layer thickness must be higher than the physical dimensions of the emulsifier molecules themselves in electrostatically stabilized systems (Tadros et al., 2004). The droplet concentration is very important in terms of physicochemical properties of emulsion and the application of emulsion. For example, increasing

droplet concentration ensures the proper encapsulation of bioactive compounds and control release of them (such as flavor and aroma).

## 5.3 Droplet Size and Size Distribution

Droplet size and size distribution has significant effect on the emulsion stability (eg, gravitational separation, flocculation, coalescence, and Ostwald ripening), control release, optical properties (eg, lightness, color, clarity) and rheology (McClements, 2005; Piorkowski and McClements, 2014). Emulsion droplet size depends on the production technique and factors involved in the production process. In high-energy approaches several factors including pressure, number of passes, homogenizer opening, homogenization time, affect droplet size of emulsion (Wooster et al., 2008). However, in low-energy approach, system composition (such as surfactant–oil–water ratio, surfactant type, ionic strength) and environmental conditions (such as, temperature–time history, stirring speeds) control the droplet size (Anton et al., 2008; Anton and Vandamme, 2009). Emulsifier properties (viscosity, charge, solubility) also have effect on emulsion droplet size and size distribution (Schubert and Engel, 2004).

In the emulsion the droplet radius is the sum of radius of inner core content and the thickness of emulsifier coating: $r_{\text{effective}} = r + \delta_s$. In o/w emulsion, during emulsification the emulsifier creates a coating around the oil droplets. The thickness of emulsifier coating around the oil droplet depends on the molecular dimension of emulsifier. In the same conditions, the emulsifier with small size molecules produce less thick coating layer compare to the emulsifier having large size molecule. There are considerable variations in the thickness of the layers developed by food-grade emulsifiers: typically, small molecule surfactants (such as Tweens and Spans) < globular proteins (eg, egg, whey, or soy proteins) < flexible proteins (such as caseinate or gelatin) < polysaccharides (gum Arabic or modified starch) (McClements, 2010, 2011).

There are a number of methods available to measure the droplet size and size distribution of emulsion including microscopic method, light scattering, measuring droplets dynamics and individual count of droplets. Among the methods, the light scattering method is reported by number of researchers for the measuring of the droplet distribution in nanoemulsion (Achouri et al., 2012; Piorkowski and McClements, 2014). In light scattering technique, light is allowed to pass through emulsion and from the refractive index value droplet size distribution is measured. The way that a particle refracts incident light depends on its size (Boom, 2008).

Along with the droplet size the width of droplet size distribution is also very important in terms of stability and effective application. The properties of two emulsions can be varied from each other, when the two have different widths of distribution, even when the average droplet size is the same. The span of a distribution can be described as:

$$Span = \frac{d(90) - d(10)}{d(50)} \tag{12.11}$$

Where, the lower decile is $d(10)$, the median value $d(50)$, and the upper decile $d(90)$.

## 5.4 Droplet Charge

Due to adsorption of ionic species (eg, proteins, ionic polysaccharides, ionic surfactants, phospholipids, fatty acids, and some small ions) at the surface, the droplets in nanoemulsion often have an electrical charge (McClements, 2005). The sign and ionic characteristics of emulsion droplets depends on the type, concentration, and organization of ionic species and physicochemical properties of the surrounding environment. If the emulsion droplets are stabilized with nonionic surfactants (eg, Tweens and Spans) then the emulsion droplets have no charge; however, it may show few charges due to impurities in the ingredients. When the droplet stabilizer is anionic surfactants have negative charge (eg, lecithin, fatty acids) (Kralova and Sjöblom, 2009), opposite the droplets possess positive charge when stabilized by cationic surfactants (eg, lauric arginate) (Asker et al., 2009). The commercial polysaccharide (eg, gum Arabic modified starch) are used as a stabilizer; it shows net negative charge because of anionic groups such as sulfate or carboxyl present on the polymer chain (Chanamai and McClements, 2002). On the other hand, when proteins (whey protein, casein, soy proteins, egg proteins) are used as a stabilizer the droplet charge depends on the solution pH relative to the isoelectric point (p*I*) of the protein (Gu et al., 2005). Protein-coated droplets consist of a net positive charge for pH < p*I*, no net charge at pH = p*I*, and a negative charge at pH > p*I*.

It is also possible to change the droplet surface charge by maintaining the proper ration of ionic or nonionic surfactant during the preparation of emulsion. The magnitude and sign (positive or negative) of the electrical charge on the droplets has a great influence on stability and functional properties of nanoemulsion, for instance, interaction with other charged species in emulsion, for example, ions (as calcium or iron), or polyelectrolytes (such as, proteins or polysaccharides), aggregation stability, rheology,

interaction with the surface of processing equipment or vessel, interaction with other food ingredients (McClements, 2010, 2011; Piorkowski and McClements, 2014).

The ionic properties of droplet in emulsion are described in terms of its surface charge density ($\sigma$), electrical potential ($\Psi_0$), and /or ζ-potential (ζ) (Hunter, 1986). The surface charge density is the quantity of charge present at the per unit surface area of the droplet (McClements, 2010). The droplet surface charge density depends on the total number of ionized groups per unit interfacial area (Piorkowski and McClements, 2014).

## 5.5 Interfacial Characteristics

The interphase between disperse and continuous phase is a very thin layer of emulsifier or stabilizer consisting of various molecules with particular structures, organizations, and interactions. The nature and molecular structure of emulsifier plays a very important role on the properties of interphase; for example, thickness, charge, permeability, rheology, and environmental responsiveness. These properties are generated and/or altered by the type, concentration, and interactions of any surface-active species present, as well as by the events that occur before, during, and after emulsion formation; such as, complexation, competitive adsorption, layer-by-layer formation (Dickinson, 2003). Control of interfacial properties is very important to get the emulsion with desired functional properties. For example, the thickness and nature of interfacial layer control the diffusion of internal core content. Interfacial region of emulsion droplet have influence on different important physicochemical and sensory properties of nanoemulsion including their stability, rheology, mouthfeel, and flavor (Piorkowski and McClements, 2014). The interfacial properties of nanoemulsion could be controlled by selecting specific types of emulsifier; for instance, surfactants, phospholipids, proteins, or polysaccharides (McClements, 2010).

## 5.6 Droplet Physical State

Usually the nanoemulsions are liquid, where oil is used for the preparation of these emulsion; however, it is also possible to form the emulsion where the oil phase is in a partially or fully crystalline state at the application temperature. In that case, during formation of emulsion the oil phase is usually maintained in liquid form by keeping the processing temperature above the melting point or by dissolving any crystalline material in an appropriate organic solvent. Then the oil droplets in emulsion could be formed partial or fully crystalline structure at the application

temperature, which can be controlled by controlling their composition and/or the preparation conditions, such as temperature and triglyceride composition (Walstra, 2003; Muller and Keck, 2004; Wissing et al., 2004; McClements, 2005, 2010). For instance, in o/w emulsion the oil phase can be turned into crystalline form when the temperature is sufficiently below the melting point of the oil phase. This approach is usually used to produce solid lipid nanoparticles (SLN) or nanostructured lipid carriers (NLCs), which are nanoemulsions, where the oil phase has been crystallized (McClements, 2010). The crystalline temperature of oil phase in nanoemulsion is different and may be appreciably below that of the same fat in a bulk phase. The nature of crystal formed in bulk fat are different from those after emulsification in droplet form in nanoemulsion. There are factors involved with this phenomenon, including curvature effects, the limited volume present in the droplets, and the lack of secondary nucleation sites (Muller and Keck, 2004; Wissing et al., 2004). The concentration, nature, and location of the fat crystals within the lipid droplets in an o/w emulsion could be maintained by proper selection of oil type (eg, solid fat content versus temperature profile), thermal history (eg, temperature versus time), emulsifier type (eg, tail group characteristics), and droplet size (Müller et al., 2000; Walstra, 2003; Muller and Keck, 2004). The formation of crystalline structure of oil droplet (eg, SLN or NLC) deduces the molecular diffusion and lowering the chemical degradation reaction. Therefore, it improves the stability and bioavailability of encapsulated lipophilic components.

# 6 Physicochemical Properties of Nanoemulsions

The bulk properties of emulsion mainly depend on the physicochemical properties of individual ingredients use for the formation of emulsion and the method of emulsification. The selection of emulsion in food processing depends on its physicochemical properties. The physicochemical properties of micro- and nanoemulsion include optical properties, stability (see Section 4), rheology, release characteristics are appreciably different from conventional emulsion and mostly desired by food manufacturers.

## 6.1 Optical Properties

The optical properties of emulsion are very important to the emulsion-based food product's overall visual appearance and acceptability by the consumer. The impact of emulsion droplet characteristics on overall appearance of an emulsion is considered as

important in terms of designing and formulation of a food product. Each type of food product is expected to have a particular appearance depending on its nature. Some food products should be totally transparent or only slightly turbid (eg, jellies, jams, soft drinks, and fruit beverages) and therefore any oil droplets added to the food products should not contribute appreciably to their opacities. On the other hand, some products should be optically opaque (eg, dressings, sauces, mayonnaise) and the oil droplets make a valuable contribution to their overall by scattering the light (McClements, 2010). Scientifically, the optical properties of emulsion could be defined and quantified in terms of their opacity and color, which can be quantitatively described using tristimulus color coordinates, such as $L^*a^*b^*$ system (McClements, 2002a, 2005; Piorkowski and McClements, 2014). In this color system, $L^*$ represents the lightness, and $a^*$ and $b^*$ are color coordinates: where, $+a^*$ indicates red direction, $-a^*$ indicates green direction; $+b^*$ for yellow direction, $-b^*$ for blue direction; low $L^*$ means dark and high $L^*$ means light. The opacity of an emulsion can therefore be characterized by the lightness ($L^*$), while the color intensity could be characterized by the chroma: $C = (a^{*2} + b^{*2})^{1/2}$. The color intensity of emulsion is oppositely related to the lightness. Therefore, the croma decrease with increasing lightness. The conventional emulsions scatter the light strongly and refractive index is used to measure the optical properties. However, nanoenulsion scatter the light very weakly (therefore, usually it looks transparent or slightly turbid) and so to measure the optical property using transmission measurement, where the intensity of light wave passes through the emulsion is measured. For monodispersed droplets the optical transmission is generally express as

$$T = \exp(-\tau L) \tag{12.12}$$

Where, $\tau$ is the turbidity and $L$ is the optical path length. The specific turbidity for a light scattering cross section is presented as

$$\frac{\tau}{c} = \frac{3\pi}{4\rho_c}\frac{Q_{av}}{r_{vs}} \tag{12.13}$$

Where, $c$ is the volume concentration of emulsion droplets, $\rho_c$ represents density of the continuous phase, $Q_{av}$ is the mean light scattering efficiency, $r_{vs}$ is the volume mean radius. The mean light scattering efficiency ($Q_{av}$) is determined by particles size, refractive indices of dispersed and continuous phases, and wavelength of light scattered.

The relative refractive index, the droplet concentration, and the droplet size are used to measure the optical properties of emulsion

(McClements, 2002a,b, 2005). The turbidity or lightness of emulsion increases with increased refractive index contrast and droplet concentration and droplets size. The turbidity becomes maximum at strongest light scattering, when the droplet size is similar to the wave length of visible light (ie, $d \approx \lambda$) (McClements, 2010). In nanoemulsion, the droplet dimensions are much smaller than the wavelength of light ($d << \lambda$), causing weak light scattering and hence low turbidity. Therefore, nanoemulsions tend to be transparent in appearance (Zhang, 2011).

## 6.2 Rheology

Rheology represents the deformation and flow properties of materials. The rheological properties of emulsion are very important for the solid, semisolid, and liquid food materials, where emulsion is incorporated during manufacturing. During processing operations rheological properties are also very important in terms of mixing, flowing through pipe, duct, or orifice, and packaging. Rheology of emulsion depends on their composition, structure, and droplet interactions; based on these, feature emulsions can show a broad range of various rheological characteristics: viscous liquids; viscoelastic liquids; viscoelastic solids; plastics; or elastic solids (McClements, 2005; Genovese et al., 2007). Usually the shear viscosity is used to characterize the rheology of comparatively dilute emulsions (McClements, 2005; Genovese et al., 2007). Generally, the shear viscosity of an emulsion is primarily determined by the continuous phase viscosity ($\eta_0$), the droplet concentration ($\phi$), and the nature of the droplet–droplet interactions (w): $\eta = \eta_0 \times f(\phi, \text{w})$. When the droplet concentration is less than about 5% ($\phi < 0.05$), then the shear viscosity can be defined by Einstein's equation:

$$\eta = \eta_0(1 + 2.5\phi) \tag{12.14}$$

The approximate viscosity ($\eta$) of concentrated emulsions could be described by a semiempirical equation, which takes into account the increased viscosity that generate due to higher droplet concentrations by droplet -droplet interactions (Quemada and Berli, 2002; Berli et al., 2005; McClements, 2005, 2010):

$$\eta = \eta_0\left(1 - \frac{\phi}{\phi_c}\right)^{-2} \tag{12.15}$$

Here, $\phi$ represents disperse phase volume fraction, and $\phi_c$ represents the critical disperse phase volume fraction above which the droplets are so closely packed together that they cannot easily

flow past each other. Typically, $\phi_c$ has a value of around 0.4–0.6 depending on the nature of the system (McClements, 2005). From this equation it is clear that with increasing droplet concentation in a emulsion its viscosity also increase.

### 6.3 Molecular Distribution and Release Characteristics

Emulsion has ability to incorporate bioactive food component such as, flavors, aroma, antioxidants, antimicrobials, vitamins, and nutraceuticals on their composition and can act as a delivery system. In the o/w emulsion a protecting layer is formed around the lipophilic compound and the interfacial layer controls the release of internal contents of the droplet. In nanoemulsion the interfacial coating layer thickness is higher compared to conventional emulsion; hence, the nanoemulsion system offers more control on the release rate of encapsulated bioactive food compounds during processing and storage. Basically, the release rate and extent of release mainly depends on the several factors that trigger mechanisms for release such as pH, ionic strength, temperature, and enzyme activity (McClements, 2010).

The fact that the smaller droplet size in nanoemulsion has important implications for the release rate of any encapsulated substances. A convenient measure of the rate of release is the time required for half of the compound to diffuse out of the droplets, $t_{1/2}$ reported by (Lian et al., 2004):

$$t_{1/2} = \frac{0.058 r^2 k_{DC}}{D} \tag{12.16}$$

Here, $D$ refers to the translational diffusion coefficient of the encapsulated substance through the oil phase. For the nanosized droplets ($d < 100$ nm), these times are usually <1 ms and therefore the release of the encapsulated components could be considered to be almost instantaneous. However, it might be possible to slow down release somewhat if there is a highly impermeable and thick coating surrounding the lipid droplets (McClements, 2005, 2010).

## 7 Techniques for Identifying the Properties of Nanoemulsion

The detection, identification, and characterization of nanoemulsion systems are very important for its potential applications and understanding benefits and possible risk or toxicity

(Luykx et al., 2008). There are number of approaches that are typically used for the identification and characterization of nanoemulsion, which have been subsectioned into three classes: separation, physical characterization, and imaging techniques (Silva et al., 2012).

*Separation Techniques*: A number of methods are applied to identify nanoemulsion; however, in most of the cases it is very difficult to detect them in the food mixture or in complex food products. So, separation of nanoemulsion is the prerequisite for their characterization in these cases. Chromatography and Field Flow Fraction (FFF) are the most commonly applied techniques. Size and/or charge are main characteristics of nanoemulsion. Hence, size-exclusion chromatography (SEC) and/or ion exchange chromatography (IEC) are the most suitable types of liquid chromatography for the separation of nanoemulsions from the food matrix (Luykx et al., 2005; Silva et al., 2012). On the other hand, FFF is a flow-assisted separation technique, and applicable for a wide range of particle size (1 nm–100 μm) (Yohannes et al., 2011). The basic principle of FFF approach is the mode of movement of particles, such as smaller particles are transported faster and eluted earlier when the flow nature is parabolic and when particles with same volume and different shape in a cross flow, then the isometric particles will be eluted first than the asymmetric particles (Jores et al., 2004).

*Physical Characterization Techniques*: These techniques are based on the physical perspective of the nanoemulsions; such as droplet size, size distribution, zeta potential, and crystallinity. There are a number of approaches under this type of technique reported by different scientist. The approaches are: (1) Dynamic light scattering (DLS) is used for rapid determination of the size distribution profile of small particles in suspensions or polymers in solution. It measures Brownian motion and relates this to the size of the particles through Stokes–Einstein equation. Through the illumination of the particles with a laser and analyzing the intensity fluctuations in the scattered light; DLS allows calculating the size of the particles (Silva et al., 2012). (2) Zeta potential is related to colloidal science. It is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. (3) Differential Scanning Calorimetry (DSC) is a thermo-analytical technique. This approach measures the difference in the amount of heat that is required to increase the temperature of a sample and a reference at a specific level throughout the experiment. (4) Fourier transform infrared (FTIR) is an approach where an infrared radiation is passed through the sample and measures the amount of rays absorbed by the sample

and transmitted through the sample. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample (Silva et al., 2012). (5) Nuclear magnetic resonance (NMR) is used for exploring the structural information regarding molecular compounds. (6) X-ray diffraction (XRD), in which a working principle of this method is detecting the scattered intensity of an X-ray beam hitting a sample as a function of incident and scattered angles, polarization, and wavelength or energy. (7) Small-angle X-ray scattering is used to measure the structural features of colloidal size particles where the elastic scattering of X-rays by a sample that has inhomogeneities in the nanometric range, is recorded at very low angles (typically 0.1–10 degree) (Silva et al., 2012).

*Imaging Techniques*: In this technique, an image of the sample is taken by microscope and analyzed. This method provides information about the size, shape, and aggregation state of the nanoemulsions. Same imaging methods, which are used for characterization of nanoemulsion are: (1) Transmission electron microscopy (TEM), which is a widely used technique and capable of a resolution on the order of the 0.2 nm (Luykx et al., 2008; Wang, 2000). It is used to check the morphology, structure, particle distribution, particle shape in nanoemulsion. (2) Scanning electron microscopy (SEM) is capable of producing images of sample surface with high resolution. The image from SEM has t3-dimensional appearance and which are useful for observing the surface structure. (3) Atomic force microscopy (AFM) is the newest technique in the area of microscopy (Ruozi et al., 2005; Edwards and Baeumner, 2006; Luykx et al., 2008). AFM is capable of achieving high resolution (±0.1 nm) and used to view single atoms or molecules that have dimensions of a few nanometers. It uses a nanometer-sized sharp probe to move over the sample and results in a high-resolution 3-dimensional profile of the surface under study.

## 8 Application of Micro- and Nanoemulsion in the Food Industry

There are huge quantities of emulsion used in wide range of food products by the food processing industries. In recent time, food manufacturers show extra interest in the use of micro- and nanoemulsion for their stability and unique physicochemical properties over conventional emulsions. Nanoemulsions become an increasingly important medium for carrying functional agents like fatty acids, polyphenols, vitamins, natural colorants,

antimicrobials, some micronutrients and flavors in different types of food (Seikikawa and Watanabe, 2008; Mao et al., 2009; McClements and Rao, 2011; Rao and McClements, 2011; Zhang, 2011; Laouini et al., 2012).

In the beverage industry micro- and nanoemulsions are used as delivery systems for color, flavor, antioxidants, and other fat soluble vitamins and bioactive component in beverages. The unique property of micro- and nanoemulsion is optical clarity, which makes them more applicable in transparent beverages as delivery systems. Nanoemulsion is also used to produce cloudy beverages. Nanoemulsions are able to retain flavor compounds from manufacturing conditions and throughout the beverage's shelf life. It is thought that nanoemulsions can cover the flavor and save it from temperature, oxidation, enzymatic reactions, and hydrolysis and are thermodynamically constant at an extensive range of pH values (NutraLease, 2011).

There are increasing trends in sales observed for the bottled waters globally in the recent years. The water includes enhanced, flavored, and fruit-flavored are very popular all over the world. Enhanced water is enriched with electrolytes, vitamins, and other nutraceuticals, while flavored and fruit-flavored waters are flavored with flavorants and fruit extracts, accordingly. Less light scattering property of micro- and nanoemulsion make it very suitable to supply oil-soluble flavors and nutraceuticals in bottled water (Piorkowski and McClements, 2014).

The baked products; such as bread, biscuits, and cake are equally popular all over the world. To enhance the quality of baked products application of nanoemulsion is very effective, in terms of retaining color, flavor, and other fortifying components. The nanoemulsions are also able to modify the internal structure of the products because their very tiny particles can work with the internal structure of the ingredients to improve product quality. Emulsions can improve texture and volume of baked products by retaining more air bubbles in the products.

Few giant food processing industries reported about the application of nanotechnology in food products in recent years. For example, Nestlé and Unilever have applied nanoemulsion in ice cream to change its functionality. The quality of ice cream has been prepared using nanoemulsion technology by Unilever without changing the original taste. The goal is to produce ice cream having lower fat content, reaching a fat decrease from the initial 16% to 1% (Martins et al., 2007a,b; Silva et al., 2012). Nestlé has a patent in w/o emulsions (10–500 nm), which focused on obtaining faster and simpler thawing by the supplement of polysorbates and other micelle-forming ingredients; these are applied to the

contribution of uniform thawing of frozen foods in the microwave (Möller, 2009).

The tiny particle size provides very effective encapsulation for the bioactive food components in food materials, which makes them more stable processed food. Most of the food-grade emulsions are o/w-type emulsion. For example, o/w nanoemulsion systems have been used as a carrier and combiner in food in order to encapsulate Omega-3 fatty acids in yogurt (Chee et al., 2005) and ice cream (Chee et al., 2007).

The other applications of micro- and nanoemulsion in food industry includes encapsulate bioactive and volatile components very effectively, control release of flavor and aroma and antimicrobial nanoemulsions for decontamination of food apparatus, packaging, or food. It also increases the bioavailability of hydrophilic or hydrophobic compounds (functional compounds) by means of very tiny particles.

## 9 Possible Risks of Nanoemulsion

There are a number of physicochemical and physiological mechanisms connected with the small particle size in nanoemulsion that may possibly create toxicity. Although standardized testing protocol precisely designed to evaluate the potential toxicity of nanoemulsions proposed for application within food products is still not available (Maynard et al., 2006).

Nanoemulsion encapsulates the lipophilic bioactive compound of small size and increases the bioavailability. Many bioactive compounds are desired by the human body, for these cases increasing bioavailability might have no negative effects on human body. Nevertheless, certain bioactive compounds occasionally show toxic effects, when consumed at greater amounts. Bioavailability of these bioactive compounds is usually low. But it is increased when incorporated with nanoemulsion. Practically, bioactive component is incorporated into a food product through nanoemulsion and regular consumption of that particular food and may cause accumulation of the compound and exhibit toxicity.

In addition, few components typically used to make nanoemulsions are toxic when consumed at adequately high amounts, such as emulsifiers and solvents. A comparatively large amount of emulsifier is essential to cover the large specific surface area connected with the droplets in nanoemulsions (McClements and Rao, 2011). Many small molecule surfactants are identified to reveal poisonous effects when consumed at greater amounts (Kralova and Sjöblom, 2009; He et al., 2010).

## 10 Conclusions

Emulsions have a long history of use in food products, especially conventional o/w emulsions are commonly and extensively used in the food industry. However, in recent years application of nanoemulsion in food processing offers a number of benefits over conventional emulsions in terms improved solubility, bioavailability, and functionality of hydrophobic compounds in food matrices. Although nanoemulsion provides several facilities, its application in the food sector is still challenging, which must be addressed both in terms of the production process, particularly their cost and characterization of both the resulting nanoemulsions and the food systems, in which it might be applied in terms of product safety and acceptance. Additionally, there are few methods of nanoemulsion production still in lab scale and have number of drawbacks in the scale-up process.

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# 13

# NANOSTRUCTURAL CHARACTERIZATION OF FOOD GRADE MICROEMULSIONS: ULTRASONIC RESONATOR TECHNOLOGY

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## 1 Introduction

Microemulsions are isotropic, transparent, nanosize (<100 nm), and thermodynamically stable oil in water (o/w), water in oil (w/o), or water–oil (bicontinuous) dispersions. They are potentially capable for use in food formulations, functional foods, and pharmaceuticals. Microemulsions have many other purposes, including nanoencapsulation, extraction, and as nanoreactors. Furthermore, microemulsions are self-emulsifying systems that can be simply produced by selecting a correct formulation without the need for external forces, for example, shearing or homogenization. In addition, their functionalities strongly depend on, and are determined by, their molecular structures. Over the past few decades, attempts have been made to characterize the nanostructures of microemulsions, but further structural characterization is required to obtain a clear perspective on functionality. Therefore, this chapter attempts to give a concise explanation regarding the history and background of microemulsions and their fundamental differences from other emulsions, their formulation and fabrication challenges, thermodynamic aspects of microemulsification and their stability, and their applications in various fields with a focus on food systems and their characterization challenges. The following section deals with ultrasound (definition, types, differences) with special focus on ultrasonic resonator

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00013-1

technology (URT). The principles of its operation, velocity and attenuation measurements, and the correlation of these parameters with thermodynamic variables (compressibility and volume functions) will be addressed. The third section reviews the applications of various URT devices for characterization of nonfood and food grade emulsions, nanoemulsions, and microemulsions.

# 2 Microemulsions

## 2.1 Definition, Background, and History

In general, emulsions consist of two immiscible liquids where one is dispersed in the other, usually as small spherical droplets. Based on the composition of the dispersed or continuous phases, emulsions can be categorized as oil-in-water (o/w), water-in-oil (w/o), water-in-oil-in-water (w/o/w), or oil-in-water-in-oil (o/w/o) systems. Most of these emulsions can be found in natural or formulated food systems (Leal-Calderon et al., 2007; Santana et al., 2013).

With regard to the size of droplets, emulsions are also generally classified as nanoemulsions (1–100 nm), miniemulsions (100–1000 nm), and macroemulsions (conventional emulsions) (0.5–100 μm). It needs to be noted that there is no strong agreement on the abovementioned ranges between all scientists in this field, and there is also some overlap among these categories. Furthermore, all of them, except for the subgroup of nanoemulsions, which are called microemulsions (the subject of this chapter), are thermodynamically unstable systems so that their kinetic stability must be improved by adding surfactants and stabilizers, or by reducing the size of droplets, and/or controlling production conditions. Nanoemulsions are kinetically much more stable than miniemulsions, while the latter is much more stable than macroemulsions (Leal-Calderon et al., 2007; McClements and Rao, 2011; McClements, 2012; Santana et al., 2013).

A microemulsion is "a system of water, oil and an amphiphile which is a single, optically isotropic, and thermodynamically stable liquid solution" (Danielsson and Lindman, 1981). These systems can be produced simply by self-assembling of surfactant molecules without or with a very low level of external energy input (mixing), hence their designation as self-emulsifying systems. In addition, sometimes supplementary components are present, that is, cosurfactants or cosolvents that are necessary to produce a stable and one phase microemulsion (Saito and Shinoda, 1967, 1970). Some scientists consider microemulsions only as minuscule types of conventional emulsions, with a size over the

range of 5−100 nm. But it needs to be emphasized that such a description is not precise, because conventional emulsions are essentially different than microemulsions. For instance, in emulsion systems the average droplet size raises continuously with time so that phase separation is an inevitable phenomenon, that is, they are thermodynamically unstable and their formation requires input of a high level of external work, while this is not the case for microemulsions. Another important difference relates to their appearances: conventional emulsions are usually cloudy or turbid while microemulsions are always clear or translucent because the wavelength of visible light is significantly larger than the droplets in microemulsions. There are also other differences that can be found in the literature (Stubenrauch, 2009; Fanun, 2009; Najjar, 2012; McClements, 2012).

In the late 19th century, Australians, for the first time, made an old-fashioned detergent, creating a microemulsion that was used to wash wool. During 1940s and 1950s, the oldest scientific reports regarding the spontaneous emulsification of water and oil in the presence of surface-active agents (surfactants), were also published starting where the systematic understanding of microemulsions (Hoar and Schulman, 1943; Winsor, 1946, 1954). For describing a system consisting of surfactant, water, oil, and alcohol, the term microemulsion was used for the first time by Schulman et al. (1959). It is worth noting that the term microemulsion is sometimes indefinite because it does not expose the nanometer range of the size of the dispersed phase droplets (Fanun, 2009; Stubenrauch, 2009; Najjar, 2012).

Microemulsions were then almost ignored for two decades (1960s and 1970s), but interest in this field really accelerated in the late 1970s and early 1980s as the crude oil price rose owing to serious conflicts in the Middle East (the Islamic Revolution in Iran and the Iran–Iraq War). During that era, it was recognized that the microemulsion technique was a feasible method for enhanced oil recovery whenever the crude oil price reached practically high levels. Since then, research into different applications for microemulsions has increased dramatically, so that in 2014 alone more than 1630 papers had been published (based on Web of Science reports).

## 2.2 Formulation, Manufacturing, and Structure

The very first step for formulation of any microemulsion system is to select the proper components including the oil phase, aqueous phase, surfactant, cosurfactant, and cosolvent. Due to toxicity and other health issues, there is special emphasis on selecting

components, which are "*G*enerally *R*ecognized *A*s *S*afe" (GRAS), as microemulsions are expected to be used in food, pharmaceutical, and cosmetic products (Flanagan and Singh, 2006; McClements and Rao, 2011; McClements, 2012; Muzaffar et al., 2013).

Generally speaking, emulsification can be performed by two different methods, namely *high* and *low energy* processes. The former one, the high energy method, is traditionally used in industrial operations due to flexible control of droplet size distribution and its capability to produce very fine emulsions, even nanoemulsions, using a wide range of materials. To do so, one needs to use various homogenization devices such as rotor and stator, colloid mill, high pressure homogenizer, microfluidizer, membrane devices or high power ultrasonic techniques. In contrast, in low energy methods, emulsification occurs under laminar and low energy conditions, which is of obvious interest from an economic point of view besides other advantages. Therefore, in the rest of this chapter we focus on describing these nanosized emulsions—so-called microemulsions—which are produced by low energy methods (Flanagan and Singh, 2006; McClements and Rao, 2011; McClements, 2012; Santana et al., 2013; Mirmajidi Hashtjin and Abbasi, 2015a,b).

The low energy method consists of *phase titration* and *phase inversion* approaches. In phase titration, microemulsions are manufactured by simply mixing the major constituents; this can be illustrated by using ternary or pseudo ternary phase diagrams (Fig. 13.1a). The formation of a thermodynamically stable one phase microemulsion system normally requires adequate ratios and matching types of components (Fig. 13.1b). It needs to be emphasized that suitable composition determination is a major challenge in this field. For this purpose, one needs to prepare mixtures with different compositions of the components, and check their physical stability as well as the type and number of phases present in the system using a phase diagram. Therefore, the phase diagram construction is a useful attitude to study the complex interactions that can take place when different components at various concentrations are mixed (Shinoda, 1970; Becher and Arai, 1968; Ruckenstein, 1981; Fanun, 2009; McClements, 2012; Sole et al., 2012; Muzaffar et al., 2013). For microemulsion systems, which consist of four or five components, the quaternary (four component system) or quinary (five component system) phase diagrams needs to be created; however, these phase diagrams are usually time consuming and difficult to interpret. Therefore, in such cases it is a good idea to construct a pseudo-ternary phase diagram to identify the one phase microemulsion formation zone. In this case, each vertex of the triangle diagram demonstrates

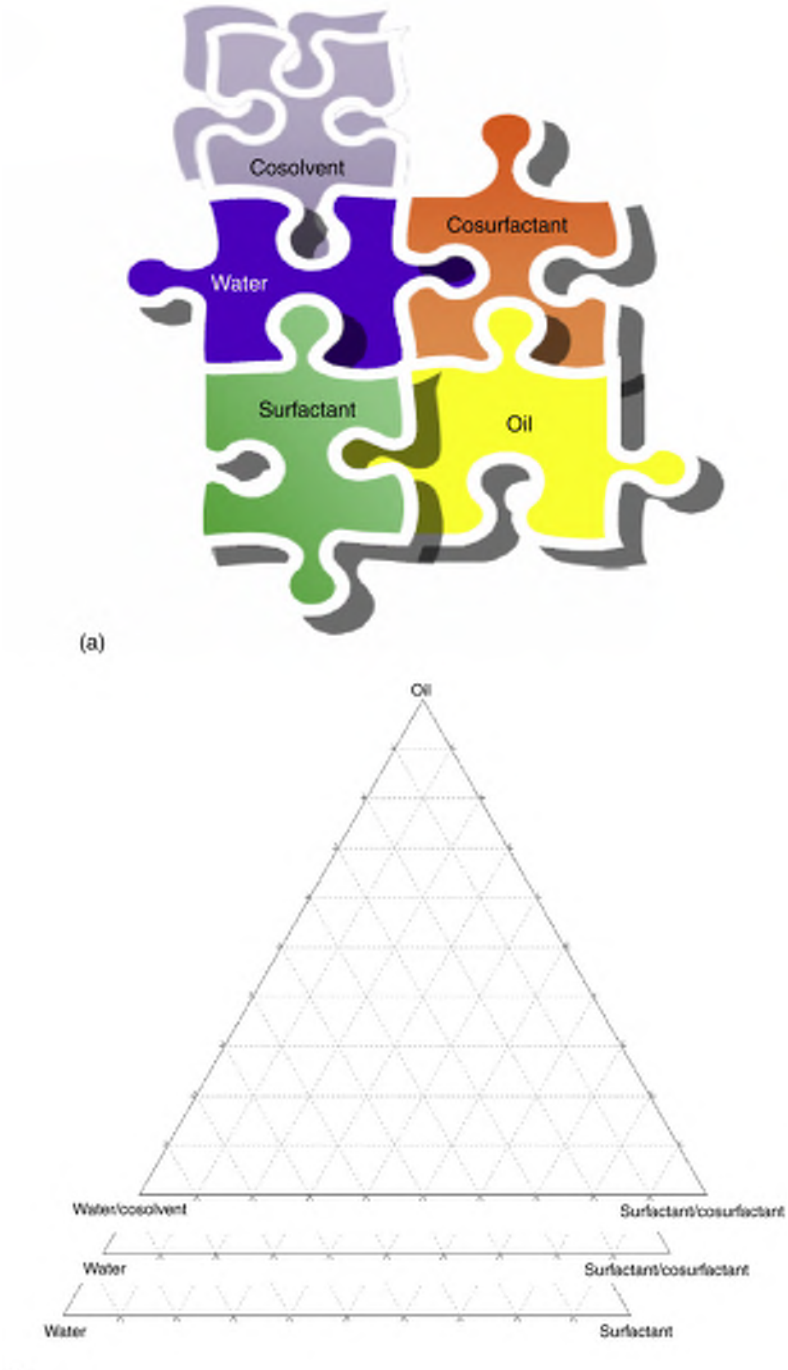


**Figure 13.1. Illustration of (a) the perfect puzzle type matching of components to produce a stable microemulsion system, (b) ternary phase diagram (lower layer), and pseudo ternary phase diagrams containing four and five components (upper layers).**

100% of a particular component or 100% of components that have been mixed at a constant ratio (Fig. 13.1b).

The second type of low energy method is called phase inversion, where a microemulsion is formed in response to either temperature change, additional dispersed phase, or pH and/or ionic strength. During this manipulation process, the curvature of the surfactant layer can change, for example, from reverse micelle to normal one and vice versa. For instance, in a system containing nonionic surfactants, by changing the temperature, there is a conversion from an o/w microemulsion, at low temperatures, to a w/o microemulsion at higher temperatures (transitional phase inversion). During cooling, the system will again pass bicontinuous phase to form an o/w microemulsion (Muzaffar et al., 2013). This method is also known as a *phase inversion temperature* (PIT) technique (Fig. 13.2). Similar transitions can be expected

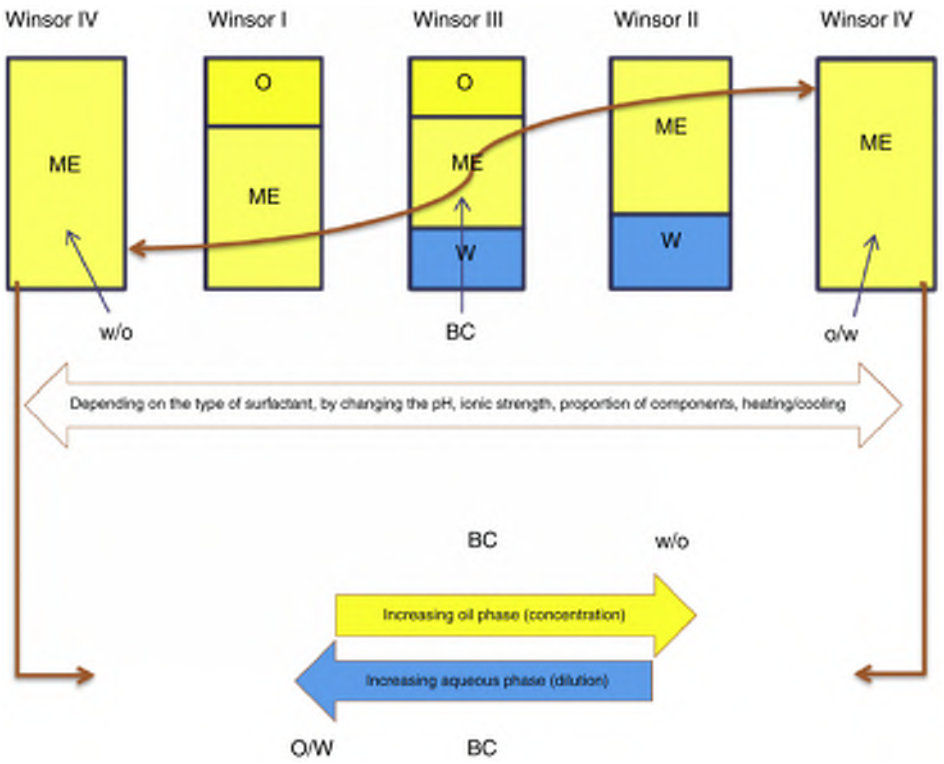


**Figure 13.2. Schematic representation of Winsor states (I, II, III, IV) as well as the influence of some variables on structural changes (normal micelle, rod-like normal micelle, hexagonal normal micelle, cubic, hexagonal reverse micelle, rod-like reverse micelle, reverse micelle) in microemulsion systems.** O, W, and ME represent oil, water and microemulsion, respectively.

by altering pH, ionic strength, and the volume fraction of oil or water phases. The latter approach is very common in microemulsion manufacturing where a w/o microemulsion, with a constant ratio of components, is prepared before it is diluted along a dilution line. A dilution line is any point on a line connected from the water corner to a point on the line directly opposite the water corner that contains various amounts of water but a constant ratio of other components (Flanagan and Singh, 2006; Sole et al., 2012; Abbasi and Scanlon, 2016).

Depending on the proportion, the chemical composition and concentration of each component, the mixtures of oil, water, and surfactants (cosurfactants and cosolvents as well if necessary) are able to form a wide variety of phases (one, two, or three) or Winsors (I, II, III, IV) and structures (w/o, o/w, BC): examples are normal and reverse micelles, lamellar, hexagonal, cubic (Eastoe and Tabor, 2014; Winsor, 1948). There are four types of mono- and multiphase microemulsions or so called Winsor phases (Winsor, 1948) that exist in equilibria (Fig. 13.2):

- Winsor I, the lower (o/w) microemulsion phase is in equilibrium with the upper surplus oil.
- Winsor II, the upper (w/o) microemulsion phase is in equilibrium with lower excess water.
- Winsor III, the middle (bicontinuous or BC) microemulsion phase is in equilibrium with upper extra oil and lower extra water phases.
- Winsor IV, in which oil, water, and surfactant are mixed homogenously as a single and translucent phase (Winsor, 1948; Sole et al., 2012).

Of the aforementioned microemulsion systems, food formulation engineers and technologists are usually interested on the thermodynamically stable single phase Winsor IV type microemulsions. From a structural point of view, they are categorized as w/o, o/w, and BC microemulsions. Similar to conventional emulsions, in w/o microemulsions, water droplets are dispersed in the oil phase while in o/w microemulsions the oil droplets are dispersed in the aqueous phase. In BC systems, the quantities of water and oil are alike (Flanagan and Singh, 2006; McClements and Rao, 2011; Sole et al., 2012; McClements, 2012).

## 2.3 Thermodynamic Aspects of Microemulsion Formation and Stability

In general, the stability of any emulsion system can occur either kinetically (high energy methods) or thermodynamically (low energy methods). Microemulsions can be simply produced

by mixing the appropriate type and ratio of components by a low energy method and their stability is thermodynamic. Regarding ideas on the formation and stability of microemulsions, there are three approaches, namely interfacial theories, solubilization theories, and thermodynamic theories, of which the latter is accepted by the majority of scientists. The reader is referred to others for further information (Schulman et al., 1959; Ruckenstein and Chi, 1975; Prince, 1975; Rance and Friberg, 1977; Flanagan and Singh, 2006; Fanun, 2009; Stubenrauch, 2009; Mehta and Gurpreet, 2011; Muzaffar et al., 2013; Santana et al., 2013). This subsection covers thermodynamic theory.

The thermodynamic state of microemulsions can be described by the microemulsion formation free energy ($\Delta G_f$) according to Eqs. (13.1), (13.2), and (13.3):

$$\Delta H_f = \gamma \Delta A \tag{13.1}$$

$$\Delta S_f = -nk_B \left[ \ln \varphi + \left\{ \frac{1-\varphi}{\varphi} \right\} \ln(1-\varphi) \right] \tag{13.2}$$

$$\Delta G_f = \Delta H_f - T\Delta S_f \tag{13.3}$$

In Eq. (13.1), $\gamma$ is the interfacial tension and $\Delta A$ is the interfacial area gained through emulsification. In Eq. (13.2), $n$ is the number of droplets of dispersed phase, $k_B$ is the Boltzmann constant, and $\varphi$ is the dispersed phase volume fraction. In Eq. (13.3), $\Delta G_f$ is the free energy of microemulsion formation, $T$ is the temperature of the system (Kelvin), $\Delta S_f$ is the change in entropy of the system, and $\Delta H_f$ is the change in enthalpy due to microemulsion formation (Fanun, 2009; Stubenrauch, 2009; McClements, 2012; Santana et al., 2013).

From a thermodynamic point of view, if $\Delta G_f$ is negative, the reaction is spontaneous, whereas when it is positive the reaction is not spontaneous so one must supply external energy. Therefore, in a microemulsion system, a high concentration of surfactant is used so that the interfacial tension tends to drop to extremely low values ($10^{-2}$–$10^{-4}$ mN/m). As a result, the enthalpy penalty for droplet formation is compensated by the very large entropy that arises from mixing of one phase in the other one, owing to the formation of a large number of small droplets. All in all, when substantial reductions in surface tension are complemented by significant favorable entropic changes then a negative free energy of formation is achieved. In such conditions, microemulsion is spontaneously formed and the resulting system is thermodynamically stable (Fanun, 2009; Santana et al., 2013; Muzaffar et al., 2013). Therefore,

the following conditions needs to be met during the preparation of a stable one phase microemulsion system:

- Selection of surfactant, or mixture of surfactants, is a very crucial step since a very low interfacial tension ($10^{-2}$–$10^{-4}$ mN/m) needs to be achieved at the o/w interface.
- The surfactant concentration or the number of surfactant molecules must be high enough to offer the requisite number of surfactant molecules for inclusive coverage of the newly formed nanosized droplets.
- The presence of cosurfactants or cosolvents is absolutely necessary to ensure the interface is sufficiently flexible or mobile to promote the formation of microemulsions (Fanun, 2009; McClements, 2012; Santana et al., 2013; Muzaffar et al., 2013).

It is noteworthy that many surfactants are unable to achieve thermodynamically stable microemulsions alone, because of their geometry and molecular structure, or their inability to sufficiently lower the interfacial tension between the two fluids. Therefore, cosurfactants or cosolvents can overcome this issue; and this is why many microemulsion formulations comprise four or five components (Fig. 13.1).

## 2.4 Applications

In the 21st century, microemulsions play an important role in everyday life. Numerous final products are available, which in principle are manufactured based on the microemulsion technique and/or in very close relation with this method. Furthermore, in some cases, the formation of microemulsion is the essential process that occurs at the ending stage of the application. In other cases, solubilization, extraction, or removal of active agents or the unwanted compounds are the primary objectives. Nonetheless, solubilization usually aids to deliver the active agents to the required sites at the requested conditions (Najjar, 2012).

In terms of applications of microemulsions at an industrial scale, there are two major concerns, namely health aspects and economic feasibility. The former has really high importance for foods, pharmaceuticals, and cosmetics, the subject of this chapter, whereas the latter has high importance in every application field, e.g., cleaning products and cleansing processes, the largest industrial application for microemulsions. Nevertheless, microemulsions have already found limited industrial applications in other areas, including formulation of cosmetics, liquid–liquid extractions, pharmaceuticals drug delivery, and analytical applications (Austad and Taugbøl, 1995; Ozawa et al., 1997; Solans and Kunieda, 1997; Capek 2001;

Mulqueen 2003; Valenta and Schultz, 2004; Xu and Gan, 2005; Santanna et al., 2009; Fanun, 2009; Stubenrauch, 2009; Najjar, 2012; Santana et al., 2013).

Apart from the aforementioned applications, microemulsions have high capability to be used in food-grade and nonfood-grade systems but with specific food interest. The solubilization of proteins, withdrawal of triglycerides from edible oil seeds and nuts, the extraction of lycopene, carotenoids, phenolics, antioxidants, flavonoids, and other colorants or bioactives from natural resources, nanoencapsulation of essential oils, enzymes, and other components are some of the potential applications in this field. In addition, over the past decade, most of the research attentions have been centered on the application of microemulsions as delivery systems for nutraceuticals, and how microemulsions can improve their bioavailability. It seems that the major concerns in food systems are the limited number of commercially available food grade or GRAS surfactants, cosurfactants, and cosolvents. The fate of consumed surfactants is another health concern that has attracted attention over the recent years (Flanagan and Singh, 2006; McClements and Rao, 2011; McClements, 2012; Santana et al., 2013; Radi et al., 2013; Amiri et al., 2013; Amiri-Rigi and Abbasi, 2016; Abbasi and Radi, 2016).

It is evident that the applicability of microemulsions in any field of interest, particularly for food and pharmaceuticals, is strongly dependent on a good characterization of their nanostructure under various formulation and application conditions. Knowledge of the nanostructure of a microemulsion system is very important because its phase behavior can control its solubilization capability, and release of bioactives, or the formation of nanoparticles (Krauel et al., 2005). However, the characterization of microemulsions is a challenging task due to the complexity and presence of a variety of structures and components (e.g., see Figs. 13.1 and 13.2). A wide range of techniques (pulsed-gradient spin-echo nuclear magnetic resonance, differential scanning calorimetry, self-diffusion NMR, small-angle neutron and X-ray scattering, dynamic light scattering, dielectric spectroscopy, cryo-transmission electron microscopy, electrical conductivity, and viscosity measurements) are potentially available for characterization purposes, but most of the techniques have their own associated limitations (Regev et al., 1996; Ezrahi et al., 1997; Fedotov et al., 1997; Feldman et al., 1997; Garti et al., 2000; Glatter et al., 2001; Yaghmur et al., 2003; de Campo et al., 2004; Amar et al., 2004; Garti et al., 2004; Amiri et al., 2013; Abbasi and Radi 2016). Therefore, matching studies using a combination

of techniques are usually required to obtain an inclusive understanding of the physicochemical properties, phase transitions, and structure of microemulsions.

The URT is a technique that has already shown considerable potential for characterization of emulsions, nanoemulsions, and microemulsions (Hickey et al., 2006, 2010; Shah et al., 2007). Despite its potential as an effective tool for structural characterization of microemulsions, it has not been widely utilized. Therefore, the following sections discuss in detail the principles of this technique as well as its application in characterization of emulsions, nanoemulsions, and microemulsions.

# 3 Ultrasound

## 3.1 Background

Sound is likely a psychophysical concept that refers to a physiological response to pressure oscillations. In contrast, the pressure oscillation is a purely physical concept and can be described by physical parameters such as amplitude and frequency. Moreover, the auditory response is frequency dependent with capability becoming insignificant outside a distinct frequency range. The typical frequency range that can be heard by human beings is about 20 Hz to 20 kHz (acoustic). Therefore, the region below 20 Hz (infrasonic), above 20 kHz (ultrasonic), and over 1 GHz (hypersonic) cannot elicit an auditory response (Mason, 1998; Martini, 2013).

The longitudinal pressure oscillations of sound invoke a wave propagation direction that is parallel with the oscillation direction, so that the medium is locally compressed and decompressed (expanded). This is true regardless of whether an auditory response is generated. In contrast, in a transversal wave, the direction of propagation is at right angles to that of the oscillation, so that the medium is exposed to shear stress. The transversal wave only appears in highly viscous and solid samples whereas low viscous liquids do not show rigidity, so that transversal waves cannot propagate (Martini, 2013). In this chapter, all our discussions are about longitudinal or compressional waves.

In terms of amplitude of the pressure oscillations, ultrasound can have various applications that can be generally divided into two different categories, namely high power and low power ultrasound. Generally speaking, the former is normally used for processing, surface cleaning, synthesis, destruction of rigid structures, nanoemulsification, and other potential applications that need high strain energy density input (Mason et al., 1996;

Mason, 1998; Martini, 2013; Ghasemi and Abbasi, 2014; Mirmajidi Hashtjin and Abbasi, 2015a,b). In contrast, low power ultrasound is a nondestructive method, which is usually used as a characterization or diagnostic technique. This ultrasonic technique has a wide range of applications in many fields ranging from physics, chemistry, biology, medicine, pharmaceuticals, food systems, as well as food emulsions. The capabilities and applications of this type of ultrasound are quite wide and complicated. For the purposes of this chapter, we focus our descriptions on velocimetry techniques.

The velocity of ultrasound in various media can be measured by various approaches, namely pulse (through transmission and pulse–echo) and continuous wave (quasi-standing and standing) techniques in narrow and broad frequency ranges (McClements, 1998; Hosoda et al., 2005; Kaatze et al., 2008; Povey, 2013). Each technique has its own advantages and disadvantages in comparison to the others. But, the continuous standing wave or URT is of special interest in this chapter due to its high accuracy and precision, its high sensitivity to minute changes in the composition and structure of solutions and dispersions, and its ability to provide results using low sample volumes (Zhang et al., 2015). Therefore, in the following subsections, the general structure of a URT device, and the principles of velocimetry using URT are discussed. To allow ultrasound velocity and attenuation to be related to thermodynamic properties, the importance of densitometry are also discussed. Finally, the applications of URT for characterization of emulsions, nanoemulsions, and microemulsions are introduced. It is worth noting that the following subsections neither provide a theoretically complete introduction to ultrasonic velocimetry nor is it meant to be comprehensive. Rather, it is meant to give the reader a general understanding about applying ultrasonic velocimetry to aqueous solutions such as emulsions, nanoemulsions and microemulsions, the subject of this chapter.

## 3.2 Structure of URT Apparatus

Dr Theodor Funck is one of the pioneer scientists who developed the URT in the 1970s. But, it was the early 21st century when commercial URT instruments were introduced. To the best of our knowledge, nowadays there are three major manufacturers in the field of lab scale URT instruments who are actively manufacturing these devices with different brands and capabilities. TF Instruments Inc. (Heidelberg, Germany) produces its devices under the brand of ResoScan®, functioning within a

narrow frequency range (7–9 MHz). Ultrasonic Scientific (Dublin, Ireland) offers its devices as High-Resolution Ultrasonic Spectroscopy (HR–US®), which are capable of working over a wider range of frequencies (2–20 MHz), as well as having software for particle sizing. Both instruments are capable of acquiring measurements within a temperature scan over the ranges 5–85 and –20–120°C, respectively. A combined density and ultrasonic velocity instrument is also available from Anton Paar (), but with lower sound velocity accuracy.

Generally speaking, a URT system consists of three major modules or compartments: the resonator, the temperature control unit, and PC based–software (Fig. 13.3a). The resonator is the most important part of the device, which normally consists of two

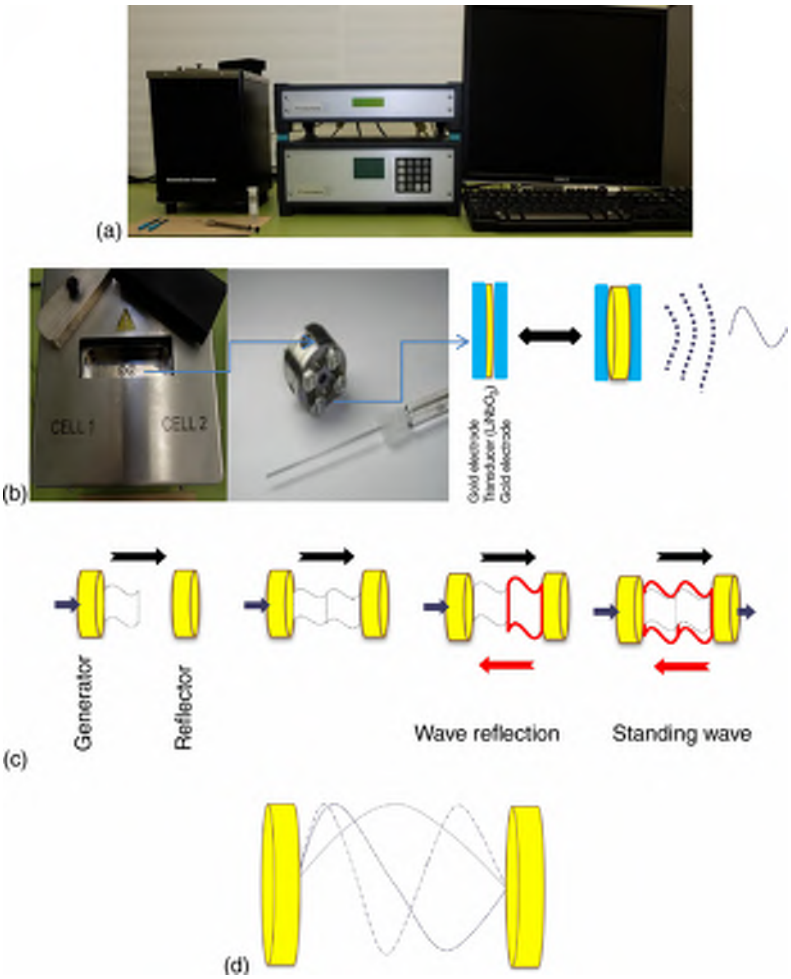


**Figure 13.3. Representation of (a) three major compartments of a lab scale URT instrument, (b) upper view of resonator, inner view of cell, and schematic view of the piezoelectric transducer operation (left to right), (c) establishment of standing waves between transducers, and (d) standing wave pattern for $n = 1, 2,$ and 3.**

closed parallel identical cells (sample and reference) with a distinct path length (eg, 7.0 mm) and with a capacity of 200 μL where evaporation from the cells is avoided by using tight sealing lids (Fig. 13.3b). In the cells, samples can be thermally treated (heating or cooling) up to 1050 mK/min using Peltier elements. The absolute accuracy of the thermostat temperature in the ResoScan® is 1 mK. The resolution of the ultrasonic velocity and attenuation are 0.1 mm/s and 1–3%, respectively [adapted from TF Instruments GmbH software].

A number of techniques can be used to generate ultrasonic waves in resonator cells. The most common technique uses piezoelectric transducers because of their wide frequency range, inertness, thermal stability, and their electromechanical conversion efficiency. The lithium niobate (LiNbO3) is the preferred piezomaterial for generating longitudinal waves due to good energy coupling between the acoustic field and the electrical circuit near the transducer's fundamental frequency (Kino, 1987; Eggers and Kaatze, 1996). It is noteworthy that the power levels employed in URT are very low, over the range $10^{-4}$– $5 \times 10^{-3}$ W/cm$^2$, which does not disturb the native structure of the samples. This is why this system is categorized as a low power and nondestructive technique (Wang et al., 2005).

In a resonator compartment (Fig. 13.3b, c), two piezoelectric transducers are precisely aligned at opposite ends of the cell. Ultrasonic waves that are generated by the sender transducer are reflected by the receiver transducer to generate ultrasonic interference phenomena in the space between the two transducers. Because the reflector is at a fixed distance ($d$) from the generator, sound waves of certain wavelengths will be enhanced by reflection and interference within the fixed distance of the cell. These standing waves then arise at defined resonance frequencies. The lowest resonance frequency that corresponds to a half wavelength of sound is called the fundamental resonance frequency, $f_1$. As shown later, with knowledge of the resonance frequencies and the fixed distance between transducers, the ultrasonic velocity in the fluid can be ascertained (TF Instruments GmbH).

Due to the existence of two cells (reference and sample cells), URTs can operate in singular or differential manners. In the differential manner, calibration can be simply made against a known liquid, such as water, so that even very small changes in the velocity of the sample of interest can be determined. In addition, the system is capable of very high precision in comparison to pulse techniques; however, accuracy is highly dependent on the precision with which temperature is measured and controlled. Creating the identical temperature environment for reference and sample cells thus affords better precision for differential measurements.

In a practice, a sample and reference fluid (200 μL each) are injected (slowly and gently for wetting purposes and to avoid bubble entrainment) into the sample and reference cells using a syringe or micropipette, measurements are carried out, so that the samples evacuated and cells are washed and rinsed with deionized water. A reference medium (plain deionized water or containing very small amounts of surfactant for wetting purposes) is usually injected in the reference cell to compare its ultrasonic measurements with the sample. All processes (except sample loading and unloading in older versions) are under computer control and all calculations are performed by professional software.

With reference to the aforementioned explanations, URT is a new method based on the high-resolution measurement of ultrasonic velocity and attenuation. The principles of the URT rely on sound waves that potentially compress and expand the sample at a molecular level. Therefore, this method is capable of determining how solutes behave in dispersion or solution, the nanostructure of dispersions, and how the dispersion changes with time or temperature. There is no need for sample dilution so that opaque or translucent samples, regardless of viscosity and concentration, can be analyzed. The defining characteristics of the dispersion are achieved by detecting changes in the speed of sound (ultrasonic velocity, $u$) and attenuation (ultrasonic attenuation, $A$) (Eggers and Funck, 1975; Wang et al., 2005; Buckin and Kumar Hallone, 2012). Therefore, in the following subsection the principles of their measurements are discussed in detail.

## 3.3 Principles of Velocity and Attenuation Measurements Using URT

In order to conduct an ultrasonic velocimetry assessment, it is necessary to have a good understanding about how the sound velocity and attenuation are measured, and how we can use these recorded ultrasonic data for interpretation at the nanolevel.

### *3.3.1 Ultrasonic Velocity*

Because the URT system measures ultrasonic velocity based on the resonance technique, the principles can be illustrated for a measuring cell whose path length ($d$) is 7 mm. In this case, an initial ultrasonic wave with a wavelength twice this distance will be reflected back to the generating transducer just as the next wave is being generated. This in-phase reflected signal strengthens the generated signal so that starting waves of resonance occurs in the cell for this particular solution. For water at 20°C, the velocity of sound is 1481 m/s, so this fundamental resonance occurs at a frequency of 105.790 kHz ($d = \lambda/2$, $u = f\lambda$).

The same in-phase augmentation of the signal will occur for waves whose wavelength is exact divisibles of 7 mm ($d = \lambda_1/2$). Thus, the second resonance frequency ($n = 2$) occurs at 211.571 kHz, the third ($n = 3$) at 317.357 kHz and so on (Fig. 13.3d).

For a solution whose ultrasonic velocity is unknown, the scanning for frequency where resonance occurs by the instrument permits ultrasonic velocity to be very precisely determined. Thus, for the 70th and 71st peak orders, with resonance at frequencies of 7.4050 MHz and 7.5108 MHz, respectively, the velocity can be determined from (Eggers and Funk, 1973; Eggers, 1997; Coupland, 2004):

$$\begin{aligned} u &= 2d[f_n - f_{(n-1)}] \\ &= 2\times(7\times10^{-3}\,\text{m})[7.5108\times10^{6} - 7.4050\times10^{6}\,\text{Hz}] \\ &= 1481.2\,\text{m/s} \end{aligned} \tag{13.4}$$

In the existing commercial ultrasonic resonator systems, these theoretical resonance frequencies are different to the measured frequencies (Funck and DeMaeyer, 2001). The actual resonances are corrected by the existing software of the URT system and this is likely much easier for peak orders of 65–85. For this reason, the working frequency range of URT systems, particularly the Reso-Scan®, is 7–9 MHz.

The significance of precise measurement of ultrasonic velocity is that it permits determination of a fundamental thermodynamic parameter of the sample, the adiabatic compressibility. In a homogeneous fluid, there is a relationship between the ultrasonic velocity, $u$, and the adiabatic compressibility coefficient, $\beta_s$, given by the Newton–Laplace equation (Wood, 1964):

$$u = \frac{1}{\sqrt{\rho\beta_s}} \quad or\ u^2 = \frac{1}{\rho\beta_s} \tag{13.5}$$

where $\rho$ is the fluid's density.

In mixtures (Urick, 1947), such as in microemulsions, the density and the compressibility of the mixture depend on the amount of each substance in the mixture. Thus, $\rho_{12} = \rho_1\varphi + \rho_2(1-\varphi)$ and $\beta_{12} = \beta_1\varphi + \beta_2(1-\varphi)$, where $\rho_{12}$ and $\beta_{12}$ are the density and adiabatic compressibility coefficient of the mixture; $\rho_1$ and $\beta_1$ are the density and adiabatic compressibility coefficient of component 1; $\rho_2$ and $\beta_2$ are the density and adiabatic compressibility coefficient of component 2; $\varphi$ *and* $1-\varphi$ are the volume fractions of components 1 and 2. Hence, the velocity of sound in the mixture is:

$$u_{12} = \frac{1}{\sqrt{\rho_{12}\times\beta_{12}}} \tag{13.6}$$

In the special case where the densities of both components and the mixture are very similar (Shah et al., 2007), the velocity of sound in the mixture is related to the two compressibilities by:

$$u_{12} = \frac{1}{\sqrt{[\beta_1\varphi + \beta_2(1-\varphi)]\rho_2}} \tag{13.7}$$

These equations are also applicable to three component mixtures (Shah et al., 2007), like microemulsions the subject of this chapter, which normally consist of as a minimum three different components (oil, water, and surfactant), and typically contains fourth and fifth components as well. In such situations, for any quantitative evaluations, one may consider the hydrated mixed phase of surfactant:cosurfactant or other mixed possibilities as one component (Hickey et al., 2006, 2010).

In aqueous solutions, the compressibility coefficient ($\beta_2$) for bulk water may not be the sole parameter, because restructuring of water around the dispersion phase can lead to a certain volume fraction of water with a different compressibility coefficient. As a result, the ultrasonic velocity of a dispersion results from the dispersed phase's intrinsic properties, but also by its water of hydration and the nature of the interaction between the solute and the aqueous solvent (Sarvazyan et al., 1979; Sarvazyan, 1991; Chalikian, 2003).

As shown from the Newton–Laplace equation (Wood, 1964; Urick, 1947), the sample's density and its precise measurement are of high importance in the field of velocimetry as well as the interpretation of adiabatic compressibility coefficient variations in relation to molecular or nanostructural changes. Therefore, the following subsection briefly discusses the importance and measuring principles for sample density.

#### 3.3.1.1 Densitometry

Density is a physical property of matter; the mass per unit volume. The most common units for density are kg/m$^3$ or g/cm$^3$. The density of any material strongly depends on its intrinsic properties and the temperature. For almost all substances, water at 0–4°C being a notable exception, the density of the liquid phase decreases as temperature increases (Povey, 1997). It has already been discussed that ultrasound velocity is also very sensitive to temperature. Therefore, in order to acquire accurate and precise information regarding the adiabatic compressibility coefficient one should measure these two parameters under very precisely controlled temperature conditions. In other words, precise control of temperature during density and velocity measurements, as well as their exact similarity, is a must. That is why there have been

quite a number of attempts over the past decades to utilize various techniques for accurate measurement of density. Apart from classical techniques, one of the most frequently used methods is the vibrating tube densitometer.

Densitometry is a proficient tool for investigating the volumetric properties of solutions over a wide range of temperatures. It can also be operated (although less commonly) at different pressures. The density is derived from precise measurements of a fluid contained within a vibrating tube in a very precisely regulated temperature environment. The density of the sample, $\rho$, is obtained by comparing the vibration period ($\tau$) of the tube when it is filled with the sample relative to the vibration period ($\tau_0$) of the reference fluid of known density ($\rho_0$), which is usually water:

$$\Delta\rho = \rho - \rho_0 = K(\tau^2 - \tau_0^2) \tag{13.8}$$

where $K$ is calibration constant obtained from an experiment with two fluids of well-known densities (Hynek et al., 1997; Zhang et al., 2015). The most important advantages of this technique are rapid temperature equilibration, highly accurate temperature control (±0.001°C or 1 mK), and a high resolution ($\pm 1 \times 10^{-6}$ g/cm$^3$) (Zhang et al., 2015). Readers can refer to the brochures of major manufacturers, such as Anton Paar, to find detailed information about this technique.

### 3.3.2 Attenuation and Attenuation Coefficient

Another parameter that can be measured by the URT system is the attenuation. Attenuation is the gradual loss in intensity (amplitude) of a sound wave as it travels through a sample. This loss can arise from absorption or from scattering. The sound energy is converted to heat due to viscous losses, thermal conduction, and molecular relaxations. Scattering is the redirection of the sound in directions different than its original direction and so it is not detected at the receiving transducer. Scattering is particularly evident in heterogeneous systems where a discontinuity interacts with the acoustic field to scatter the sound. The amplitude change of a plane wave that is attenuated can be expressed as:

$$A = A_0 e^{-\alpha d} \tag{13.9}$$

where $A_0$ is the initial amplitude of the propagating wave, $A$ is the amplitude after traveling a distance, $d$, and $\alpha$ is the attenuation coefficient (McClements, 1995; Povey, 1997, 2013).

The magnitude of attenuation (sound energy decay rate) in pure and homogeneous samples strongly depends the intrinsic

absorption of the components in the mixture but also on the ultrasonic wave frequency, particularly with respect to the size of dispersion droplet sizes. For an ideal dispersion, the intrinsic absorption depends on attenuation in the continuous phase and the dispersed phase (solute) and the volume fraction of each phase (Hickey et al., 2010). However, any discontinuities in the elastic and density properties of the solution, for example, droplets in the microemulsions, can give rise to inhomogeneities in the medium, which cause scattering that affect the attenuation. Therefore, the ultrasonic attenuation measurements arising from the scattering of ultrasonic waves within the sample can point to structural changes in the solution (McClements, 1995; Wang et al., 2005).

How the scattering from droplets affects ultrasonic velocity and attenuation depends strongly on the particle size of the droplets and their concentration and the wavelength of the sound. In the long wavelength limit ($\lambda \gg$ particle diameter), expressions are available to define the ultrasonic scattering in dispersions of spherical particles. In the long wavelength limit, thermo-elastic (heat wave) and visco-inertial (shear wave) scattering are two primary considerations, although radiative scattering may also be a concern (Povey, 1997; Pinfield et al., 2011). In emulsions the density difference between the continuous and the dispersed phase is not large, so the thermo-elastic mechanism dominates (Povey, 1997). Because of this reduced analytical complexity, ultrasonic scattering theories permit the sizes of dispersed droplets to be determined from measurements of attenuation, provided that the physical properties of the continuous medium and the droplets are known (Hickey et al., 2006, 2010; Buckin and Kumar Hallone, 2012).

Smyth et al. (2004) showed how the URT would utilize measurements in the 2–20 MHz frequency range to calculate the size of particles in an undiluted water-in-oil emulsion and how the particle size changed during dilution. This analysis also showed that existing techniques, which require dilution of the concentrated emulsion, would not correctly measure the size of droplets. Hickey et al. (2006) also utilized this technique to measure droplet sizes of microemulsion systems and its comparison with a light scattering technique confirmed its accuracy.

Phase transition is another parameter that can change the ultrasound velocity and attenuation (McClements et al., 1993; Povey, 1997). Therefore, any phase transition is a potential cause of attenuation change and this permits ultrasound to be a proficient tool in the study of phase transitions, which is directly relevant to microemulsions that attain various structures (Fig. 13.2)

under different formulation conditions (refer to earlier subsection in this chapter). It needs to be noted that as the ultrasonic attenuation is influenced by many factors, a lot of physicochemical parameters are required for a full interpretation of the ultrasonic attenuation. On the other hand, for complex systems these parameters are often unknown. Therefore, the interpretation for a complex system is not as simple as it is for pure and well defined systems.

## 3.4 Correlation of Ultrasonic Parameters (*u* and *A*) with Compressibility and Volume Functions

The relationship of ultrasonic velocity and ultrasonic attenuation, the ultrasonic parameters measured by URT, to thermodynamic parameters (compressibility and volume functions) are discussed in this subsection. As mentioned earlier, with knowledge of the density of a solution, the compressibility can be determined from the ultrasonic velocity. It is noteworthy that there are two different types of compressibility, namely isothermal (constant temperature) and isentropic or adiabatic (constant entropy). As the propagation of ultrasound in a liquid can change its temperature, due to repeated compression and decompression cycles, therefore the compressibility determined by the ultrasonic method is adiabatic (Sarvazyan, 1991; Pffefier and Heremans, 2005). Thus, the thermodynamic relation covering the extent of the volume change in the solution in response to the pressure waves of the transducer is given by:

$$\beta_s = -\frac{1}{V} \times \left( \frac{\delta V}{\delta P} \right)_s \tag{13.10}$$

where $V$ is volume, $P$ is pressure, $\beta_s$ is the adiabatic compressibility coefficient, subscript "$s$" represents adiabatic (constant entropy) conditions, and the negative sign indicates a volume decrease. Because the right-hand side of this equation $\left( k_s = -\left( \frac{\delta V}{\delta P} \right)_s \right)$ is a second derivative of the molar Gibbs free energy, its values are mainly sensitive to any compositional change of the solution (Sarvazyan et al., 1979; Sarvazyan, 1991).

It has already been stated that ultrasonic velocity, or more precisely compressibility, is affected by intrinsic properties, hydration, and solute–solvent interaction (see earlier). Of these, hydration has great importance in aqueous solutions such as microemulsions, the subject of this chapter. In this regard, compressibility of water can vary substantially: the compressibility of bulk water at 25°C is $45 \times 10^{-11}$ $Pa^{-1}$, while the compressibility of water

in a hydration shell is $18 \times 10^{-11}$ $Pa^{-1}$ (Gavish et al., 1983; Eden et al., 1982; Gekko and Nugguchi, 1979). Therefore, hydration has an adverse contribution to the compressibility and any increase in hydration in the system can potentially increase the sound velocity (Nölting et al., 1993; Gekko 2002; Fuchs et al., 2010).

Another important parameter is temperature. It has been reported that in water the ultrasonic velocity varies by approximately 3 m/sK (Povey, 1997). Moreover, water usually shows an irregular adiabatic and isothermal compressibility coefficient in comparison to other liquids at higher temperatures (below and above 70°C). This anomaly is attributed to variation in the structural and dynamic properties of the water's hydrogen bonding network when its temperature changes (Chalikian et al., 1994; Perven, 2012).

The ratio of solutes in a mixture such as microemulsions or dispersions is another parameter that contributes to compressibility to alter the adiabatic compressibility, as well as the density (see Eq. (13.4) and following descriptions). However, how the solute molecules affect their interactions with the aqueous phase can complicate additive approaches to determining solute ratio information from URT measurements.

Apart from the compressibility parameter, one can calculate a number of other parameters from density and ultrasonic velocity measurements. Examples are partial specific compressibility, apparent specific volume, partial specific volume, excess volume, and others. These parameters can reveal compositional changes, structure modifications, interaction between components, phase transitions, and nanoscale characterizations, and how they change over time or with temperature changes. This is not only useful for defining intramolecular forces and elastic properties in dispersions, emulsions, and microemulsions for fundamental studies, but also for numerous technical applications (Urick 1947; Sarvazyan 1991; Povey 1997; Wang et al., 2005; Negredo et al., 2007; Kaatze et al., 2008; Born et al., 2010; Zhang and Scanlon, 2011; Perven, 2012; Maya Desdier, 2012; Zhang et al., 2015).

## 4 Characterization of Emulsions, Nanoemulsions, and Microemulsions Using URT

URT has already shown significant potential in analysis and characterization of emulsions, nanoemulsions, and microemulsions (Buckin and Kumar Hallone, 2012). Nevertheless, its application was often restricted mostly due to low resolution, requiring large volume of samples and limited range of measuring systems.

Hence, in the early 21st century the URT apparatus was introduced and these limitations were overcame and nowadays, it has found innumerable applications in various areas. Here, we briefly demonstrate the applications of different URT systems categorized as narrow band frequency (7–9 MHz), broad band frequency (2–20 MHz), and combined systems (density and sound velocity meter) for characterization of emulsions, nanoemulsions, and microemulsions in chronological order, respectively. Finally, we briefly discuss our work on the nanostructural characterization of food-grade nano- and microemulsion systems using URT approaches.

## 4.1 Narrow Band Frequency

In one of the first reports, Shah et al. (2007) aimed to characterize nanoemulsions using ultrasonic properties. For preparation of nanoemulsions, cyclosporine A, sweet orange oil (oil phase), emulphor EL 620 and capmul (surfactant:cosurfactant) were mixed in various ratios. The mixtures were mixed and heated to ensure good dispersion. Then, the mixture (surfactant:cosurfactant:oil phase) was carefully weighed and diluted with water by vortexing. The velocity and attenuation of samples were measured at 25°C using a URT. The authors did not find any reasonable fit of the data with Eq. (13.5) (Urick equation). However, they declared when the extended Urick equation (Eq. (13.7)), as described earlier, was used for the determination of the adiabatic compressibility, a reasonable interpretation of the experimental data was likely. Based on the compressibility comparisons, they recognized the nanoemulsion droplets as oil-in-water (o/w) type of emulsions. Sound velocity was also reduced as a function of oil content in the formulation, which confirmed the higher compressibility of the oil phase. Moreover, as surfactant:cosurfactant ratio increased, the droplet diameter and ultrasonic velocity considerably increased and decreased, respectively. They concluded that attenuation data modification using scattering theory, and its combination with accurate ultrasonic velocity data, could be promising for determination of droplet sizes by URT measurements even at a single frequency.

The phase transitions between different phases of monoglyceride emulsifier systems and pearlescence (a desired luster resembling that of mother-of-pearl) in cosmetic creams were investigated using URT with variation of temperature by Alberola et al. (2007). They reported that the slope of the ultrasound velocity curve versus temperature showed direct correlation with the bound water content in the different phases. They also stated that these insights made it possible to study the pearlescent effect of

the coagel (phase of the cream). In addition, the URT was capable of determining the short time reversibility of the pearlescence as well as how long it takes the coagel to form upon cooling. They concluded that these data (time and temperature of phase transition) were very useful for optimization of the formulation of the pearlescent creams. In other words, they used the time and temperature sweep capability of the URT and the sensitivity and dependency of ultrasound velocity to temperature, for elucidation of desirable structural transitions in the cosmetic creams.

Kudla et al. (2010) also used the URT to analyze the effect of fatty alcohols:surfactant ratio (9.5:0 to 0:9.5) in the presence of constant amount of water and additives (89.3 and 1.2% w/w) on the phase behavior and microstructure of a mixture of liquid–crystalline emulsion systems during the ripening process. URT measurements revealed that structure formation of the system took up to at least 2 weeks after production. They explained this behavior by a slow water bonding process in the lamellae (a thin plate-like structure or system, which can be formed when fatty alcohols, cationic surfactants, and water are mixed), which was verified by an exponential growth in the ultrasonic velocity. They observed that the ripened system does not form to a reasonable extent before a certain rest time, that is, 3 days, after production, based on their URT results.

In another study (Preetz et al., 2010), a nanoemulsion (medium-chain triglycerides 5% v/v and negatively charged emulsifier, OSA starch 4.75% w/w) was prepared by a high energy emulsification method. Afterward, capsules were obtained by injecting a negatively charged polyelectrolyte (chitosan or carrageenan) to create layers around the droplets. Then, URT measurements were performed to investigate changes in ultrasound parameters by the polyelectrolyte nanocapsule dispersion as capsule wall composition was varied. Comparison of the absolute ultrasonic velocity values showed that ultrasound propagation velocity increased with increasing number of layers around the oil droplets. It was concluded that the mechanical properties of the shell had a great influence on sound propagation. By transformation of emulsion droplets into polyelectrolyte nanocapsules with a shell composed of three or five layers, a higher wall stiffness with increasing number of shell layers led to the velocity changes. They explained that the increase in wall stiffness was evidence for the solidification of the deposited oppositely charged polyelectrolytes. They concluded that despite the sensitivity of URT to salt molecules, it showed a very fast capacity for differentiation of nanoemulsions from nanocapsules.

In a most recent report (Niederquell and Kuentz, 2013), there was an attempt to introduce stability categories for

nanodispersions by investigating the physical stability of aqueous dispersions of 20 different pharmaceutical nanoemulsifying formulations. All the formulations (oil components, surfactant, cosolvent) were stirred to produce homogenous single phase mixtures. In a following step, the preconcentrates were diluted to 1:10 and 1:100 (v/v). Then, thermo-reversibility (heating–cooling cycles) of the emulsions and nanoemulsions was studied using a URT. Changes in the ultrasonic attenuation were considered as an indicator of microstructural changes in the emulsions, and the attenuation assessments of instability were correlated to changes in light scattering. Because dispersions with droplet sizes between 15–100 nm are difficult to categorize, the URT was a useful tool for identifying changes in rather unstable nanoemulsions.

Stillhart et al. (2013) also attempted to understand how drug supersaturation and precipitation can result from aqueous dilution of Pouton type IV systems. They intended to achieve a better understanding of the dispersion process to provide a basis for the selection of dilution levels in early formulation screening by use of the URT in conjunction with other techniques. They used two different surfactant:cosolvent model systems at various aqueous dilution ratios that were practically relevant. One of the Pouton type IV formulations contained Cremophor® RH 40 and ethanol (1:1 w/w, cremophor system), and the other consisted of polysorbate 80 and ethanol (1:1 wt, polysorbate system). They were mixed on a magnetic stirrer to attain a homogeneous and clear solution then the model drug was added. Then, the Cremophor and polysorbate were dispersed in demineralized water (1:1 to 1:100). The ultrasonic velocity of all dispersions was measured in comparison to water using a URT. The change in the ultrasonic velocity difference was similar for the two systems and $\Delta u$ (velocity difference of sample and reference cells) peaked at a dilution factor of 1:2 before decreasing at larger dilution ratios. Moreover, when they plotted the ultrasonic velocity difference as a function of the formulation concentration, these dilute samples essentially exhibited a linear response. The largest concentration (undiluted, 1:1) did not conform to linearity, and it was for this formulation that a lamellar liquid crystalline (LLC) structure formed. In contrast, the density of the diluted systems was essentially linear for the whole series of dilution levels including 1:1. Based on the Newton–Laplace equation, a change in ultrasound velocity is attributed to a difference in density or compressibility of these systems. Therefore, they concluded that in the most concentrated systems the microstructure was different than those present at low dilution levels, based on the lower compressibility of the concentrated system, likely due to coherent liquid–crystalline structure. The decrease in $\Delta u$ upon

dilution indicated the system was more compressible, a result that is not inconsistent with the formation of isolated micelles in water. Therefore, URT analysis was capable of revealing a transition of the formulation structure as a function of dilution level.

## 4.2 Broadband Frequency

Smyth et al. (2004) demonstrated the application of URT to evaluate the thermal stability of two different emulsion systems (consecutive heating 0–50°C and cooling 50–0°C cycles). They only observed reversible ultrasonic parameter changes in one of the emulsions during the consecutive heating and cooling cycles. The first emulsion profile indicated that its structure was changed with temperature, but all the changes were recoverable, and the structure was not affected by the thermal history. In contrast, a sharp drop in ultrasonic velocity and attenuation in the second emulsion was observed at the first heating cycle, which did not recover during subsequent cycles. They pointed out that the decrease in ultrasonic parameters can be explained by the temperature-induced coagulation of the oil particles and the aggregation of the original particles into larger particles following the phase separation between oil and water phases. They concluded that high-resolution ultrasonic spectroscopy could provide new tools for analysis of the chemical and microstructural characteristics of emulsions and suspensions.

Smyth et al. (2005) were also the first research group to report the application of a URT over a wide frequency range (2–20 MHz) for characterization of microemulsions. In a commercial report, a microemulsion system consisting of isopropyl myristate (oil phase), lecithin (surfactant), *n*-propanol (cosurfactant), and water was studied. Based on ultrasonic measurements they stated that dissolution of water in the diluted mixtures (up to 2% w/w), which was accompanied by hydration of lipid and cosurfactant, led to a fixed ultrasonic velocity increase. Microstructural reorganization was also observed as shown by a further increase in ultrasonic velocity over the range 2–6% w/w. Formation of the microemulsion was seen ≥8% w/w, where ultrasonic attenuation rapidly increased, owing to the scattering of the ultrasonic wave from the particles. These findings showed the potential of the technique to provide information about the structure and phase transition during dilution.

Neither of the above two reports were scientific research papers. Therefore, the first comprehensive research in the field of microemulsions was published in 2006 by Hickey and coworkers. In this report, they attempted to take advantage of a broad range

URT (2–20 MHz) to construct a pseudoternary phase diagram for mixtures consisting of isopropyl myristate:lecithin:*n*-propanol. Changes in the ultrasonic velocity and attenuation were observed when the mixtures were diluted with water. The ultrasonic velocity and dilution profiles did not show a significant dependence on frequency. Moreover, ultrasonic velocity (at a constant frequency) and attenuation changes were related to changes in the microstructure. For instance, the ultrasonic velocity curve versus water volume fraction exhibited a number of abrupt changes, which enabled the characterization of various phases (hydration of surfactant, microaggregation, microemulsion, coarse emulsion, and pseudo-bicontinuous). They also argued why the addition of water, at first, led to a decrease in velocity in the mixture. They attributed this behavior to water's high specific heat capacity so that at low concentrations a large apparent adiabatic compressibility and therefore low ultrasonic velocity was feasible. The state of water in the mixtures was assessed by the partial concentration increment of the ultrasonic velocity. It is noteworthy that their microemulsions seemed to be multiphase in the presence of higher water contents which make any characterization potentially very difficult. This is likely why they did not identify the type of structures for their systems beyond the bicontinuous phase.

In their next study on microemulsion characterization, Hickey et al. (2010) analyzed a pseudo-ternary phase diagram for mixtures consisting of ethyl oleate (oil phase)/Tween-80: Span 20 (3:2), without using any cosurfactant. They studied three different ratios of oil:surfactants (75:25, 50:50, 25:75% w/w) or three dilution lines. The ultrasonic velocity and attenuation changes upon diluting oil/surfactants mixtures with water (up to 60% w/w) indicated that a number of well-defined transitions (surfactant hydration, surfactant nanoaggregation, liquid crystal formation and pseudo-bicontinuous stage) took place, and this allowed construction of a phase diagram for the system. Finally, they compared the state of water in this study with their earlier work (Hickey et al., 2006) where they revealed three different states of water in the microemulsion corresponding to hydration water (stage I), water in swollen reverse micelles (stage II), and bulk water in aqueous droplets surrounded by surfactant (stage III). In this study, however, only two microemulsion stages (I and II) were detected. They concluded that the end of stage II approximately corresponded to minimum hydration of surfactant and cosurfactant in both systems. They proved that the URT is a useful technique for characterization of microemulsion systems.

Buckin and Kumar Hallone (2012) in a book chapter in the field of microemulsions stated that previous applications of URT

for analysis of microemulsions (Hickey et al., 2006, 2010) demonstrated the exceptional sensitivity of ultrasound to the structure of microemulsions and to the state of their components. Nevertheless, more research was necessary to clearly link ultrasonic data to the microscopic structure and the molecular characteristics of dispersions. Therefore, they focused on interpretation of the compressibility of microemulsions obtained from ultrasonic measurements. They also discussed fundamental relationships between compressibility and structural and physical characteristics of microemulsions. As with other contributions from this group, the assessment of the state of water in w/o microemulsion droplets was demonstrated from previous research (Hickey et al., 2006, 2010). Their calculations on previously reported microemulsion systems demonstrated that the ultrasonic technique allows efficient mapping of microemulsion phase diagrams where water occurs in different states, including water in nanodroplets.

## 4.3 Combined System

New generations of combined ultrasonic velocimetry and densitometry instruments are capable of measuring the velocity and density of one sample in a single run. The main disadvantage of this system is its low resolution on sound velocity (0.5 m/s) in comparison to the aforementioned URTs that are capable of 0.001 m/s. Despite its low resolution, it has found a role in research laboratories because of its many other advantages. For instance, Fanun et al. (2012) studied *n*-propanol:sucrose laurate:allylbenzene micellar systems, which were diluted at various ratios (33:67:0 to 3:7:90) with water (up to 100% w/w). They measured ultrasonic velocity and density of the different microemulsion samples with a combined ultrasonic velocimetry and densitometry instrument. The microemulsions were characterized using density, excess volume, ultrasonic velocity, and adiabatic compressibility. Based on their findings, the densities increased with increase in the water volume fraction. In contrast, excess volumes decreased for water volume fractions $< 0.2$, leveled off between 0.2 and 0.6 and increased $> 0.6$. Adiabatic compressibility also increased with temperature for water volume fractions $< 0.8$ and decreased above 0.8. On the basis of these data they elucidated nanostructural transitions from w/o to bicontinuous to o/w systems along the dilution lines.

## 4.4 Experiments on Food-Grade Microemulsions

As discussed earlier, there are only a limited number of investigations regarding the application of URT for the characterization

of microemulsion systems. All of these were on pharmaceutical formulations and their dilution levels were either less than 60% w/w or they were not stable as a single-phase microemulsion system at higher levels, and so could not be studied for structural purposes. Therefore, in a very recent attempt we aimed to characterize the structural changes and phase transitions in two different food-grade microemulsions (four and five component) systems over a wide range of dilution (0 to 100% w/w). Briefly, in the four component microemulsion system, T80, as surfactant, is mixed with 2-propanol, as cosurfactant (surfactant:cosurfactant ratio of 1:1) at ambient temperature. Then, this mixture is blended with orange peel oil (OPO), as the oil phase (10:0 …0:10), and diluted stepwise with water (0 to 100% w/w). The final mixtures were gently shaken for 5 min and kept for 24 h at room temperature to ensure equilibrium. It was ascertained that microemulsions were one phase based on their visual transparency and physical stability (Amiri et al., 2013).

For preparation of the five-component microemulsions, the one phase channel (de Campo et al., 2004) was chosen (OPO instead of pure limonene) for which the mixture of OPO:ethanol (1:3) was mixed with T80 (56:44) and final mixtures were diluted using water:glycerol (3:1).

Nanostructural investigations were conducted on both systems using densitometry, ResoScan (7–9 MHz), and viscosity measurements. In order to elucidate the impact of the presence or absence of the oil phase (OPO), identical blanks for dilution lines without OPO were also prepared.

Based on ultrasonic velocity, attenuation, and density measurements as well as the adiabatic compressibility coefficient, and the excess volume along dilution line (10% w/w water addition intervals), significant changes were observed in the excess volume and attenuation at distinct concentrations of water (Fig. 13.4a). The excess volume is calculated by subtracting the measured specific volume of the microemulsion from the sum of the specific volume of each component times its corresponding volume fraction. Changes by rheological measurements (viscosity changes) in excess volume mirrored those assessed.

The abrupt points on Fig. 13.4a from left to right (lower to higher water volume fractions) were correlated to surfactant hydration, surfactants nanoaggregation, and transformations between w/o, BC, and o/w regions. It seems that attenuation was even more sensitive to nanostructural changes and transitions than excess volume. Owing to the nanosize of these systems, except BC, it can be concluded that URT was quite capable of elucidating nanostructural changes in food-grade or any other microemulsion systems over

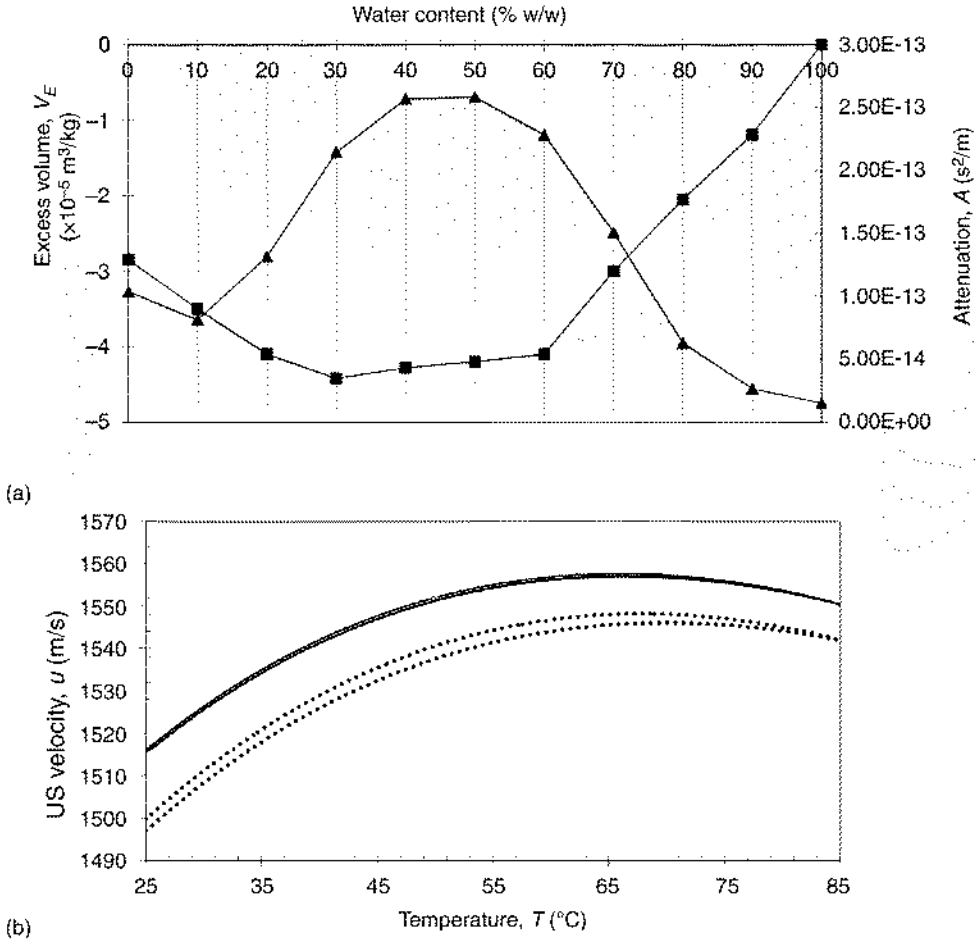


**Figure 13.4. Illustration of (a) the effect of dilution (water content) on ultrasound attenuation (▲) and excess volume (■) of OPO microemulsions [containing T80:2-propanol (1:1) (90 %w/w):OPO (10 %w/w)] across dilution line at 25°C, and (b) comparison of heating and cooling processes on the ultrasonic velocity of microemulsion [T80 (2%w/w):2-propanol (2%w/w):OPO (1%w/w): water (95%w/w) *solid line*] and nanoemulsion [T80 (2%w/w): OPO (1%w/w): water (97%w/w) *dotted line*]. Upper and lower lines represent cooling and heating cycles, respectively.**

a wide range of concentrations. The URT was also capable of distinguishing the stability of an OPO nanoemulsion [water:T80:OPO (97:2:1) prepared by high energy method (Mirmajidi Hashtjin and Abbasi, 2015a)] and a microemulsion [water:T80:2-propanol:OPO (95:2:2:1) prepared by low energy method (Amiri et al., 2013)] over temperature sweep tests (5 to 85 and 85 to 5) following several heating and cooling cycles (Fig. 13.4b). The only difference between these two samples in terms of formulation is the presence

of 2-propanol in the microemulsion (and instead slightly more water in the nanoemulsion); they contained similar amounts and ratios of surfactant and oil (2:1).

As can be seen (Fig. 13.4b), during the heating cycle the ultrasonic velocity gradually increased between 25 to 65°C but then slightly declined. This behavior is quite similar to pure water, which was expected as they both contained over 95% of water. However, their cooling curves were interestingly at higher velocities than their heating curve and a hysteresis loop was observed whose size was significantly larger in the nanoemulsion. This difference can be attributed to the stability mechanisms of nano and microemulsions as the former one is kinetically stable whereas the latter one's stability is thermodynamic. Furthermore, when the heating and cooling processes were repeated consecutively it was seen that the hysteresis loop of the microemulsion disappeared whereas the loop for the nanoemulsion persisted and was still visible after 3 cycles. Therefore, it shows that the URT was capable of differentiating nano- and microemulsions based on their thermal stability. Full information on this subsection can be found elsewhere (Abbasi and Scanlon, 2016).

## 5 Conclusions

Microemulsions are very complicated mixtures of various components that are formed, mostly using a low energy method, in the presence of appropriate types and very precise ratios of incorporating components, as thermodynamically stable systems. Apart from challenges in their formulation and manufacturing, due to their applications in very diverse fields as well as the strong dependency of their nanostructure and functionality, it is very important to understand and characterize their structure under lightly and highly diluted forms. For this reason, analyses of nanostructure, phase transitions, and droplet size in nanoemulsions and microemulsions have been an attractive and challenging field of study for a number of scientists worldwide. Furthermore, the majority of the existing analytical techniques either oblige dilution or manipulation of the microemulsion. These steps are time consuming or expensive and interfere with accurate detection of nanostructural transitions and droplet sizing. Because dilution of any microemulsion system regularly leads to the complete devastation of the system, it is crucial that microemulsions to be measured as they are. Another limitation is the effect of the measuring technique itself on the structure of microemulsions. Therefore, in this chapter the capability of ultrasonic resonator technology (URT) was discussed and examined to address these issues. The URT is a very precise ultrasonic velocity

and attenuation measurement system, extremely sensitive to the nano- and molecular level organization of structure, requiring only small sample volumes (less than 1 mL), is nondestructive, and requires no markers, dilution, or preparation. It showed good capacity for the nanostructural characterization of pharmaceutical, industrial, and food-grade microemulsion systems. However, utilization of this technique and its information still requires further developments for considering of ultrasonic data and their relationship with the nanostructural characteristics of microemulsions.

## Acknowledgments

The authors gratefully acknowledge support from the Discovery grants program of NSERC (Canada). SA also would like to thank Tarbiat Modares University (TMU) for their financial support during his sabbatical leave.

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14

# APPLICATION OF SELF-EMULSIFYING DELIVERY SYSTEMS FOR EFFECTIVE DELIVERY OF NUTRACEUTICALS

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## 1 Introduction

In the current health scenario, when a paradigm shift is being observed from treatment to prevention, the preventive potential of nutraceuticals is the focus of attention of the health-care industry. To ensure compliance for their long-term use, designing their oral delivery systems becomes a prerequisite. Oral routes are noninvasive in nature and offer ease of administration leading to long-term patient compliance. Use of the oral route of administration, however, is limited by the problem of low bioavailability from the gastrointestinal (GI) tract and in vivo stability of many of the nutraceuticals, for example, vitamins, enzymes, certain antioxidants, and phytoconstituents like curcumin, lycopene. A number of novel formulation strategies are being developed for overcoming the problems related to oral absorption and bioavailability of these nutraceuticals. These novel formulations have been utilized not only for enhancing the solubility and bioavailability of these bioactives but have also proved effective in enhancing their physical, chemical, biological, and photo stability. These formulations offer an added advantage of controlling the release of nutraceuticals for a prolonged period of time and also of delivering them at the required site, thus preventing their wastage (Ajazuddin and Saraf, 2010). Many nutraceuticals and phytoconstituents like curcumin, flavonoids, and vitamins have been reported to show enhanced therapeutic effect at lower doses when incorporated into novel delivery systems vis-à-vis that from their conventionally

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00014-3

delivered counterparts (Gosangari and Dyakonov, 2013; Joshi et al., 2013; Wahlang et al., 2012).

A number of lipid-based novel delivery systems like liposomes, niosomes, tranferosomes, ethosomes, phytosomes, nano emulsions, solid lipid nanoparticles, self-emulsifying delivery systems have been developed in recent years, which provide enhanced solubility, bioavailability, and stability (Kalepu et al., 2013). Despite such a long list of novel formulations that can be administered through various routes and offer a number of advantages, oral delivery systems are still the most preferred ones because of their convenience. One such novel system that has been successfully explored for oral delivery is that of Self-emulsifying delivery systems (SEDS). SEDS were first reported by Pouton (1982). These delivery systems offer advantages like improved solubility, increased bioavailability, increased absorption via lymphatic system, and increased P-gp efflux. These are basically isotropic mixtures of bioactives, lipids, and emulsifiers, with or without cosolvents/coemulsifiers (Singh et al., 2009). SEDS have been broadly classified based on their dispersion with droplet size up to few microns. If the droplet size of dispersion is in range of 100–250 nm then the SEDS are termed as SMEDS, while those having droplet size below 100 nm are called SNEDS (Singh et al., 2009; Tarate et al., 2014). However, it is important to note that there is no consensus on the droplet size of SMEDS and SNEDS and they may vary according to literature (Singh et al., 2009; Tarate et al., 2014).

Based on the type of constituents, lipid formulations are categorized in four different types as described later:

## 1.1 Type I

These are basically nondispersing systems. They consist of oils like triglycerides or mixed glycerides and form coarse dispersion on dilution. As they are nondispersing themselves, their absorption takes place through digestion via gastric enzymes. These systems are suitable for molecules exhibiting higher solubility in oils so that their required dose can be incorporated in the optimum quantity of oil (Porter et al., 2008; Pouton and Porter, 2008; Singh et al., 2009; Tarate et al., 2014).

## 1.2 Type II

These are composed of single or mixed triglycerides along with lipophilic surfactants having HLB (hydrophilic lipophilic balance) values less than 12 (Pouton, 2000). Self-emulsification usually occurs when surfactant concentration is between 20 and 60%. On dispersion, large interfacial areas are generated, which lead to optimum partitioning of the biomolecules between both the phases (Hauss et al., 1998; Porter et al., 2008; Pouton and Porter, 2008).

### 1.3 Type III

These consist of Oils and hydrophilic surfactants with HLB values more than 12 (Sapra et al., 2012). These form SMEDS (Singh et al., 2009). Cosolvents may also be added to improve the formulation characteristics. Commonly used cosolvents include ethanol, propylene glycol, and polyethylene glycols. Type III emulsions may further be classified as type III A and type III B. Type IIIA consists of more amount of lipids (40–80%) while type IIIB contains less amount of lipids (up to 20%) and more surfactants. Type IIIB formulations are capable of achieving greater dispersion rates as compared to Type IIIA although in their case, the risk of drug precipitation on dispersion of the formulation is higher because of their lower lipid content (Kumar, 2013).

### 1.4 Type IV

These are emulsions without oils. They constitute water soluble and insoluble surfactants along with cosolvents. The resultant droplet size on dispersion is generally less than 50 nm. These are generally opalescent or transparent in nature (Singh et al., 2009).

## 2 Composition of SEDS

Selection of lipid, surfactant, and cosolvent is done based on solubility of drug in lipid, surfactant, and cosolvent (Kohli et al., 2010). A ternary phase diagram of the three components is plotted by keeping oil, surfactant, and cosurfactant on three different axes. Based on the clarity of dispersion, emulsification time, and droplet size, the entire plot is divided into four regions namely phase separation, SEDS, SMEDS, SNEDS, respectively. A ratio should be selected from a particular relevant region of this plot to achieve the desired type of formulation (Singh et al., 2009). For selection of oil, surfactants, and cosurfactants, solubility and affinity of drugs in each of these components, their compatibility with each other at concentration in which they are selected are the important prameters to be evaluated. However, the most important parameter is the self-emulsification ability of the resultant formulation (Rahman et al., 2013).

Various ingredients used are discussed in the next sections.

### 2.1 Oils/Lipids

Lipids form one of important components of SEDS, which not only provide solubilization of lipophilic drugs but also protect the drugs from chemical and enzymatic degradation. They also enhance the lymphatic delivery of drugs. Blood and lymph distribution of drug depends on the HLB value, chain length, saturation

degree as well as the amount of lipids used. Various natural oils comprising of mono-, di-, or triglycerides have been used in formulation of SEDS (Mandawgade et al., 2008). These triglycerides can be hydrogenated to decrease the unsaturation, which, in turn, increases their resistance against oxidation. Triglycerides have proved to be an ideal candidate for SEDS owing to their natural origin, use in day-to-day life in food, easy digestibility and absorption in the GI tract (GIT). Moreover, they are generally recognized as safe (GRAS). Modified long and medium chain triglycerides with varying degrees of saturation or hydrolysis as well as synthetic oils like different grades of Capmul, Captex, Labrafac, and Miglyol have been widely used for the design and optimization of SEDS (Dash et al., 2015).

Capmul is series of synthetic oils and emulsifiers prepared via glycerolysis of different oils. It consists of mono-, di-, and triglycerides. They can be used as emulsifiers, cosurfactant, emollient, oils, and also have antimicrobial properties. They provide stability to emulsion and also act as solubilizer and carrier in a emulsion. Monodiglyceride medium chain esters are particularly recommended for the dissolution of difficult compounds such as sterols (Rao et al., 2013; Li et al., 2015). Captex is a family of medium chain ester frequently used in preparation of SEDS (Patel et al., 2012). Labrafac is a series of oil substances like propylene glycol dicaprylate/dicaprate NF. Possessing an HLB value of 2, they are commonly used as oil in formulation of self-emulsifying drug delivery systems (Shah et al., 2013). Miglyols are neutral oils marketed by Cremer care. These are medium chain triglyceride commonly containing a mixure of decanoyl- and octanoyl glycerides (Rajot et al., 2003).

Oils used for formulation of SEDS are selected from the class of biodegradable oils. Selection of suitable oil can be done based on composition, potential utilities, physical state, and HLB of the resulting formulation (Porter et al., 2008; Pouton and Porter, 2008; Singh et al., 2009). There are reports, where some nonconventional oils like melon oil and omega-3 containing oils have been used as excipients as well as cotherapeutics (Abo Enin, 2015; Obitte et al., 2011). Thermo-softening excipients, which melt in the range of 26–70°C and exist as waxy semisolids at ambient room temperature, are typically filled into capsules in the molten state, with the excipient melting temperature limiting their use to hard gelatin capsules. Table 14.1 enlists different oils that have been used for preparation of SEDS.

## 2.2 Emulsifiers

Surfactants that are safe on oral administration are used as emulsifiers for the preparation of SEDS of nutraceuticals. These

# Table 14.1 Oils/Lipids Used for SEDS

| Lipid/Oil | Chemical/General Name | References |
|---|---|---|
| Bean phospholipids | — | Lv et al. (2012) |
| Capmul MCM EP | Glyceryl caprylate/caprate | Jain et al. (2014a,b) |
| Caprylic/capric triglyceride | Caprylic/capric triglyceride | Shao et al. (2013) |
| Capryol 90 | Propylene glycol monocaprylate (type II) NF | Inugala et al. (2015) |
| Captex 355 | Glyceryl tricaprylate/tricaprate | Inugala et al. (2015) |
| Capmum MCM C8 | Glyceryl monocaprylate | Inugala et al. (2015) |
| Castor oil | Castor oil | Tran et al. (2014) |
| Chuanxiong oil | | Cai et al. (2007, 2008) |
| Cinnamon oil | Cinnamon oil | Zhang et al. (2008a) |
| Cotton seed oil | Cotton seed oil | Kang et al. (2012) |
| Corn oil | Corn oil | Kang et al. (2012) |
| Cradamol GTCC | Caprylic/capric triglyceride | Yao et al. (2008) |
| CremophorEL castor oil | Macrogolglycerol ricinoleate Ph.Eur., polyoxyl 35 castor oil USP | Inugala et al. (2015) |
| Ethyl oleate | Ethyl oleate | Cui et al. (2005) |
| Gelcire 44/14 | Lauroyl macrogol-32 glycerides EP, lauroyl polyoxyl-32 glycerides NF | Mandawgade et al. (2008) |
| Isopropyl myristate | Myristic acid isopropyl ester | Wang et al. (2009) |
| Labrafac PG | Propylene glycol dicaprylocaprate EP, propylene glycol dicaprylate/dicaprate NF | Setthacheewakul et al. (2010) |
| Lauroglycol FCC | Propylene glycol mono laurate | Rao and Shao (2008) |
| Labrafac CC | Caprylic/capric triglyceride | Inugala et al. (2015), Kang et al. (2012) |
| Mineral oil | Higher alkanes from mineral source | Kang et al. (2012) |
| Maisine oil | Glyceryl monolinoleate | Parmar et al. (2011), Zhang et al. (2008a) |
| Miglyol 812 | Liquid lipids/C8/C10 triglycerides | Ma et al. (2012) |
| Myvacet 9–45 | Myvacet 9–45K NF | Kommuru et al. (2001) |
| Methyl decanoate | Decanoic acid methyl ester | Wang et al. (2009) |
| Methyl oleate | Oleic acid methyl ester | Wang et al. (2009) |
| Oleic acid | Octadececenoic acid | Qi et al. (2011), Rao and Shao (2008) |

(*Continued*)

## Table 14.1 Oils/Lipids Used for SEDS (*cont.*)

| Lipid/Oil | Chemical/General Name | References |
|---|---|---|
| Olive oil | Olive oil | Qi et al. (2011) |
| Peanut oil | Peanut oil | Kang et al. (2012) |
| Peceol | Glycerol monooleate | Rao and Shao (2008) |
| Phosal 53 MCT | Lecithin in caprylic/capric triglycerides, alcohol, glyceryl stearate, oleic acid, and ascorbylpalmitate | Shanmugam et al. (2011b) |
| Polyoxyethylene castor oil | Polyoxyethylene castor oil | Mekjaruskul et al. (2013) |
| Sesame oil | Sesame oil | Kang et al. (2012) |
| Sunflower oil | Sunflower oil | Kang et al. (2012) |
| Soybean oil | Soybean oil | Qi et al. (2011, 2014) |
| Trilaurin | Glycerol trilaurate | Elgart et al. (2013) |

surfactants are amphiphilic in nature and help in keeping both oil and water phase together in an emulsion. As safety is a major criterion during the selection of emulsifiers, compounds of natural origin are given preference especially in SEDS for nutraceuticals. However, low emulsification ability is one of the limitations of natural emulsifiers. Selection of emulsifiers for SEDS is mainly dependent on their HLB value. Emulsifiers with higher HLB value are best suited for SEDS because they would promote instant emulsification when they come in contact with aqueous medium in GIT.

The bioavailability of the active constituents in the SEDS is increased by surfactants by various mechanisms. One of the major reasons of increased bioavailability is an improvement in the dissolution of the drug (Constanitinides et al., 1994). Surfactants are able to increase the permeability of the drugs across the intestinal epithelial membrane as well as across the tight junctions (Koga et al., 2006). Decrease in the glycoprotein mediated efflux is another factor that can be attributed to the presence of surfactants in the formulation (Eaimtrakarn et al., 2002).

Nonionic surfactants with high HLB value like Tween-80 are considered suitable and are preferred over ionic surfactants. Surfactant concentration in SEDS is normally kept between 20 and 60%, as higher concentrations may lead to GI irritation. Moreover, the long-term stability of the formulations may be disturbed by the presence of their high concentration. Popular emulsifiers used for formulation of SEDS include polyethylene glycol (PEG), polyoxyethylene, ethoxyl esters, fatty acids, and lecithins

## Table 14.2 Surfactants Used for SEDS

| Surfactant | Chemical Name | References |
|---|---|---|
| Capmul | Mono-diglyceride of medium chain fatty acids | Basalious et al. (2010) |
| Cremophor RH40 | PEG-40 hydrogenated castor oil | Rao and Shao (2008) |
| Cremophor-EL | PEG-35 castor oil | Parmar et al. (2011) |
| Labrafil M 2125 CS | PEG-6 corn oil | Inugala et al. (2015), Kang et al. (2012) |
| Labrafil M1944CS | PEG-6 apricot kernel oil | Inugala et al. (2015), Kang et al. (2012) |
| Labrasol | Caprylocapryl macrogol glycerides | Inugala et al. (2015), Parmar et al. (2011), Rao and Shao (2008) |
| Polysorbate 80 | Polyoxy ethylene 20 sorbitan mono oleate | Rao and Shao (2008) |
| Polysorbate 20 | Polyoxy ethylene 20 sorbitan mono laurate | Rao and Shao (2008) |
| Polyoxamer 407 | Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) | Date and Nagarsenker (2007) |
| Polyoxamer 188 | Pluronic F-68 solution | Date and Nagarsenker (2007) |
| Solutol HS 15 | Macrogol (15)-hydroxystearate | Date and Nagarsenker (2007) |
| Span 20 | Sorbitan monolaurate | Kang et al. (2012) |
| Span 80 | Sorbitan monooleate | Kang et al. (2012), Qi et al. (2011) |
| Span 85 | Sorbitan trioleate | Qi et al. (2011) |
| Tween-20 | PEG-20 sorbitan monolaurate | Date and Nagarsenker (2007) |
| Tween-80 | PEG-20 sorbitan monooleate | Date and Nagarsenker (2007), Qi et al. (2011) |
| Tween-85 | PEG-20 sorbitan trioleate | Singh et al. (2009) |

(Balakumar et al., 2013; Chistyakov, 2001; Devraj et al., 2013; Porter et al., 2008; Pouton and Porter, 2008; Tarate et al., 2014). Various surfactants (emulsifiers) that are commonly reported in the SEDS formulations are listed in Table 14.2. The majority of these are derivatives of PEG with HLB values ranging between 4 and 14.

Polyglycolyzed glycerides (PGGs) are successfully used as emulsifiers with different oil combinations (Shah et al., 1994). Based on their varying fatty acid and polyethylene glycol (PEG) chain lengths, these PGGs can be used in varying proportions with different combinations of oils and surfactants.

Certain surfactants having bioenhancing action were tried in SNEDDS for improving the dissolution and oral absorption of lacidipine. SNEDDS prepared using Labrafil/Capmul and

Cremophor/Tween-80 mixture showed a remarkable increase in dissolution rates and were found to be stable under accelerated stability conditions of 40°C/75% RH for 3 months (Basalious et al., 2010).

### 2.3 Cosolvents

To improve the formulation characteristics like droplet size, stability, and payload of active ingredients, cosolvents like transcutol, ethanol, propylene glycol, and polyethylene glycol (PEG) may be used in formulation of SEDS. These help in dissolution of hydrophilic surfactants in SEDS. Due to the volatile nature of some of these cosolvents, there are chances of their evaporation even from the capsule shell. This, in turn, may lead to a compromise in the stability of the formulation.

Moreover, in certain cases, addition of cosolvents led to a decrease in the solubility of drug, for example, in case of SNEDDS of Cinnarizine, prepared for optimizing its oral bioavailability, the presence of propylene glycol as a cosolvent reduced the solubility of the drug to a remarkable extent (Shahba et al., 2012).

Various cosolvents or coemulsifiers used in SEDS have been listed in Table 14.3 (Pouton, 2000; Pouton and Porter, 2008; Singh et al., 2009).

## 3 Mechanism of Formation of SEDS

Self-emulsification is a phenomenon that occurs spontaneously during the formation of SEDS. It occurs when entropy change that favors dispersion is greater than energy required to increase the surface area of emulsion (Kohli et al., 2010; Singh et al., 2009). Free energy of an emulsion is considered as a direct function of the energy required to create a new surface between any two immiscible phases. The two immiscible phases of an emulsion exhibit a tendency to separate so as to reduce interfacial area to minimum and thus to minimize free energy of system. These systems are stabilized by use of emulsifying agents that reduce the interfacial tension (Kohli et al., 2010; Parmar et al., 2011; Singh et al., 2009).

Thus, for SEDS, such kinds of emulsifiers and cosolvents need to be selected that will be able to reduce the interfacial tension. This, in turn, will lower the free energy required by SEDS so that when they come in contact with aqueous medium in the GIT, the self-emulsification process sets in. Fig. 14.1 depicts the mechanism of SEDS formation (Kohli et al., 2010; Singh et al., 2009).

## Table 14.3 Cosolvents Used for SEDS

| Cosurfactant | Chemical Name | HLB | References |
|---|---|---|---|
| 1,2 octane diol | 1,2 octane diol | | Wang et al. (2009) |
| Akoline MCM | Caprylic/capric glycerides | 5–6 | Date and Nagarsenker (2007) |
| Akomed | Oil containing triacylglycerols of caprylic and capric acid | | Date and Nagarsenker (2007) |
| Capmul MCM-C8 | Glycerylcaprylate | 5–6 | Singh et al. (2009) |
| Caproyl 90 | Propylene glycol mono caprylate | 6 | Kang et al. (2012), Parmar et al. (2011), Rao and Shao (2008) |
| HCO-60 | PEG-60 hydrogenated castor oil | 14 | Singh et al. (2009) |
| Imwitor 742 | Caprylic/capric glycerides | 4 | Date and Nagarsenker (2007) |
| Labrafil 1944 CS | PEG-6 apricot kernel oil | 4 | Date and Nagarsenker (2007) |
| Lauroglycol 90 | Propylene glycol monolaurate | 5 | Inugala et al. (2015), Parmar et al. (2011) |
| Lauroglycol FCC | Propylene glycol monolaurate | 4 | Rao and Shao (2008) |
| Lutrol F127 | PolyoxamersPh Eur., polyoxamers USP | | Beg et al. (2014) |
| PEG 400 | Polyethylene glycol 400 | 11.6 | Rao and Shao (2008) |
| PG | Propylene glycol | | Date and Nagarsenker (2007), Rao and Shao (2008) Shahba et al. (2012) |
| Plurol oleique CC 497 | Polyglyceryl-3 dioleate NF, Polyglyceryl-3 oleate (USA FDA IIG) | 3 | Date and Nagarsenker (2007) |
| Transcutol P | Diethylene glycol mono ethyl ether | — | Date and Nagarsenker (2007), Parmar et al. (2011) |

# 4 Categorization of SEDS

## 4.1 Liquid SEDS

These are self-emulsifying isotropic mixtures of oil, surfactant, and cosolvent in liquid state. These offer the advantages of enhanced solubility of drugs and their increased lymphatic absorption. However, due to their liquid state, they are difficult to be dispensed as dosage form. To make the dosage form more convenient, they need to be incorporated into soft gelatin capsules. This, in turn, adds to the cost of formulation (Singh et al., 2009).

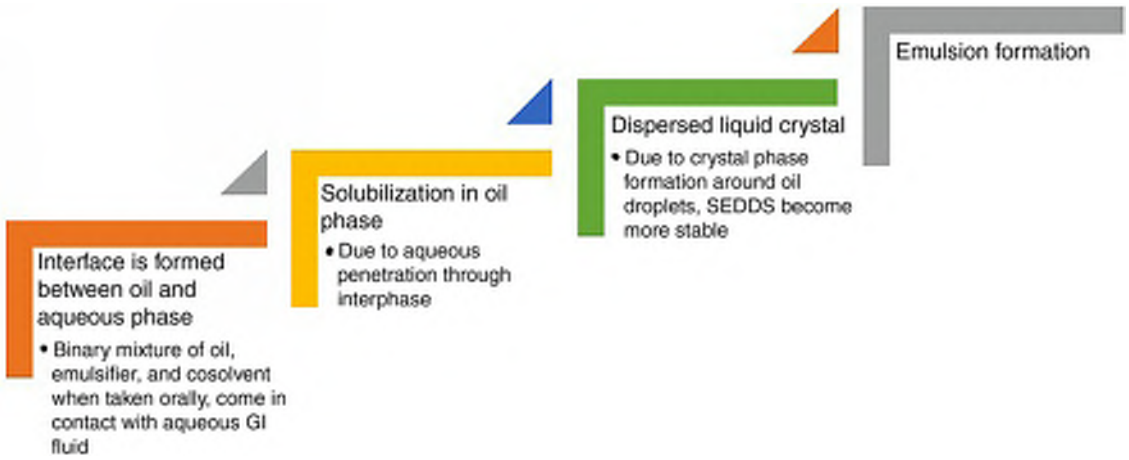



**Figure 14.1. Mechanism of SEDS formation.**

## 4.2 Supersaturable SEDS

Concentration of surfactants in the SEDS formulation is usually in the range of 20–60%. From safety point of view, use of such high concentration of surfactant becomes a concern for the formulator, as their higher concentration may lead to some adverse effect in GIT. To overcome this problem, the concept of supersaturable SEDS was created. In these, the concentration of surfactants is reduced by the inclusion of water soluble polymeric precipitation inhibitor (PPI). These formulations maintain a supersaturable metastable state in vivo by reducing precipitation of drug using PPI. Hydroxypropyl methylcellulose (HPMC) of different grades of viscosity have been widely reported to prevent crystallization as PPI in supersaturable SEDS (Gao and Morozowich, 2006; Gao et al., 2003; Raghavan et al., 2000).

## 4.3 Solid SEDS (S-SEDS)

Development of SEDS as solid dosage form was undertaken to overcome the limitations associated with the liquid SEDS. Solid SEDS offer the advantages of low production cost, high stability, and convenience leading to high patient compliance. To prepare solid SEDS, liquid SEDS are converted into powder form and then formulated as different dosage forms like tablets, pellets (Chen et al., 2011; Tarate et al., 2014; Yan et al., 2011). S-SEDS can be prepared by different methods like extrusion-spheronization, melt granulation, spray drying, lyophilization, adsorption on solid support (Chen et al., 2011; Kang et al., 2011; Lei et al., 2011; Onoue et al., 2012; Shanmugam et al., 2011a; Singh et al., 2011a).

### 4.4 Positively charged SEDS

Most of the absorptive cells present in the human body carry a negative charge. Due to this reason positively charged SEDS have been reported to show better bioavailability as compared to conventional SEDS (Singh et al., 2011a). Oppositely charged SEDS have more time to interact with gastric mucosa via increased adhesion. This leads to higher uptake of drug at the absorption site. Ethyl oleate is generally used as lipid carrier in positively charged SEDS while oleylamine is generally used as charge inducer. It falls under the category of GRAS and induces a charge of 30–35 mV. Apart from oleyamine, chitosan, and stearylamine have also been reported as charge inducers (Cevc, 1997; Jain et al., 2009; Rojanasakul et al., 1992; Singh et al., 2009; Tarate et al., 2014).

## 5 Drug Transport Mechanism of SEDS

SEDS offer oral administration of water insoluble drugs also. Once they reach the GIT, they undergo three processes; that is, digestive, absorptive, and circulatory. These three phases are depicted in Fig. 14.2. During digestion, SEDS form a coarse emulsion, which undergoes enzymatic hydrolysis at oil water interphase and thereby gets ready for absorption phase. After formation of mixed micelles, due to interaction of fatty acid with bile, digestion process stops. The next phase of drug absorption then starts. These colloids are taken up by passive diffusion or active transport through enterocyte membrane. Some drugs may get absorbed via lymphatic circulation through chylomicrons. In circulatory phase, drug is released from chylomicrons and the residual lipid is used in body (Charman and Porter, 1996; Stremmel, 1988).

## 6 Applications in Nutraceuticals

The technique of formation of SEDS to achieve the optimum delivery of nutraceuticals has been widely used. A number of bioactives and functional foods have been prepared in the form of various types of SEDS using variable combinations of the available excipients to achieve their optimum delivery.

A single dose of self-emulsifying formulation of vitamin E resulted in quicker and higher absorption as compared to the conventional formulation available as soft gelatin capsules. This was attributed to finer dispersion size and the resultant larger surface area in self-emulsifying systems (Julianto et al., 2000). Raut et al. (2015) studied the drug precipitation inhibition and supersaturation mechanism of vitamin E from SEDS. It was found that drugs

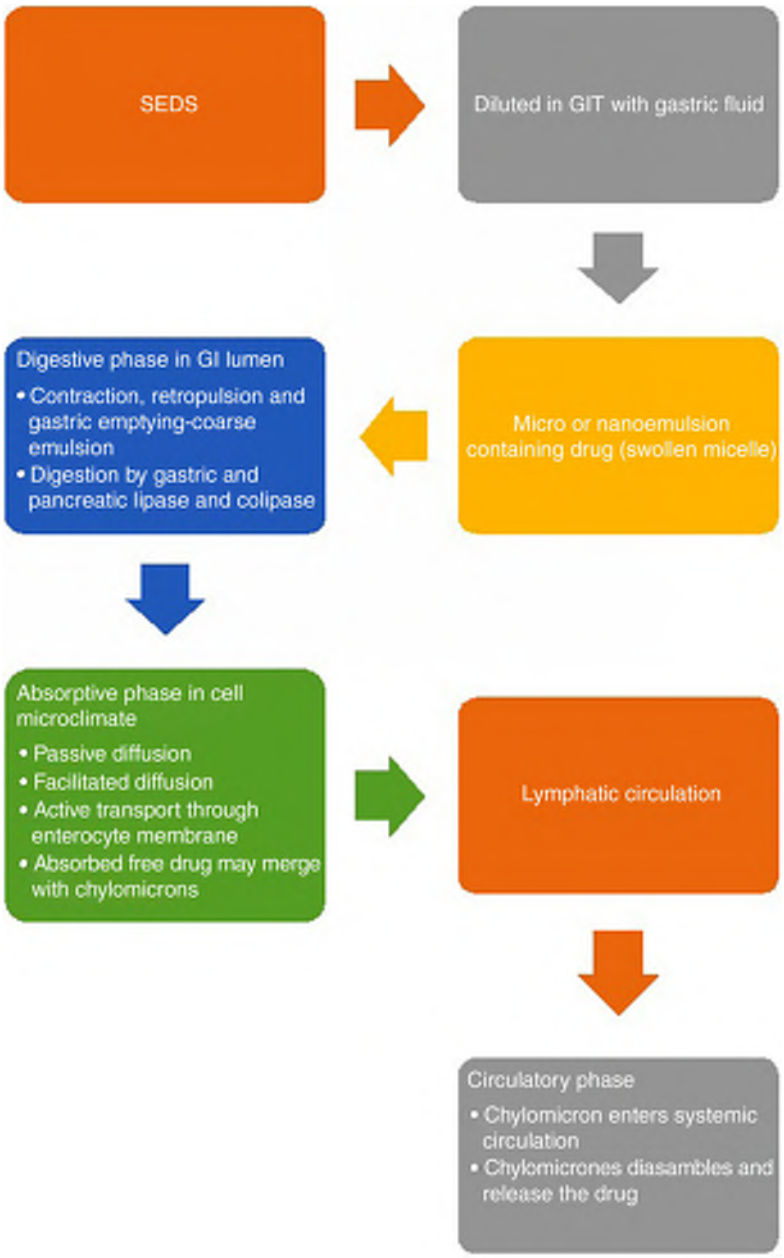



**Figure 14.2. Drug transport mechanisms of SEDS** (Singh et al., 2009).

(indomethacin) precipitated from Labrasol® SEDS whereas TPGS was able to inhibit precipitation and achieve high drug supersaturation levels (Raut et al., 2015).

Coenzyme $Q_{10}$, an antioxidant, is used in the treatment of cardiovascular disorders such as angina pectoris (Mortensen, 1993). However, poor solubility and bioavailability of the molecule have limited its application and use. Kommuru et al. in 2001, reported the delivery of coenzyme $Q_{10}$ through SEDS. Optimized formulation consisting of Myvacet 9-45 (40%), Labrasol (50%), and lauroglycol (10%) was prepared. Increased bioavailability achieved by the formulation as compared to that from the conventional one suggests that SEDS can be successfully used for oral absorption of lipophilic drugs (Kommuru et al., 2001). In a subsequent study, eutectic based semisolid self-nanoemulsified drug delivery system was prepared and characterized using lemon oil, cremophor, and capmul (Nazzal et al., 2002). Three different methods were used to prepare SEDDS of coenzyme $Q_{10}$ for evaluating various oils used (Palamakula and Khan, 2004). The methods used included suspension method, homogenization method, and nanoemulsions of oils in DMEM. In a study carried out in Caco-2 cells, nanoemulsion-based method was found to result in least nontoxic preparations. Food grade oils, surfactants, and cosurfactants were employed to prepare self-emulsifying formulations of coenzyme $Q_{10}$ (Thanatuksorn et al., 2009). The prepared formulations were found to increase the bioavailability of the drug as compared to that of the conventional formulation. A simple, one-step method was devised to prepare self-emulsifying delivery systems of coenzyme $Q_{10}$ using Labrafil M 1944 and Labrafil M 2125 as oil phase, Labrasol as surfactant and Lauroglycol FCC and Capryol 90 as cosurfactants (Balakrishnan et al., 2009). In the bioavailability studies carried out in rodent model, the prepared SEDS were found to enhance the maximum plasma concentration as well as the area under the curve as compared to the powdered formulation.

Cui et al. in 2005 worked to enhance solubility and bioavailability of *Pueraria lobata* isoflavone by formulating it as SMEDS. A threefold increase was observed in the in vitro dissolution of the bioactive with the use of SMEDS as compared to that from the tablets. Results of bioavailability study conducted on dogs, further confirmed the superiority of the SEDS dosage form over the conventional one (Cui et al., 2005).

SMEDS of puerarin, a protein molecule having cardiovascular and antidiabetic action, were developed by Quan et al. (2007) to achieve its oral formulation. Oleic acid (17.5%), Tween-80 (34.5%), and propylene glycol (34.5%) were used as oil, surfactant, and cosurfactant, respectively. Absolute bioavailability of puerarin

in beagle dogs after oral administration was found to be 24.8%. This proved the ability of SEDS to deliver this protein molecule successfully through the oral route (Quan et al., 2007).

Taha et al. (2007) carried out the bioavailability assessment of liquid and solid SNEDS of vitamin A and compared it to that of the conventional oil filled capsules of vitamin A. An optimized oily formulation containing a mixture of vitamin A, soybean oil (16.17 mg), Cremophor EL (43.62 mg), and Capmul MCM C8 (42.53 mg) were used in liquid form. Solid SNEDS were prepared using Avicel PH105 as absorbent, 4% talc powder as lubricant and compressing the SNEDS in the form of tablets. Vitamin A SNED-filled capsules and compressed tablets showed a significant increase in the rate and extent of drug absorption. Bioavailability was reported to be much higher compared to that obtained for capsule filled with an oily solution of vitamin A (Taha et al., 2007).

β-Artemether (BAM), a lipid-soluble antimalarial derivative of artemisinin, on oral administration shows rapid but incomplete absorption and a short half-life (Karbwang et al., 1997). Mandawgade et al. (2008) developed its SMEDS based on indigenous natural lipophile (N-LCT) and commercially available modified oil. N-LCT or Capryol 90 with Plurol Oleique CC 497 (1:1) was selected as oil phase, Cremophor EL or Tween-80 as surfactant and Gelucire 44/14 as cosurfactant. Both the BAM–SMEDS showed excellent self-microemulsification efficiency and released >98% of the drug in just 15 min whereas marketed formulation showed only 46% drug release at the end of 1 h (Mandawgade et al., 2008). In another study, SMEDS were prepared using Suppocire, Gelucire, and Transcutol in the form of suppositories (Gugulothu et al., 2010). The antimalarial effect of the prepared suppositories was compared to that of the conventional suppositories against malarial parasite Plasmodium berghei in murine model. Self-microemulsifying suppositories exhibited increased antimalarial activity as well as better survival rate as compared to the conventional formulation.

Ginkgo biloba extract (GBE) was formulated in the form of SNEDS prepared from Tween-80, Cremophor EL 35, 1,2-propanediol and ethyl oleate. Dissolution rate of SNEDS was found to be significantly higher in this formulation as compared to conventional tablets. Relative bioavailability of SNEDS for bilabolide and ginkgolide A and B was reported to be 162.1, 154.6, and 155.8%, respectively, as compared to the reference tablets in dogs (Tang et al., 2008).

Yao et al. studied the intestinal permeability in rats of nobiletin SNEDS. Nobiletin was mixed in isotropic mixture of Tween-80,

PEG 400, PEG 35 castor oil in ratio 7:2:1. Estimated absorption of nobiletin in human volunteers for the SNEDS was found to be higher than that for submicron emulsions ($p < 0.01$) and similar to that for the micelles ($p > 0.05$) (Yao et al., 2008).

Certain protein compounds have been formulated as SNEDDS for achieving the oral delivery of these molecules by providing protection against gastric enzymes (Rao and Shao, 2008). The peptide fluorescent labeled beta-lactamase, was studied for its formulation which was subsequently tested for its in-vitro transport and in vivo oral absorption (Rao et al., 2008).

Curcumin, a naturally active constituent has been used as antitumor, antiinflammatory, antiviral, antioxident, and anti HIV, with promising clinical application. But low aqueous solubility, poor bioavailability, and low stability have been perceived as major limitations in its formulation into a clinically useful dosage form. Cui et al. (2009) developed curcumin loaded SMEDS using 57.5% surfactant (emulsifier OP:Cremorphor EL = 1:1), 30.0% cosurfactant (PEG 400), and 12.5% oil (ethyl oleate). In vivo study showed that absorption of curcumin from SMEDS was through passive diffusion across the lipid membranes (Cui et al., 2009). In a recent study, a combination of four naturally occurring enzyme inhibitors (piperine, quercetin, tangeretin, and silibinin) was coformulated with curcumin to prepare its SMEDS (Grill et al., 2014). Both ex vivo and in vivo studies indicated the superiority of the codelivery of enzyme inhibitors with curcumin in the form of self-emulsifying delivery system.

Gentiopicrin, an antiinflammatory phytochemical obtained from roots of gentians was complexed with phospholipid and the complex so prepared was formulated as SMEDDS (Gao et al., 2009). The SMEDDS prepared from glyceryl monolinoleate, sasol, capric trigyceride, Labrasol, caprylocaproyl macrogolglycerides, Cremophor EL, and Transcutol exhibited 703.62% relative bioavailability as compared to Gentiopicrin alone in rat model.

Vinpocetine, the water insoluble active alkaloids of vinca were formulated into SMEDDS to increase its bioavailability (Cui et al., 2009). The formulation prepared using ethyl oleate, Solutol HS and Transcutol P was found to increase the bioavailability of the active constituent by approximately 1.7-fold. Another SMEDS formulation prepared using Labrafac, oleic acid, Cremophor EL, Transcutol P, and gum acacia was compared to a solid dispersion of the drug for its dissolution as well as pharmacokinetic parameters (Chen et al., 2009). The SMEDS formulation was reported to be superior to the solid dispersion formulation in terms of its solubility, dissolution, permeability, absorption, and oral bioavailability.

Flavones of Hippophae rhamnoides, which have proven health benefits, have been successfully formulated into stable SMEDS, reported to exhibit better dissolution properties than its pure form (Xie et al., 2009). In a further study, the authors found that the formulation possessed good self-emulsification properties and was able to release almost 90% of the contents in 20 min (Li et al., 2012). The in vivo studies indicated more than fourfold increase in bioavailability.

Setthacheewakul et al. prepared pellets of curcumin loaded SMEDS. The liquid SMEDS were prepared using 70% mixtures of two surfactants: Cremophor EL and Labrasol (1:1), and 30% mixtures of oil: Labrafac PG and Capryol 90 (1:1). Extrusion/spheronization (E/S) technique was used to convert this oily liquid (36% w/w) into pellets after mixing with solid pharmaceutical excipients (62.4% w/w) like silicon dioxide and glyceryl behenate. Increased absorption (up to 10-fold) was observed in in vivo (rats) with liquid SMEDS while the pellets showed 14-fold increase as compared to its aqueous suspension. Moreover, degradation study under intermediate and accelerated stability conditions for 6 months indicated that both the formulations were stable (Setthacheewakul et al., 2010). As a modified application of SEDDS, the same group prepared the self-emulsifying floating drug delivery systems of tetrahydrocurcumin for controlled release on oral administration (Setthacheewakul et al., 2011). By virtue of possessing a floating efficiency of 93% for 6 h, the delivery system was able to provide a sustained release of drug for a period up to 8 h.

An alkaloidal drug obtained from Sophora roots, matrine has been reported to exhibit antitumor activities in a variety of cancer models (Liu et al., 2014). However, the use of drug is limited by its poor bioavailability. To enhance its oral bioavailability, a two-pronged approach of preparing its phospholipid complexes and then formulating these complexes as SNEDDS was adopted (Ruan et al., 2010). A substantial increase in the bioavailability of the drug was achieved in rat models with the SNEDDS formulation, prepared by using Lauroglycol FCC, Cremophor EL, and Transcutol HP. The use of this technique was also made in preparing the SNEDDS of morin wherein the phospholipid complexes of the drug were prepared (Zhang et al., 2011). These complexes were then formulated as SNEDDS using Labrafil, Cremophor RH, and Transcutol. In this case also, a substantial increase in the bioavailability of the drug could be achieved.

Self-emulsifying microemulsions of silymarin were prepared for improving its bioavailability (Woo et al., 2007). On oral administration of these SMEDS prepared with glyceryl monooleate, polysorbate 20, and HCO-50 in rodent model, much higher

bioavailability could be achieved as compared to that from the reference capsules. SMEDS of silymarin were reported by Li et al. (2010). Optimal formulation could be formulated using 10% (w/w) of ethyl linoleate, 30% of Cremophor EL, and 60% of ethyl alcohol. In vitro release and oral bioavailability of this formulation was found to be significantly higher in dogs (Li et al., 2010). Taking further the SEDS approach, Iosio et al. (2011), prepared self-emulsifying pellets for the milk-thistle plant extract containing silymarin using Akoline MCM, Miglyol, Tween-80, soy lecithin, and propylene glycol as ingredients of SEDS and microcrystalline cellulose and lactose monohydrate as solid support for the preparation of pellets. The pharmacokinetic data generated in rats indicated a 100-times enhancement in bioavailability as compared to that obtained from the conventional formulation.

SEDS of supersaturable nature of silybin were prepared using Labrafac CC, Cremophor RH40, Labrasol, and 5% HPMC (Wei et al., 2012). The formulation exhibited excellent self-emulsification properties and approximately threefold increase in bioavailability as compared to the conventional formulation.

SMEDS of Daidzein (4.7-dihydroxylisoflavone) were prepared in an attempt to increase its solubility and bioavailability. Optimized formulation consisting of ethyl oleate (10%), cremophor RH 40 (60%), and PEG 400 (30%) showed and improved dissolution profile as compared to conventional tablets (Shen et al., 2010).

Lutein is a carotenoid reported for prevention of eye diseases like macular degeneration and cataract (Trumbo and Ellwood, 2006). Yoo et al. in 2010 reported the preparation of lutein loaded SNEDS containing 25% oil (Phosal 53 MCT), 60% surfactant (Labrasol), and 15% cosurfactant (Transcutol-HP or Lutrol-E400). Dissolution of lutein from the solid SNEDS (physical mixture of the optimized SNEDS and Aerosil 200) took place in less than 5 min in distilled water, and once dissolved, no precipitation or aggregation of the drug could be observed. In contrast, no drug release was reported from lutein powder or from the commercial product (Eyelac) until 3 h of the study duration (Yoo et al., 2010).

Shanmugam et al. (2011a) prepared solid SNEDS of lutein by spray drying of liquid SNEDS using colloidal silica as solid carrier. Optimized formulation of SNEDS contained Phosal 53 MCT/ labrasol/transcutol HP, oil/ surfactant/cosurfactant, (25/60/15, w/w/w) respectively, with 4% of lutein. Solid state characterization of S-SNEDS by SEM (Scanning Electron Microscopy), DSC (Differential Scanning Calorimetry), and XRPD (X-ray powder diffraction) revealed the absence of any crystalline lutein in the S-SNEDS. The maximum plasma concentration for S-SNEDS was found to be 21-folds and 8-folds compared with lutein powder

(LP) and commercial product (CP), respectively (Shanmugam et al., 2011a).

Zhao et al. (2010) developed SNEDS for the oral delivery of Zedoary turmeric oil (ZTO). Optimized formulation consisting of ZTO, ethyl oleate, Tween-80, Transcutol P (30.8:7.7:40.5:21, w/w) and loaded with 30% drug was prepared. On oral administration of ZTO-SNEDS in the rat model, both bioavailability indicators, that is, area under curve (AUC) and Cmax of germacrone (a representative bioactive marker of ZTO) was found to increase by 1.7-fold and 2.5-fold, respectively, when compared with the powder form of ZTO (Zhao et al., 2010).

Persimmon (*Diospyros kaki*) leaf extract (PLE) consisting of flavonoids, oligomers, organic acids, tannins, phenols, chlorophyll, vitamin C, and caffeine has been reported to be effective in many cardiac disease (Li et al., 2011). Li et al. (2011) optimized the formulation of PLE extract containing SNEDS having 44.48 mg/g PLE total flavonoids. These were prepared from Cremophor EL, Transcutol P, Labrafil M 1944 CS (56:34:10, w/w). In vitro release as well as in vivo study in beagle dogs indicated that formulation in the form of SNEDS could be a potential option for the delivery of PLE extract (Li et al., 2011).

As a next step in the development of SEDS, self-double emulsifying delivery system (SDEDS) for Hydroxysafflor yellow A (HSYA) was developed to overcome its poor bioavailability. SDEDS consist of w/o emulsions and hydrophilic surfactants. These are capable of self-emulsification into w/o/w double emulsions on coming in contact with the aqueous gastrointestinal fluid. HSYA in 0.5% gelatin solution constituted the inner water phase. The oil phase contained bean phospholipids, medium chain triglycerides, Tween-80, oleic acid, and labrasol. SDEDS were found to improve the absorption of HSYA mainly through inhibition of p-gp expression and Papp improvement of this water-soluble drug (Lv et al., 2012).

Rhizoma corydalis decumbentis (RCD), a famous traditional Chinese herbal medicine with poor aqueous solubility has also been incorporated into SNEDS by Ma et al. (2012). The optimized formulation consisted of 45% Solutol, 40% ethyl oleate, and 15% Transcutol P. An average droplet size of less than 100 nm could be achieved. Bioavailability study in rats with commercial tablets and SEDS established the superiority of SNEDS over conventional tablets (Ma et al., 2012).

Berberine is an isoquinoline alkaloid whose therapeutic potential has not been fully exploited due to its low bioavailability. To improve its solubility and bioavailability, SMEDDS were prepared using ethyl linoleate and oleic acid, Tween-80 and glycerol

(Zhu et al., 2013). In an in vivo study in rat model, the bioavailability of the prepared formulation was found to be more than twice as that of the commercially available tablets. On similar lines, SNEDDS of berberine were prepared using Acrysol K-l 50, Capmul MCM, and polyethylene glycol 400 (Pund et al., 2014). The efficacy of the prepared formulation was checked using chick chorioallantoic membrane assay as well as in rat model. The prepared formulation showed improved in vitro as well as in vivo efficacy. In a latest study, the SNEDDS of berberine prepared using castor oil, Tween-20, and glycerol have also been reported to enhance the bioavailability of the drug (Ke et al., 2015).

Methoxyflavones isolated from Kaempferia parviflora (KP) have been reported to possess antiinflammatory, antimicrobial, and antiulcer activity (Patanasethanont et al., 2007). However, the bioavailability of the active constituents is limited because of its hydrophobic nature. Mekjaruskul et al. (2013) developed SMEDS and cyclodextrin (CD) complex formulations of its methoxyflavones. Polyoxyethylene castor oil (53.3%), propylene glycol (26.7%), and triglyceride of coconut oil (20%) were combined to form SMEDS. Increased dissolution rate of methoxyflavones by the use of SMEDS in both 0.1 N HCl and 0.2 M PBS pH 6.8 could be obtained compared to methoxyflavones dissolved in solutions of various solvents like propylene glycol, PEG 400, ethanol, and water. Even oral bioavailability of SMEDS formulations was found to be higher than that of standard (Mekjaruskul et al., 2013).

Shao et al. (2013) developed solid granules of SMEDS containing Brucea javanica oil (BJO). Optimized SNEDS consist of Cremophor RH-40, PEG400, Caprylic/capric triglyceride (GTCC) along with BJO. Methyl thiazolyl tetrazolium assay demonstrated that BJO SMEDS had a significant effect on cancer cells. Antitumor activity studies also showed a remarkable inhibition of S180 tumors. In vitro dissolution studies results were reported to be better than those for conventional dosage form and authors reported SMEDS as promising strategies for the oral delivery of the poorly water-soluble BJO (Shao et al., 2013).

SEDS of rutin were prepared using Triton/Acconon/Labrafac and then transformed into solid dosage forms by adsorption onto various solid supports like Neusilin, Fujicalin, and F-melt. The prepared dosage forms exhibited good loading efficiency, improved dissolution profile and good flowability (Kamel and Basha, 2013).

Apigenin, a natural product belonging to the flavone class, has been reported to exhibit antioxidant, antimutagenic, anticarcinogenic, antiproliferative, and antiinflammatory activities (Kelloff et al., 2000). Zhao et al. (2013) designed SNEDS of apigenin, to increase its dissolution potential. Optimal formulation of SNEDS

was prepared using 60% CremophorEL, 30% TranscutolHP, and 10% Capryol 90. Developed SNEDS were reported to have mean droplet size of 17.1 nm and dissolution within 10 min. Thus, this leads to increased solubility of apigenin (Zhao et al., 2013).

Oleanolic acid, which exhibits a poor bioavailability due to its poor aqueous solubility, was formulated into SEDS which were subjected to in vitro bioavailability studies in rat model. SNEDDS formulation was prepared using Sefsol218 as the oil, Labrasol, and Cremophore EL as primary surfactants and Transcutol P as cosurfactant. Oral delivery of these resulted not only in an enhanced bioavailability but also increased the plasma retention time of oleanolic acid as compared to that of the conventional tablet in rat model (Xi et al., 2009). In a later study, formulation of oleanolic acid in the form of SMEDS, was able to achieve more than fivefold increase in bioavailability on oral administration as compared to the marketed formulation (Yang et al., 2013).

Many natural essential oils that are used as food additives act as green preservatives due to their inherent antibacterial activities. However, being thermolabile and photolabile, their use as a food additive is limited (Turek and Stintzing, 2013). To increase the stability of such constituents, the technique of self-emulsifying delivery systems has been used successfully. Carvacrol, a green preservative commonly used in foods was formulated as SEDS using medium chain triglycerides and various grades of Tween (Chang et al., 2013). Good physical stability and antimicrobial efficacy could be achieved using this technique. Another essential oil obtained from *Swietenia macrophylla* having antimicrobial as well as antiinflammatory properties has also been incorporated into SNEDDS (Eid et al., 2013). The formulation prepared using Tween-20, Labrafil, Labrasol, and Capmul MCM were found to possess excellent self-emulsifying properties. In a further study, the antiinflammatory activity of the prepared SNEDS was compared to that of the oily solution in carrageenan-induced rat paw oedema model (Eid et al., 2014). The self-emulsifying formulation was found to be more effective in reducing the inflammation. Self-emulsifying nanoemulsions of cinnmaldehyde were prepared using medium chain triglycerides (Tian et al., 2016). These were found to be stable with variation in pH as well as salt content. However, the instability in response to high temperature could not be overcome. The SNEDS were found to retain their antimicrobial activity against *Escherichia coli*.

Quercitin is a dietary flavonoid with potential chemoprotective effects but its use is limited by poor solubility, less intestinal absorption, and poor bioavailability. Self-emulsifying delivery systems of quercetin were prepared using ethyl oleate,

Cremophor, and butanol (Hu et al., 2007). More than twofold increase in solubility of quercetin could be achieved in the formulation as compared to the pure drug. Tran et al. (2014) prepared SNEDS of quercetin using castor oil, Tween-80, Cremophor RH 40, and PEG 400. Fluorescence imaging after 40 min of oral administration as well as other pharmacokinetic parameters like area under the concentration curve and maximum plasma concentration demonstrated an increased bioavailability on the use of SEDS in rats (Tran et al., 2014). Jain et al. (2014a,b) worked on the combination of tamoxifen and quecitin SNEDS and solid SNEDS. Optimized formulation was developed using oil (Capmul MCM), surfactant (Cremophor RH 40), and cosurfactant (Labrafil 1944CS) to prepare liquid SNEDS, which were reported to solubilize high amounts of tamoxifen (10 mg/g) and quercetin (19.44 mg/g). Increased bioavailability of about 4–8 times could be achieved as compared to its aqueous solutions (Jain et al., 2014a,b).

To prevent its intestinal presystemic metabolism, resveratrol, a polyphenolic nutraceutical was formulated as its SMEDS. This not only aimed at increasing its bioavailability but also at reducing its intestinal toxicity. The prepared formulation was evaluated for its flux through Caco cells and intestinal cells and cytotoxicity. Excellent self-emulsifying properties and rapid release could be achieved through the SMEDS formulation (Seljak et al., 2014).

On similar lines, baicalin was formulated into SMEDS and further combined with phospholipids to prepare complexes, with the aim to improve its bioavailability. The resultant formulation, prepared by solvent evaporation method, exhibited an enhanced bioavailability in in vitro, ex vivo as well as in vivo studies. An increase in bioavailability to the tune of 220.37% was achieved by the combination of the techniques of SMEDS and phospholipid complexation (Wu et al., 2014).

SEDS of tocotrienol were prepared and evaluated for their enhanced bioavailability. The formulation was evaluated using a three-way crossover design using six healthy human volunteers. The SEDS exhibited a faster absorption and higher bioavailability of tocotrienol as compared to its soluble counterpart, an oily solution (Yap and Yuen, 2004). Alqahtani et al. (2014) formulated SEDS of tocotrienol and evaluated then for both in vitro and in vivo bioavailability. SEDS were prepared using cremophor EL (40.7% w/w) as the primary surfactant, labrasol (40.7% w/w) as a cosurfactant, captex 355 (7.2% w/w) as an oil, and ethanol (11.4% w/w) as a cosolvent. Tocotrienol incorporated in SEDS showed 2 times increase in solubilisation during in vitro lipolysis experiment as compared to Tocovid (marketed formulation). Thus in vitro cellular uptake and in vivo oral bioavailability

studies have shown that enhanced solubilization and passive permeability lead to increased cellular uptake and bioavailability (Alqahtani et al., 2014).

Use of a ellagic acid, a very popular natural polyphenolic, is limited by its poor aqueous solubility and the resultant low bioavailability. SNEDS of ellagic acid-phospholipid complex were prepared and tested for enhancement in its bioavailability. The in vitro as well as ex vivo studies indicated an improved flux of ellagic acid (Avachat and Patel, 2015).

Tables 14.4 and 14.5 enlist the work done in the area of SEDS formulation in nutraceuticals and herbal drugs.

## Table 14.4 Application of SEDS in Nutraceuticals

| Drug | Oil | Surfactant | Cosurfactant | Type of Emulsion | References |
|---|---|---|---|---|---|
| Apigenin | CremophorEL | Capryol 90 | TranscutolHP and 10% | SMEDS | Zhao et al. (2013) |
| Baicalin | Ethyl oleate | Tween-80 | Glycerol | SMEDS-PL complex | Wu et al. (2014) |
| Berberine | Ethyl linoleate and oleic acid | Tween-80 | Glycerol | SMEDS | Zhu et al. (2013) |
| | Acrysol KL-50 | Capmul | PEG 400 | SNEDS | Pund et al. (2014) |
| | Castor oil | Tween-20 | Glycerol | SNEDS | Ke et al. (2015) |
| Bruceajavanica oil | Caprylic/capric triglyceride | Cremophor RH-40 and Solutol HS-15 | PEG400 | SMEDS and its granules | Shao et al. (2013) |
| Carvacrol | Medium chain triglycerides | Tween-20, 40, 60, 80, and 85 | — | SNEDS | Chang et al. (2013) |
| Coenzyme Q10 | Myvacet 9-45 and Captex-200 | Labrafac CM-10 and Labrasol | Lauroglycol | SEDS | Kommuru et al. (2001) |
| | Lemon oil | Cremophor | Capmul | SNEDS | Nazzal et al. (2002) |
| | Labrafil M 1944 and Labrafil M 2125 | Labrasol | Lauroglycol FCC and Capryol 90 | SEDS | Balakrishnan et al. (2009) |

## Table 14.4 Application of SEDS in Nutraceuticals (*cont.*)

| Drug | Oil | Surfactant | Cosurfactant | Type of Emulsion | References |
|---|---|---|---|---|---|
| Curcumin | Capryol 90, Labrafac PG | Cremophor EL | Labrasol, PEG 400 | SMEDS and SMEDS pellets | Setthacheewakul et al. (2010) |
| | Ethyl oleate | Emulsifier OP and Cremorphor EL | PEG 400 | SMEDS | Cui et al. (2009) |
| Curcumin and Docetaxel | Lauroglycol FCC | Labrasol | Transcutol HP | SEDS | Yan et al. (2012) |
| Curcumin, piperine, quercetin, tangeretin and silibinin | Captex | Cremophor | Carbitol | SMEDS | Grill et al. (2014) |
| Daidzein | Ethyl oleate | Cremophor RH 40 | Peg 400 | SMEDS | Shen et al. (2010) |
| Ellagic acid-phospholipid complex | Captex 500 | Cremophor RH 40 | PEG 400 | SNEDS | Avachat and Patel (2015) |
| Flavones of Hippophaerhamnoides | Miglyol | Cremophor EL | 1,2-Propylene glycol | SMEDS | Xie et al. (2009), Li et al. (2012) |
| Gentiopicrin | Glyceryl monolinoleate | Labrasol, Cremorphor EL | Transcutol | SMEDS | Gao et al. (2009) |
| Ginkgo bilova extract | Ethyl oleate | Tween-80-Cremophor (1:1) EL 35 | 1,2-Propanediol | SEDS | Tang et al. (2008) |
| Hydroxysafflor yellow A | Bean phospholipids, medium chain triglycerides, | Tween-80, oleic acid | Labrasol | Self-double-emulsifying drug delivery system (SDEDDS) | Lv et al. (2012) |
| Jiaotai Pill actives (Berberine hydrochloride) | Cinnamon oil | OP | Propanediol | SMEDS | Zhang et al. (2008a) |
| β-Lactamase | Lauroglycol | Cremophor EL | Transcutol P | SNEDDS | Rao and Shao (2008) |
| Ligusticumchuanxiong (a volatile oil) | Chuanxiong oil | Nonionic surfactant | — | SMEDS | Cai et al. (2008), Cai et al. (2007) |

(*Continued*)

## Table 14.4 Application of SEDS in Nutraceuticals (*cont.*)

| Drug | Oil | Surfactant | Cosurfactant | Type of Emulsion | References |
|---|---|---|---|---|---|
| Lutein | Phosal 53 MCT | Labrasol | Transcutol HP | Solid SNEDS | Shanmugam et al. (2011b) |
| Matrine | Lauroglycol | Cremophor EL | Transcutol P | SNEDS | Ruan et al. (2010) |
| Methoxy-flavone | Polyoxyethylene castor oil | triglyceride of coconut oil | Propylene glycol | SNEDS | Mekjaruskul et al. (2013) |
| Morin | Labrafil | Cremophor R | Transcutol P | SNEDS | Zhang et al. (2011) |
| Nobiletin | Cradamol GTCC | T80, Cremophor EL 35 | PEG 400 | SMEDS | Yao et al. (2008) |
| Oridonin | Maisine 35-1, Labrafac CC | Cremophor EL | Transcutol P | SMEDS | Zhang et al. (2008b) |
| Oleanolic acid | Sefsol 218; Ethyl oleate | Cremophor EL and Labrasol | Transcutol P and ethanol | SMEDS, SNEDS | Xi et al. (2009), Yang et al. (2013) |
| Pueraria Lobata Isoflavon | Ethyl oleate | Tween-80 | Transcutol P | SMEDS | Cui et al. (2005) |
| Puerarin | Oleic acid | Tween-80 | Propylene glycol | SMEDS | Quan et al. (2007) |
| Persimmon leaf extract | Labrafil M 1944 CS | Cremophor EL | Transcutol P | SNEDS | Li et al. (2011) |
| Quercetin | Castor oil | Tween-80, CremophorRH 40, | Transcutol P | SNEDS | Jain et al. (2013), Tran et al. (2014) |
| | Ethyl oleate, | Cremophor | Butanol | SEDS | Hu et al. (2007) |
| Quercetin and Tamoxifen | Capmul MCM EP | Cremophor RH 40 | Labrafil 1944 | Solid SNEDS | Jain et al. (2014a,b) |
| Resveratrol | Castor oil/ Capmul | Kolliphor EL | Kolliphor RH | SMEDS | Seljak et al. (2014) |
| Rhizoma corydalis decumbentis extracts | Miglyol 812 and Ethyl oleate | Tween-80 | Transcutol P | SEDS | Ma et al. (2012) |
| Rutin | Labrafac | Acconon | Triton | SSEDS | Kamel and Basha (2013) |
| Silybin | Labrafac CC | Cremophor RH40, Labrasol | HPMC | SEDDS | Wei et al. (2012) |

## Table 14.4 Application of SEDS in Nutraceuticals (*cont.*)

| Drug | Oil | Surfactant | Cosurfactant | Type of Emulsion | References |
|---|---|---|---|---|---|
| Silymarin | Ethyl linoleate, glycerol monooleate | Tween-80, Polysorbate 20 | Ethanol Transcutol P | SMEDS | Wu et al. (2006); Li et al. (2010) |
| | Glycerol monooleate | Polysorbate 20 | HCO-50 | SMEDS | Woo et al. (2007) |
| | Akoline MCM, Miglyol | Tween-80, soy lecithin | Propylene glycol | Pellets of SEDS | Iosio et al. (2011) |
| Swietenia macrophylla oil | Swietenia macrophylla oil | Tween-20, Labrafil | Labrasol, Capmul | SNEDS | Eid et al. (2013, 2014) |
| Tetrahydrocurcumin | Capryol 90, Labrafac PG | Cremophor EL | Labrasol, PEG 400 | SEFDDS | Setthacheewakul et al. (2011) |
| Tocotrienol | Captex 355 | Cremophor EL | Labrasol | SEDS | Yap and Yuen (2004), Alqahtani et al. (2013, 2014) |
| Vitamin E | Palm oil | Tween-80, Span 80 | — | SEDS | Julianto et al. (2000) |
| Vitamin E TPGS and Indomethacin | Lauroglycol FCC | Labrasol and Vitamin E TPGS, | Transcutol P | SEDS | Raut et al. (2015) |
| Vinpocetine | Ethyl oleate | Solutol HS Transcutol P | Transcutol P | SMEDS | Cui et al. (2009) |
| | Labrafac, oleic acid | Cremophor EL | Transcutol P | SMEDS | Chen et al. (2009) |
| Vitamin A | Soybean oil | Cremophor EL | Capmul MCM | SNEDS | Taha et al. (2007) |
| Zedoary essential oil | Ethyl oleate | Tween-80 | Transcutol P | SNEDS | Zhao et al. (2010) |
| Zedoary turmeric oil | Ethyl oleate | Tween-85 | — | Self-emulsifying microsphere | You et al. (2005) |
| Artemether | N Light chain triglyceride, Caproyl 90, Gelucire 44/14 | Cremophor EL, Tween-80 | Plurol Oleique CC 97 | SMEDS | Mandawgade et al. (2008) |
| | Suppocire | Gelucire | Transcutol P | Self-emulsifying supoositories | Gugulothu et al. (2010) |

## Table 14.5 Patents on SEDS of Nutraceuticals and Herbal Drugs

| Drug Category | Patent No. | Summary of Patent | References |
|---|---|---|---|
| Vitamin E | EP1578408A2 | SEDS of tipranavir, vitamin E, and solvents were prepared. Helpful for paediatric patients as ease of swallowing | Abelaira et al. (2010) |
| Vitamin A, D, E K, tocotrienols and β carotene | EP1170003B1 | Absorption of toternols increased up to 3 times than conventional formulations | Ho et al. (2006) |
| Taxoids | EP1499143A1 | SMEDS were formulated to enhance solubility and bioavailability of toxoids | Cote et al. (2005) |
| Vitamin E as cosolvents | EP1340497A1 | Vitamin E as cosolvent in SMEDS increased bioavailability and solubility of drugs like paclitaxel and docetaxel | Tarate et al. (2014) |
| Curcuminoids | U20110294900A1 | SNEDS prepared increased solubility and bioavailability. Drug loading was also increased than emulsion. | Kohli et al. (2011) |
| Buthylpthalide | EP1787638A1 | Increased solubility and bioavailability | Liu et al. (2007) |
| Omega-3-fatty acids | US20120232141A1 | SEDS tablets 40 % increased bioavailability in pigs | Hustvedt et al. (2012) |
| Coenzyme $Q_{10}$ | US20100166873A11 | SNEDS having Co$Q_{10}$, lemon oil and camphol release 93.4 % of Co$Q_{10}$. | Khan and Nazzal (2010) |

# 7 Characterization of SEDS Containing Nutraceuticals

## 7.1 Equilibrium Phase Diagram

Although self-emulsification is a nonequilibrium process, information on equilibrium phase behavior is still not completely available about self-emulsification. Phase behavior of three components can be represented by ternary diagrams. There is a correlation between emulsification efficiency and region of solubilization in water and phase inversion region. These diagrams help in comparison of different surfactant and cosurfactants. To characterize a phase diagram phase solubility study and pseudo

ternary phase diagram study should be carried out. In phase solubility study, ratio of oil to surfactant or cosurfactant is varied (w/w) and emulsion is prepared under vigorous stirring by addition of water. Pseudo-ternary phase diagrams helps in identification of microemulsion region. They can be prepared by using oil, surfactant: cosurfactant and water via titration method. The SEDS (specific ratio of oil, surfactant, and cosurfactant) along with drugs that should be titrated with water and observed visually for phase separation. Micro- or nanoemulsion region can be selected from phase diagram. A typical example of phase diagram is shown in Fig. 14.3 (Kohli et al., 2010; Singh et al., 2009, 2011b; Trotta et al., 2003).

## 7.2 Dispersibility Test

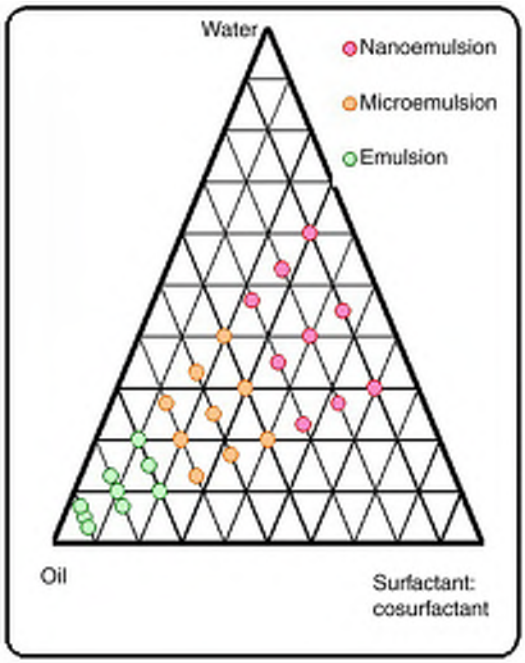
It is conducted to check the phase separation and clarity. In dispersibility test, USP dissolution apparatus II is used. SEDS (1 mL) is added to 500 mL of water at 37 ± 0.5°C at 50 rpm. Emulsion formed will be examined visually and graded accordingly, that is, emulsion, microemulsion, nanoemulsion, or no emulsion (Singh et al., 2009).

## 7.3 Droplet Size, Morphology, and Zeta Potential

Droplet size has an important role in the stability of an emulsion. It not only affects the bioavailability but also influences drug loading. Droplet size also helps in categorization of emulsions like SEDS, SMEDS, and SNEDS. Various techniques used to measure droplet size are zeta sizer using dynamic light scattering (DLS), static light scattering, multiangle light scattering, and so on. Emulsion droplet polarity helps in determining affinity of drug for emulsion, oil, or water. Electron microscopy studies like SEM, TEM, and cryo-TEM to generate information on sample topography, composition, morphology, shape, texture, size, and so on. Zeta potential is measured using DLS. Charge plays an important role in stability of emulsion. In convention SEDS usually negative charge is present, however, depending on the requirements, desirable charge can be added by the selection of proper emulsifier (Kohli et al., 2010; Muller and Muller, 1984; Singh et al., 2009; Van den Bergh et al., 1999).

## 7.4 Turbidity

Turbidity helps in determining whether equilibrium has reached in a dispersion. It helps in the calculation of emulsification time. Turbidity meters like Hach turbidity meters are popularly used to measure turbidity. These meters can also be connected




| SNo. | Oil | Surfactant | Cosurfactant | Emulsion type |
|---|---|---|---|---|
| 1 | 10 | 45 | 45 | |
| 2 | 20 | 40 | 40 | 1 |
| 3 | 30 | 35 | 35 | 1 |
| 4 | 40 | 30 | 30 | 1 |
| 5 | 50 | 25 | 25 | 2 |
| 6 | 60 | 20 | 20 | 2 |
| 7 | 70 | 15 | 15 | 3 |
| 8 | 80 | 10 | 10 | 3 |
| 9 | 90 | 5 | 5 | 4 |
| 10 | 10 | 30 | 60 | 1 |
| 11 | 20 | 27 | 53 | 1 |
| 12 | 30 | 23 | 47 | 1 |
| 13 | 40 | 20 | 0 | 2 |
| 14 | 50 | 17 | 27 | 2 |
| 15 | 60 | 13 | 7 | 3 |
| 16 | 70 | 10 | 20 | 3 |
| 17 | 80 | 7 | 13 | 4 |
| 18 | 90 | 3 | 7 | 4 |
| 19 | 10 | 60 | 30 | 1 |
| 20 | 20 | 53 | 27 | 1 |
| 21 | 30 | 47 | 23 | 1 |
| 22 | 40 | 40 | 20 | 2 |
| 23 | 50 | 33 | 17 | 2 |
| 24 | 60 | 27 | 13 | 3 |
| 25 | 70 | 20 | 10 | 3 |
| 26 | 80 | 13 | 7 | 3 |
| 27 | 90 | 7 | 3 | 4 |

**Figure 14.3. Pseudo ternary phase diagram.**

to dissolution apparatus and can measure turbidity at frequent intervals to know the clarity of micro- or nanoemulsion with respect to time (Kohli et al., 2010; Singh et al., 2009).

## 7.5 Rheological Study

As SEDS emulsify in the GIT rheological parameters are very important to study. These affect the bioavailability of drug. In GIT oil and emulsifier form an interface with GIT called as intermediate liquid crystalline phase. Rheological properties can be determined using cup and bob, cone and plate, or rotational viscometer.

Decrease in viscosity and increase in absorption of SEDS, after dilution in GIT can be determined from the viscosity studies (Patil et al., 2012; Singh et al., 2009).

## 7.6 Stability Study

Physical and chemical stability of emulsions containing drug should be studied via phase separation and clarity study. Emulsions are centrifuged for a specific time and stored at different temperature (5, 15, 25, and 37°C). Normally emulsions should be kept at 15°C or higher. Usually at 5°C, emulsions show turbidity and phase separation (Kohli et al., 2010; Singh et al., 2009; Verwey, 1947). Thermodynamic stability can be studied by keeping the samples a number of times between 4 and 45°C and then centrifuging them for 30 min at 3500 rpm followed by freeze thaw cycles between −21°C and +25°C in triplicate. All formulations should be kept at each temperature for not less than 48 h (Singh et al., 2009, 2011b).

Dilution robustness is an important stability parameter for SEDS. SEDS should form micro- or nanoemulsion with any dissolution media simulating GIT at any volume. There should be no change in the emulsion even after dilution. It should neither show phase separation nor drug precipitation even after 12 h (Singh et al., 2009).

## 7.7 Liquefaction Time

This test is conducted for solid SEDS. It estimates the time taken by solid SEDS to melt in vivo in absence of agitation to simulated GI conditions. The solid SEDS wrapped in polyethylene film placed in 250 mL of simulated gastric medium maintained at 37°C. Time taken for liquefaction is noted.

## 7.8 Determination of Emulsion Phase

It is also important to confirm which type of emulsions (o/w or w/o) SEDS are preparing in the GIT. This can be confirmed via conductivity test. In this test first SEDS are added to 500 mL of water to form an emulsion. Now electricity is passed via electrodes. If emulsion is able to conduct the electric charge then it is an o/w emulsion. On the other hand, if no conductivity, then it is a w/o emulsion as oil is a bad conductor of electricity while water is a good conductor of electricity (Shao et al., 2013, 2014).

Fig. 14.4 summarizes all the characterization parameters of SEDS in nutraceuticals.

## 7.9 Permeation Studies

Permeation of SEDS through intestine helps in determining bioavailability. It can be done either via different in vitro/ex vivo models or via cell models as depicted in Fig. 14.5.

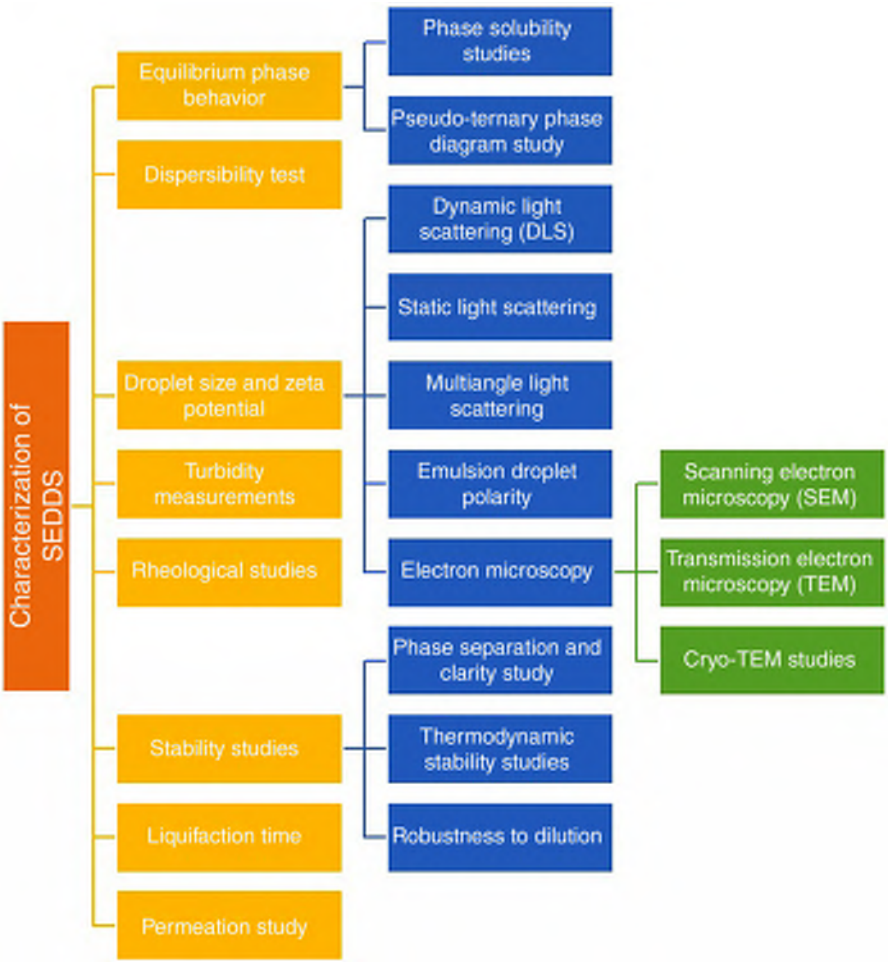



**Figure 14.4. Characterization of SEDS in nutraceuticals.**

## 8 Conclusions

The well-demonstrated preventive and curative potential of many nutraceuticals has remained unrealized because of the limitations faced in their formulation as convenient oral dosage forms. This limitation has been attributed to a number of factors such as solubility, bioavailability, and solubility. A number of attempts have been made to circumvent these bottlenecks by the use of self-emulsifying delivery systems. The technique has shown promising results in terms of dissolution rate enhancement, bioavailability improvement, targeting, extending circulation half-life, and enhanced stability. However, exploration of the SEDS

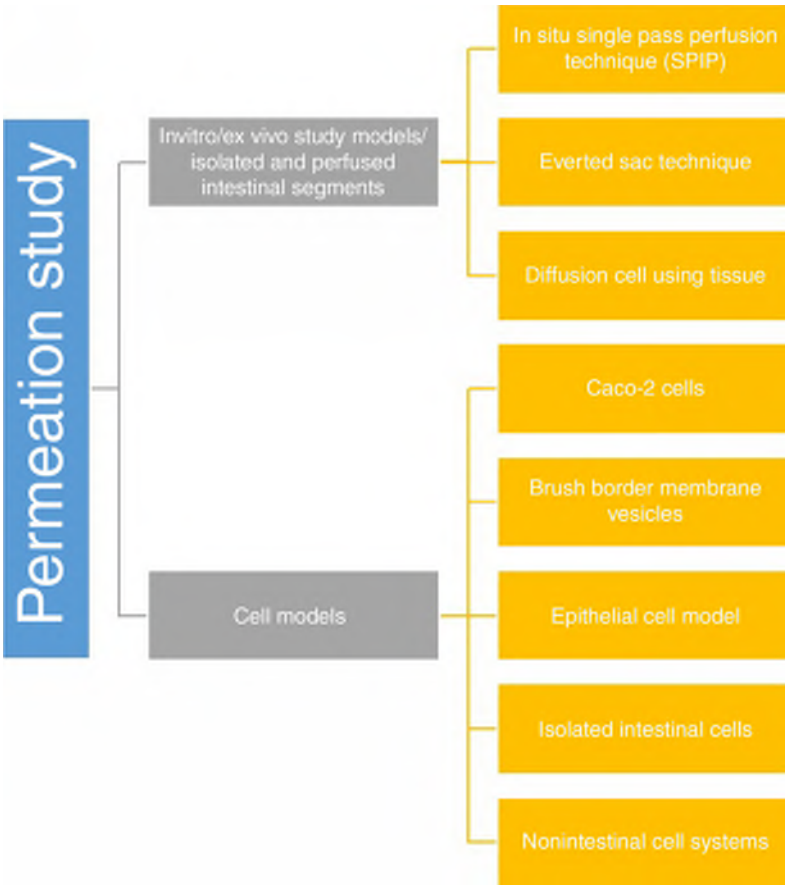



**Figure 14.5. Permeation study of SEDS in GIT** (Singh et al., 2009).

technique using more categories of nutraceuticals is required so that the optimum delivery vehicles would be designed from the available excipients to achieve the desired bioavailability profiles for the bioactives depending on their physicochemical characteristics like molecular size, aqueous solubility, partition coefficient, and inherent stability.

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15

# THE SYNTHESIS AND APPLICATION OF VITAMINS IN NANOEMULSION DELIVERY SYSTEMS

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## 1 Introduction

Nanotechnology is a modern discipline which is developing innovative pharmaceutical carriers such as nanoemulsions to improve drug design, delivery, and efficiency. Nanoemulsions are an example of efficient pharmaceutical nanocarriers. Due to various applications of nanoemulsions in pharmacy and medicine, many improvements in drug discovery have been made. Due to the great importance of vitamins in health and cellular processes, nanoemulsion carrier research in areas of biochemistry, organic and physical chemistry can advance the methodology of vitamin delivery. Vitamin delivery systems that have potential for use are: oil-in-water emulsions, microspheres, self-emulsifying drug delivery systems, lipid nanoemulsions, microemulsions, solid emulsions, water-in-oil in water emulsions, oil-in-water-in-oil emulsions, microspheres containing fat soluble drugs or water soluble drugs. Often an emulsifying or stabilizing agent such as a surfactant or cosurfactant added to nanoemulsions can improve nanoemulsion properties (Tadros and Becher, 1983; Warren et al., 2009; Qingrong et al., 2010). The choice for the formulations of nanoemulsions should be based on the physicochemical properties of the solvents (interfacial tension, viscosity, vapor pressure, and water miscibility) and surfactant. High and low energy methods are used for the emulsification process to form nanoemulsions (Wang et al., 2008). Nanoemulsion

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00015-5

vitamin delivery systems are formulated with surfactants which are safe, nontoxic, nonirritant, and approved for human consumption. The most often used drug carriers are liposomes, polymeric micelles, and phospholipids. The controlled delivery of vitamins using nanoemulsion carriers can be achieved to deliver vitamins to specific locations in the body. Nanoemulsions have improved vitamin solubility and bioavailability, vitamin degradation, and enhanced uptake and release of vitamins. Vitamin delivery systems can also control vitamin concentration and reduce side effects.

The function of a nanoemulsion is to act as a specific drug carrier. A drug carrier is added to the drug and administered to obtain the desired effect in the human body by carrying the drug to a specific target. The drug delivery system, which includes a drug carrier, can allow for enhanced solubilization of hydrophobic drugs to enable their therapeutic effect. Moreover, the human body always produces hydrophilic compounds at the end of many metabolic processes. Hydrophylic and hydrophobic drug/metabolite properties can be improved with the incorporation of nanoemulsion carriers. Drugs, like vitamins, are divided into water and fat soluble groups whose properties may be adjusted. Nanoemulsion vitamin delivery systems are suitable for human and veterinary therapeutic purposes. The advantages and therapeutic potential of nanoemulsions have led to major changes in pharmacy and medicine over the past few years.

## 2 Applications of Nanoemulsions to Drug Delivery

Researchers have developed drug delivery systems to improve drug administration and efficacy. The discovery of a new drug delivery method is expected to provide significant therapeutic benefit for a drug and improve monitoring of treatment. When a drug carrier is added to the drug, the desired effect is to carry the drug to a specific location. Despite many efforts, drug discovery research still produces molecules with poor physicochemical properties (such as lack of solubility in vivo) for which drug delivery systems are necessary (Adair et al., 2010). Many hydrophobic drugs (anticancer, antidepressants, and vitamins) require a solubilization process to enable their therapeutic effect. Therefore, the physicochemical properties of drugs such as solubility, acidity, lipophilicity, and stability are possible to improve using a drug carrier. The use of drug delivery systems in pharmacy now plays a major role in improving drug efficiency (Date and Nagarsenker, 2008; Liang et al., 2013). The major advantages of these systems include

targeted localization and targeted delivery of drugs at a constant kinetic rate. Moreover, drug delivery systems minimize side effects, and enhance efficacy of treatment (Del Valle et al., 2009). Over the years, the form of drugs has changed from simple to highly sophisticated drug delivery systems. However, control of delivery must be achieved to deliver drugs to targets more precisely. Currently, the options available for synthesis of drug delivery systems include various methods which result in emulsion formation. By definition, an emulsion is a dispersion of two liquids which are immiscible. Therefore, one liquid must be dispersed as droplets in the phase of other liquid to create emulsion.

In this chapter, we give an overview of the drug delivery systems that make use of nanoemulsions as vitamin carriers. Vitamins are defined as organic substances that must be obtained as nutrients (McClements, 2012b). Vitamin-derived coenzymes represent types of compounds which are formed from precursors that must be also obtained as a nutrient. Based on current literature, vitamin delivery systems such as nanoemulsions represent a promising approach to a more specific and efficient delivery. Nanoemulsions as drug delivery systems are also suitable for most routes of administration into the human body. The ability to apply nanomaterials as targeted delivery agents for vitamins and drugs is very promising for a wide variety of diseases, including many types of cancer (Adair et al., 2010). Nanoemulsions can be defined as oil-in-water emulsions with mean droplet diameters ranging from 50 to 1000 nm. There is evidence corroborating the notion that, for nanoemulsions which contain oily hydrophobic drugs as the dispersed phase in water, the addition of an emulsifying or stabilizing agent is necessary (Devarajan and Ravichandran, 2011; Lawrence and Rees, 2000). Therefore, the formation of a nanoemulsion is often mixtures of compounds such as oil, water, surfactant, and cosurfactant, with at various concentration ratios (Heuschkel et al., 2008). Due to droplet size, nanoemulsions have higher surface areas and higher free energies than macroemulsions, which make them an effective carrier in drug delivery system. There is sufficient research done on formulation methods which are attractive due to their wide application and stability aspects. Further research supports the formulation of transparent nanoemulsions that exhibit optical transparency at high droplet volume fractions, strong elasticity, and enhanced diffusive transport (Kreilgaard, 2002). However, the applications of nanoemulsions are still limited by the instability. Nanoemulsion stability is the function of concentration of surfactant and cosurfactant and type of oil (Date and Nagarsenker, 2008). Carrier-based drug delivery systems are recognized as an alternative approach to improve the

bioavailability of drugs. Recent advances in drug delivery design provide the ability to use the optically transparent nanoemulsions. In all types of nanoemulsions, the surfactants and cosurfactants used to stabilize such systems are nonionic, cationic, or anionic surfactants (Shinoda and Lindman, 1987; Shinoda et al., 1991; Attwood et al., 1992; Aboofazeli et al., 1994; Angelo et al., 1996). As an additional benefit, the release profile of encapsulated drugs can be tailored by choosing an appropriate encapsulation material. Studies showed that the use of alcohol additions to emulsions provide an optimum solubility for vitamins and enhance evaporation from the completed form of emulsion. The research provides support that oil-in-water emulsions could be a core of nonpolar material suspended in a polar solvent. In nanoemulsion research, the most often studied carriers include liposomes, phospholipids, nonionic lipids, surfactants, polymeric micelles formed by self-assembly of charged or neutral block copolymers, and nano- and microparticulate carriers formed by various processes (Lasic, 1996). In addition to this, the available evidence in the literature suggests that nanoemulsions can persist over many months or years due to the presence of a stabilizing surfactant that inhibits the coalescence of the small sized droplets (Walstra and Becher, 1996). The concentration of surfactant and cosurfactant in the solution of emulsion can also play an important role in determining the saturation radius of the droplets resulting from emulsification. A cosurfactant is commonly used to lower the interfacial tension and fluidize the interfacial surfactant in nanoemulsions (Kreilgaard, 2002; Kogan and Garti, 2006; Heuschkel et al., 2008). Structurally, nanoemulsions are composed of two phases and their effectiveness in drug carrier research depends on the phase ratios (Pershing, 1992; Pershing et al., 1993; Kreilgaard, 2002; Heuschkel et al., 2008). On the basis of the evidence currently available, medium chain length alcohols have the effect of further reducing the interfacial tension in nanoemulsions (Pershing et al., 1990; Eccleston and Swarbrick, 1994; Lawrence, 1996). Alcohols with low molecular weight are more mobile than long chain alcohols. Low molecular weight alcohols can evaporate faster from emulsions (Eccleston and Swarbrick, 1994; Lawrence, 1996). Liposomes have many advantages as drug delivery carrier agents. They are biocompatible and biodegradable, and they can be functionalized with surface molecules such as poly(ethylene glycol) to increase their circulation time or with targeting ligands to direct their attachment to specific cells.

It is difficult to characterize these systems, as a nanoemulsion is in a dynamic state and phases. As these nanoemulsion systems have water and oil phases, both hydrophilic and lipophilic drugs

can be delivered using nanoemulsions (Friedman et al., 1995; Amselem and Friedman, 1998; Labor et al., 2000; Heuschkel et al., 2008). For the encapsulation of hydrophilic species, numerous studies have been done over the past few years on the generation of aqueous core nanocapsules dispersed in an aqueous bulk phase. Nanoemulsions possess specific kinetic stability and optical transparency and may affect the permeability of drugs in the skin (Clares et al., 2014). Transdermal drug delivery offers many benefits including noninvasiveness, accessibility, avoidance of metabolism, and controllable drug delivery rates (Azeem et al., 2009). In this case, the components of nanoemulsions serve as permeation enhancers during transdermal drug delivery (Gasco et al., 1991; Liu et al., 1991; Kim et al., 1992; Ktistis and Niopas, 1998; Tenjarla, 1999; Kreilgaard et al., 2000). This generates an increased thermodynamic activity toward the skin (Trotta, 1999; Kreilgaard et al., 2000; Alvarez-Figueroa and Blanco-Méndez, 2001). Nanoemulsions improve the transdermal delivery of several drugs over the conventional topical preparations (Ktistis and Niopas, 1998; Kreilgaard et al., 2000) and gels (Kriwet and Müller-Goymann, 1995).

Many studies have reported that using nanoemulsions as vehicles can enhance the transportation of drugs through the skin over conventional topical products such as ointments, gels, and creams (Fouad et al., 2013; El Maghraby et al., 2013; Mostafa Moslehi et al., 2014). Nanoemulsion with small-sized droplets allow effective transport of the active agents to skin, increasing drug penetration (Fouad et al., 2013; El Maghraby et al., 2013; Mostafa Moslehi et al., 2014).

On the other hand, nanoemulsions have also been shown to penetrate through the hair follicles (Wu et al., 2001; Izquierdo et al., 2007). Nanoemulsions demonstrated great potential in nasal drug delivery, increasing the absorption and the bioavailability of many drugs. The nasal mucosa is an attractive site for vaccination, as it is very accessible and low on proteolytic enzymes compared to the oral route (Slütter et al., 2010). Therefore, nasal delivery of drugs has become a growing area of interest for drug administration (Comfort et al., 2015). The solubility of hydrophobic drugs is significantly enhanced by coadministration with lipids in oral or nasal formulations. Drugs coadministration with lipids enhances drug solubilization and improves absorption. A solvent is always a free vehicle in the emulsion droplets of submicron size and is more efficacious in terms of percutaneous absorption. In addition, the large internal hydrophobic core of emulsion droplets allows high solubilization capacity for water insoluble drugs. Topical application of emulsion containing a mixture of lidocaine and prilocaine

is promising and was studied clinically (Tamilvanan, 2004). Recently, a novel pressurized aerosol system has been devised for pulmonary delivery (Lovelyn and Attama, 2011). Oil-in-water emulsion is being studied as a drug carrier for lipophilic drug delivery to the eye (Tamilvanan, 2004). In summary, nanoemulsions can deliver the drug orally, to skin or mucous membranes. The transport of lipophilic compounds is another feature of nanoemulsions used extensively in cosmetics (Amselem and Friedman, 1998). In a study performed by Acharaya, coconut oil, polyoxyethylene 2-cetylether, isopropanol, and ethanol were used to form a stable nanoemulsion (Acharya et al., 2002). In other studies, antimicrobial nanoemulsions were formed by using oil-in-water droplets that range 200–600 nm. The positive charge of the nanoemulsion and the negative charge of bacteria causes the interaction between them. The energy released during this reaction results in cell lysis and bacteria death (Tamilvanan, 2004; McClements, 2012a; Pouton, 1985). To summarize, in both types of nanoemulsions, oil in water or water in oil, the most common droplet size is between 100 and 500 nm (Ravi and Padma, 2011). Generally, the droplet sizes less than 100 nm flow much easily (Heuschkel et al., 2008). The droplet sizes usually below 140 nm in diameter makes the nanoemulsion a transparent liquid (Mitchell and Ninham, 1981; Carlfors et al., 1991; Tenjarla, 1999). The transparent behavior and small-sized droplets are achieved by adding a cosurfactant, which helps to form a thermodynamically stable nanoemulsion. However, ultrasonicators can be used to achieve the desired size range 140 nm droplet. During emulsion formulations, oil-and-water soluble ingredients are generally dissolved in the oil and aqueous phases, respectively. Oil and aqueous phases are adequately heated and then mixed (Malmsten et al., 1999). Nanoemulsions with nanoscale droplet size possess stability, which helps against droplet sedimentation. The nanoscale droplets are formed due to external force, which results in a lipophilic core separated from the surrounding aqueous phase by a monomolecular layer of emulsifier. Nanoemulsion has also shown great potential for carriers of lipophilic and hydrophilic drugs. For efficient delivery of water insoluble drugs, the formation of complex soluble in water is a challenge (Morales et al., 2003; Dixit and Nagarsenker, 2008). Another line of important evidence is that the methods of emulsification include high pressure homogenizations, jet dispersion, microfluidization, sonication methods, and ultrasonic systems, phase inversion methods with temperature techniques and phase inversion composition methods. The preparation of nanoemulsions requires high pressure homogenization. Emulsion stability is usually monitored by incubating the nanoemulsions at 4 and 25°C

## Table 15.1 The General Methods of Emulsion Formulation

| Method | |
|---|---|
| High pressure Homogenization | The example for high pressure homogenization method is jet disperser technique. The method uses high pressure to create an emulsion with nanosized droplets by flowing the liquid through micro channels (Harshal and Jyotsna, 2013). Microfluidization is a homogenization technique where oil and water are combined together to yield a nanoemulsion (Friedman et al., 1995; Fang et al., 2001). |
| Sonication method | The droplet size of emulsions is reduced with the use of sonication (Shinoda and Saito, 1968). The sonicator generates mechanical vibration and is able to produce physical and chemical changes such as emulsification (Walstra and Becher, 1996). |
| Phase inversion method Temperature technique | In the phase inversion temperature technique, temperature is used to dissolve oil in a microemulsion (Shinoda and Saito, 1968; Sheikh, 2007). A transitional inversion takes place from water in oil to an oil in-water morphology. In the phase inversion composition method nanoemulsions with droplet size (~50 nm) can be generated by the stepwise addition of water into solution (Allouche et al., 2004). |

under sealed conditions for 3–6 months. The incorporation of surfactants to the nanoemulsions can be done by using high energy equipment during manufacturing of nanoemulsions (Sintov and Shapiro, 2004). Table 15.1 lists the methods of emulsion formulation discussed in this paragraph (Date et al., 2010). This table is focused on recent advances in the formulation, characterization, and application of nanoemulsions in drug delivery.

As reviewed in this chapter, a variety of emulsions can be formed. All types of emulsions offer a wide number of applications in interdisciplinary fields of research. Low vitamin solubility in water is associated with poor absorption. Controlled vitamin delivery can be achieved by using drug delivery systems. This advanced methodology improves bioavailability by preventing vitamin degradation, enhancing uptake and release of vitamins, maintenance of vitamin concentration, and reduction of side effects, improving site-specific delivery of vitamins and improving therapeutic outcomes of vitamin administration. Among the types of useful emulsions with potential for the use in controlled vitamin delivery systems are: oil-in-water emulsion, microspheres, water-in-oil emulsions, self-emulsifying drug delivery systems, lipid

nanoemulsions (nanospheres), microemulsions, solid emulsions, water-in-oil-in-water emulsion, oil-in-water-in-oil emulsions, microspheres containing fat soluble drugs, microspheres containing soluble in water drugs. Oil-in-water emulsions are prepared for poorly soluble drugs. Oil-in-water emulsions serve as carriers of many soluble in oil drugs (Lukyanov and Torchilin, 2004). For example, therapeutic application of emulsions has been employed to deliver drugs by dissolving drugs in soybean oil. Thus soybean oil emulsions are one type of oil in water emulsions (Takenaga, 1996; Mizushima, 1996). Oil-in-water emulsions containing drugs are called lipid microspheres (Rokstada et al., 2014). Water-in-oil emulsions can serve as carriers for water soluble drugs by dissolving drugs in a water phase. However, the water-in-oil emulsion is limited because of high viscosity of oil, which makes transport difficult. Self-emulsifying drug delivery systems are also known. These systems consist of oils and surfactants and cosolvents. Lipid nanoemulsions are often prepared from oil and egg yolk lecithin.

In the literature, there are three main types of nanoemulsion formulations: (1) self-emulsifying, (2) self-emulsifying drug delivery system, and (3) self-nanoemulsifying drug delivery systems. Self-emulsifying formulations are mixtures of oil, surfactant, cosurfactant, solvents, and cosolvents. These compounds result in a transparent isotropic solution, which emulsify under gentle agitation. Self-emulsifying drug delivery system is used to solubilize and deliver the hydrophobic drugs. Self-nanoemulsifying drug delivery systems are composed of oil, surfactant, cosurfactant, and drugs that form fine oil in water nanoemulsion. Advantages of the use of self-emulsifying drug delivery systems and self-nanoemulsifying drug delivery systems in drug delivery systems are listed in Table 15.2.

## Table 15.2 Advantages of the Use of Self Emulsifying Drug Delivery Systems and Self Nano-Emulsifying Drug Delivery Systems

| Advantages of Self-Emulsifying Drug Delivery Systems | Advantages of Self-Nanoemulsifying Drug Delivery Systems |
|---|---|
| Absorption of poorly water soluble drugs | Protection of sensitive drug substances |
| Easy absorption of drug | Good bioavailability |
| Ease process of preparation | Localized targeting of drugs |

Nanoemulsions are commonly used in cosmetics for the delivery of active ingredients to skin layers. Due to advances in nanoemulsion technology, it is possible to alter the optical and physical properties of particles sized 15–3500 nm (Tanojo et al., 1997).

While it has been possible to administer water soluble vitamins by injection, practical procedures for injecting water insoluble vitamins such as vitamins A, D, E, and K are needed. Vitamin concentrations in the effluents are the result of a complex interaction of several factors, including flow rates, tubing arterials and sizes, intensity of light exposure, environmental humidity and temperature as well as the relative content of each vitamin. Therefore, the role of the drug delivery systems in pharmacy has undergone major changes in the past few years (Hussein et al., 2009; Bhandari and Van Berkel, 2012; Hartman and Shamir, 2015).

Oil-in-water nanoemulsions have been considered to be efficient delivery systems for hydrophobic compounds by dispersing the lipid phase as a colloidal dispersion. After encapsulation, the lipophilic bioactive components can be easily incorporated into foods and beverages by increasing the water dispersibility, protecting them from degradation, oxidation, or interactions with the other food ingredients (Jian, 2014). Nanoemulsions are characterized by scattering of visible light. The light can be refracted many times through droplets of nanoemulsions.

The number of papers reporting nanoemulsions for vitamin delivery published during 1947–2014, has increased 62 times. Fig. 15.1 presents the data collected using PubMed database 2015.

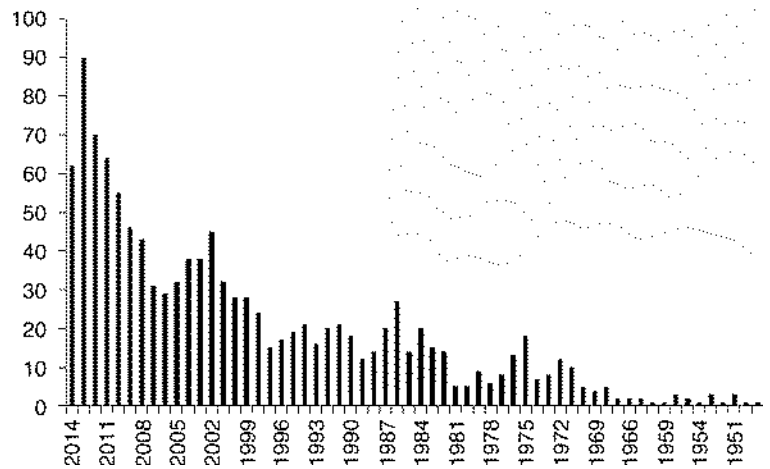


**Figure 15.1.** The number of papers reporting nanoemulsions for vitamin delivery by year.

Nanoemulsions offer several advantages for the delivery of drugs and are receiving increasing attention as drug carriers. Current applications of nanoemulsions in drug delivery include:

- Nanoemulsions applications for oral drug delivery
- Nanoemulsions in vaccines and immunotherapeutics delivery
- Nanoemulsions in cell culture technology
- Nanoemulsions applications in cosmetics
- Nanoemulsions in ocular drug delivery
- Nanoemulsions as a carrier for transdermal delivery
- Nanoemulsions and intranasal drug delivery
- Nanoemulsions for pulmonary drug delivery
- Antimicrobial nanoemulsions

## 3 Vitamins and Their Role

In 1795, lime juice was used to prevent scurvy in sailors. In 1930, the active compound vitamin C (ascorbic acid) was isolated and proven to be essential dietary component. In 1827 English physician William Prout defined the three essentials of the human diet, which are fats and oils, carbohydrates, and proteins. In 1906, an English biochemist, Frederick Hopkins, discovered that mice fed on a pure diet of the three essentials could not survive unless they were given supplementary small amounts of milk and vegetables. A Polish scientist, Casimir Funk, used the term vitamin to describe substances from rice husks that cured beriberi. Funk first believed that the vitamins were chemically related amines, thus *vita* (life) plus amines. Between 1925 and 1955, the known vitamins were all isolated and synthesized. Research continues today on the function of the various vitamins (Wagner and Folkers, 1977). The major period of discovery began in the early 19th century and ended at the mid-20th century. Table 15.3 lists vitamins discussed in this chapter and years of discovery (Funk, 1912; Bing, 1937; Wagner and Folkers, 1977).

There is a rapidly growing opinion on the role of vitamins. From the early years of the 20th century vitamins played an important role in transforming the pharmaceutical industry. As the commercial applications of nanotechnology have increased over the past several years, there has also been an increase in the potential uses of nanoparticles in cosmetic products as well as other pharmaceutical products.

Vitamins are essential for various roles throughout the human body. Thus, we must obtain them from the foods we eat, or via vitamin supplements. The supplements are synthetic or natural substances such as pills, tablets, capsules, wafers, powders, or liquids.

## Table 15.3 Discovery of Vitamins

| Year of Discovery | Vitamin |
|---|---|
| 1910 | Vitamin $B_1$ (Thiamine) |
| 1920 | Vitamin C (Ascorbic Acid) |
| 1920 | Vitamin D (Calciferol) |
| 1920 | Vitamin $B_2$ (Riboflavin) |
| 1922 | Vitamin E (Tocopherol) |
| 1931 | Vitamin $B_5$ (Panthenic acid) |
| 1931 | Vitamin $B_7$ (Biotin) |
| 1936 | Vitamin $B_3$ (Niacin) |

In general, vitamins are divided into two groups: eight are soluble in water and they are thiamin, riboflavin, niacin, $B_6$, pantothenic acid, biotin, folic acid, and vitamin C. The next four are fat soluble vitamins, which include vitamins A, D, E, and K. Water soluble vitamins need regular replacement in the body, travel freely through the body, and excess amounts usually are excreted by the kidney. As a result, vitamins soluble in fat are stored in the liver and fatty tissues, and are eliminated much more slowly than vitamins soluble in water. Vitamins soluble in fat are more likely to be toxic.

Chemists have always been interested in improving nutrition (Martini and Phillips, 2009). Many new branches of research were developed to overcome agricultural and food problems. Therefore, much attention has been given to the vitamin and mineral levels of fruits and vegetables. Many researches have shown that different climates, soil, and fertilizers greatly influence the amounts of carotene, riboflavin, ascorbic acid, and thiamine in plant material. Table 15.4 lists water soluble vitamins and Table 15.5 lists fat soluble vitamins.

Vitamins soluble in fat are stored in the body's cells and are not excreted as easily as soluble in water vitamins. The absorption efficiency of lipophilic vitamin is highly variable and is dependent on a range of factors.

Absorption of lipophilic vitamins can be enhanced from delivery systems with high bioavailability. Table 15.6 lists the differences between water and fat soluble vitamins.

All essential vitamins needed for normal metabolism and regulation of cell functions are listed and discussed. These vitamins can be found in food sources as well as supplemented materials. Fat

## Table 15.4 Water Soluble Vitamins

| Nutrient | Sources |
|---|---|
| Vitamin $B_1$ (Thiamine) | Natural sources are: pork, whole-grain, enriched breads, cereals, legumes, nuts, and seeds. |
| Vitamin $B_2$ (Riboflavin) | Widespread in foods, also produced in intestinal tract by bacteria. |
| Vitamin $B_3$ (Niacin) | Natural sources: sweet potatoes, squash, and corn. |
| Vitamin $B_5$ (Panthenic acid) | Natural sources: fruits, vegetables, especially citrus fruits, vegetables in the cabbage family, cantaloupe, strawberries, peppers, tomatoes, potatoes, lettuce, papayas, mangoes, and kiwifruit. |
| Vitamin $B_6$ (Pyridoxine) | Natural sources are: eggs, milk, cheese, milk products, meat, fish, potatoes, bananas, meat, fish, poultry, liver, soybeans, chickpeas, lentils, pistachio, nuts, and sunflower seed. |
| Vitamin $B_7$ (Biotin) | Natural sources: sweet potatoes, yogurt peanuts, almonds, eggs, liver, soy protein egg yolk, soybeans, and nuts. |
| Vitamin $B_9$ (Folic acid) | Natural sources: asparagus, broccoli, cabbage, cauliflower, and egg yolk. |
| Vitamin $B_{12}$ (Cobalamins) | Natural sources are: green vegetables and legumes, seeds, orange juice, milk, cheese, yogurt, fortified soy or rice, meat, fish, liver, eggs, and soy products. |
| Vitamin C (Ascorbic acid) | Natural sources are: Citrus fruits, oranges, grapefruits, kiwi, strawberries, mangoes, papaya, peppers, broccoli, cabbage, tomatoes, fish, cereals, vegetables, and peanut butter. |

## Table 15.5 Fat Soluble Vitamins

| Nutrient | Sources |
|---|---|
| Vitamin A (and its precursor β-carotene) | Vitamin A (retinol): milk, cheese, cream, butter, eggs, liver fortified margarine, eggs, and liver.<br>β-carotene sources are: dark green vegetables; dark orange fruits (apricots, cantaloupe) and vegetables (carrots, winter squash, sweet potatoes, pumpkin). |
| Vitamin D | Sources are: egg yolks, liver, fish, fortified milk, margarine. When exposed to sunlight, the skin can make vitamin D. |
| Vitamin E | Found in polyunsaturated plant oils (soybean, corn); vegetable oils, leafy green vegetables, avocados, sunflower seeds, some nuts, peanut butter meat, and fish. |
| Vitamin K | Found in leafy green vegetables and vegetables in the cabbage family, milk; also produced in intestinal tract by bacteria. |

## Table 15.6 The Differences in Properties between Water Soluble and Fat Soluble Vitamins

| Process | Water Soluble | Fat Soluble |
|---|---|---|
| Absorption process | The changes in absorption of water soluble vitamin can be directly observed in blood. | In the case of absorption of fat soluble vitamins, the absorption can be directly observed in lymph. |
| Internal transport | The transport of water soluble vitamins does not require a carrier in the body. | The transport of fat soluble vitamins require a carrier in the body. |
| Storage and circulation of vitamins | Free circulation is observed for water soluble vitamins. | Fat soluble vitamins are stored in fat. |

soluble vitamin do not need to be consumed as often as soluble in water vitamins. Vitamins are usually consumed via food or dietary supplements (Sizer and Ellie, 2008). Many natural hydrophobic liquid oil products are obtained from plants by hydrodistillation or solvent extraction. These compounds have been used for a wide variety of purposes including cosmetics, nutrients, and medicines (Edris, 2007; Dorman and Deans, 2000; Jones and Herbs, 1996). In a review paper by Adrianza de Baptista, relationships between nutrition and cancer were discussed. The data showed that the carcinogenesis process can be influenced by nutrition. Also, deficiency of macro and micronutrients correlate with tumor location and stage (Adrianza de Baptista and Murillo Melo, 2014).

## 4 Vitamin A

According to presented research, vitamin A (called retinol) is an essential micronutrient. This vitamin is required for normal vision, reproduction, embryonic development, cell and tissue differentiation, and immune function (Lammer et al., 1996; Wiegand et al., 1998; Labor et al., 2000). Dietary forms of vitamin A are found as retinal esters and carotenes (α-carotene, β-carotene, γ-carotene and β-cryptoxanthin (Johnson et al., 1992). The numbers of the natural sources of vitamin A are animal sources such as eggs, meat, cheese, milk, liver, kidney, cod, and halibut fish oil and plant sources such as sweet potatoes, cantaloupe, pink grapefruit, apricots, broccoli, spinach, and pumpkin (Table 15.7). The deficiency of vitamin A is influencing resistance to infections and extremely dry skin, hair, or nails.

## Table 15.7 Foods High In Vitamin A

| Foods High In Vitamin A in Daily Values (DV) Units | |
|---|---|
| Carrot (1 raw) | 410% DV |
| Mango (1 raw) | 160% DV |
| Sweet Potatoes (1/2 c) | 150% DV |
| Cantaloupe (1 c) | 100%DV |
| Vegetable soup (1 c) | 60% DV |

This data also suggests that vitamin A metabolism has two biological functions: (1) providing appropriate retinoids to tissues throughout the in vivo production of retinoic acid, (2) and providing retinol to the retina for adequate production of 11-cis retinal (Sauvant et al., 2012). Vitamin A is necessarily linked to protein metabolism. The concentrations of vitamin A influences vision, cellular differentiation, organ development during embryonic and fetal growth (Hathcock and Rader, 1990). Current research indicates that Vitamin A has an essential role in normal differentiation and maintenance of epithelial cells. Also, adequate immune function depends on vitamin A. However, as shown in research, Vitamin A cannot be synthesized and has to be provided by food. Vitamin A is soluble in fat and poorly soluble in water and is unstable during food processing or storage (Sauvant et al., 2012). The research papers illustrated that diets rich in vitamin A and β-carotene can lower the risk for cancer and prevent neurodegenerative diseases. This is associated with the use of vitamin A in food supplements (Krasinski et al., 1989). In 2007, a study showed that the self-nanoemulsified drug delivery vitamin A formulations increased the drug absorption compared to the oily drug solution. The research demonstrated that self-nanoemulsified drug delivery vitamin A optimized formulations, either as filled capsules or as compressed tablets (Taha et al., 2007). Yoshida and coworkers reported stability studies of vitamin A in three different environments that were the following emulsions (1) oil in water, (2) water in oil, and (3) oil in water in oil. The reported stability of vitamin A at 50°C after 4 weeks were: 56.9% in oil in water in oil, 45.7% in water in oil, and 32.3% in oil-in-water emulsion (Yoshida et al., 1999). Dizaj developed the oil-in-water stable emulsion of vitamin A for a drug delivery system. The emulsion consists of sunflower oil, surfactant, vitamin A palmitate, different cosurfactants, and water. The cosurfactants studied were anhydrous glycerol, sucrose, ethanol, and 1-propranol. Sucrose was

selected as the best cosurfactant for this emulsion (Dizaj, 2013). In recent studies Thongchai used a high performance liquid chromatographic procedure for separation of retinol and α-tocopherol from avocado oil. The values of retinol and α-tocopherol in avacodao oil in water nanoemulsion were found to be 0.002 mg $g^{-1}$ and 0.033 mg $g^{-1}$, respectively (Thongchai, 2014). A research review by Chen showed that retinoic acid used for cancer treatment is promising for suppression of breast, ovarian, lung, bladder, skin, and prostate cancers. Retinoic acid is a metabolite of vitamin A (Chen et al., 2014). Priyadarshani reviewed research regarding the anticancer functions of carotenoids, which are precursors of vitamin A. Moreover, the potential role carotenoids play in prevention of cancers, coronary heart diseases, age-related macular degeneration, and cataract were discussed (Priyadarshani, 2015).

## 5 β-Carotene

Carotenoids belong to a large group of tetraterpenoid organic pigments. β-carotene is crystalline with poor uptake and low bioavailability. β-carotene is one of many hundreds of food carotenoids (Mathews-Roth, 1986; Simon et al., 2013). Only a few β-carotenes have been studied in relation to their impact on human physiology. First, β-carotene is insoluble in water and only slightly soluble in oil at room temperature. β-carotene is the most abundant form of provitamin A in fruits and vegetables (Bendich, 1988; Hathcock et al., 1990; Sauvant et al., 2012).

The literature shows that diets rich in carotenoids are decreasing the risk of chronic diseases. Properties of β-carotene oil-in-water nanoemulsions were investigated by Liang and coworkers. β-carotene retention in nanoemulsions was higher compared to that of the β-carotene dispersed in bulk oil (Liang et al., 2013). It was also observed that β-carotene possess the chemical abilities to quench singlet oxygen and to inhibit peroxyl free radical reactions (Qian et al., 2012; Diplock, 1995; Xu et al., 2014).

The aim of work done by Salvia-Trujillo was the incorporation of β-carotene into foods. For this purpose, the author used oil composition (medium-chain triglyceride to long-chain triglyceride ratio and total carrier oil concentration. For the study the variations of oils compositions, different ratios of the long-chain triglyceride was used. The results showed that the total fraction of triacylglycerols converted to free fatty acids decreased. This behavior was observed when the percentage of long-chain triglyceride ratio within the lipid phase increased. This result suggests the influence of particle size on lipid digestion and β-carotene bioaccessibility (Salvia-Trujillo et al., 2013).

## 6 Vitamin D

In 1913, McCollum and Davis for the first time discovered vitamin D (Steenbock, 1924). To date, we know that vitamin D helps the body absorb calcium and is an essential for bone formation. In general, vitamin D maintains calcium and phosphorus homeostasis together by increasing intestinal absorption of calcium and phosphorus by affecting the renal reabsorption of phosphorus and to a lesser extent calcium (Chesney, 1989). Vitamin D is available in two forms which are ergocalciferol (known as vitamin $D_2$) and cholecalciferol (known as vitamin $D_3$). Both vitamin forms, $D_2$ and $D_3$, are available as supplements that can be taken to reduce vitamin D deficiency. However, vitamin $D_3$ is more effective in sustaining vitamin D levels in the blood (Marx et al., 1989; Trang et al., 1998). Riverin and coworkers review of research indicates that vitamin D supplementation is related to the reduction of asthma exacerbations (Riverin et al., 2015). Vitamin D has been described as a prohormone or sunshine-dependent vitamin. Some dietary vitamin $D_2$ comes from plants. It is proved that the largest amount of the vitamin $D_3$ is located in fish liver oils, milk, eggs, and liver (Fomon et al., 1966; Hathcock et al., 2007). However, the sun is the most recognized source of vitamin D. Vitamin D can be safely administered through topical or oral forms. Topical vitamin D is used to treat skin disorders and may also protect the skin from UV induced damage. Studies have shown that topical vitamin D can restore permeability and the skin's natural antimicrobial barriers that are often disrupted by topical corticosteroid (Marx et al., 1989; Trang et al., 1998). After a review of literature comparing the different routes of vitamin D administration, including oral, transdermal, topical, and injections, it was found that oral administration of vitamin D significantly improves serum vitamin D levels. The spontaneous emulsification method is simple and inexpensive to carry out and therefore has great potential for forming nanoemulsion based delivery systems for food, personal care, and pharmaceutical applications (Gloth et al., 1995; Guttoff et al., 2015). In a recent study, encapsulation of vitamin D in lipid-based carrier systems was investigated by using sonication techniques. The system was used to preserve native properties against oxidation. Vitamin D was used together with nanoliposomes to prepare thin film hydration (Mohammadi et al., 2014). The authors of a systematic review of vitamin D summarized that parathyroid hormone level response is reduced due to vitamin D supplementation and calcium administration (Moslehi et al., 2015). A paper by Saraff reviews the various factors that influence the synthesis of vitamin D due to exposure to sun. Also, the dietary supplementation to

achieve adequate vitamin D levels for optimal bone health is discussed (Saraff and Shaw 2016). On the basis of a review of the literature, vitamins such as vitamin A, vitamin B complex, vitamin C, vitamin D, vitamin E, and vitamin K are essential nutrients for human metabolism. Vitamin D deficiency is one of the most common nutritional deficiencies. Vitamin D, the sunshine vitamin, is essential for bone health and the prevention of chronic diseases, including autoimmune diseases, cancers, heart disease, type II diabetes, and infectious diseases (Holick, 2013). Research showed that vitamin D in the diet with antioxidant supplementation is used in breast cancer prevention. Several studies have shown that a high proportion of women at risk for breast cancer or affected by the disease have deficient vitamin D levels, that is, 250H-D $< 20$ ng $mL^{-1}$ or 50 nmol $L^{-1}$ (Lazzeroni et al., 2011). The research showed that to prevent vitamin D deficiency, all infants should receive 400 IU/day of vitamin D. Based on research, children and adolescents age $>1$ year may require 600 IU/day of vitamin D. All newborns should receive 1 mg of vitamin K at birth to prevent vitamin K deficiency bleeding (Lauer and Spector, 2012). Higher serum levels of the main circulating form of vitamin D are associated with substantially lower incidence rates of colon, breast, ovarian, renal, pancreatic, aggressive prostate and other cancers. Based on research, 40–60 ng $mL^{-1}$ of vitamin D would prevent approximately 58,000 new cases of breast cancer and 49,000 new cases of colorectal cancer each year (Garland et al., 2009).

## 7 Vitamin E

Vitamin E is of plant origin, nonpolar hydrophobic compound that has been shown to be essential to human health in several ways. The biological role of vitamin E is to protect polyunsaturated fatty acids and low-density lipoproteins from oxidation by free radicals. Vitamin E is a peroxyl radical scavenger (Frankel and Finley, 2008). The name of vitamin E refers to a family of eight naturally occurring homologues. There are two groups of vitamin E: tocopherols and tocotrienols. Tocopherols are composed of four known forms with a saturated phytol side chain: the α-, β-, γ-, and Δ-tocopherols, the tocotrienols are composed of six forms with 3 double bonds in the side chain: α-, β-, γ-, and Δ -tocotrienols. In vivo vitamin E can be absorbed efficiently during regular fat absorption. Conditions for absorption can be summarized in four steps: (1) efficient emulsification, (2) solubilization within mixed bile salt micelles, (3) uptake by enterocytes, and (4) secretion into the circulation via the lymphatic system

(Gallo-Torres, 1970; Reboul et al., 2006). Morais and coworkers characterized encapsulation efficiency of vitamin E in the emulsion as a function of time and evaluated formulation stability (Morais Diane and Burgess, 2014). A growing number of studies indicate that vitamin E can effectively induce apoptosis in cancer cells suggesting a potential role for antioxidants. Recent review by Neophytou and Constantinou provides a summary of the anticancer effects of the vitamin E isoforms and an overview of the various formulations developed to improve their efficacy (Neophytou and Constantinou, 2015). In study by Peng et al. the mechanisms of drug transport by vitamin E loaded silicone hydrogel contact lenses were investigated. The results show that drug molecules adsorb and diffuse along the surface of the vitamin E barriers, reducing the barrier effect for both hydrophobic and hydrophilic drugs (Peng et al., 2012). In the study by Laouini and coworkers, liquid dispersions encapsulating vitamin E were prepared using various methods based on membrane contactor. Vitamin E was chosen as a hydrophobic drug for the preparation of drug-loaded micelles. The dispersions were nebulized and aerodynamic characteristics of the generated aerosols were assessed (Laouini et al., 2014). The aim of the study by Alqahtani and coworkers was to evaluate the in vitro and in vivo performance of γ-tocotrienol incorporated in a self-emulsifying drug delivery system (Alqahtani et al., 2014). The objective of another study by Mehmood was to prepare canola oil based vitamin E nanoemulsions by using food grade mixed surfactants to replace some concentration of nonionic surfactants with natural soya lecithin and to optimize their preparation conditions (Mehmood, 2015). Physicochemical stability of vitamin E in nanoemulsions were studied by fabrication of by a low energy emulsification method known as emulsion phase inversion (Hategekimana et al., 2015). In summary, vitamin E is clearly essential to life (Bjelakovic et al., 2007; Morais Diane and Burgess, 2014). Accordingly, it appeared that diffusion across the interfacial film was the rate-limiting step for in vitro release from these nanoemulsions. This demonstrates that sustained release of vitamin E was observed and could be explained based on the high partition coefficient and on the nanoemulsion interfacial film proprieties (Kim and White, 1996). This demonstrates that oil-in-water nanoemulsions is finding increasing use as delivery systems to encapsulate lipophilic bioactive components in functional food, personal care ingredients, and pharmaceutical products. Gong et al. (2012) worked on nanoemulsion formulations of natural vitamin E to increase oral bioavailability. The natural vitamin E nanoemulsion was prepared by a modified emulsification technique. This demonstrates that the overall results have promising potential

for clinical application (Gong et al., 2012). The authors of several references have indicated future research using nanoemulsions with polyphenolic compounds (Covas, 2007; Floury et al., 2003; Huang et al., 2010; Perona et al., 2009; Marquardt et al., 2013).

A research paper published by Chiua presents a method of determining the stability of vitamin E emulsions by using anionic, zwitterionic, and cationic surfactants in the presence and absence of NaCI. The addition of NaCl increased the stability of vitamin E (Chiua and Jianga, 1999). In the most recent study, vitamin E emulsions were formed by adding vitamin E acetate in an octenylsuccinic starch solution with water. The result showed that lower total concentrations of starch and oil and lower ratio of oil to starch resulted in more stable emulsions (Qiu et al., 2015).

A study performed by Yang showed the influence of oil phase composition (vitamin E to medium chain triglyceride ratio), aqueous phase composition (glycerol to water ratio), and surfactant type on the size of the droplets produced by high pressure. The results showed that due to high viscosity, emulsions can be formed when ≥20% medium chain triglycerides are used (Yang and McClements, 2013). Recently it has been shown that emulsion phase inversion methods can be used to produce food-grade nanoemulsions enriched with vitamin E acetate. The method is titrating water into an oil and surfactant. The first formed water-in-oil emulsion then inverts into an oil-in-water emulsion (Mayer et al., 2013). Vitamin E supplementation showed differences in antioxidant effect due to variations in dose, route of administration and treatment duration (Bessell et al., 2015).

To prepare vitamin E delivery systems from vitamin E acetate and lecithin or quillaja saponin, high pressure homogenization was used. In these studies, factors such as pH, temperature, ionic strength, and stability were also investigated. The results showed that emulsions were unstable at $> 100$ mM sodium chloride for lecithin and $\geq 400$ mM sodium chloride for quillaja saponin (Ozturka et al., 2014). Vitamin E nanoemulsifying was used for improved delivery of cyclosporine A. The solubility and stability of emulsion were evaluated in a series of oils and surfactants. The results were optimized (Jain et al., 2015). Natural forms of vitamin E scavenge reactive nitrogen and oxygen species, inhibit cyclooxygenase- and 5-lipoxygenase-catalyzed eicosanoids. Research evidence implicates oxidative damage in development of various diseases and as the major fat soluble vitamin E is protective against oxidative damage. Also, natural forms of vitamin E have influence on metabolism, and antiinflammatory activities (Qing, 2014).

Vitamin E was used to prepare oil-in-water nanoemulsions using the nonionic surfactant by means of a high pressure

homogenization technique. During the emulsification process the variable factors including pressure, temperature, and concentration of the emulsifying agent were investigated. The relation between pressure and mean droplet diameter was derived and described. It was observed that the droplet size decreased by increasing the vitamin E concentration. Increased fat content had a slight influence on the droplet size and the mean droplet diameter of the nanoemulsion (El Kinawy et al., 2012).

The next study showed that vitamin E acetate nanoemulsions used with mustard oil can improved bioactivities. Vitamin E acetate nanoemulsion was fabricated using surfactant during 15 days (Qiu et al., 2015). The droplet size and size distribution were studied. It was observed that a stable nanoemulsion was formed at droplet size of 86.45 ± 3.61 nm (Dasgupta et al., 2015). A growing number of studies indicate that vitamin E can effectively induce apoptosis in cancer cells suggesting a potential role for antioxidants. About 450 papers were published about delivery of vitamin E in nanoemulsion form, far higher than research produced on vitamin A, D, or K.

## 8 Vitamin K

The discovery of a vitamin is almost invariably due to the recognition of a new syndrome that depends on the absence of an unknown substance from the diet. Vitamin K was first discovered in the early 1930s by the Danish biochemist Henrik Dam who observed, while studying cholesterol metabolism in chickens, that chicks fed with a diet free of sterols and low in fat tended to develop subcutaneous and intramuscular hemorrhages (Henrik, 1935).

The concept proposed here can be generalized that vitamin K is a group of fat soluble vitamins that are essential for biosynthesis of proteins involved in blood coagulation and metabolic pathways in bone and other tissues. This group includes two natural vitamins: vitamins $K_1$ (phylloquinone) and $K_2$ (menaquinone). Three synthetic types of vitamin K are known: vitamins $K_3$, $K_4$, and $K_5$. Although the natural $K_1$ and all $K_2$ homologs have proven to be nontoxic, the synthetic $K_3$ (menadione), $K_4$, and $K_5$ have shown toxicity (Kanai et al., 1997; Rasmussen et al., 2005). The importance of vitamin K in hemostasis arises from the fact that all vitamin K–dependent coagulation factors require γ-carboxylation of glutamic acid residues at their Gla domains to enable binding of calcium and attachment to phospholipid membranes.

Vitamin $K_1$ is lemon yellow oil at room temperature. At −70°C it separates from acetone or ethyl alcohol in light yellow rosettes, which melt at about −20°C into oil plus solvent. As the

temperature rises the oil gradually passes into solution. The vitamin is soluble in the ordinary fat solvents ethyl alcohol, acetone, hexane, benzene, chloroform, and dioxane. It is insoluble in water and only sparingly soluble in methyl alcohol.

Vitamin $K_2$ is lemon-yellow crystalline melting at 53.5–54.5°C. It may be crystallized from ethyl alcohol, acetone, or mixture 1:1 of methyl alcohol and chloroform. In general it is less soluble than vitamin $K_1$. The diacetates of dihydrovitamin $K_1$ and dihydrovitamin $K_2$ melt at 62–63°C and 59–60°C, respectively. The dibenoate of dihydrovitamin $K_1$ melts at 85–86°C. Only 47 papers in the Pubmed source refer to delivery of vitamin K in nanoemulsion form. Back in 1955, Howell discussed dosimetry for emulsions of vitamin $K_1$ given intravenously for the treatment of anticoagulant induced hemorrhage (Howell, 1955). Diet supplementation with vitamin K appears to aid in proper blood coagulation and bone formation (Jagannath et al., 2015). Vitamin K, similar to all vitamins, is necessary to maintain human health. Carboxylation of vitamin K dependent proteins has been implicated in soft tissue calcification and insulin resistance. The deficiency of vitamin K resulted in bleeding (Harshman et al., 2014). Studies suggest that a diet low in vitamin K is associated with the risk of hip fractures. Supplementation with vitamin $K_1$ and $K_2$ may reduce the risk of fractures (Hamidi et al., 2013). When calcium increases and accumulates at the vessel wall, vascular calcification occurs. Vitamin K supplementation was proposed in vascular calcification. Review by Shea presented the discussion about vitamin K and vascular calcification (Shea and Holden, 2012). Increased vitamin K may have substantial influence on bone physiology and prevention of atherosclerosis. Vitamin K is involved in bone remodeling, cell signaling, apoptosis, arterial calcification, chemotaxis, and it has antiinflammatory effects (Falcone et al., 2011).

## 9 Vitamin C

Vitamin C (ascorbic acid) displays high water solubility (Afroz et al., 1975). Among the various functions of vitamin C, we can find it is used for the synthesis of carnitine, which is necessary for the cellular transport of fat (Baer et al., 1994; Bendich, 1988; Bendich and Langseth, 1995). This vitamin is also required for the synthesis of collagen. Collagen is known to be a component of blood vessels, ligaments, and bone (Aronow, 1993). Vitamin C is involved in many biochemical reactions, for example, biosynthesis of collagen, L-carnitine, and certain neurotransmitters (Karrer, 1934; Carr and Fre, 1999; Li and Schellhorn 2007). Vitamin C is also an important physiological antioxidant (Frei et al., 1989) and has been

shown to regenerate other antioxidants within the body, including alpha-tocopherol (vitamin E) (Jacob and Sotoudeh, 2002). As an antioxidant, vitamin C can block production of nitrosamines, which are found in increasing concentration during cancer disease (Herbert, 1993, 1994; Curhan et al., 1996; Ekvall et al., 1981; Hathcock et al., 1990; Menkes et al., 1986). Vitamin C protects the body from free radicals, helps form connective tissue that hold bones, muscles, and tissues together (collagen), aids in the healing of wounds, aids the body in absorbing iron from plant sources, helps to keep your gums healthy, helps your body to fight infections, aids in the prevention of heart disease, helps prevent some forms of cancer, toxic to viruses, bacteria, and some malignant tumor cells.

Farahmand and coworkers demonstrate study the stability of vitamin C in multiple phase emulsions. Studied emulsions include oil-in-water-in-oil emulsion. The results showed that about 14% of vitamin C released from oil-in-water-in-oil emulsions during the first half hour (Farahmand et al., 2006). Khodaeian reviewed the current data on the influence of vitamin C and vitamin E supplementation on insulin resistance in type 2 diabetes mellitus (Khodaeian et al., 2015).

## 10 Vitamin B

Vitamin $B_1$ (Thiamin $B_1$) is an organosulfur compound, known as vitamin F. This vitamin is a sulfur-containing member of the B complex family. Thiamin $B_1$ is water soluble, essential for normal development, growth, and physical performance. Vitamin $B_1$ is involved in releasing energy from macronutrients that provide energy from carbohydrates. Uptake of vitamin $B_1$ by cells of the blood and other tissues occurs via active transport and passive diffusion. Vitamin $B_1$ is an essential vitamin that cannot be synthesized and must come from the diet. Vitamin $B_1$ is especially sensitive to the antinutritive effects of excess alcohol consumption, which decreases the absorption of thiamin and increases its excretion. Alcohol also inhibits the activation of thiamin to its coenzyme forms. Thiamin Vitamin $B_1$ is important in: producing energy from carbohydrates, proper nerve function, stabilizing the appetite and promoting growth (Tanphaichitr, 1999).

Vitamin $B_2$ (riboflavin), like thiamin and some other B vitamins that are a part of the B complex of vitamins is also essential for normal growth and physical performance. Vitamin $B_2$ is involved in an essential oxidation and reduction (redox) reactions. Vitamin $B_2$ is important in: energy transfer, carbohydrate and fat

metabolism, protein metabolism. The main vitamin $B_2$ functions include formation of antibodies and red blood cells, maintenance of good vision, skin, nails, and hair. Vitamin $B_2$ is widely distributed in small amounts in many foods, and milk is one important dietary source. Riboflavin-based nanoemulsion system (riboflavin 5-phosphate and riboflavin base) was developed and studied in corneal stroma. Riboflavin nanoemulsion was able to penetrate the corneal epithelium. The riboflavin-5-phosphate nanoemulsion diffused better into the stroma than the riboflavin base nanoemulsion (Bottos et al., 2013).

Vitamin $B_3$ (niacin) has fundamental roles as part of reduction and oxidation coenzymes involved in energy and amino acid metabolism. Vitamin $B_3$ is important in energy production, maintenance of skin and tongue, can improves circulation, maintenance of nervous system and health of the digestive track. Vitamin $B_3$ helps the body to use protein, fat, and carbohydrate to make energy and helps enzymes work properly in the body. Niacin is recognized in several forms: nicotinic acid, nicotinamide, and other derivatives such as inositol hexanicotinate (Miller and Hayes, 1982). During study of the antioxidant properties of honey samples, vitamin $B_3$ was likely to be in the high concentration, which covered for 69–80% of the total vitamin content (Chua et al., 2013). Niacin was used to preparation of niacin-ethyl cellulose microspheres by water in oil in oil double emulsion. The release of niacin was 85% for particle size range 405–560 μm (Maravajhala et al., 2009).

The next member of the vitamin B complex family is vitamin $B_6$ (pyridoxin). To date three forms of vitamin $B_6$ are known: pyridoxine (pyridoxol), pyridoxal, and pyridoxamine. Vitamin $B_6$ helps the body to make and use protein and glycogen, which is the stored energy in muscles and liver and helps form hemoglobin, which carries oxygen in blood. This vitamin plays a role in the metabolisms of carbohydrate, lipid, and amino acids. Vitamin $B_6$ is extensively involved in the metabolism of nitrogen-containing compounds, including serotonin, dopamine, gamma-aminobutyric acid, and the heme component of hemoglobin. Pyridoxine, as pyridoxal phosphate, also has an important role in the conversion of tryptophan to nicotinic acid. Vitamin $B_6$ interacts with drugs, which may either decrease the activity of the drug or might adversely affect vitamin $B_6$ levels.

In another study compounds such as α-tocopherol vitamin E and retinyl acetate vitamin A, pyridoxine-Vitamin $B_6$, and ascorbic acid vitamin C were transformed to oil-in-water or water-in-oil emulsions and penetration to skin using topical administration were investigated (Valgimigli et al., 2012; Clares et al., 2014).

Vitamin $B_9$ (folic acid or folate) is responsible for prevention of anemia. Folic acid is involved in many biochemical reactions and is reduced to dihydrofolate and tetrahydrofolate forms for biological activity.

Emulsions containing folic acid and sodium dodecyl sulfate was prepared and used for interacting with cancer cells. The presence of a folate receptor on the surface of cancer cells allowed for the interaction of folic acid and cancer cells. This work showed potential for the use of folic acid emulsions for detection and treatment of cancer cells (Yoon et al., 2014). Folic acid was used to target emulsions of all-trans retinoic acid to folate receptor-overexpressing tumor cells. In this study, folic acid shows the potential for effective and selective delivery of anticancer agents to the receptor in carcinoma cells (Kim et al., 2008). A study by Pillai showed a synthesis of folic acid conjugated crosslinked acrylic hydrogels for the delivery of hydrophobic drugs to cancer cells (Pillai et al., 2014).

Vitamin $B_{12}$ (cobalamin) is the coenzyme that affects the cellular metabolism and works together with the vitamin $B_9$ folate to synthesize nucleic acids, helps to maintain healthy blood cells, maintains the nervous system, and helps prevent types of anemia. Its primary role is related to methylation reactions, the key step in such processes as homocysteine conversion to ethionine, or synthesis of the succinyl-CoA, a metabolite of the Krebs cycle. In this respect, vitamin $B_{12}$ is the direct cofactor for methionine synthetase, the enzyme that recycles homocysteine back to methionine (Schoenen et al., 1998). In the adenosylcobalamin form, vitamin $B_{12}$ is the cofactor in methylmalonyl-coenzyme A mutase. Both reactions are involved in promoting the rapid growth and proliferation of bone marrow cells and ultimately red blood cells (Carlson et al., 1968; Schoenen et al., 1998; Spector et al., 1995). Its primary role is related to the methylation reactions, the key steps in such processes as homocysteine conversion to ethionine, or synthesis of the succinyl-CoA, a metabolite of the Krebs cycle vitamin $B_{12}$ participates in regulation of the brain and nervous system functioning as well as in blood and nucleic acid formation. For these reasons there is a large cobalamin uptake by highly proliferating cells. This fact has extensive consequences in terms of the applicability of vitamin $B_{12}$ in the diagnosis and treatment of tumors. Gupta demonstrated the biological stability of vitamin $B_{12}$. The prepared formulations were evaluated for their strength, dissolution rate, and size distribution and compared for the biological stability of vitamin $B_{12}$ (Gupta and Rao, 1985). The next study provided a new water oil emulsion of vitamin $B_{12}$ for topical applications. In these experiments, amounts of water, surfactant

concentration, oil and surfactant ratio and physicochemical properties of cosurfactants had an influence on emulsion (Salimi et al., 2013). Vitamin $B_{12}$ emulsions in poly (e-caprolactone) were prepared by using oil in oil, melt encapsulation, and water in oil in water methods. Process was controlled by using various polymer concentrations, solvent ratios, and external oil phase volumes. The release of vitamin $B_{12}$ from water in oil in water in oil was 35 to 40% and about 90% was released in 28 days. The release of vitamin $B_{12}$ from oil in oil and melt encapsulation was about 100% (Rames, 2009). In the current research, the roles of vitamin B in bone health were studied. The results showed that individual B vitamins, particularly, $B_2$, $B_6$, folate, and $B_{12}$, influences bone structure, quality, and mass (Dai and Koh, 2015). Research reviews provide evidence that Vitamins B is linked to abiotic and biotic stress responses (Colinas and Fitzpatrick, 2015). The roles of different vitamins in various gastrointestinal diseases were reviewed. The use of vitamins $B_{12}$ and D showed potential in therapy of chronic hepatitis C (Masri et al., 2015). The deficiency of vitamins C, A, E, $B_1$, or $B_6$ in the diet has negative effect on the antioxidant defense system (Bhandari and Van Berkel, 2012). The high doses of vitamin C, E and β-carotene show prooxidant effects (Kodentsova et al., 2013).

Biotin is a B vitamin and a coenzyme for carboxylase enzymes and is involved in the synthesis of fatty acids and amino acids and in gluconeogenesis. Biotin is present in at least small amounts in all cells of plants and animals. D-biotin is synthesized by the intestinal flora.

The function of biotin is to support the health of the skin, nerves, digestive tract, and lungs. Functional deficiency of biotin has occurred through genetic defects in the enzymes and is resulting in damage to the enzyme systems associated with respiration (Chase et al., 1990; Clementz and Holmes, 1987; Schoenen et al., 1998; Spector et al., 1995).

Ongoing efforts have been made to develop coenzyme Q10 nanoemulsions. Coenzyme Q10 is a soluble in oil vitamin-like substance that is present in mitochondria in most eukaryotic cells. In 2012, Belhaj and coworkers formulated a nanoemulsion consisting of salmon oil, salmon lecithin, CoQ10 and water. In these studies a commercial oily mixture was used for comparison. Mean droplets size of the control and CoQ10 nanoemulsions were 164 and 167 nm, respectively. The nanoemulsion formulation increased the bioavailability of CoQ10 at least two fold as compared to the conventional oily formulations (Belhaj et al., 2012). There are various examples in the literature that indicate vitamin delivery is clearly moving to the nanoemulsion scale.

Despite many efforts to improve delivery of vitamins, symptoms of vitamin deficiency are still known to cause multiple problems. Table 15.8 presents the results of vitamin deficiencies problems and symptoms. Vitamin deficiency is also called avitaminosis, which is a lack of the recommended blood levels of essential vitamin (McDowell, 2000).

Plants are a source of new drugs. Plant-based drug discovery resulted mainly in the development of anticancer and antiinfectious agents and continues to contribute to new leads in clinical trials. The endogenous antioxidants are glutathione, superoxide dismutase, peroxidases, and catalase. The exogenous antioxidants in the diet are β-carotene, vitamin C, and vitamin E.

Many studies have been performed on vitamin E, which is the most important antioxidant in the human diet. However, recent studies confirm that carotenoids interact with vitamins E and C and can protect lipoprotein against oxidative damage even when vitamin E levels are low (Niwano et al., 2011). In a study done by Lobo and coworkers the composition and physicochemical stability of vitamins in neonatal parenteral nutrition was studied. The composition of vitamins in emulsions was as follow: vitamin A, 1.3 μg $mL^{-1}$; vitamin D, 5 μg $mL^{-1}$; vitamin E, 10 μg $mL^{-1}$; vitamin $B_1$, 3 μg $mL^{-1}$; vitamin $B_2$, 3.6 μg $mL^{-1}$; vitamin $B_3$, 40 μg $mL^{-1}$; vitamin $B_5$, 15 μg $mL^{-1}$; vitamin $B_6$, 4 μg $mL^{-1}$; vitamin $B_7$, 60 μg $mL^{-1}$; vitamin $B_9$, 400 μg $mL^{-1}$; vitamin $B_{12}$, 5 μg $mL^{-1}$; and vitamin C, 100 μg $mL^{-1}$ (Lobo et al., 2012). Mechanisms of vitamin action are described in relation to deficiency signs or symptoms that are observed when such compounds are limiting in the diet (Rucker et al., 2002). Vitamins play an important role as coenzymes or enzymes. In recent years data showed that vitamins can have an important role in the prevention and treatment of cancer (Mamede et al., 2011). Supplements can treat a vitamin deficiency (Bhandari and Van Berkel, 2012). When a food is supplemented with vitamins, it means that the amount of vitamins already present in food is increased (Harris, 1959). To reduce vitamin deficiencies, synthetic vitamins and supplements are used in daily diet. Supplements and synthetic vitamins can effectively treat vitamin deficiency. When a diet is supplemented with vitamins, it means that the daily amounts of vitamins already present in food are increased. In an article by Zhou, vitamins from the perspective of vitamin homeostasis are discussed (Zhou et al., 2015). Vitamin A, D, K, E, and C were used for chemoprevention and therapy of melanoma in the preclinical and clinical studies (Russo et al., 2015). In summary, natural product drugs and vitamins play a dominant role in pharmaceutical care.

# Table 15.8 Vitamin Deficiency

| Vitamin | Deficiency |
|---|---|
| Vitamin A (retinol) | Based on low vitamin A levels. Vitamin A deficiency can result in liver disorders. Deficiency causes rashes and typical ocular effects. |
| Vitamin $B_1$ (Thiamin) | Vitamin $B_1$ (Thiamin) deficiency refers to the following disorders: dry beriberi (1), Wernicke-Korsakoff syndrome (2), cardiovascular beriberi (3).<br><br>(1) Dry beriberi are neurologic deficits that cause muscle wasting, polyneuropathy, which can eventually affect the arms.<br>(2) Wernicke-Korsakoff syndrome is a type of apathy causes also nystagmus, ataxia, ophthalmoplegia.<br>(3) Cardiovascular (wet) beriberi is myocardial disease. |
| Vitamin $B_2$ (Riboflavin) | Vitamin $B_2$ (Riboflavin) deficiency refers to the most common signs of the mucosa infected with *Candida albicans*. Rarely vitamin $B_2$ deficiency causes neovascularization and photophobia. |
| (Vitamin $B_3$) Niacin | Vitamin $B_3$ (Niacin) deficiency refers to pellagra. Pellagra is skin disease with mucous membrane symptoms, photosensitive rash and mental aberrations. |
| Vitamin $B_6$ (Pyroxidol) | Vitamin $B_6$ (Pyroxidol) deficiency causes peripheral neuropathy and a pellagra-like syndrome with seborrheic dermatitis. Vitamin $B_6$ deficiency in adults, can result in depression, confusion, seizures, normocytic, microcytic, and anemia. |
| Vitamin $B_9$ (Folic acid) | Folate deficiency is common and may result from inadequate intake, malabsorption, or use of various drugs. Deficiency of Vitamin $B_9$ causes megaloblastic anemia (indistinguishable from that due to vitamin $B_{12}$ deficiency). |
| Vitamin $B_{12}$ (Cobalamin) | Deficiency of Vitamin $B_{12}$ (Cobalamin) causes megaloblastic anemia, damage to the white matter of the spinal cord and brain, and peripheral neuropathy. Diagnosis of deficiency is usually made by measuring serum vitamin $B_{12}$ levels. |
| Vitamin C (ascorbic acid) | Vitamin C deficiency can occur as part of symptoms such as depression, and connective tissue defects (eg, gingivitis, petechiae, rash, internal bleeding, impaired wound healing). |
| Vitamin D | Vitamin D deficiency impairs bone mineralization causing osteomalacia and possibly contributing to osteoporosis. Treatment usually consists of oral vitamin D, calcium and phosphate. Vitamin D deficiency causes chronic kidney disease, various renal tubular disorders and mineralization of bone matrix. |
| Vitamin E | Dietary vitamin E deficiency is common reason of fragility of and degeneration of neurons, particularly peripheral axons and posterior column neurons. |
| Vitamin K | Vitamin K deficiency decreases levels of prothrombin and other vitamin K–dependent coagulation factors, causing defective coagulation and, potentially, bleeding. |

## 11 Conclusions

Nanoemulsions are effective vitamin and drug carrier transport systems. A nanoemulsion vitamin delivery system can be formulated in variety of forms such as liquids, foams, creams, and sprays.

Nanoemulsion vitamin delivery systems are safe, nontoxic, and nonirritant and hence can be easily applied to tissue, skin, and mucus membranes. Nanoemulsion vitamin delivery systems have been shown to improve vitamin absorption. Nanoemulsion may be excellent carriers of lipophilic, active compounds in food products. Nanoemulsion stability improved substantially with a decrease in droplet size. The emulsion droplet size and oil concentration significantly influenced their biological applications. An appropriate combination and proportion of nanoemulsion vitamin delivery system includes the type and concentration of oil, surfactant, cosurfactant, and water.

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# 16

# EMULSIFIED PROTEIN FILAMENTS: TYPES, PREPARATION, NUTRITIONAL, FUNCTIONAL, AND BIOLOGICAL PROPERTIES OF MAYONNAISE

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## 1 Introduction

There has been an increasing consumer demand for healthier and more natural food products with an increasing focus on foods with better nutritional and health promoting properties (McClements and Demetriades, 1998). Worldwide, one of the most widely consumed products for use in condiments or sauces is mayonnaise (Harrison and Cunningham, 1985). In North America, mayonnaise is used normally as a sandwich spread (McClements, 2004). It was first commercially produced in the early 1900s, becoming famous in North America from 1917 to 1927 (Harrison and Cunningham, 1985; Brabant, 1992). In the Mediterranean area, in countries such as Syria, Jordan, and Iraq, mayonnaise is used in several food commodity products such as Shawarma and poultry products as food ingredients. Mayonnaise is prepared by mixing several food ingredients, which include egg yolks, oil, vinegar, and spices, especially mustard (Liu et al., 2007; Gaonkar et al., 2010). Mayonnaise is a semisolid product with a total fat content around 70–80% produced by mixing an

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00016-7

oil phase with water phase using egg yolk as surfactant (Kerkhofs et al., 2011; McClements, 2004; Depree and Savage, 2001). The emulsion is formed by slowly blending oil with a premix that consists of egg yolk, vinegar, and mustard. Eggs are important food components due to their multifunctional properties such as gelling, foaming, and emulsifying characteristics. The lipid content of mayonnaise is composed of 66% triglyceride, 28% phospholipids, and 5% cholesterol. According to the standards of the US Food and Drug Administration (FDA), mayonnaise must contain at least 65% vegetable oil (Garcia, 2006). Mayonnaise is sensitive to spoilage because of its high polyunsaturated fatty acid content that can readily undergo autooxidation (Wills and Cheong, 1979). Peroxide value is used to express the degree of oxidation or rancidity (Pandey and Carney, 2008) and is one of the most frequently determined quality parameters during oil production, storage, and marketing (Saad et al., 2006). Wills and Cheong (1979) found that the peroxide values were increased by 3.5 meg/kg after 15 days of mayonnaise storage. Stability of emulsification in mayonnaise is dependent on several factors, including the amount of added oil and egg yolk, viscosity, volume of oil an aqueous phase, method of mixing, water quality, and temperature (Harrison and Cunningham, 1985). Protein, oils, lecithin, solid fat, cephalin, cholesterol, and pigments are the main organic constituents of egg yolk (Seli et al., 1935). Egg yolk contributes about 48% water, 16.4% protein, 2.7% carbohydrate, and 32.9% lipid (Privett et al., 1962). Phospholipids contain approximately 79% lecithin from the egg yolk (Privett et al., 1962).

Mayonnaise is a relatively microbiologically stable product due to its high fat content and the addition of acid ingredients. Acid ingredients contribute to the desirable flavor, decrease final pH of product (<4.8), and are toxic to food-borne pathogens (Depree and Savage, 2001; Karas et al., 2002). *Salmonella*, *Escherichia coli* O157:H7, *E. coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Yersinia enterocolitica* do not survive when they are inoculated into mayonnaise (Smittle, 2000). On the other hand, the milder acid content of homemade mayonnaise, which can remain in unchilled storage for hours or days before consumption, has been associated with outbreaks of food poisoning due to the presence of *Salmonella* in the raw eggs, which is the principal vehicle of infection (Radford and Board, 1993). In Jordan, use of unpasteurized eggs is considered as the major health problem in the manufacture of mayonnaise due to *Salmonella* risk. Commercial mayonnaise produced using pasteurized eggs, however, does not represent a health risk from pathogenic bacteria (Smittle, 2000).

Recently, consumers have demanded food products with enhanced nutritional and functional food properties, including products containing lower amounts of fat. To circumvent the caloric content of mayonnaise, low fat mayonnaise is manufactured using ingredients such as modified starch, pectin, microcrystalline cellulose, carrageenan gum, and some thickeners to stabilize the emulsion and to increase the viscosity of light mayonnaises, in addition to producing a decrease in the dispersed phase and increased water content. There is little information, however, regarding the use of plant proteins instead of carbohydrate components as stabilizing and emulsifying agents. Generally, the functional properties of proteins are divided into three major groups according to their gelation, hydration, and surface properties (Fennema, 1993). In that regard, replacement of animal protein by plant protein in preparation of mayonnaise could change the microbiological, physicochemical, and nutritional properties of mayonnaise due to the differing emulsification properties of plant proteins.

This chapter focuses on an overview of the preparation of different types of mayonnaise and their chemical composition and physicochemical, nutritional, and sensory properties. The final chapter section examines the biological properties of different legume proteins that could be used as egg yolk replacement to enhance the nutritional properties of mayonnaise.

# 2 Types of Mayonnaise

## 2.1 Mayonnaise Prepared From Palm, Soybean, Mustard, and Olive Oil

### *2.1.1 Preparation of Mayonnaise From Palm, Soybean, Mustard and Olive Oil*

The ingredients used in preparation of mayonnaise are palm, soybean and mustard oils, egg (whole), salt, powder milk, sugar, mustard seeds, corn starch, carboxymethyl cellulose (CMC), vinegar, ethylene diamine tetra acetic acid (EDTA), and potassium sorbate. Eggs (20%) are mixed in a mixer until the mixture is lightly thickened, followed by the addition of sugar (2%), mustard powder (1%), milk powder (2%), salt (1%), carboxymethyl cellulose (1.5%), corn starch (5%), and water (25.4%). The resulting mixture is well mixed until the ingredients are evenly distributed. Afterward, vinegar (12%) is added to the mixture and the mass is blended extensively. Oil is then added carefully (drop by drop) to the mixture with continuous stirring. Ethylene diaminetetraacetic acid (EDTA)

and potassium sorbate are added to the finished product in the required quantities (Palma et al., 2004).

### 2.1.2 Chemical Composition of Mayonnaise Prepared From Palm, Soybean, Mustard, and Olive Oil

The moisture content of mayonnaise made from palm oil, soybean oil, mustard oil, and olive oil was reported as 43.64, 41.94, 46.21, and 46.95%, respectively (Palma et al., 2004). Prescott and Proctor (1937) found that the moisture content ranged from 42.64% to 57.25% in the mayonnaise produced from the aforementioned oils. The same authors reported that the protein content of mayonnaise made from palm oil, soybean oil, mustard oil, and olive oil was 1.48, 1.62, 1.395, and 1.09%, respectively (Palma et al., 2004), which is within the range of early studies by Prescott and Proctor (1937) who stated that the protein content of such mayonnaise was ranged from 1.00% to 1.88%. The fat content in different mayonnaise samples (palm, soybean, mustard, and olive oils) were reported as 33.40, 31.16, 32.60, and 30.29%, respectively (Palma et al., 2004). Similarly, Dudina et al. (1992) found that the fat content in mayonnaise ranged from 30% to 40%. The ash content of different mayonnaise samples (palm, soybean, mustard, and olive oils) was shown to range from 1.15% to 1.8% (Palma et al., 2004) whereas another study reported that the ash content was in the range of 1.04–2.04% (Prescott and Proctor, 1937).

### 2.1.3 Physicochemical Properties of Prepared Mayonnaise From Palm, Soybean, Mustard, Fish, and Olive Oil

The color and texture of mayonnaise samples that included palm, soybean, mustard, and olive oils were 23.1, 21.1, 21.3, and 21.4, respectively (Palma et al., 2004). The mayonnaise prepared from palm oil had the highest values of color and texture, while the soybean oil mayonnaise had the lowest color and texture values. The mayonnaise prepared from palm oil had the highest value of flavor, whereas the mayonnaise prepared from mustard oil had the lowest value of flavor (Palma et al., 2004). The mayonnaise prepared without vegetables and tuna or shrimp had more oxidative stability as compared to fish oil-enriched mayonnaise (Sorensen et al., 2010). The mayonnaise containing either 65% or 78% soya oil had shelf life of 40 and 32 days, respectively, while the mayonnaise with either 65 or 78% groundnut oil had shelf life of 34 and 26 days, respectively. Mayonnaise produced from 65% soya and groundnut oils was preferred to that produced from 78% soya and groundnut oils according to the sensory evaluation (Chukwu and Sadiq, 2008).

## 2.2 Mayonnaise Prepared From Eggs

### *2.2.1 Preparation of Mayonnaise From Eggs*

Abu-Salem and Abou-Arab (2008) reported similar protein (47.09%), carbohydrate (4.03%), and lipid (45.10%) content in prepared mayonnaise from ostrich eggs as compared to prepared mayonnaise from chicken eggs, 47.14, 4.71, and 45.13%, respectively. On the other hand, the ash content in prepared mayonnaise from ostrich eggs (3.79%) was higher as compared to chicken eggs (3.10%). Makhlouf et al. (1996) reported that the content of lipids, protein, carbohydrates, and ash in prepared mayonnaise from chicken eggs was 44.99, 46.68, 5.31, and 3.02%, respectively. Di Meo et al. (2003) stated that the lipid, protein, and ash content in prepared mayonnaise from ostrich eggs ranged from 43.8% to 44.2%, 47.7% to 48.2%, and 5.2% to 5.5%, respectively.

### *2.2.2 Chemical Composition of Mayonnaise From Eggs*

Abu-Salem and Abou-Arab (2008) reported similar protein (47.09%), carbohydrate (4.03%), and lipid (45.10%) content in prepared mayonnaise from ostrich eggs as compared to prepared mayonnaise from chicken eggs, 47.14, 4.71, and 45.13%, respectively. On the other hand, the ash content in prepared mayonnaise from ostrich eggs (3.79%) was higher as compared to chicken eggs (3.10%). Makhlouf et al. (1996) reported that the content of lipids, protein, carbohydrates, and ash in prepared mayonnaise from chicken eggs was 44.99, 46.68, 5.31, and 3.02%, respectively. Di Meo et al. (2003) stated that the lipid, protein, and ash content in prepared mayonnaise from ostrich eggs ranged from 43.8% to 44.2%, 47.7% to 48.2%, and 5.2% to 5.5%, respectively.

Abu-Salem and Abou-Arab (2008) showed that the iron and calcium content was lower in prepared mayonnaise from chicken eggs as compared to prepared mayonnaise from ostrich eggs. In contrast, the content of zinc, sodium, and potassium was found to be higher in prepared mayonnaise from chicken eggs as compared to prepared ostrich egg mayonnaise. The phosphorus and iron content in prepared mayonnaise from ostrich and chicken eggs did not differ (Abu-Salem and Abou-Arab, 2008). Makhlouf et al. (1996) who reported that the mineral contents of Fe, K, Ca, Zn, P, and Na in prepared mayonnaise from chicken eggs were 9.85, 539.62, 192.45, 5.41, 720.75, and 407.55 mg/100 g, respectively.

The titratable acidity of unpasteurized mayonnaise made from ostrich eggs was higher as compared to mayonnaise made from pasteurized eggs during storage. The pH of pasteurized mayonnaise made from ostrich eggs was stable during storage as compared to mayonnaise made from unpasteurized eggs

that was unstable during storage due to the growth of lactic acid bacteria, which decreased the pH of mayonnaise made from unpasteurized eggs (Worrasinchai et al., 2006). Titratable acidity and pH in mayonnaise made from chicken eggs was steady in pasteurized or unpasteurized mayonnaise during storage. Thiobarbituric acid reactive substances, as a measure of lipid oxidation, increased as storage period increased in both pasteurized and unpasteurized mayonnaise prepared from both ostrich and chicken eggs (Abu-Salem and Abou-Arab, 2008). Kaur et al. (2011) reported that peroxide values were decreased in lycopene-treated mayonnaise as compared with control mayonnaise samples following storage. Park et al. (2010) described emulsion stability from the supplementation with 20, 40, 60, 80, and 100 ppm of 1-monocaprin for preparing mayonnaise that using soybean oil, egg yolk, sugar, salt, and vinegar. Mayonnaise containing 60 ppm of 1-monocaprin had the highest emulsion stability followed by mayonnaise containing 40, 20, 80, and 100 ppm 1-monocaprin and viscosity decreased at a slower rate with higher 1-monocaprin concentrations.

### 2.2.3 Sensory Evaluation of Mayonnaise

The color, flavor, taste, and appearance were shown to be better for mayonnaise made from ostrich egg mayonnaise than for chicken egg mayonnaise (Abu-Salem and Abou-Arab, 2008). Mayonnaise made from ostrich egg was also more resistant to microbial spoilage and stable during storage due to a decrease in pH values compared to mayonnaise made from chicken egg (Abu-Salem and Abou-Arab, 2008).

### 2.2.4 Microbiology of Mayonnaise Prepared From Egg

Zhu et al. (2012) showed that the *Salmonella* mixtures survived longer in mayonnaise made with vinegar than with lemon juice during storage at 4°C. The numbers of *Salmonella* in two mixtures of either acetic acid or citric acid solutions containing preservatives were significantly lower than those in vinegar or lemon juice solutions without preservatives. Zhu et al. (2012) suggested that mayonnaise contaminated with *Salmonella* could survive in the mayonnaise-making process but use of vinegar and lemon juice leads to inhibition of *Salmonella* growth due to the organic acids and chemical preservatives. Glass and Doyle (1991) reported that no *Salmonella* was detected at 48 h in mayonnaise made with 0.7% acetic acid. The organism was also not detectable in samples containing 0.3% acetic acid after at 2 weeks.

## 2.3 Mayonnaise Prepared From Polysaccharide Gums

Development of low-fat mayonnaise is an important issue for the food industry and for consumers (McClements and Demetriades, 1998). Fat replacers could improve processing functionalities and also contribute nutritional benefits. Polysaccharide gels (guar, pectin, and xanthan gums) are good replacers for fat (Laneuville et al., 2005; Ward, 1997; ADA, 2005; Warrand, 2006). Xanthan gum has been used in mayonnaise either alone or together with other gums in salad dressings to produce the desired rheological properties (Ward, 1997; Ma and Barbosa-Canovas, 1995). Citrus fiber could also be used in mayonnaise as this has been used as a fat replacer, emulsifier, and stabilizer in ice cream processing without adverse effects on ice-cream properties (viscosity, overrun, or sensory properties) (Dervisoglu and Yazici, 2006).

### *2.3.1 Preparation of Mayonnaise From Polysaccharide Gum*

Full fat mayonnaise and polysaccharide gum containing low fat mayonnaise were prepared by Su et al. (2010) using egg yolk, apple vinegar, sugar, and salt. Su et al. (2010) prepared mayonnaise from polysaccharide gums (citrus fiber, xanthan gum, and guar gum), xanthan gums, and citrus fiber by dissolving these ingredients together in deionized water. The citrus fiber (100 g/kg) was mixed with 0, 2.5, 5.0, or 7.5 g/kg guar gum. The xanthan gums (15 g/kg) were mixed with 0, 5.0, 7.5, 10.0, or 12.5 g/kg guar gum. Shen et al. (2011) prepared low fat mayonnaise by adding oat dextrin as fat substitute using of 10.6% egg yolk, dextrose, and 27.9% oat dextrin. El-Bostany et al. (2011) studied the development of light mayonnaise using potato powder mash as fat replacement to reduce fat content to 50%.

### *2.3.2 Chemical Composition of Mayonnaise Prepared From Polysaccharide Gum*

The ash and protein content for mayonnaise prepared from eggs and polysaccharide gums (xanthan gum to 10 g/kg guar gum, and citrus fiber to 5 g/kg guar gum) demonstrated values of 11.7, 12.5, 12.4 g/kg for ash, and 22.1, 21.4, 22.3 g/kg for protein, respectively (Su et al., 2010). The content of moisture and carbohydrate was higher in low fat mayonnaise when compared to full fat mayonnaise. Also, the low digestibility of mayonnaise prepared with fat replacers (guar gum, xanthan gums, and citrus fiber) further decreased the caloric of the low fat mayonnaise in relation to the full fat mayonnaise (Su et al., 2010). Polysaccharide gums contain a high level of dietary fiber as fat replacers (xanthan gums +10 g/kg

guar gum and citrus fiber +5 g/kg guar gum) resulting fiber values of 6.8 and 28.7 g/kg, respectively (Su et al., 2010).

### 2.3.3 Physicochemical Properties of Mayonnaise

The brightness (L value) of low fat mayonnaise was shown to be higher than full fat mayonnaise (Su et al., 2010). The higher L value maybe due to larger lipid droplets observed in low fat mayonnaise mayonnaises with xanthan gums + guar gum and citrus fiber + guar gum (Su et al., 2010). Chantrapornchai et al. (1999) found that the emulsion changed from a gray color to a bright-white color when the droplet size decreased due to an increase in light scattering. The water activity of low fat mayonnaise mayonnaises is higher than full fat mayonnaise due to the increased of water holding capacity of the formulations (Su et al., 2010). Chirife et al. (1989) found that the water activity (Aw) of full fat mayonnaise (77–79% oil) was about 0.93 whereas low fat mayonnaise samples (37–41% oil) showed a higher Aw, that is, close to 0.95.

The pH in xanthan gums + 10 g/kg guar gum group was found to be equal to full fat mayonnaise whereas the citrus fiber + 5 g/kg guar gum had a lower pH than fat mayonnaise, which was likely due to the acetic acid residue remaining in the citrus fiber preparation after acid extraction (Su et al., 2010). Hathcox et al. (1995) reported that the pH of mayonnaise made from fat replacer was higher than that of the full fat mayonnaise due to the dilution of acetic acid in the aqueous phase of the full fat formulations. Shen et al. (2011) concluded that the low-fat mayonnaise had a higher viscosity and a lower caloric value compared to full-fat mayonnaise and had an acceptable sensory evaluation. Gaonkar et al. (2010) found that the mayonnaise prepared from egg protein components had smaller oil drops producing a creamier mayonnaise compared to whey protein concentrate and whey protein isolate-based mayonnaise. El-Bostany et al. (2011) found that all light-fat mayonnaise had lower energy content, but higher water content than the full fat mayonnaise. The low fat mayonnaise formula had higher color values than the commercial light fat mayonnaise. The xanthan gum mayonnaise had higher heat stability, consistency coefficient, viscosity, firmness, adhesiveness, adhesive force, and overall acceptance by consumers as compared to guar gum-based mayonnaise (Nikzade et al., 2012). An increased viscosity and adhesion was seen with model mayonnaise having an increased concentration of food gums with the greatest increase when guar gum was applied alone and the lowest increase seen with xanthan gum addition (Bortnowska and Makiewicz, 2006).

### 2.3.4 Sensory Evaluation of Mayonnaise From Polysaccharide Gum

The physical and sensory properties closest to traditional mayonnaise has been obtained by applying food gums at 0.11% (Bortnowska and Makiewicz, 2006). Su et al. (2010) summarized the influence of fat replacers on the sensory characteristics of mayonnaise. The aroma, appearance, greasiness, taste, and overall acceptance score of xanthan gums + 10 g/kg guar gum was the same when compared to full fat mayonnaise. However, taste, appearance, and overall acceptance of citrus fiber + 5 g/kg guar gum treatment was lower as compared to both xanthan gum mayonnaise (+10 g/kg guar gum) and full fat mayonnaise. This latter outcome was attributed to the rougher appearance of the citrus fiber + guar gum treatment. Reduced-fat mayonnaise with 3.8 or 5.6% of 4αGTase-modified rice starch treated starch and xanthan gum showed smaller droplets compared to full fat mayonnaise with gum (Mun et al., 2009).

## 2.4 Mayonnaise From Plant Protein

### 2.4.1 Biological Properties of Protein Isolates

Legumes play an important role in supporting the nutritional health of populations, especially in developing countries with low annual incomes as they are a low cost food staple that provide high biological value proteins as well as key vitamins and minerals (Tharanathan and Mahadevamma, 2003; Singh and Singh, 1992). Regular intake of pulses (ie, chickpea, lupin, and beans) has been associated with reduced risk of some chronic diseases, particularly diabetes and cardiovascular disease (Roy et al., 2010), which has been related to the presence of peptides and phenolics with antihypertensive and hypoglycemic properties. Hence, incorporation of these pulse proteins could impart functional food properties to foods such as mayonnaise.

Preparation of mayonnaise from a mixture of broad bean and chickpea protein isolates is recommended to be used in the food industry due to the superior values of fat, protein, lightness, redness, antihypertensive, and antidiabetic and thermal denaturation temperature properties(Tawalbeh, 2012). Preparation of mayonnaise from a mixture of broad bean and chickpea protein isolates enhance the value of fat and protein contents and lightness (Tawalbeh, 2012). Mayonnaise prepared from either broad bean or chickpea protein isolates had antihypertensive and antidiabetic properties (Tawalbeh, 2012).

### 2.4.2 Antihypertensive Properties (Angiotensin I-Converting Enzyme [Ace] Inhibitory Activity)

Hypertension (defined as high systolic and diastolic blood pressures) is one of the major independent risk factors for cardiovascular disease (Fitzgerald and Murray, 2006). Blood pressure is partly controlled by the renin–angiotensin system (Chen et al., 2009; Erdmann et al., 2008). Angiotensin I-converting enzyme (ACE) is an enzyme associated with the renin–angiotensin system, which hydrolyzes angiotensin I (adecapeptide) to the octapeptide angiotensin II, a potent vasoconstrictor, resulting in arterial constriction and blood pressure elevation. ACE works via several mechanisms to elevate blood pressure including breakdown of bradykinin, a vasodilator. Inhibition of ACE is considered as an important mechanism for lowering blood pressure (Chen et al., 2009; Erdmann et al., 2008; Fitzgerald and Murray, 2006). Hypertension can be controlled by dietary modifications, exercise, calcium channel agonists, angiotensin II receptor blockers, diuretics, and ACE inhibitors (Fitzgerald and Murray, 2006). Owing to the potential side effects of pharmaceutical drugs, such as cough, skin rashes, and angioedema, there is increased interest in identifying foods that naturally contain ACE-inhibitory peptides and phenolics with hypotensive properties (Roy et al., 2010).

The peptides obtained from hydrolysates of chickpea have shown strong ACE inhibitory activities (Yust et al., 2003; Pedroche et al., 2002). Medina-Godoy et al. (2012) reported that the ACE inhibitory concentrations (IC50) of hydrolyzed peptides obtained from chickpeas were in range of 0.101–37.33 μg/mL. The ACE inhibitory activity of chickpea hydrolysates, however, was affected by the enzyme type used for the hydrolysis (Barbana and Boye, 2010). Extracted protein isolates from lupin showed ACE inhibitory activity; however, the activity of the extracted protein isolate was higher at neutral pH as compared to acidic pH (Yoshie-Stark et al., 2006). High temperature treatment of the lupin proteins at 125°C also decreased their ACE inhibitory activities (Yoshie-Stark et al., 2006). Phenolic compounds extracted from pulses also have shown exert ACE inhibitory activities as extracted phenolics from faba bean exhibited significant ACE inhibitory activities (Siah et al., 2012). The extracted phenolic compounds from *Lupinus mutabilis* of SLP-1 and H-6 varieties also have shown ACE inhibitory activity (Ranilla et al., 2009). Phenolic extracts from chickpea flour have also shown ACE inhibitory activities (Sreerama et al., 2012).

### 2.4.3 Antidiabetic Properties (α-Glucosidase and α-Amylase Inhibitory Activities)

Diabetes is one of the major medical complications the world faces today (Kumar et al., 2012). It is characterized by hyperglycemia

and alteration in carbohydrate, protein, and lipid metabolism caused by defects in insulin production or action (King et al., 1998). The management of blood glucose levels is a critical strategy in the control of diabetes complications. Inhibitors of carbohydrate hydrolyzing enzymes such as α-glucosidase and α-amylase have been useful as oral drugs for the control of hyperglycemia, especially in patients with type II diabetes mellitus (Kumar et al., 2012). Intestinal α-glucosidase is located at the epithelium of the small intestine and is a key enzyme in carbohydrate digestion. It catalyzes the hydrolysis of 1,4-α-glycosidic bonds within carbohydrates, resulting in the release of α-glucose to promote an increase of blood glucose levels after a meal. α-glucosidase has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality that occurs in Type II diabetes mellitus (Skeggs et al., 1956; Koyasu et al., 2010; Yao et al., 2010).

α-glucosidase inhibitors antagonize the activity of α-glucosidase and so delay intestinal carbohydrate absorption thereby slowing the sharp rise in blood sugar levels that diabetic patients typically experience after meals (Koyasu et al., 2010; Kumar et al., 2012). α-glucosidase inhibitors including acarbose, voglibose and miglitol are clinically used as oral antihyperglycemic agents, but their prices are high and clinical side effects occur (Scott and Spencer, 2000; Frantz et al., 2005; Shimabukuro et al., 2006). Pancreatic α-amylase is located in the brush border of the small intestines and is a key enzyme in the digestive system. Amylase breaks down the carbohydrates into a mixture of small oligosaccharides consisting of maltose, maltotriose, and a number of α-(1–6) and α-(1–4) oligoglucans. Glucosidase enzymes including lactase, maltase, and sucrose, complete the breakdown to monosaccharide units (glucose), which upon absorption enters the bloodstream. Degradation of dietary starch proceeds rapidly and leads to elevated postprandial hyperglycemia (ADA, 2008; Bowles, 1990). Alpha amylase inhibitors prevent starch digestion by completely blocking access to the active site of the α-amylase enzyme (Barrett and Udani, 2011). Rahimi-Alangi and Bandani (2012) reported that the protein extracts from chickpea showed an inhibitory effect of α-glucosidase activity. Phenolic extracts from chickpea flour also showed the inhibitory activities of α-amylase and α-glucosidase as well as significant antioxidant activity (Sreerama et al., 2012). The extracted phenolic compounds from faba bean have shown to exhibit the inhibitory α-glycosidase activities (Siah et al., 2012). Prathapan et al. (2011) found that the sprouting of Bengal gram decreases the inhibitory activity α-glucosidase and α-amylase as compared to nonsprout of Bengal gram. The sprouting of Bengal gram also enhanced its antioxidant activity as compared to nonsprouted Bengal gram (Prathapan et al., 2011).

In summary, since legumes can be used as a functional ingredient to improve the texture and stability of foods (Makri et al., 2005), future studies should investigate their use as a replacement of egg proteins in mayonnaise toward its development as a functional food. The addition of legume proteins and associated phytochemicals could provide health benefits such as antihypertensive, hypoglycemic, and antioxidant properties. Replacement of egg protein in mayonnaise with legume proteins could also be useful for people with dietary restrictions such as vegans, vegetarians, and individuals with egg allergies (Arozarena et al., 2001; Bennion and Bamford, 1997).

## 3 Conclusions

Future studies recommend to study the effect of mayonnaise prepared from protein isolates on the contents of essential fatty and amino acids and to study the sensory attributes and consumer acceptability of mayonnaises prepared from plant protein isolates. In vivo studies are required to evaluate the effect of mayonnaise prepared from protein isolates on biological properties.

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# 17

# TRENDS AND METHODS FOR NANOBASED DELIVERY FOR NUTRACEUTICALS

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## 1 Introduction

The past couple of decades have seen an enormous development of dietary supplements, functional foods, and herbal products. This popularity is driven by the increasing consumer demand for products of natural origin that have nutritional and therapeutic value. Such products are aptly named as "nutraceuticals," a term that combines "nutrition" and "pharmaceuticals." Nutraceuticals have been projected as formulations that offer health benefits besides providing basic nutrition. Many of the nutraceuticals have also demonstrated therapeutic benefits. Over the years, alternative therapy using nutraceuticals has gained popularity as they are perceived to be a safer alternative/adjuvant to the conventional therapy that uses pharmaceutical drugs. Nutraceuticals have been explored for their ability to improve human health and wellness. Nutraceuticals are used for preventing or delaying a number of age-related diseases, such as Alzheimer's disease, arthritis, osteoporosis, cataracts, cardiovascular, and metabolic diseases (Espin et al., 2007). Many of the chronic ailments have an adjuvant therapy of nutraceuticals that can boost immunity, augment the efficacy of certain drugs, and resist damaging effects of chronic therapy.

A recent market survey and analysis revealed that many factors are driving the demand for a more unique and personalized health solution (www.bccresearch.com). People are becoming increasingly aware and concerned about their health, especially in the developing counties as the per capita income is increasing, many governments are encouraging the use of the safer alternatives to

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00017-9

expensive drug therapies by providing various subsidies, and that there has been a steady increase in the aged populations. According to this market research, there is an exponential growth in the nutraceutical market in the past few years, and it projects that the growth is expected to continue. In 2013, the global nutraceutical market was $160.6 billion and by 2019, it is expected to grow to nearly $241.1 billion. The estimated compound annual growth rate (CAGR) for the nutraceutical market has been projected to rise by 7% from 2014 to 2019 (www.bccresearch.com).

Numerous nutraceuticals are currently widely used. The efficacy and delivery of these many of these nutraceuticals has been the topic of research studies. Examples of popular nutraceuticals include polyvitamins, omega-3 fatty acids, polyphenols from plant sources like curcumin, resveratrol, epigallocatechin gallate, anthocyanins, proanthocyanidins, flavanones, isoflavones, and ellagic acid and carotenoids like lycopene, β-carotene, lutein, zeaxanthin (Espin et al., 2007). Curcumin and its derivatives are best characterized among the nutraceuticals. Curcumin is extracted from the turmeric plant, *Curcuma longa*, belonging to the family zingiberaceae. Curcumin has demonstrated antioxidant, anti-inflammatory, and anticancer properties. Extensive studies have indicated that the anticancer effect is due to the activation of apoptosis signaling and inhibition of many cell proliferation signaling pathways (Hong et al., 2004; Shaikh et al., 2009; Tang et al., 2010). The delivery of curcumin and bioavailability has been extensively studied and these studies indicate that nanoparticle encapsulation of curcumin improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin that is administered with piperine as absorption enhancer (Narayanan et al., 2009; Prajakta et al., 2009; Shaikh et al., 2009). Coenzyme Q10 is another popular nutraceutical that is widely used. It is a benzoquinone derivative that is produced endogenously in the human body and has demonstrated antioxidant properties (Kendler, 1999). Many foods such as liver, heart, and muscles of fish, beef, vegetables, and nuts contain Coenzyme Q10 (Borek, 2006). Thymoquinone is a beneficial phytochemical that is extracted from *Nigella sativa*. This compound acts as a potent therapeutic agent having antioxidant, antiinflammatory, bronchodilator, and anticancer effect (Shah et al., 2010; Singh et al., 2013; Woo et al., 2012). Multiple mechanisms have been proposed for the activity of thymoquinone such as inhibition of proliferation, induction of apoptosis, cell cycle arrest, generation of reactive oxidative species, and antimetastasis/antiangiogenesis (Woo et al., 2012).

The plethoras of nutraceutical formulations that are available in the market today deceptively indicate that the formulation of

nutraceuticals is relatively straightforward. However, it is quite a challenging task to obtain formulations that exhibit satisfactory bioavailability for such nutraceuticals (Zaki, 2014). The poor bioavailability is due to one or more of the following reasons: poor solubility in gastrointestinal fluids, low tissue permeability, low serum levels, limited tissue distribution, apparent rapid first pass metabolism, short circulation half-life, and stability related issues.

One of the options to enhance bioavailability is to increase the dosage of the nutraceutical. However, dose escalation is not always a solution to address limited oral bioavailability, and may increase the risk of adverse events, most frequently in the gastrointestinal system. Thus, the nutraceutical industry is looking out for new technologies to improve not only the bioavailability of nutrients, but also to explore methods that can improve the nutritional value, shelf life, and traceability of their products. They are also aiming to develop improved tastes, reduce the amount of salt, sugar, fat, and preservatives, address food-related illnesses (eg, obesity and diabetes), develop targeted nutrition for different lifestyles and aging population, and maintain sustainability of production, processing, and food safety.

There is a major research impetus for the use of nanotechnology for nutraceutical, pharmaceutical, and cosmetic applications as a novel approach to enhance stability, solubility, and/or permeability. A number of new processes and materials derived from nanotechnology can provide answers to many of unmet needs in the delivery of nutraceuticals, as they offer the ability to control and manipulate properties of substances close to molecular level (Gokce et al., 2010). Fig. 17.1 shows various possibilities for application of nanotechnology in the field of nutraceuticals. Indeed, nanotechnology can provide an avenue to improve not only the products but also the methods used to prepare nutraceuticals with improved biopharmaceutical characteristics.

The small size in combination with their chemical composition and surface structure gives nanoparticles unique features and huge potential for applications. Research indicates that the nanosize will allow easier absorption after oral administration as some particles can get absorbed by endocytosis and these nanoparticles can easily move between cells of the gastrointestinal tract (Chaudhry et al., 2008). While the nanoformulations have clearly demonstrated increased bioavailability of drugs, the detailed mechanism by which these nanobased formulations lead to enhanced absorption of the active ingredient is still a challenge. More studies are required to prove the existing theories of increased solubility, increased rate of mass transfer, increased


**Figure 17.1. Various possible applications of nanotechnology in the field of nutraceuticals.**

retention time, and/or direct uptake of the nanoparticles (Hussain et al., 2001). It has been theorized that an increase in the surface area due to nanoparticles can explain increased absorption, the entrapment of the nanoparticles in the mucosal tissues may lead to longer retention time, or due to the small size the nanoparticles may be directly absorbed (des Rieux et al., 2006).

In fact, the advent of nanotechnology in the field of pharmaceutical sciences has led to novel pathways for addressing the issues with solubility, stability, and/or bioavailability enhancement of problematic nutraceuticals (Zaki, 2014). Pharmaceutical companies continue to strive with a vision to develop the most advanced chemistry of nanosized delivery systems. These are aimed at formulating bioactives for obtaining maximum therapeutic efficacy. In this regard, novel nanostructured biomaterials and formulations are being studied for site-specific targeting and controlled release of not only small molecules that are used as drugs but also for larger biomolecules like the recombinant proteins, vaccines, nucleic acids, and nutraceuticals and functional foods.

With this background, this chapter addresses:

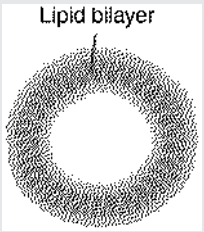
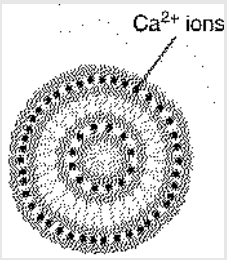
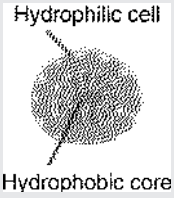
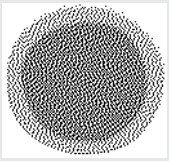
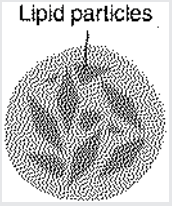
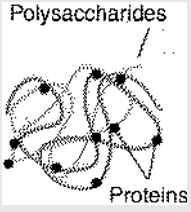
- Many of the nanotechnology ideas and methods that are used for the delivery of pharmaceutical dosage forms are being applied to nutraceuticals, also. These nanotechnology-based delivery systems are discussed along with examples of nanonutraceuticals.
- Emphasis is on nanotechnology applications for increasing bioavailability or addressing the solubility issues of bioactives and nutraceuticals.
- Current marketed products in the nanonutraceuticals category.
- Challenges with regard to safety of nutraceuticals for the human body and the environment.
- Regulatory issues of using nanotechnology-based delivery in nutraceuticals.

## 2 Delivery Systems

During the Experimental Biology 2009 symposium entitled "Nanotechnology Research: Applications in Nutritional Sciences" the potential for application of nanotechnology for targeted delivery of nutrients was the main focus for discussion and presentation (Srinivas et al., 2010). Nanotechnological approaches can be used for encapsulation and controlled-release of active food, drugs, and nutrients. The design of nanoparticles can be in diverse structural forms. These nanoparticles can have different physicochemical properties depending on the materials and methods used. These various structural forms and/or their formulations are shown in Table 17.1.

As shown in Table 17.1, these different structural formulations include nanoemulsions, nanoliposomes, micelles, nanolipid dispersions, nanospheres, nanocapsules, nanocochelates, and coacervates. Nanotechnology-based systems in the absence or in the presence of carriers have been attempted. When a carrier molecule is not used and the bioactive is formulated in the nanoform, it directly enhances wettability and dissolution rates of the bioactive due to increased surface area. However, in the presence of a carrier, biocompatibility and biodegradability-related issues become crucial. Thus, biodegradable FDA-approved polymers are generally preferred by formulators while designing nutraceutical-loaded nanoparticles. The most common methodology used is polymer-based encapsulation that not only modulates the release of the bioactives but also protects the bioactives from degradation, modifying their biodistribution and altering their transport across biological membranes from a passive diffusion process to endocytosis (Mundargi et al., 2008; Patil and Panyam, 2009;

# Table 17.1 Types of Nanobased Delivery Systems and Their Characteristics

| Type-Description | Structure | Size (nm) | Applications | Limitations |
|---|---|---|---|---|
| *Nanoliposomes*<br>• Small vesicles surrounded by lipid bilayer<br>• Consists of phospholipids and cholesterol |  | 10–300 | • Encapsulation of lipophilic and hydrophilic compounds<br>• Controlled release of bioactive compounds | • Physical instability<br>• Chemical degradation |
| *Nanocochelates*<br>• Small vesicles surrounded by solid lipid bilayer<br>• Consist of phosphatidylserine, cholesterol and calcium ions |  | 50–500 | • Encapsulation of lipophilic and hydrophilic compounds<br>• Increased mechanical stability<br>• Better protection of encapsulated compounds | Expensive |
| *Micelles*<br>Consist of surfactant molecules with a hydrophobic core and a hydrophilic shell |  | Less than 100 | Encapsulation of amphiphilic and lipophilic compounds | Large amounts of surfactants are used in the |
| Nanoemulsions<br>Colloidal dispersion of two immiscible liquids (oil and aqueous) with submicron size droplets |  | 10–100 | Encapsulation of amphiphilic and lipophilic compounds | Thermodynamically unstable |
| *Lipid Nanoparticles*<br>Consist of solid lipid core |  | 100–200 | • Increased stability<br>• Controlled release of bioactive compounds | • Crystallization of fat globules<br>• Particle size cannot be controlled |
| *Cocervates*<br>Biopolymer complexes of two oppositely charged protein and/or polysaccharides via electrostatic interactions |  | 10–600 | • Encapsulation of small lipophilic molecules like flavoring oils<br>• Controlled release of bioactive compounds | • Expensive<br>• Complex procedure and structure |

*Source: Adapted with kind permission from Functional Foods and Dietary Supplements: Processing Effects and Health Benefits, Athapol Noomhorm, Imran Ahmad, Anil K. Anal (Eds.), Wiley.*

Ravindran et al., 2010; Song et al., 2009). Additionally carrier molecules can be attached with the targeting moiety on its surface. This leads to target and/or endocytic uptake of nanoparticles, thereby maximizing their intracellular delivery. Targeted delivery is extremely useful and it is one of the most desired attributes in the formulation of a pharmaceutical or a nutraceutical as therapeutic efficiency and toxicity of bioactives are significantly affected (Zaki and Tirelli, 2010; Zaki et al., 2011). This aspect has been demonstrated in the case of Coenzyme Q10. Formulation of Coenzyme Q10 with an aim to target subcellular organelle like mitochondria is beneficial and this has been achieved by a special targeting moiety, namely lipophilic triphenylphosphonium cation. The cation can either be chemically conjugated to the nanocarrier or to the coenzyme Q10 directly (Cochemé et al., 2007). A similar conjugation has been investigated for resveratrol molecule (Biasutto et al., 2008). In case of resveratrol, conjugating resveratrol to the membrane-permeable lipophilic triphenylphosphonium cation provided transient protection against metabolic conjugation. However, conjugated resveratrol accumulated in mitochondria and showed cytotoxic activity on fast-growing cells but not for slower-growing cells (Biasutto et al., 2008). These studies emphasize the importance of such mitochondrial targeting of antioxidant nutraceuticals as a powerful tool to mediate mitochondrial and cellular redox processes that can have pathophysiological consequences (Porteous et al., 2010).

"Polymer conjugates" is a novel approach where a bioactive is chemically linked to a polymer. These polymer conjugates can be designed to control the physicochemical, pharmacokinetic, and therapeutic properties of a bioactive compound. Water-soluble polymer conjugates of anticancer curcumin have been synthesized and analyzed for enhancing the bioavailability of curcumin. These studies showed altered biodistribution and improved anticancer efficacy as it combines the twin advantage of enhanced aqueous solubility and internalization of the drug molecule in presence of the polymer (Safavy et al., 2007).

Another approach to enhance bioavailability is through lipid-based formulations. Lipid-based formulations increase the absorption by enhancing solubilization, prolonging gastric residence time, stimulating the intestinal lymphatic transport pathway, altering intestinal permeability, reduced activity of efflux transporters and reduced metabolism (Chime and Onyishi, 2013). Lipid-based formulations present a large range of optional systems such as solutions, suspensions, self-emulsifying systems, and nanoemulsions. These are discussed in the next sections in detail.

## 2.1 Nanoemulsions

Nanoemulsions are isotropic, thermodynamically stable transparent (or translucent) systems of oil, water, and surfactants with a droplet size usually in the range of 10–100 nm. Their long-term stability, ease of preparation (spontaneous emulsification), and high solubilization of active molecules make them promising as a delivery tool for drugs and nutrients. They are widely applied for oral drug delivery to enhance the solubility and bioavailability of the lipophilic drugs. Nanoemulsions contain continuous phase, dispersed phase, and emulsion stabilizer, the emulsifier or also called as surfactant (Solans et al., 2005). Micelles are lipid molecules that form spherical aggregates of 5 nm diameter in aqueous solutions. These are amphiphilic in nature with lipid core and polar groups on the surface. When lipid molecules enter the micellar core, these aggregates swell to produce spherical particles with diameter of 100 nm or more. The use of lipid-based delivery systems like the micro/nanoemulsions and micelles, offers many advantages over the conventional methods micronizing/milling, cosolvent addition, spray drying, and salt formation (McClements and Rao, 2011). Some of these include relatively higher kinetic or thermodynamic stability over conventional emulsions and suspensions, which provide significantly better stability, applicable for either hydrophilic or lipophilic active component. The incorporation of the nanoparticles as nanoemulsions leads to enhanced tissue distribution due to the decreased size of the droplets in the emulsions. Thus, leading to the improved transport of active ingredients across cell membranes that results in higher bioavailability compared to microemulsions. In particular, nanoemulsions are an attractive form for nutraceuticals because nanoemulsions usually have better stability to particle aggregation and gravitational separation (Tadros et al., 2004). Nanoemulsions contain particles that only scatter light waves weakly, and so they are suitable for incorporation into products that need to be optically clear or only slightly turbid (Mason et al., 2006; Velikov and Pelan, 2008). Nanoemulsions can be designed to form highly viscous or gel-like systems at much lower droplet concentrations than conventional emulsions (Mason et al., 2006; Sonneville-Aubrun et al., 2004; Tadros et al., 2004), which may have interesting applications in nutraceuticals.

The stability of nanoemulsions is due to high interfacial tension and due to considerable surface energy (which is the surface tension times the surface area) (Mason et al., 2006). Nanoemulsions may possess high kinetic stability; however, they are thermodynamically unstable systems because of their characteristic size. The issue related to creaming and sedimentation is not as

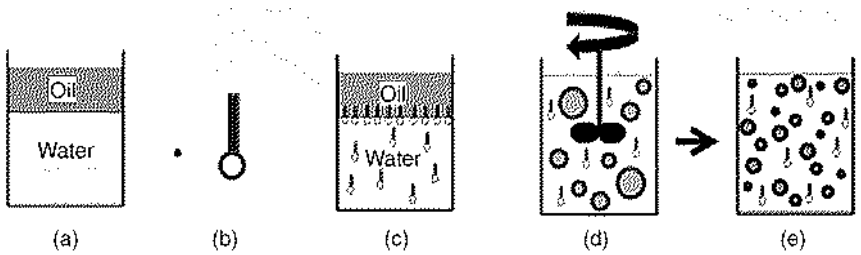



**Figure 17.2. Steps in the formation of nanoemulsions.** (a) Addition of oil to water; (b) addition of surfactant; (c) surfactants primarily at interface; (d) emulsification using energy; and (e) formed nanoemulsions. From Dash et al., 2013, with the kind permission of Elsevier.

significant as the Brownian motion. This is advantageous in the formulation of phytochemicals as the active ingredients are protected and act as a reservoir for the phytochemicals.

The basic process for the preparation of nanoemulsions is shown in Fig. 17.2 (Dash et al., 2013).

The common methods for the preparation of nanoemulsions involve either high- or low-energy emulsifications.

High shear homogenization, microfluidization, high-pressure homogenization, ultrasonic homogenization (Solans et al., 2005), and electrified coaxial liquid jets (Loscertales et al., 2002) are some of the common methods that involve high-energy emulsification processes. Although concepts involving ultrasonic homogenization and electrified coaxial liquid jets have been proven on a laboratory scale, their application for large-scale production needs to be demonstrated (Mason et al., 2006; Solans et al., 2005). Protein and peptide molecules cannot be formulated using high-energy methods as they are temperature sensitive molecules. Thus, use of low-energy emulsification methods is necessary. Phase inversion is one such method that relies on changes in solubility of nonionic surfactant with temperature (Lovelyn and Attama, 2011). In order to obtain smaller and more uniform (lower polydispersity) emulsion systems response surface methods may be used (Yuan et al., 2008). Other methods for the preparation of nanoemulsions involve the preparation of colloidosomes (Dinsmore et al., 2002), cubosomes (Spicer, 2004), and microfluidic channels (Xu et al., 2005).

The choice of oils and emulsifiers used will affect the formulation of nanoemulsions. The type and concentration of oil used for the preparation of nanoemulsions. The contraction of the nanoemulsion components are optimized using pseudo ternary phase diagrams. A phase diagram is a graphical representation that is generally used to determine the optimal formulation by varying

the concentrations of water, oils, emulsifiers, coemulsifiers, and/or cosolvents at different mass ratios. Typically the HLB (hydrophilic lipophilic balance) of the emulsifiers are used as a reference point for optimizing the formulations. Both emulsifiers and coemulsifiers are amphiphilic in nature with an affinity for both oil and water phases. They partition to certain degrees into the interfacial layer of the emulsions. Generally votexing and magnetic stirring is employed in the preparation of these nanoemulsions. Occasionally, the compositional variables such as temperature, shear rate, and pressure are also studied. Four or more components used for a nutraceutical formulation are usually optimized by the construction of pseudoternary diagrams. Typically in these diagrams, each corner represents a mixture of two or more components such as emulsifier/coemulsifier, water/cosolvent/phytochemicals, or oil/cosolvent/phytochemicals (Garti et al., 2003). As the optimization procedures using ternary phase diagrams can be prolonged and extensive, a series of pseudobinary mixtures are prepared initially and then titrated with the third component. It has been observed that for many of the food-grade emulsifiers there is an only limited region for the formation of nanoemulsions.

A wide variety of techniques have been used to characterize the morphological and physical properties of nanoemulsions. Rheometer, conductivity meter, and pendant drop tensiometer are generally used to study the macroscopic properties such as rheological properties, interfacial tension, and conductivity respectively (Boonme et al., 2006). Dynamic light scattering (DLS) techniques are routinely used to characterize the size and shape of the emulsion droplets (McClements, 2005). One of major disadvantages of DLS is that it requires dilution of emulsion samples, which is usually necessary to reduce multiple scattering and interdroplet interactions. Any dilution will affect the pseudoternary phases of the nanoemulsions. Thus, the native structure and composition of the samples could be modified while the nanoemulsions are evaluated by DLS. Other methods such as small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), and microscopy methods like cryotransmission electron microscopy (TEM) (Borne et al., 2002; Spicer et al., 2001) are also commonly used for characterizing the size and shape of droplets in nanoemulsions.

Some of the examples of phytochemicals that have improved characteristics of bioavailability when administered as nanoemulsions are discussed in the next section.

Curcumin nanoemulsions using medium chain triglyceride (MCT), Tween-20, and water at a ratio of 10:10:80 have been successfully prepared using high-pressure homogenization method

by Wang et al. (2008). Their studies demonstrated that application of multiple cycles of high-pressure homogenization can lead to smaller sized nanoemulsions with less polydispersity. Oil droplet nanoparticles of about 79.5 nm in diameter were obtained and this method effectively encapsulated 1% curcumin in the nanoemulsions. It was also demonstrated that the curcumin nanoemulsions had better biological activity in a 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear inflammation model in comparison with curcumin solution. Studies indicated that the size of the nanoparticles in the nanoemulsions also affected the therapeutic efficacy. Curcumin nanoemulsions containing 79.5 nm particles inhibited 85% mouse ear inflammation as compared to curcumin nanoemulsion containing 618.6 nm-sized particles, which showed 43% inhibition. Similar but improved nanoemulsion formulation of curcumin was prepared by Jiang in 2009. This study demonstrated 100% inhibition of inflammation after oral administration of these new curcumin nanoemulsions in the TPA-induced mouse ear inflammation model (Jiang, 2009). Studies with dibenzoylmethane (DBM) nanoemulsions have also demonstrated an enhanced oral bioavailability (Lin et al., 2011). DBM is a constituent found in licorice roots and is a beta-diketone analog of curcumin. This natural phytochemical has shown anticancer activities. Studies have shown that oral bioavailability of DBM can be increased 3-fold by using nanoemulsion over the conventional emulsion (Lin et al., 2011). Pharmacokinetic studies of DBM nanoemulsions showed a mean plasma concentration of DBM reached its peaks at 1.6 h ($t_{max}$). However, the conventional emulsion showed a mean plasma concentration reached peak at of 3.3 h. Thus, nanoemulsion formulations appear to be better as they are absorbed much faster than the conventional emulsions.

## 2.2 Micelles

Micelles are aggregates of amphiphilic molecules that have both hydrophilic and hydrophobic functional groups. In an aqueous solution, as the concentration of the amphilies reach critical micellar concentration (CMC), a number of these amphiphiles spontaneously assemble to form special structures called micelles. The outer shell of these micelles are hydrophilic while the core region is hydrophobic. The lipophilic bioactives are generally trapped in the hydrophobic core of the micelles. Micelles are thermodynamically stable as there is no exogenous energy input required for their formation. This is a key feature that is different from the nanoemulsions. Thus, encapsulation using micelles is relatively simple compared to nanoemulsion preparation. The method

involves an aqueous solution, amphiphilic compound (either of low molecular weight or high molecular weight) with concentration above CMC, and the bioactive. The emphasis is usually on the loading process in micellar encapsulation as it could be tricky and affect the encapsulation capacity and efficiency. A few of the commonly used methods to encapsulate bioactives are listed below.

*Solvent dialysis*: This method involves initial solubilization of amphiphiles and bioactives in a common water-miscible solvent. The resultant solution is dialyzd against water or aqueous solution to remove the solvent.

*Solvent evaporation*: In this technique the amphiphile and the bioactive are dissolved in a common volatile solvent and then the dissolved solution is mixed with an aqueous solution. Later, the volatile solvent is evaporated.

*Coprecipitation*: The amphiphile and bioactive are dissolved in a common solvent and then the solvent is evaporated to form a mixture. This is then mixed with aqueous solution.

*Emulsification*: This popular method involves separate solubilization of amphiphile in an aqueous solution and the bioactive in water-immiscible volatile organic solvent. The organic mixture is then added to the aqueous solution to form emulsion followed by the evaporation of the organic solvent. The major advantage of this method is that it generates higher encapsulation yields (Soleymani Abyaneh et al., 2014; Lavasanifar et al. 2007).

Nanodispersions that are based on biopolymer micelles is a fast-growing area in the delivery of nutraceuticals or drugs. The main advantage is the extended release of the bioactives through nanodispersion formulations. Studies by Yu and Huang showed that micelles can be generated from hydrophobically modified starch (HMS) in aqueous solution (Yu and Huang, 2010). A comparison of the curcumin-loaded HMS micelles with the free curcumin showed almost a 1700-fold increase in the solubility of curcumin in pure water. This can be correlated to the observed higher bioactivity of encapsulated curcumin than that of free curcumin as demonstrated in the in vitro anticancer model studies. Similarly, casein micellar formulations of curcumin have also shown increased bioavailability of curcumin (Sahu et al., 2008; Yu and Huang, 2010). However, it should be noted that not all micellar formulations enhance the delivery of encapsulated bioactives. For example, encapsulation of curcumin using poly(ethylene oxide)-b-poly(epsiloncaprolactone) and methoxypoly(ethyleneglycol)-palmitate did not increase the bioavailability of curcumin as tested in cancer cells (Ma et al., 2008; Sahu et al., 2008). Thus, these studies may indicate that natural components may be better in terms of facilitating the cellular uptake of micelles.

Extensive research is also focused on obtaining better biodegradable and biocompatible surfactants. Huang and others synthesized a chitosan-based amphiphile, namely, octanoyl chitosan-polyethylene glycol monomethyl ether (acylChitoMPEG). This was synthesized using both hydrophobic octanoyl and hydrophilic polyethylene glycol monomethyl ether (MPEG) substitution (Huang et al., 2010). These novel chitosan-based amphiphiles exhibited good solubility both in water-based solutions and in common organic solvents such as ethanol, acetone, and chloroform. Higher concentration of acylChitoMPEG at 1 mg/mL exhibited negligible cytotoxicity. The ability to alter hydrophilic–lipophilic balance (HLB) values by controlling the amount of hydrophobic octanoyl or hydrophilic PEG moieties is a major advantage of this synthetic method. This flexibility offers possibilities to encapsulate a wide range of nutraceuticals or drugs.

β-Carotene is a well-established natural antioxidant agent that has been used as a nutraceutical. However, its poor water-solubility, low chemical stability, and low bioavailability limits its application in food, pharmaceuticals, and cosmetics industries. Wang and coworkers synthesized amphiphilic chitosan-graft-poly (lactide) (CS-g-PLA) copolymer via a homogeneous ring-opening polymerization in ionic liquid. They showed that the CS-g-PLA copolymer was able to self-assemble into about 14 nm nanomicelles in water at low concentration. The self-assembled nanomicelles of CS-graft-PLA copolymer were investigated as the capsules of β-carotene. The stability and antioxidant activity of β-carotene encapsulated by CS-g-PLA micelles. These for β-carotene loaded nanomicelles had improved in vitro antioxidant activity in comparison with free β-carotene.

The micelles have been good candidates to encapsulate nutraceuticals as generally they exhibit enhanced bioactivities. Although these micellar formulations are usually tested in cell culture assays or by intravenous injection into experimental animals (Jones and Leroux, 1999), it is still a challenge to evaluate these bioactive micelles on oral administration. More research needs to be done to obtain a comprehensive understanding about the pharmacokinetic properties of the micellar formulations.

## 2.3 Nanoliposomes

Liposomes are lipid bilayered membrane structures composed of phospholipids, which have hydrophilic heads and hydrophobic fatty acid tails. Lipsomes have a biphasic character and thus can be used as carriers for both hydrophilic and hydrophobic compounds. The application of liposomes spans a variety of areas such as in drug delivery, cosmetic formulations, diagnostic agents, and

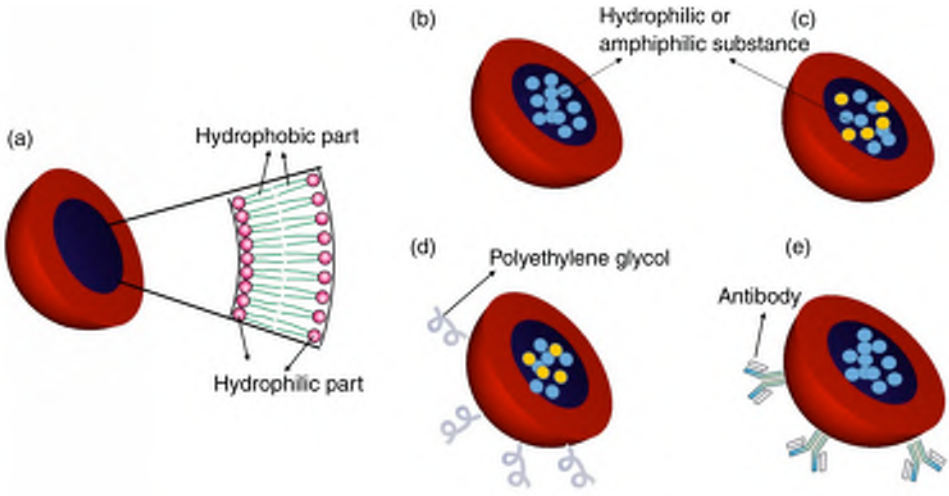



**Figure 17.3.** (a) Liposome structure with the bilayer; few examples liposomes as carrier of hydrophilic and amphiphilic substances (b) mono substance and (c) two substances; liposomes as targeted delivery systems using (d) PEG (polyethylene Glycol) and (e) Antibody.

food industry (Allen and Cullis, 2013). The US Food and Drug Administration (FDA) has approved liposome-based drugs and they are available in the market for treating different diseases (Harrison et al., 1995). The various types of liposomes for possible applications in drug delivery are shown in Fig. 17.3.

Nanoliposomes are essentially nanometric versions of liposomes. They are colloidal structures that are formed when a phospholipid is mixed into an aqueous solution using high-energy input. Pharmaceutical, cosmetic, and food industries have carried out extensive research on nanoliposomes as they have a great potential to be developed into effective nanocarrier systems. They are advantageous as they protect and aid in the delivery of bioactive agents. Liposomes and nanoliposomes have the same chemical, structural, and thermodynamic properties; however, the smaller size of nanoliposomes can lead to a larger interfacial area of encapsulated compounds. Thus, nanoliposomes can penetrate biological tissues providing higher potential to increase the bioavailability of encapsulated compounds (Mozafari et al., 2009). The lipid vesicles consist mainly of phospholipids, which are the main components of the naturally occurring lipid bilayers of physiological membranes. The important characteristic of bilayer-forming molecules is their amphiphilic nature. Liposomes exhibit varied shapes such as lamellar, hexagonal, micellar, or cubic phases (Siegel and Tenchov, 2008). A point to be noted is that liposomes are closed, continuous, vesicular structures composed mainly of

phospholipid bilayer(s) in an aqueous environment (Mozafari and Mortazavi, 2005). The main aim of developing these vesicular structures has been for specialized applications, such as ultradeformable vesicles for transdermal drug delivery (Dubey et al., 2008) or arseno-liposomes for anticancer therapy (Zagana et al., 2008).

Natural ingredients such as eggs, soy, or milk can be used in the manufacture of nanoliposomes (Thompson et al., 2007). Preparation of nanoliposomes in aqueous solution requires higher energy input (Mozafari et al., 2008). The commonly used methods for nanoliposome synthesis include sonication, extrusion, freeze-thawing, ether injection and microfluidization. On a laboratory scale, sonication and extrusion techniques have been widely used (Mozafari, 2010; Mozafari et al., 2008 ); whereas microfluidization method is commonly used for industrial manufacturing. Microfluidization involves high pressure and high force technologies using a device called a microfluidizer to produce a flow stream passing through a fine orifice in order to reduce particle sizes of liposomes. A schematic representation of the microfluidizer and a small extruder commonly used in the manufacture of nanoliposomes is shown in Fig. 17.4. The process begins when the product enters the inlet reservoir. An intensifier pump generates extremely high pressures to accelerate the product to the interaction chamber at velocities over 400 m/s. Inside this Y-type chamber, the stream separates into microchannels, which are as narrow as the cross-section of a human hair. The

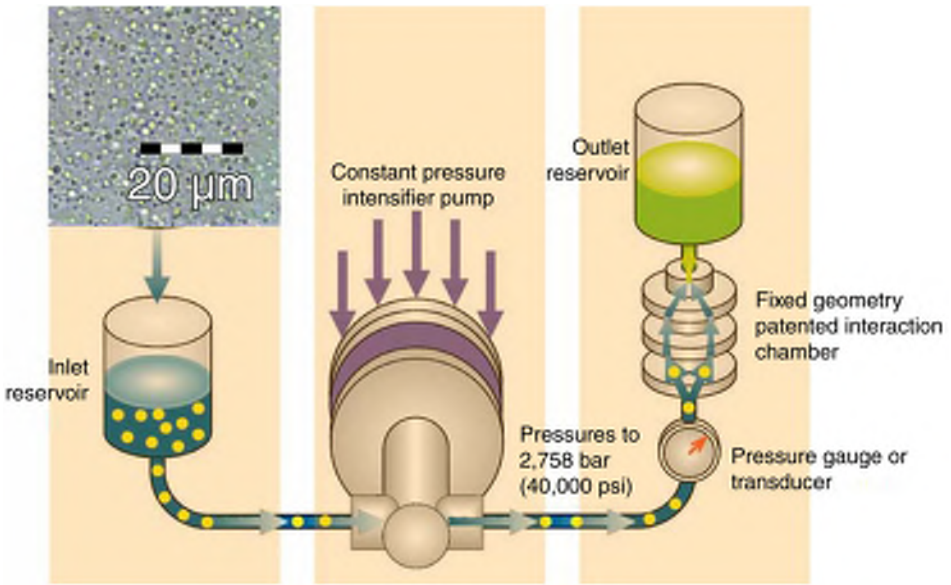



**Figure 17.4. Schematic representation of a microfluidizer.** Courtesy of Microfluidics International Corporation.

product is then forced to collide upon itself due to the impinging jet design, which produces incredible forces of impact and shear. Finally the finished product is cooled to prevent loss of actives.

Nanoliposomes have many distinct advantages. The methods used for the preparation provide control over particle size, and are highly reproducible for large-scale preparation, and do not involve use of toxic solvents (Mozafari, 2010). The major drawback of nanoliposomes is that in the circulatory system nanoliposomes are recognized as foreign particles and are rapidly cleared by the reticuloendothelial system (Tang et al., 2013). Additionally, electrostatic, hydrophobic, and van der Waals forces can disintegrate nanoliposomes (Lasic et al., 1991). Therefore, steric stabilization is required and can be achieved by coating the nanoliposomes with inert polymers (Momekova et al., 2007; Tang et al., 2013). The FDA approved the first liposomal drug, a PEG-lated liposomal formulation of doxorubicin (Doxil in the United States and Caelyx outside the United States), for the treatment of Kaposi's sarcoma in 1995 (Harrison et al., 1995). The success of liposomal doxorubicin has initiated development of many liposomal formulations that are currently under test in clinical trials. Among nutraceuticals, nanoliposomes suspensions of multichain fatty acids with vitamin C that were prepared and freeze dried for long-term storage show promising approach (Yang et al., 2013). Vitamin D3 was formulated as nanoliposomal product that can be used for fortification of beverages (Mohammadi et al., 2014).

## 2.4 Lipid Nanoparticles

The variation of the nanoemulsion where liquid lipid in emulsion is replaced by high melting point solid lipid opens a new area for the delivery of drugs and nutraceuticals. In the pharmaceutical field, lipid nanoparticles based on solid matrix have been extensively studied as potential drug carriers to improve gastrointestinal (GI) absorption and oral bioavailability of several lipophilic compounds (Mehnert and Mader, 2002; Radtke et al., 2005). The formulations are designed for sustained drug release (Muller et al., 2000). As oral formulations, both solid lipid nanoparticles (SLN) and the newer generation of lipid nanoparticle, called nanostructured lipid carrier (NLC), have been studied for their capability to act as drug carriers (Shidhaye et al., 2008). The use of biodegradable, biocompatible, and physiological lipids is generally favored in the preparation of these nanoparticles. Thus, toxicity problems related to the polymeric nanoparticles can be minimized. Studies have also demonstrated that formulations containing SLNs have increased stability in comparison to other

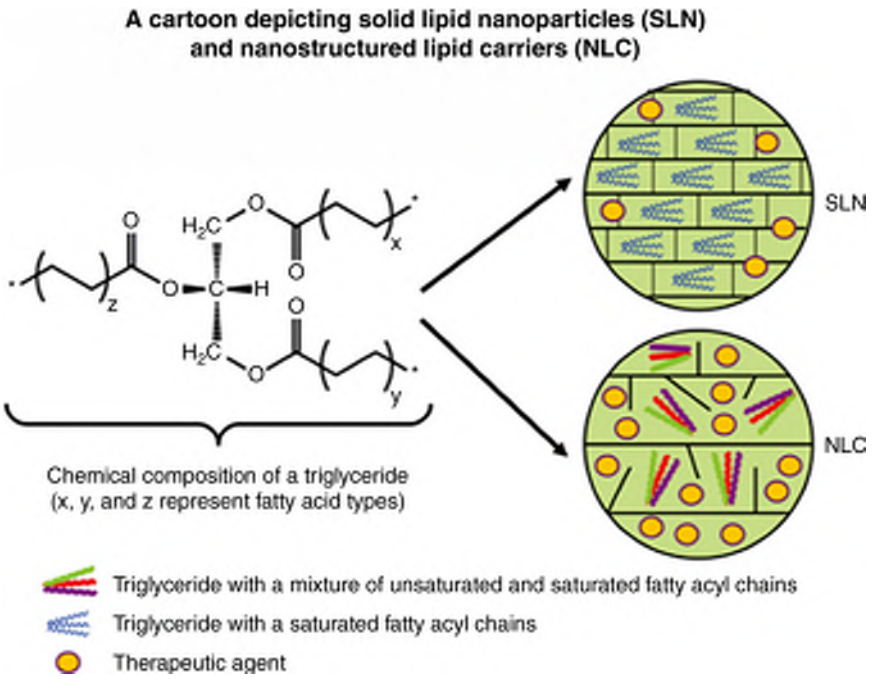



**Figure 17.5. Schematic representation of solid-liquid nanoparticles (SLN) and nanostructured lipid carriers (NLC).**
From Puri et al., 2009, with the kind permission of the National Center for Biotechnology Information (NCBI).

liquid nanocarriers. This can be attributed to the presence of the solid matrix in these lipid nanoparticles (Hu et al., 2004). Fig. 17.5 shows the structure of SLN and NLC.

SLNs have distinct advantages:

- Better control over release kinetics of encapsulated compound
- Lipid composition and size can be engineered to suit the needs
- The release of the active ingredient can be triggered by the melting of the SLN
- Enhanced bioavailability of entrapped bioactives
- Chemical and environmental protection of labile compounds
- Much easier to manufacture than biopolymeric nanoparticles
- Nontoxic solvents used
- Ease of manufacturing
- Very high long-term stability
- Can be subjected to commercial sterilization and freeze drying

These nanoparticles can be produced by different formulation techniques. The major approaches in the preparation of SLNs are hot homogenization (homogenization at elevated temperatures), hot microemulsification (formation of microemulsion at elevated temperatures) and cold homogenization (homogenization at

low temperatures using milling processes) (Muchow et al., 2008). These procedures can be easily scaled up for production from lab scale to industrial scale. It has been well documented that reasonably high drug encapsulation efficiency of the nanoparticles can be achieved. Drugs, nutrients, and cosmetics have been formulated as solid lipid nanoparticles (Shidhaye et al., 2008).

Although SLNs have shown tremendous potential there are several disadvantages associated with SLNs. Some of these are that SLN dispersions contain high amount of water, the crystalline structure of the solid lipid limits the drug-loading capacity of SLNs, particle growth or gelation issues along with release of the drug during storage can affect the release profile of drug, and polymorphic transitions of the solid lipids are possible (Müller et al., 2002a,b).

The research work by Neves et al. focused on the development of lipid nanoparticles for the incorporation of the poorly soluble lipophilic resveratrol to improve its oral bioavailability. Resveratrol-loaded lipid nanoparticles (SLNs and NLCs) were successfully produced by a modified hot homogenization technique (Neves et al., 2013). This study showed that resveratrol formulated as SLNs and NLCs was protected against physical and chemical degradation and had a desirable controlled release profile as indicated in the simulated in vitro studies.

Triptolide is a purified compound of a traditional Chinese medicine with antiinflammatory, immunosuppressive, antifertility, and antineoplastic activity (Chen, 2001). Mei et al. showed that solid lipid nanoparticles prepared for transdermal delivery increased triptolide penetration into the skin and its antiinflammatory activity (Mei et al., 2003). It is assumed that 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.

## 2.5 Nanocapsules

Lipid nanocapsules are submicron particles made of an oily liquid core surrounded by a solid or semisolid shell. Earlier studies have demonstrated a lipid-based solvent-free formulation process for the preparation of nanocapsules. Huynh et al. have developed new nanocargos, the lipid nanocapsules, with sizes below the endothelium fenestration (size range of less than 100 nm) that solve the disadvantage of liposomes. They are prepared according to a solvent-free process and they are stable for at least 1 year in suspension ready for injection, which should reduce considerably the cost and convenience for treatment. Moreover, these new nanocargos have the ability to encapsulate efficiently

lipophilic drugs, a much needed characteristic of a pharmaceutical solution for intravenous administration. The lipid nanocapsules (LNCs) have been prepared according to an original method based on a phase-inversion temperature process developed and patented (Sawant and Dodiya, 2008). Their structure is a hybrid between polymeric nanocapsules and liposomes because of their oily core, which is surrounded by a tension active rigid membrane. They have a lipoprotein-like structure. Their size can be adjusted below 100 nm with a narrow distribution. Importantly, these properties confer great stability to the structure (physical stability of more than 18 months). Blank or drug-loaded LNCs can be prepared, with or without PEG that is a key parameter that affects the vascular residence time of the nano cargos. Other hydrophilic tails can also be grafted. Different anticancer drugs (paclitaxel, docetaxel, etoposide, hydroxyl tamoxifen, doxorubicin, etc.) have been encapsulated. They all are released according to a sustained pattern. Preclinical studies on cell cultures and animal models of tumors have been performed, showing promising results (Huynh et al., 2009).

Markman and Livney prepared separate nanocapsules of vitamin D and epigallocatechin-3-gallate using casein -maltodextrin Maillard reaction conjugates. Clear solutions were obtained with nanoencapsulated particle size of vitamin D-loaded nanoparticles being smaller than that maltodextrin caseinate mixture with vitamin D. The nanocapsules had good solubilization, stabilization, and protection against degradation during shelf life, and gastric digestion. It was observed that conjugation considerably improved the protection against both oxidation and low pH for vitamin D. This is a desirable feature for acid drinks and for stability against gastric digestion (Markman and Livney, 2011).

## 2.6 Nanospheres

Nanospheres are the spherical particles with a diameter of 10–200 nm. These nanospheres have demonstrated enhanced-size dependent properties in comparison to larger spheres of the same material. Nanosphere is a delivery system where a drug is dissolved, entrapped, encapsulated, or attached to the matrix of a polymer. The drug/nutraceutical is uniformly dispersed in the matrix system of polymer. Nanospheres can be amorphous or crystalline in nature. The main advantage of the nanosphere is that they protect the encapsulated product from enzymatic and chemical degradation (Mohanraj and Chen, 2006). Lipid emulsion based nanosphere carriers are dynamic structured. The dispersed

nanodroplets are generated from natural lipids that possess an outer phospholipid layer and an encapsulated inner lipid core (Iqbal et al., 2012; Radtke et al., 2005). It has been shown that the hydrophobic surfaces of these particles are highly susceptible to clearance by the reticulo-endothelial systems (RES). Attempts have been made to alter the surface of nanoparticles by adsorbing various surfactants to the particle surface including poloxamine, poloxamer, and other polymers (Mueller and Wallis, 1993).

Nanospheres can be envisaged as tiny spherical entities, in which drugs are wrapped by polymer materials or dispersed in polymer materials. These tiny capsules act as a store house for drugs. These structures are called vesicles and the solid skeleton structure is called nanospheres. As a novel system for delivery of the bioactives, nanospheres are advantageous because they can be ingested or injected giving a choice for mode of administration of the bioactives. Additionally, they can be tailored for desired release profiles and are applicable for site-specific delivery of bioactives. They have a potential for organ-targeted release of the bioactives, also.

Nanospheres can be prepared into biodegradable nanospheres and nonbiodegradable nanospheres. Albumin nanospheres, modified starch nanospheres, gelatin nanospheres, polypropylene dextran nanospheres, and polylactic acid nanospheres, are examples of biodegradable nanospheres. Polylactic acid polymer-based nanospheres are approved to be used as a controlled-release agent in drug formulations. Immune nanospheres and magnetic nanospheres have also been prepared as special application delivery systems. Antibody and antigen is coated or adsorbed on the polymer nanospheres in immune nanospheres whereas in magnetic nanospheres, magnetic polymer nanoparticles are generally coated with protective shells.

There are several distinct advantaged of nanospheres drug delivery systems. Studies have demonstrated that they can easily pass through the smallest capillary vessels due to their tiny volume (Illum, 2007; Jung et al., 2000). They are well suited for application as slow release or controlled release of bioactives as they can avoid the rapid clearance by phagocytes prolonging their duration in bloodstream. Another advantage is the potential for application as organ-targeted delivery as they can easily penetrate the cells and tissue gap to arrive at target organs, for example, liver, spleen, lungs, spinal cord, and lymphs. Site-specific targeting can be achieved with nanospheres as small ligands can attached to the surface of the spheres. They can be designed for various routes of administration including oral, nasal, parenteral, and so on (Mohanraj and Chen, 2006). Generally they have lower

toxicity-related issues. However, there are a few challenges in the development of the nanosphere delivery systems. It is difficult to handle the liquid as well as the dry forms. Aggregation is another factor that can affect the formulation. The smaller size and larger surface area can lead to difficulties in maintaining dosage and burst release can also affect the dosage (Zhang et al., 2001).

There are various types of methods by which nanospheres are prepared. These include polymerization (emulsification polymerization), solvent evaporation, solvent displacement technique, and phase inversion temperature-based methods. These methods are similar to a large extent to the procedures used for the preparation of encapsulation discussed earlier in the chapter.

Indeed, nanospheres have been projected as a convenient dosage forms specifically for many dietary supplements, nutraceuticals, and phytonutrients because they provide a much needed greater stability, bioavailability, bioactivity, and efficacy. NanoSphere Health Sciences has a patented technology that claims that NanoSphere delivery system enables formulas up to 6 times more bioavailable than traditional supplements (http://www.nutraceuticalbusinessreview.com/technical/article_page/Futureproofing_nutraceuticals_with_nanotechnology/103574). In their patented technology, nanospheres are prepared from natural phospholipids (phosphatidylcholine) and fatty acids. The process involves dispersion and homogenization techniques that protect nutraceuticals and generate a highly concentrated NanoSphere gel.

## 2.7 Nanocoacervation

The chemical method of coacervation involves dispersion of the bioactive core material in a nonsolvent continuous phase to generate an emulsion or dispersion (the continuous phase must be a nonsolvent for the dispersed phase). Generally the shell is created by a chemical reaction. This is followed by precipitation of one or more shell-precursor materials that are dissolved in the continuous or discontinuous phase. It is possible to have different components of the shell dissolved in both phases.

The basic procedure involved in the preparation of microcapsules prepared by coacervation relies on the fact that oppositely charged polymers condense in solution and can attach to the surface of an emulsified oil droplet. The polymers can be natural or synthetic polymers. As said earlier, in coacervation encapsulation, a shell material precursor can be dissolved in the continuous phase. Gelatin or Arabic gums are the classic shell materials used and these are dissolved in an aqueous continuous phase.

The solubility of the dissolved shell material can be altered by varying the conditions of the continuous phase. This can be done either by altering the pH or by adding a water-miscible solvent. The slight alteration of solubility of the shell material causes the shell material to migrate to the surface of the dispersed core. As it reaches the surface, it can coat the noncontinuous droplets. The shell material can be chemically crosslinked by adding another. Thus, a hard shell is formed around the active core (Baruch and Machluf, 2006). A wide range of encapsulated products can be produced by this method. Encapsulation provides added advantage of flavor-masking, payload degradation protection, or ease of handling a liquid as a solid (the dried capsules can be handled as a powder and the liquid contents evaporate only very slowly).

Phase separation can be used as an alternative technique for the preparation of microcapsules from a wide range of hydrophobic polymers, such as poly(methylmethacrylate)—Plexiglass®.6. This method allows for use of polymers whose properties are dependent on pH. Thus, pH-sensitive delivery systems can be designed using these methods.

## 3 Characterization of Nanoparticle-Based Delivery Systems

In order to understand the nature and properties of nanoparticle-based formulations and the risks involved in using the nanoparticle-based delivery systems, it is essential to detect, identify, and characterize nanoparticles with good analytical techniques. Besides concentration, other characteristics of nanoparticles such as particle size and shape, particle size distribution, surface charge, and chemical characteristics, dissolution rate, and mass of the particles should also be studied in detail to efficiently design an optimized delivery system for biomedical applications. To evaluate effectiveness and success of developing an ideal nanoparticle delivery system, efficient characterization tools need to be employed. Although there is no single analytical tool that can provide all the relevant information about the nanoparticles, a range of validated methods can be adopted for analyzing the nanoparticles. These methods are based on the nature of the material from which the nanoparticles are prepared or based on the final formulation that is being used. Table 17.2 provides an overview of the techniques that can be used for characterising specific features of the nanoparticles.

Some of the commonly used methods are discused below. Fig. 17.6 show the images of microscopy andspectroscopy data.

## Table 17.2 The Different Data That Can Be Obtained Using Various Methods for Characterization of Nanoparticles

| Method | Particle Size/Size Distribution | Aggregation | Dissolution | Chemistry | Shape | Structure | Concentration | Mass | Surface Area |
|---|---|---|---|---|---|---|---|---|---|
| DLS spectroscopy | + | + | + | + | | | + | | |
| Raman spectroscopy | + | | | + | | + | | | |
| XRD | | + | | + | | + | | | |
| Microscopy | + | + | + | + | + | + | | | + |
| Chromatography | + | | + | | | | | | |
| Ultracentrifugation | + | + | + | + | + | + | | + | |

*Source: Adapted from Nair et al., 2010 with the kind permission of Elsevier.*

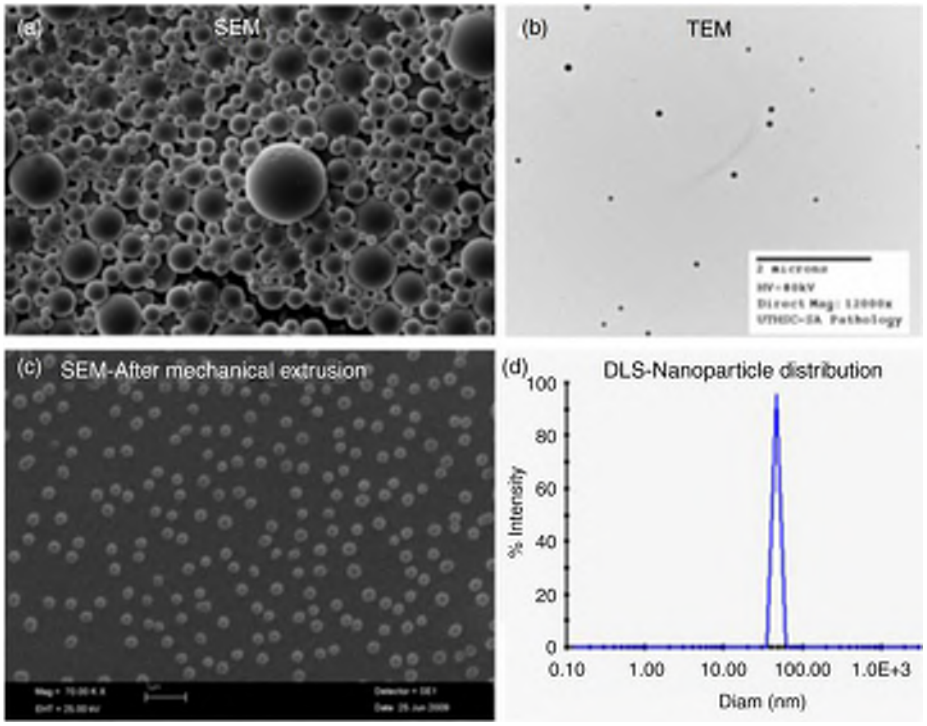



**Figure 17.6. Some of the common methods used in the characterization of nanoparticles.** (a) Scanning Electron Microscopy (SEM) image of nanoparticles; (b) transmission Electron Microscopy (TEM) image; (c) SEM image of nanoparticles after mechanical extrusion; (d) determination of size distribution of nanoparticles using dynamic light scattering (DLS). From Nair et al., 2010 with the kind permission of Elsevier.

## 3.1 Spectroscopy-Based Techniques

Spectroscopy is the most common method that is widely applicable for a range of materials. It effectively provides data about the concentration, size, and structure of nanoparticles. Apart from the common UV-visible absorption spectroscopy, light scattering spectroscopy is generally used for the charaterization of the nanoparticles. Light scattering spectroscopy measuures the amount of light scattered at certain wavelengths, incident angles, and polarization angles can be used for measuring particle size of nanoparticles. Dynamic light scattering (DLS) is the most widely used technique that can measure particle size in the range of 3–1000 nm (Brar and Verma, 2011). Another method is the nanoparticle tracking analysis (NTA), which is based on tracking the Brownian motion of the individual particle positional changes using light scattering techniques in two dimensions (Kendal et al., 2009). Zeta potential measurements can also be done on

the DLS and NTA instruments that involves measurement of the nanoparticle mobility upon application of an electric field across the nanoparticle dispersions. Although both methods require minimum quantity of the sample, are cost effective, and provide information about the size distribution, the methods are nonspecific and they do not provide any information about the chemical characteristics of the nanoparticles.

Raman spectroscopy involves both light scattering and laser techniques that can be used for molecular identification, oxidation state, structure, and size analysis of nanoparticles (Wong et al., 2009). The basis of this technique is the Raman Effect where the laser light scattered by a particle causes a change in the energy of the laser photons which can be analyzed to obtain information about the nanoparticle characteristics.

X-ray diffraction (XRD) technique is based on the observation of scattering of X-ray beam by the nanoparticles. This method can provide information about the crystallographic nature, chemical composition, and the particle size of the nanoparticles. Variation of the XRD, namely small angle X-ray scattering (SAXS) and small angle neutron scattering (SANS) are also used to study the size and shape of nanoparticles (Zabar et al., 2008).

## 3.2 Microscopy-Based Techniques

Imaging methods have become indispensable for the identification and characterization of nanoparticles. Microscopy techniques provide data about the morphology characteristics such as size and shape of the nanoparticles, particle size distribution, and chemical composition/purity of the sample, agglomeration of particles and presence of associated substances. Electron microscopy techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are very commonly used. In electron microscopy, an electron beam, which is accelerated with high voltage, is allowed to pass through the nanometric layer of the sample. Interaction of these electrons with the sample surface results in generation of secondary electrons that are knocked out from the sample material. Analysis of these electrons and the electrons that are transmitted through the sample gives information about the structure, topography, and composition of the material. Although SEM does not give higher resolution than TEM, coupled techniques such as scanning transmission electron microscopy (STEM) and focussed ion beam scanning electron microscopy (FIB-SEM) can be used to enhance the imaging sensitivity.

The fluorescence characteristics of nanoparticles can be used for detection and characterization using confocal laser scanning

microscopy (CLSM) and X-ray fluorescence (SRF) microscopy. Atomic force microscopy (AFM) can be used to probe the morphological characteristics and the particle size distribution of nanoparticles in sample.

### 3.3 Chromatography-Based Techniques

These methods are generally used for size separation of nanoparticles. Some of the examples are size exclusion chraomatography(SEC), hydrodynamic chromatography (HDC) and field flow fractionation (HFF). Coupling of the chromatographic instruments with other spectroscopic techniques like light absorption or light scattering methods can be used to analyze the concentration and the chemical composition of the separated particles. SEC enables separation of nanoparticles by their size and molecular mass. On the other hand HDC separates particles based on the hydrodynamic radium of the nanoparticles. FFF instruments are more sensitive and have better resolution as they can be used to separate particles ranging in size from 1 nm to a few micrometers.

### 3.4 Other Methods

Mass spectrometry is used for chemical and molecular mass characterization. Centrifugation, filtration, and dialysis techniques can be used for size fractionation. Thermogravimetric analysis and differential thermal analysis can be used to analyze nanomaterial characteristics, degradation temperature, and moisture content. They can also be used for measuring phase changes, melting, purity, aggregation, and so on.

The application of various methods listed earlier are shown in Table 17.2. Typically a combination of these methods are generally employed to characterize nanoparticles. A point to be noted is that more of the samples need pretreatment of the samples to suit the requirements of the instrument used for the analysis. Thus, care should be taken to ensure that the protocol involves minimal disturbance of the nanoparticles in the native or in the formulation form so that the data obtained will be as accurate as possible to the native form of the nanoparticles. Thus, there is a need to focus on developing methods for in situ analysis along with standardization and calibration materials for some of these methods.

## 4 Nanonutraceuticals in the Market

Indeed there are many studies that have shown that nanoscale delivery of nutraceuticals will provide better bioavailability and efficacy. Some of these are listed in the Table 17.3.

## Table 17.3 Some of the Nutraceuticals That Have Been Formulated as Nanobased Delivery System

| Bioactive Agent | Polymer Used | Size in nm | Target in the Body | Reference |
|---|---|---|---|---|
| Curcumin | PGLA | 80.9 | Lung, prostrate, breast cancer cells | Anand et al., 2009 |
| Curcumin | Alginate-Chitosan | 100–120 | Cervical cancer cells | Das et al., 2009 |
| Dibenzoyl methane | Polylactic acid | 77–96 | Cervical cancer cells | Contreras et al., 2010 |
| Ellagic acid | PGLA-polycaprolactone | 120 | Kidney | Sonaje et al., 2007 |
| Ferulic acid | Bovine Serum albumin | 100–200 | Liver | Li et al., 2009 |
| Quercetin | Polylactide | 50 | Brain | Das et al., 2008 |
| Resveratrol | Poly caprolactone PEG | Less than 100 | Neuronal cells | Lu et al., 2009 |
| Thymoquinone | PLGA | 150–200 | Leukemia cells | Ravindran et al., 2010 |
| Triptolide | Poly(D,L-lactic acid) | 149.7 | Skin | Liu et al., 2005 |

*Source: Adapted from Nair et al., 2010 with the kind permission of Elsevier.*

There are a few food and nutrition products containing nanoscale additives that are already commercially available. The increasing number of patents applied for in the field of nanotechnology related to food, drugs, and cosmetics are proof that many companies are conducting research on the application of nanotechnology to engineer, process, package, and deliver better nutraceuticals/bioactives by applying nanotechnology. According to a study conducted by Helmut Kaiser Consultancy in 2009 on nanobased products inventory and commercialization, there were 2580 nanobased products in the market, in more than 220 of them in the food/nutraceutical category (http://www.hkc22.com/nanobasedproducts.html). Among them are the giant food and beverage corporations, as well as tiny nanotechnology start-ups. In addition to a handful of nanofood products that are already in the market, more than 135 applications of nanotechnology in food industries (primarily nutrition and cosmetics) are in various stages of development (Ravichandran, 2010). According to Helmut Kaiser, more than 200 companies worldwide are engaged in nanotech research and development related to food. Among the 20 most active companies are 5 companies that rank among the world's top 10 largest food and beverage corporations, namely, Australia's leading Food Corporation, and Japan's largest seafood producer and processed food manufacturer. Some of the world's largest food

manufacturers, including Nestĺe, Altria, H.J.Heinz, and Unilever, are ahead with hundreds of smaller companies following their lead. In Australia, nanocapsules are used to add omega-3 fatty acids to one of the country's most popular brands of white bread. According to the manufacturer, nanocapsules of tunafish oil are added to TipTop Bread to provide valuable nutrients, whereas the encapsulation prevents the bread from tasting fishy. NutraLease, a start-up company of the Hebrew University of Jerusalem, has developed novel carriers for nutraceuticals in food systems. The nanosized self-assembled structured liquid (NSSL) technology allows for encapsulation of nutraceuticals, cosmeceuticals, and essential oils and drugs in food, pharmaceuticals, and cosmetics. Another advantage to the NSSL technology is that it allows the addition of insoluble compounds into food and cosmetics. One of the first products developed with this technology—a healthier version of canola oil—is already available to consumers in Israel.

## 5 Challenges Ahead

Unlike pharmaceutical drugs, nutraceuticals are widely available and not monitored with the same level of scrutiny by the food and drug authorities. There are still broad-based definitions of nutritional supplements. Added to this are the undistinguishing standards and functions between nutraceuticals and drugs, which creates inconsistent credibility to nutraceuticals. A number of safety, ethical, environmental, policy, and regulatory issues have been raised in view of the rapid proliferation of nanotechnologies in a wide range of consumer products (Maynard, 2006). The lack of knowledge with regard to molecular or physiological effects and their potential harm to individuals as well as the community at large are one of the main concerns in the field of nanotechnology today. Nanotechnology-based products will have a direct impact on consumer's health and the environment. Although many different delivery systems are now available to deliver bioactive components in nutraceuticals, clear in vitro and in vivo evidences of their biological efficacies are still limited. The small size of these particles allows them to gain easy access to the organs within the body. Normally, many of the organs such as placenta, brain, retina, and thyroid in the human body are protected, thus preventing the passage of the extraneous particles into them. But nanoparticles, due to their smaller size, can gain access to these organs that can lead to toxicity issues. As an example, the presence of the blood-brain barrier prevents the passage of streptomycin into the brain. However, if it does cross into the brain it will result in convulsions. Similarly, nanoparticles may become teratogenic by

crossing the placental barrier and affect the development of fetus. Some major health risks associated with such nanoparticle-based delivery systems includes: cytotoxicity, translocation to undesired cells, acute and chronic toxicity; some unknown, unpredictable, and undefined safety issues; environmental impacts of nanomaterials and nonbiocompatibility. These issues need to be addressed in an extensive and methodical approach. It is very possible that the slow absorption rate of a substance may be the reason for the safety of some of the bioactives. A rapid absorption of the active ingredient in a formulation may lead to toxicity concerns.

Indeed, from a more specific toxicological perspective, applications of nanotechnology in food, drugs, or nutraceuticals can have serious limitations. These include

*tendency for aggregation*: as the surface energy is high, nanoparticles can aggregate in biological systems

*reduced biological half-life*: the RES may be activated leading to quick scavenging of the nanoparticles

*solubility issues*: synthesis nanoparticles may have reduced biocompatibility due to poor solubility in the biological system

*diffused distribution*: due to the smaller size the nanoparticles can distribute very quickly to various organs leading to poor target and site specificity

*acute and chronic toxicity*: deposition into various no-targeted organs often raises concerns over acute and chronic toxicity related problems

Despite these shortcomings, nanoparticulate systems have demonstrated remarkable potential for applications in various pharmaceutical and biomedical, which has led to market applications. Pharmaceutical nanotechnology has provided fine-tuned diagnosis and focused treatment of disease. However, some ethical, scientific, social, and regulatory issues would pose various challenges in practical realization of pharmaceutical/nutraceutical nanotechnology. Besides, some ethical issues would alter gene expression, ultimate fate and alterations would bring permanent anomaly in cell behavior/response on short-/long-term exposure. There are no specific FDA directives to regulate nutraceutical nanotechnology-based products and related issues. Altogether these challenges call for an urgent need to regulate these nanotechnology-based products and delivery devices. The characterization, safety, and environmental impact are the three main elements that need to be regulated. The lack of adequate and conclusive research on the health risks of nanobased substances demand the need for a dialogue on regulatory adequacy, inadequacy, or possible alternatives. Well-tuned, coordinated, and sincere efforts of government, industries, academia, and researchers over guidelines for regulation must be

drawn in order to utilize the benefit of nanobased technology without hampering its development.

Besides the biological and environmental factors, consumer acceptance and perception is equally important. In recent years consumer acceptance of new products or products produced with new technologies has had serious setbacks. The introductions of food irradiation technology and genetic modification technology have led to public debates and controversies. Thus, it is highly essential to address both risk evaluation and consumer perception in the process of development and application of new technologies, especially nanotechnology. Any disregard in this area could have dramatic negative effects not only on the introduction of nanotechnology but also in general public perception of new technologies and product innovation can be dampened.

## 6 Concluding Remarks

The advances in the field of nutraceuticals, functional foods, dietary supplements, and phyto-chemicals along with consumer preferences have prompted the search and implementation of novel formulation strategies. The central focus is to develop nutraceutical products that can overcome low bioavailability and improve therapeutic efficacy. The prospect of the production of nutraceuticals at the nanoscale and nanoencapsulation of dietary supplements will be intensively explored in the future taking advantage of three types of nanoscale materials present in food: naturally occurring nanoscale substances (such as nanoscale protein, fat, or sugar molecules or micelles); a proportion of nanosize materials in the distribution of particle sizes derived from conventional processing techniques; and substances deliberately engineered to confer novel properties as a result of their nanoscale size. As the first two types of nanomaterials are those created naturally from food substances, they are less likely to pose a threat to human health compared to nanoparticles or nanomaterials that have been deliberately engineered to confer different properties. There is a rapid progress toward next generations of nanomaterials leading to more sophisticated nanobased products and processes. This implies that legislation and risk assessment must keep pace with the development of these technologies. Although it may currently appear futuristic, we can predict that the field of nutraceuticals/functional foods will not only be influenced by new nanomaterials and processes, but attempts will be also made to ensure a correct and specific cell/tissue-delivery of the desired nutraceutical/functional food products. An engineered nanoparticle carrying a specific nutraceutical compound at the

right concentration and able to trigger a unique tissue and/or cell type will not only ameliorate the bioavailability of such molecule, but will also hit the metabolic or signal transduction pathways of interest, reducing undesired effects and making the nutraceutical intervention more specific. If this prediction will become reality, the entire field will benefit. The strong criticisms to nutraceutical/functional food products regarding the lack of specificity and cause-effects relationship can be addressed when their effects are systematically evaluated in humans. This goal can be reached taking advantage of a mutual and synergic interaction between biomedical and food sectors. In fact, delivery of drugs and nutraceuticals by nanomaterials share common interests and approaches. Certainly the application of nanotechnology to nutraceuticals/functional foods is intended to revolutionize the field not only in terms of new products and market, but also to shed light on the molecular mechanisms behind nutraceutical consumption. It is hoped that all these may open up a larger market for these materials, besides giving motivation to scientists in the areas of food and pharmaceutical sciences.

## Acknowledgments

The authors are grateful to Prof Alexandru Mihai Grumezescu, editor of this multi volume set entitled *Nanotechnology In the Agri-Food Industry*, published by Elsevier, for inviting us to contribute this chapter and to the publishers Elsevier for cooperation. One of the authors (Prof Anupama Rangan) would like to thank the M/s Janatha Education Society, Rajajinagar, Bangalore (India) for their motivation and encouragement for research. The other author (Dr K.G. Satyanarayana) would like to express sincere thanks to Poornaprajna Institute for Scientific Research (PPISR), Bangalore with whom he has been associated, for their encouragement.

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# 18

# NANOEMULSIONS AS DELIVERY VEHICLES FOR FOOD AND PHARMACEUTICALS

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## 1 Introduction

Science is a growing picture that has evolved over time. With the advancement in science, the scientific community, too, has graduated from emulsions to microemulsions and then to nanoemulsions (Given, 2009; Chiu and Yang, 1992; McClements, 1998) but the chronology will not end here. Something else exists beyond existence. Beyond that existence it is only the infinity we will ever interact with. Today, scientists, formulation chemists, medical technologists are all working in convergence to come out with models to take their relevant industry to the next level of its existence. It is an obsession with them and it can be summed up in the words of Masson Cooley "Cure for an obsession: get another one."

Nanoemulsions (NEs) hold promise for different fields because they form a class of convenient systems, for they provide delivery vehicles which are economical, efficient, and reliable (Silva et al., 2012; Ravi and Padma, 2011; Amarendra et al., 2013). They allow dilution of active ingredients to an ideal concentration. They have their own inherent benefits. A host of industries, such as food, drug, cosmetic, and agriculture, rely heavily on the use of NEs (Silva et al., 2012; Ravi and Padma, 2011; Amarendra et al., 2013). Many inks and paints are based on NEs. In agricultural industry, NEs are used as delivery vehicles for fungicides, insecticides, and pesticides. In cosmetics NEs serve as delivery vehicles for many skin and hair care products (Given, 2009; Chiu and Yang, 1992; McClements, 1998; Silva et al., 2012; Ravi and Padma, 2011; Amarendra et al., 2013; Sharma and Sarangdevot, 2012).

Many foods are in the form of emulsions. NEs are also known for enhancing the flavor of food. They protect their ingredients

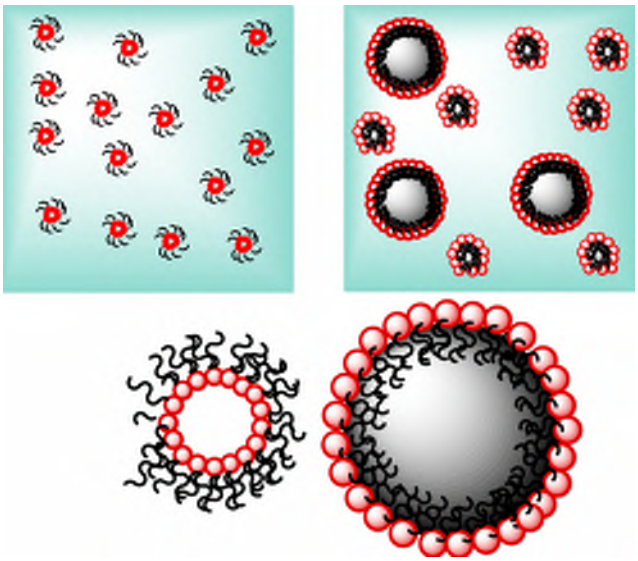
**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00018-0

from degradation. Products such as cold drinks, juices, butter, milk, cream, salad dressings, mayonnaise, soups, sauces, dips, and margarine are all in the form of NEs. They will definitely be neoharmful lipid minute droplets approved for human consumption in addition to a normal choice of food ingredients (Given, 2009; Chiu and Yang, 1992; McClements, 1998; Silva et al., 2012; Ravi and Padma, 2011; Amarendra et al., 2013; Sharma and Sarangdevot, 2012). The pharmaceutical industry also uses NEs to improve the efficacy of drugs. Several syrups, nasal sprays, eye drops, and so on, are examples of NEs in the pharmaceutical industry (Given, 2009; Chiu and Yang, 1992; McClements, 1998; Silva et al., 2012; Ravi and Padma, 2011; Amarendra et al., 2013; Sharma and Sarangdevot, 2012).

## 2 Synopsis of Earlier Studies

Before we move further let us reflect on emulsions, microemulsions, and NEs. Generally, an emulsion consists of at least two immiscible liquids (usually oil and water, but not always) with one of the liquids being dispersed as small spherical droplets in the other. An emulsion in general has large droplet size ranging 100 nm–100 μm. It is relatively unstable because of the large interfacial tension between the two phases. Emulsions are usually opaque because the droplets have similar dimensions to the wavelength of light (Given, 2009; Sherman, 1968; Becher, 1957). Emulsions are useful tools in the industry directly impacting many phases of human life. NEs on the other hand are clear, thermodynamically stable, isotropic mixtures of oil, surfactant (sometimes cosurfactant/cosolvent), and water. The aqueous phase may contain salt(s) and/or other ingredients, and the oil phase may actually be a complex mixture of different hydrocarbons and olefins. They have relatively small particle ($r < 50$ nm) size compared to wavelength of light so they scatter light only weakly and appear to be transparent. They are easier to be prepared as compared to emulsions and NEs because of high surfactant concentration in them.

The oil-in-water emulsions (o/w) with mean droplet size on the scale of 50–1000 nm are referred to as NEs. (The representative droplet structures of microemulsion/nanoemulsion are depicted in Scheme 18.1 (Hategekimana et al., 2015; Mason et al., 2006). They are a class of transparent/translucent systems that cannot be formed spontaneously by simple mixing. They are nonequilibrium systems having high solubilization capacity for lipophillic drugs and have better resistance to droplet collisions induced by Brownian movement. This gives them excellent kinetic stability. But they are thermodynamically unstable and unfavorable



**Scheme 18.1. Representative droplet structures of microemulsion/nanoemulsion.**

systems. This can be attributed to free positive energy associated with forming the oil–water interface. Due to gravitational separation, flocculation, coalescence, and Ostwald ripening they have a tendency to break down over a period of time (Friberg et al., 2004; McClements, 2005; Dickinson, 1992). However, the rates at which these processes occur are often considerably different in NEs than in conventional emulsions because of particle size and curvature effects. NEs are often more stable to gravitational separation, flocculation, and coalescence, but are less stable to Ostwald ripening. A major focus of emulsion scientists, therefore is to create NEs that have a sufficiently long kinetic stability suitable for commercial applications. The kinetic stability of NEs can be improved by controlling their composition (eg, oil, surfactant, and water phase) and microstructure (eg, particle size distribution), or by incorporating additives known as stabilizers such as emulsifiers, texture modifiers, weighting agents, or ripening retarders (Kabalnov and Shchukin, 1992; Capek, 2004).

## 3 Nanoemulsions

"Submicron sized emulsions," that is, NEs are a class apart (Kabalnov and Shchukin, 1992; Capek, 2004; Peng et al., 2011; Rao and McClements, 2011; Codex Alimentarius Commission, 1985, 1991) Codex Alimentarius Commission, Codex *1-1985*.

Therefore, the focus of present discussion is on the role of NEs in foods and drugs where they are now being widely used. The principle reasons supporting this understanding are:

1. They have high encapsulation efficiency, low turbidity, high physical stability, and high bioavailability.
2. They easily incorporate lipophilic bioactive compounds, stabilize bioactive compounds that tend to undergo hydrolysis, and reduce side effects of potent drugs.
3. They are biodegradable and can be used for large scale production.
4. They can be administered by almost all available routes, including parenteral, ocular, nasal, oral, topical, and even aerosolization to the lungs.

Traditionally, o/w emulsions formulated with an emulsifier were used for this purpose but these emulsifiers usually faced issues related to cost, stability, bioavailability, and compatibility with other ingredients (Peng et al., 2011; Rao and McClements, 2011). The conventional method of producing emulsions also limited their stability to pH, salt heating, dehydration, freezing, and chilling (Ravi and Padma, 2011; Amarendra et al., 2013; Sharma and Sarangdevot, 2012; Sherman, 1968; Becher, 1957; Hategekimana et al., 2015; Mason et al., 2006; Friberg et al., 2004; McClements, 2005; Dickinson, 1992). An awkward scenario prompts invention aimed at reducing the difficulty. The importance of critical insoluble chemical ingredients has prompted scientists to develop alternative methods for improving emulsion stability by developing emulsifier-based novel strategies. This has also resulted in improved interest among the scientific community for the utilization of emulsions as vehicles for the stabilization and delivery of bioactive food components and lipophillic drugs.

## 4 Effect of Controls and Regulations on Food Fortification With NEs

Governments of different countries enact laws on food fortification from time to time specifying the types of stabilizers that can be used in food applications (Codex Alimentarius Commission, 1985, 1991). Cost considerations and other practical factors (such as ease of utilization, reliability of source, and matrix compatibility) also have a bearing on food fortification processes. This poses a real limitation on the type of ingredients that may be used for the fabrication of NE.

It is the quality of stability and bioavailability inherent to NEs that is creating increased interest among formulation scientists to synthesize new and biocompatible NEs for use in food and drug delivery systems that may be compatible with the laws of the concerned country.

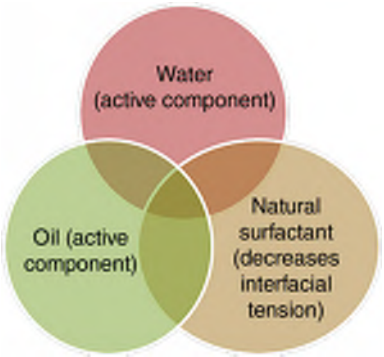



**Figure 18.1. Diagrammatic representation of components and their roles used in the fabrication of NEs.**

## 5 Methods of Preparation of NEs

Four components, namely, oil, water, natural surfactant, and energy are generally required for fabrication of NEs (Tadros et al., 2004; Jennifer and McClements, 2015). The role of each component has been indicated in Fig. 18.1. The formation of extremely fine emulsion droplets is always a challenge. This is attributable to the fact that the pharmacological properties of NEs are largely influenced by physiochemical parameters such as droplet size, size distribution, morphology, viscosity, stability, color, appearance, texture. Nanoemulsions (NEs) are a class of kinetically stable nonspontaneous systems as has been discussed earlier. Their formulation by simple mixing is not possible (Manickam et al., 2014; Lovelyn and Attama, 2011; Sekhon, 2010) because high energy is required to break large droplets into nanosizes so as to overcome the Laplace pressure. Traditional methods such as simple mixing, grinding, colloidal mills, static mixers, therefore cannot be used to fabricate NEs.

## 6 Formulatory Processes

Two methodologies are employed in the formulation of food/pharmaceutical NEs:

- One is the low-energy emulsification method. It is comprised of phase inversion temperature, solvent-diffusion, and spontaneous emulsification. Phase inversion temperature (PIT) technique is the commonest of all the formulation systems. The use of surfactants helps it generate chemical potential. But this method has been found to have its own limitations.

These include use of a large quantity of surfactant, selection of both, that is, surfactant-cosurfactant ratio, high polydispersity index (PDI) values, induced instability after long-term storage and relatively heavy and tedious workload to identify the system inversion temperature (Anton et al., 2007; Anton and Vandamme, 2009; Gutierrez et al., 2008).

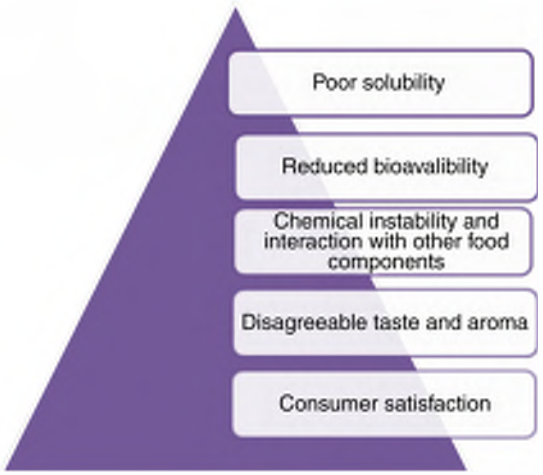
- The other is high-energy emulsification method. This method requires larger mechanical energy generated by high-pressure homogenizer (Schubert and Engel, 2004), ultrasonicator (Leong et al., 2009; Jafari et al., 2007; Abismail et al., 1999) and microfluidizer (Jafari et al., 2006). Homogenization is a two-step process where first a coarse emulsion is fabricated by homogenizing of the organic and aqueous phases with a high-speed blender for 2 min. And then, coarse emulsions are immediately passed 5–7 times through a high-pressure homogenizer/microfluidizer (Schubert and Engel, 2004; Jafari et al., 2006) so that various constituents do not separate as in soft drinks like cola products. Homogenization is also a fluid mechanical process that effects the subdivision of particles or droplets into micron sizes to create a stable emulsion for further processing. Homogenization of milk can be quoted as a classic example where milk fat globules are reduced in size and dispersed uniformly through the rest of the milk. Homogenization is an expensive and a sensitive method. This has made ultrasonic cavitation technology popular (Leong et al., 2009; Jafari et al., 2007; Abismail et al., 1999). NEs prepared by ultrasonic cavitation offer a greater improvement in terms of stability with lower Ostwald ripening rate vis-à-vis those prepared by using PIT method as referred to earlier. High-energy input originating from microturbulent implosion of cavitation bubbles generates enormous energy. That is enough to deform and break off droplets into nanometer scale. But this is possible only if the Laplace pressure is overcome (Leong et al., 2009). In terms of droplet size, the NEs formulations made with ultrasonic probe sonicator are far more superior than those prepared with homogenizer and microfluidization (Abismail et al., 1999). Small sized droplets of NEs can be produced with microfluidization, but nevertheless this is not a friendly technique vis-à-vis the use of ultrasonic probe sonicator. The use of ultrasonic probe sonicator is simple because of three major factors: (1) the probe it uses is much easier to clean and is free from line blockage whereas in case of microfluidizer defilement remains a major cause of concern (Schubert et al., 2003); (2) they provide price advantage over competitors; and (3) are energy efficient (Leong et al., 2009). This makes ultrasonic

cavitation the most efficacious and favorable method in the preparation of food/pharmaceutical NEs.

## 7 NEs in the Food Industry

With the advent of modern technology, the concept of food/daily diet has changed from being just hunger satisfiers and taste bud entertainers to a source of better health and well-being. The world contains an unimaginable vast amount of digital information, which is getting vaster even more rapidly. This has made possible many things that previously could not be done. The information now available at the click of a button can provide fresh insight into science and can guide the scientific community. As the information has gone from scarce to superabundant, people have now started valuing the positive effect of diet on general well-being. Increased interest among consumers has led to the emergence of a specialized category of food products, commonly known as the functional foods, which involve the fortification of food products with micronutrients (ie, vitamins and minerals) or useful ingredients from normal resources (eg, phytochemicals). It may also include food blending among original constituents such as addition of fish oil to bread or dairy foods may be treated with human gut bacterial culture (Patel and Velikov, 2011). But loading additional functional ingredients to food products often cause problems ranging from formulation difficulties, taste issues, product stability, product appearance, and decreased bioaccessibility (Scheme 18.2) (Huang et al., 2010; Velikov and Pelan, 2008). What makes it even more challenging is the interaction of these functional ingredients with the complex matrix of the product. Currently, trial and error methods are being adopted for food fortification. At times they work fine while in some cases they often fail (Ubbink and Krüger, 2006). Apart from food fortification, low fat, and the quantity of NaCl have also become consumer desires on the food product labels. This is fueling a lot of research to identify ways of formulating functional ingredients without compromising with the overall product functionality (ie, appearance, taste, stability, texture, and bioaccessibility).

Surfactants play a very important role in emulsion stability. Functionalization of surfactants in the fabrication of NEs is the linchpin for potential application in the field of food and pharmaceuticals. However, the choice of surfactant is always a tedious task while developing NE suitable for food formulations because the nature of surfactant forms the basis of size of the droplet and droplet stability. Commonly used surfactant is Tween 80. But it has




**Scheme 18.2. Problems related to addition of functional ingredients to food products.**

its own limitations in controlling droplet size, biocompatibility, and so on. Therefore, a recent study by Yang et al. (2013) reported the replacement of Tween 80 usually with a natural surfactant, Q-Naturale. It is extracted from the bark of the *Quillaja saponaria* Molina tree. The results indicated that Tween 80 was more effective in producing small droplets at relatively low vitamin loadings (<40%), whereas Q-Naturale was more effective in relatively high vitamin loadings (60–80%). The comparison has been indicated in Fig. 18.2. The size of droplet was found to decrease with the decrease in vitamin concentration in the oil phase and increase in amount of glycerol in oil phase. This is due to the change in viscosity of the dispersed phase and the continuous phase because these parameters are known to influence droplet disruption and coalescence during homogenization.

Further, food can be fortified with functional ingredients in two ways, one a soluble form as solution and the other in insoluble form as dispersion (Patel and Velikov, 2011; Huang et al., 2010; Velikov and Pelan, 2008; Ubbink and Krüger, 2006). Adding ingredients in soluble form is far easier and advantageous in terms of formulation ease where the components can be added to the product by simple dissolution. Fortification of food with soluble ingredients also leads to easy dissolution and enhanced bioaccessibility of the soluble food component. However, the soluble form has its own inherent taste characteristics. The chemical reactivity will be higher for a soluble form leading to undesired interactions with product components, which may cause discoloration and precipitation. Sometimes addition to soluble form changes the composition of

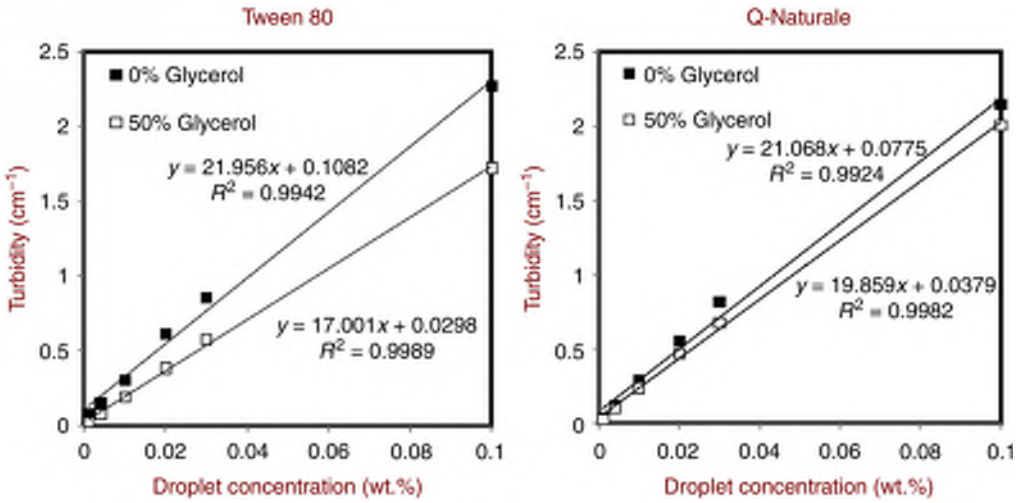



**Figure 18.2.** Impact of glycerol content and surfactant type on turbidity of 10 wt.% oil in water emulsions (60 wt.% vitamin E acetate in oil phase) diluted to different droplet concentrations (1 wt.% Tween 80 or Q-Naturale, 9000 psi, 4 passes).

the added component causing degradation. Insoluble form can be considered for addition to components that impart undesirable taste, or to components that demonstrate high chemical reactivity. However, on the flip side, it causes change in the texture due to alteration in consistency and can lead to physical instability of the product due to creaming or sedimentation. The presence of large insoluble particles may also result in sandiness or chalkiness, altering the mouth feels of the product. There may also be a potential risk of decreasing bioaccessibility due to the insoluble characteristics of added functional ingredients. Thus, the challenges faced by the food industry for fortification of food have been ever increasing.

Agriculture is an important occupation. It provides most of the world's food and fabric. Researchers are trying to develop food-grade NEs to extend the shelf life of fresh foods or to develop food that has consumer acceptability. Some of the benefits of NEs have already been noticed in the agrifood sector (Sekhon, 2014) while for others the research is still at the conceptual stage. The benefits already seen in the agrifood sector include:

- sensory improvements (flavor/color enhancement, texture modification);
- increased absorption and targeted delivery of nutrients and bioactive compounds;
- stabilization of active ingredients such as nutraceuticals in food matrices;
- packaging and product innovation to extend shelf-life;

- sensors to improve food safety;
- antimicrobials to kill pathogenic bacteria in food; and
- lowering the risk of the multiple deficiencies that can result from seasonal deficits in the food supply or a poor quality diet.

## 7.1 Challenges for Food Fortification

Innovative ideas take time to fructify. Food fortification is one such idea. It has not yet achieved its full potential. Experience in many parts of the developing world has shown that food fortification offers a cost-effective and sustainable solution to the problem of micronutrient malnutrition, vitamin deficiencies, and various diseases. All stakeholders from industry to government have started responding positively to the call of those who are advocates of nutritious food to adopt fortification as a strategy and it is the advances in science and technology that is helping them further in the discharge of their obligations toward society. On the other hand, challenges still remain in many countries because where and how its subjects live also influences what they eat. The government faces the challenge of providing the enabling environment for all stakeholders and to incorporate in the fortification efforts. Industry faces the challenge of tuning its production system with the requirements of fortification so that they can contribute to social objectives while at the same time pursuing their commercial interests. The international and bilateral aid agencies need to seek adoption of tried and innovative ways to support the multiple players associated with food fortification, as these players in turn face the challenges that confront them. Having said so, formidable challenges still remain in many countries in Asia, constraining the widespread adoption of this strategy. The science and technology community needs to provide adequate scientific and technological information as basis for planning and decision making [http://www.nap.edu/openbook.php?record_id(10872&page(2, Institute of Medicine (IOM). Dietary Reference Intakes: A risk assessment model for establishing upper intake levels for nutrients, http://www.nap.edu/openbook.php?record_id(10872&page(2].

### *7.1.1 Regulatory Framework for Stable Food Fortification to Improve Public Health*

Recommended dietary allowances (RDAs) have been established by the Food and Nutrition Board of the National Academy of Sciences (Health Canada, 2010; Food and Nutrition Board, Institute of Medicine, 1997; Freeland-Graves and Nitzke, 2002) The amount of vitamins and minerals added to a specific food is usually set at an individual's daily requirements which are usually less

than one-third of the total RDA. It has enormous benefits. These numerical values provide nutrition guidance to health professionals and to the general public. RDAs were set for different age groups, for men and women, for pregnant and nursing mothers. The board also established Estimated Safe and Adequate Daily Dietary Intakes (ESADDIs) for seven nutrients where available data were insufficient to set an RDA (Escott-Stump, 2005)

It is generally accepted by the scientific community that it is possible to consume too much of a given nutrient (http://www.nap.edu/openbook.php?record_id(10872&page(2). However, this does not mean that risk of excessive intake of these nutrients does not exist. This formed the basis for the creation of the tolerable upper intake levels (ULs) for several nutrients beginning in the late-1990s [Institute of Medicine (IOM). Dietary Reference Intakes: A risk assessment model for establishing upper intake levels for nutrients, http://www.nap.edu/openbook.php?record_id(10872&page(2]. ULs are the highest intake level known to be safe. The toxicity of intakes above UL level is unknown. ULs have not been established for all nutrients. For some nutrients the UL only applies to one form of the nutrient, or only applies when the nutrient is consumed through food fortification or as a dietary supplement [Institute of Medicine (IOM) Dietary Reference Intakes: A risk assessment model for establishing upper intake levels for nutrients, http://www.nap.edu/openbook.php?record_id(10872&page(2]. Different countries are governed by different laws. The US Food and Drug administration (FDA) has a policy about fortification of food and is codified in 21 C.F.R. 102.20. The policy statement urges the manufacturers to follow the same if they elect to add nutrients to a manufactured or processed food.

> *The codified policy includes situations and conditions in which the fortification of food with nutrients listed in the policy is considered appropriate:*
> - *(1)….to correct a dietary insufficiency that is recognised by the scientific community to exist and known to result in nutrient deficiency disease…;*
> - *2)... to restore such nutrient(s) to a level representative of the food prior to storage, handling and processing…;*
> - *3)….in proportion to the total caloric content of food, to balance the mineral, and protein content…; and*
> - *4)… that replaces traditional food in the diet to avoid nutritional inferiority… (FDA, 1980, p. 6323).*

Reportedly it is only a policy statement, not regulation because there are a number of qualifications listed with each condition of fortification (http://www.nap.edu/openbook.php?record_id(10872&page(2). In Canada, voluntary food fortification has

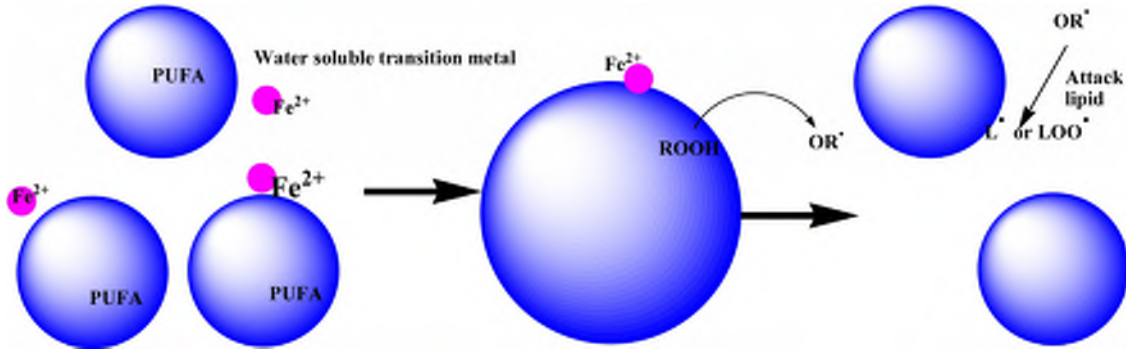
historically been tightly regulated (http://www.nap.edu/openbook.php?record_id(10872&page(2). With restrictions on the types and amounts of added nutrients permitted, very few foods have been permitted to be voluntarily fortified in Canada. However, Health Canada (2005) proposed a discretionary food fortification policy, which would have allowed for much broader voluntary food fortification practices in Canada, permitting manufacturers to add nutrients to food at their discretion (www.hc-sc.gc.ca/fn-an/nutrition/vitamin/fortification-final-doc-1-eng.php). Although the proposed policy was not formally implemented, alternate regulatory amendments increased opportunities for voluntary vitamin and mineral fortification of food, with products approved on a case to case basis (www.hc-sc.gc.ca/fn-an/nutrition/vitamin/fortification-final-doc-1-eng.php, http://www.hc-sc.gc.ca/dhpmps/prodnatur/index-eng.php; Health Canada, 2012).

Policies and regulations on fortification in United States and Canada are still different in a number of ways. Despite different regulatory environments, voluntary food fortification practices appear to be expanding in both the United States and Canada. This is evident from the recent introductions of entirely new categories of fortified foods, such as energy drinks and vitamin waters, and from the continued expansion of fortification in products such as breakfast cereals and beverages (Watkins et al., 2000).

Therefore, there is a need to have a policy that is commensurate with the requirements of vulnerable population. There is also a need to have everyone in the population consume the RDA (recommended dietary allowance) or AI (adequate intake) of each nutrient without exceeding its UL (http://www.fda.gov; Food and Nutrition Board, Institute of Medicine). Before making a policy decision the policy makers also need to analyze the impact of such a decision on the public health.

### *7.1.2 Oxidation*

Potential health benefits are associated with fortified food, which cannot be obtained from the staple diet. The consumer is now more and more aware of the health benefits of fortified foods. This involves consumption of food rich in omega-3 fatty acids, vitamins, flavanoids, minerals, and so on. However, because of the presence of lipids, food rich in the earlier mentioned components are subject to rapid oxidation. Lipid oxidation causes multiple problems. It impacts the stability, shelf life, safety, nutritional value, functionality and flavor, and so on, and any change is rapidly observed by the consumers (Garg et al., 2006; Arab-Tehrany et al., 2012; Dacaranhe and Terao, 2001; McClements and Decker, 2000). Oxidation occurs in three steps, that is, induction, propagation, and termination this leads to the production of free




**Scheme 18.3. Mechanism depicting the lipid oxidation.** *PUFA*, Polyunsaturated fatty acids; *ROOH*, lipid hydroperoxide; *RO*, alkoxy radicals; *L*, lipid radical; *LOO*, lipid radical.

radicals in the food (Dacaranhe and Terao, 2001; McClements and Decker, 2000, 2008; Waraho et al., 2011). As a result thereof undesirable sensory attributes are noticed by the consumer in food products even at very low levels. The mechanism for lipid oxidation has been depicted in Scheme 18.3.

Oxidation of food can be prevented by controlling the physicochemical characteristics of the food formulation such as droplet charge, thickness and permeability. All these factors are known to influence the formation of free radicals and oxygen interactions with the lipids in the droplets. Biocompatible nonionic surfactants with a larger head group are usually preferred for the fabrication of NEs for use in food fortification as surfactants with larger heads showing better protection than with small ones. The surfactant chain length has been shown to have minor effect on oxidation stability.

Food oxidation can also be prevented by the addition of free radical scavengers (Dacaranhe and Terao, 2001; McClements and Decker, 2000, 2008; Waraho et al., 2011; Lee et al., 2011). This includes flavonoids and metal chelations. Metal chelation is a process by which an antioxidant reduces the reactivity of transition metal by physically blocking it from interacting with lipid. Flavonoids act as scavengers by donating hydrogen from their hydroxyl groups. The chemical degradation of NEs can also be prevented by controlling the factors such as pH, ionic strength, temperature, droplet size, and emulsifier type.

However, lipid oxidation is also promoted by exposure of unsaturated lipids to air, light, heat, and irradiation. Undesirable sensory attributes are observed in food products even at very low levels and once the initiation phase has begun the rate of oxidation increases exponentially and the food is spoiled.

Therefore, it is necessary that oxidation of food is prevented. Lower temperatures, reduced oxygen exposure, and addition of antioxidants can all be crucial to solving the problem of their oxidation.

Oxidation also depends on other factors such as composition, structure, and organization of the oil, water, and interfacial phases, as well as the type, amount, and location of any antioxidants present when NEs are used in food fortification (Lee et al., 2011). This is because the presence of surfactants and nanosized droplets increase the surface area and hence aids oxidation. Relative intensity of lipid to still dissolve more because of the presence of multiple bonds, significant surface area of venerable lipids and larger permeation of light can be some of the reasons, which make fish oil NEs vulnerable to lipid oxidation (Lee et al., 2011). Protein stabilized NEs have been shown to be venerable to faster lipid oxidation vis-à-vis conventional emulsions with similar composition. The studies carried out on the subject have attributed the same to greater lipid surface area (McClements and Rao, 2011). Therefore, it necessitates further steps for stabilizing omega-3 oils captured within NEs.

### *7.1.3 Physical Stability*

The primary consideration in food fortification is the physical stability of the formulated NE. This is known to impact the shelf life, appearance, functionality, and acceptability of fortified food among consumers.

One of the major reasons for the instability of NEs is Ostwald ripening. It is a process stimulated by the degree of water solubility of the oil phases in the aqueous phase (Wooster et al., 2008; Gulotta et al., 2014). Therefore, oils which have the quality of higher water solubility shall be more prone to Ostwald ripening vis-à-vis oils like long chain triglycerides, which are water lesser soluble. It is because of the Ostwald ripening that fish oils, which contain long-chain triglycerides, are resilient to droplet growth (Wooster et al., 2008; Gulotta et al., 2014).

Physical stability is the primary factor that must be taken into consideration before employing NEs for food fortification. Therefore, utmost care is required to be taken while designing them. This can be done by: (1) fabricating NEs with more water soluble oils (such as fish, flaxseed, or algae oil) to avoid Ostwald ripening; (2) by adding ripening inhibitors; (3) by an appropriate choice of surfactant, such as surfactant type and concentration used to create a NE because it impacts its susceptibility to flocculation and coalescence (Wooster et al., 2008; Qian et al., 2012a,b; Yang et al., 2012). It is documented in literature (Saberi et al., 2013a,b)

that NEs formed by spontaneous emulsification suffered coalescence within 1 month of their storage when the surfactants used had intermediate hydrophilic/lipophilic balance (HLB) numbers. These surfactants had the tendency to solubilize both in water and oil and formed lamellar structures rather than forming micelles because of their optimal curvature. This behavior does not permit NEs to stabilize very effectively.

Nonionic surfactant stabilized NEs may also coalesce upon heating due to changes in the optimum curvature of the surfactant monolayer at elevated temperatures, that is, dehydration of the head group (Saberi et al., 2013a,b; Lee and McClements, 2010). Nonionic surfactants are sterically hindered due to protein and polysaccharide coatings. Therefore, they tend to be more stable over a wide range of salt and pH conditions. This is contrary to phospholipid-coated and protein-coated droplets, which tend to be highly vulnerable to changes in pH and ionic strength. This can mainly be attributed to electrostatic interactions that stabilize them. Apart from electrostatic interactions nonionic surfactants are also influenced by other factors like surfactant characteristics and temperature.

High salt levels or at pH values close to their isoelectric point (pI) renders protein-coated lipid droplets highly susceptible to flocculation due to reduction in electrostatic repulsion between droplets (Troncoso et al., 2012). It will, therefore be appropriate to use protein-stabilized NEs in such conditions that support electrostatic repulsion between droplets. Low ionic strength and/or pH far from pI can be the answer to this phenomenon. On the flip side highly viscous or gel-like products should be used for their fusion because in such a scenario even if the aggregation occurs, gravitational separation will not allow the nanoparticles to separate from the product. The various physiochemical factors affecting food fortification with NEs have been presented in Scheme 18.4.


**Scheme 18.4. Physiochemical factors affecting food fortification.**

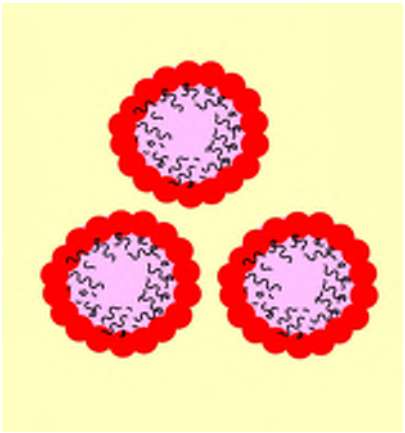
## 8 Maintaining Food Flavor

"Ultimately, food choice is determined by sensory attributes such as taste and pleasure, and consumers will not sacrifice these in favour of nutritional goodness," said consumer market analyst Michael Hughes. It will also hold true even if the consumer is aware of the potential health benefits of functional foods. So maintaining the flavor of food is very essential. As mentioned earlier fish is a rich source of omega-3 fatty acids. However, even the high-quality refined fish oil has little or no flavor. Therefore, by adding fish oil NEs to food, the consumer can receive the benefit of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) while maintaining the taste of the food (Tur et al., 2012; Ruxton et al., 2004).

## 9 Fabrication of NEs for Food Formulations

Bioactive compounds are food components or food ingredients that provide health benefits to the human body. They are known to help in preventing cardiovascular diseases, hypertension, diabetes, and some cancers, also. Some bioactive lipids with health benefits include omega-3 fatty acids, carotenoids, fat soluble vitamins (eg, vitamin E) and phytosterols. However, they have their inherent challenges of stability, degradation, lipid oxidation, and so on. Therefore, food fortification becomes challenging and requires the use of a specific delivery system.

NEs find potential applications in food and beverages to encapsulate lipophillic bioactive components including vitamins, flavors, colors, preservatives, and antioxidants that are otherwise difficult to be incorporated in liquid food products. They can be mixed with oil phase prior to emulsification or they can be mixed with organic solvents when solvent displacement method is to be used (Saberi et al., 2013a,b; Lee and McClements, 2010; Troncoso et al., 2012). Therefore, these bioactive compounds are incorporated in NEs that prevent them from degradation and the product is readily absorbed in the body because of the small size and increased surface area of NEs. However, if the NE is not completely transparent then the addition thereof to the food product may be limited by the changes in the optical properties of the food. Typically, if the NE is more transparent and has smaller droplet size then more of the same can be incorporated with food till the system turns opaque (Lee and McClements, 2010; Troncoso et al., 2012). However, care needs to be taken to check the growth of droplets in size after the food has been fortified. Otherwise, turbidity will grow during storage and the food product will get spoiled and start to decay.



**Scheme 18.5. The probable nanoemulsion structure.**

A recent study (Hatanaka et al., 2010) has reported increased bioavailability of bioactive lipids, that is, α-tocopherol by incorporating it in NEs. Fabricated NE composed of α-tocopherol and medium-chain triglycerides, decaglyceryl monooleate, lecithin, glycerol, and deionized water has also been reported in literature. When it was administered in rats it was found that the rats administered with 10% α-tocopherol (w/w) loaded NEs had higher blood plasma level of α-tocopherol than those fed with control mixture of oil and α-tocopherol. Oral administration of NE formulation resulted in significant attenuation of oxidative stress in experimental diabetic model rats. It was evidenced by the marked reduction of thiobarbituric acid reactive substance (TBARS) levels in the liver, kidney, and brain. Such studies have facilitated their use in food fortification.

An NE fabricated with palm oil and coated with galatolipids from oat oil is commercially available (Fabuless™). The probable structure has been depicted in Scheme 18.5. It delays digestion until lower regions of the small intestine stimulates satiety and reduce food intake and thus helps control obesity. There was no evidence that eating this NE altered eating behavior at a subsequent meal even after 3.5 h. It increased the feeling of fullness only when incorporated into semiliquid fermented yogurt. The increase in fullness was rapid, occurring immediately after the breakfast meal, and dissipated in nearly 2 h later. It did not alter any measured indicator of satiety when taken with water. When taken with a solid food, meal, or as a snack alone, it also did not alter the measured indicator of satiety either. Food consumption also did not get reduced significantly when treated with emulsion (Chan et al., 2012). Desserts, salads, and mayonnaise are other

classes of attractive foods that find consumer attention and have been reported to be fortified with NEs. Frozen yogurt (FY) is a dessert characterized by its having the textural properties of ice cream combined with the acidic taste of yogurt (Marshall et al., 2003; Tamime and Robinson, 2007; Alfaro et al., 2015). Its popularity has increased and continues to grow. It provides a low-fat replacement for ice cream besides probiotic benefits. Recently, fortification of FY with an NE containing purple rice bran oil (NPRBO) has been reported. NPRBO with fat droplets with size in the range of 150–300 nm was mixed with the FY ingredients to produce a frozen yogurt containing NPRBO (FYNRO). In general, its processing consisted of mixing natural stirred yogurt with stabilizers/emulsifiers and sugar before freezing the mix in a conventional ice-cream freezer. It was seen that plain frozen yogurt (PFY), frozen yogurt with sodium caseinate (FYSC), and FYNRO had similar hardness. The apparent viscosity of the FYNRO mix was similar to the PFY mix with values of 0.19 and 0.17 Pas, respectively. All the frozen yogurts mixtures were pseudoplastic shear thinning fluids. The melting % of the FYNRO was significantly lower than FYSC and PFY. It indicated that the addition of NPRBO increased the melting resistance of the FY. Moreover, the addition of NPRBO did not affect Lactic acid bacteria survival during the 6 weeks of frozen storage at 22°C. This was evidenced by counts of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* being similar for all FY. It was found that FYNRO contained 1.22 mg of total vitamin E and 266.01 mg of g-oryzanol per every 3.78 kg. FYNRO had higher peroxide value than FYSC and PFY at the end of storage period. However, all the FY had acceptable peroxide values. Overall, the study demonstrated that FY can be fortified with an NE containing purple rice bran oil to create a product with unique marketing potential in the dairy industry.

## 10 Formulation of Food With NEs Containing Omega-3 Fatty Acids

Consumption of polyunsaturated omega-3 fatty acids, such as eicosapentaenoic acid (EPA), docosapentaenoic (DPA), and docosahexaenoic (DHA) have been reported to have beneficial physiological effects. It includes reduction in the incidence of cardiovascular disease, cancer, diabetes, arthritis, central nervous system (schizophrenia, depression, Alzheimer's disease), as well as in brain and retina development in the foetus and infants (Kris-Etherton and Grieger, 2009; Zhang et al., 2015). Currently available liquid or semisolid food products that have been

enriched with omega-3 fatty acids using NE-based delivery systems include table spreads, yogurts, and milk (Tamime and Robinson, 2007; Alfaro et al., 2015; Porter, 1993). None of these products require the delivery system to be optically transparent and, therefore NEs can be used easily. However, these formulations may have some stability and bioavailability issues. Therefore, for food applications we need to develop a system of NEs which are physically and chemically stable.

But food fortification with omega-3 fatty acid is beset with a number of challenges. Oxidation of food containing omega-3 fatty acid results in offensive odor and foul smell. There are issues concerning the extent of acceptance of the food rich in omega-3 fatty acids. There can also be issues of bioavailability of omega-3 fatty rich foods (yogurts, mayonnaise) over fish oil capsules. However, the issue of oxidative deterioration of sensitive fatty acids has been addressed. The fatty acid molecules are surrounded by proteins and are emulsified with plant antioxidants and rapeseed oil. This prevents the omega-3 fatty acids coming into contact with oxygen. It helps them retain their tasteless and odor-free properties over a long period. Sometimes antioxidants are also added to prevent oxidation. However, the efficiency of the added antioxidant depends on their partitioning into different phases (oil/water/interface). This relationship between antioxidant partitioning and antioxidant efficacy is also termed as "the polar paradox" (Frankel et al., 1994; Qian et al., 2012a,b). According to the polar paradox theory, polar antioxidants such as ascorbic acid are more active in nonpolar media like neat oils vis-à-vis their more nonpolar counterparts (ascorbylpalmitate). On the contrary nonpolar antioxidants are more active in more polar systems like emulsions.

Other factors, such as the interactions between the antioxidants, iron, emulsifier and pH, also influence their efficacy in some food emulsions (Chaiyasit et al., 2007). Another important factor usually considered is the concentration of added antioxidant. At high concentrations, some antioxidants, such as tocopherol may have the opposite effect and become prooxidants (substances that induce oxidative stress). Added antioxidants or ingredients may act synergistically with endogenous antioxidants. However, prooxidant effects of endogenous and added antioxidants may also occur if the total concentration density becomes too high. Importantly, case studies have shown that the same antioxidant can exert markedly different effects in the different food systems. Ethylene diamine tetra acetate (EDTA) has been reported to work well in mayonnaise and dressings, but was less efficient in milk. EDTA has also been reported to have prooxidative effects in fitness bars. Ascorbyl palmitate promotes oxidation in mayonnaise and fitness

bars, and has some effect on dressings. However, in the case of milk it is less efficient because it has prooxidative effect in fitness bars. In contrast, gamma-tocopherol or tocopherol mixtures either do not have any effect or have slight prooxidative effects on dressing and mayonnaise. Taken together, it clearly shows that there is a need for detailed mathematical modeling of the kinetic rates on the dependence of different antioxidants in real-food systems. Such mathematical modeling can lead to a better prediction of the effect of antioxidants in foods. This demonstrates that lipid oxidation can be reduced by a rational design of oil–water interface.

Research in this area calls for collaboration between food chemists and experts in advanced microscopy and imaging techniques. Unified efforts can enrich variety of foods with these beneficial fatty acids without affecting the taste or odor of the food. A number of sausage products enriched with omega-3 fatty acids NEs are already commercially available. The enrichment of other popular foods such as pizza, noodles, and bread with omega-3 fatty acids is witnessing further development. The bioavalibility of omega-3 fatty acids encapsulated NEs from yogurts and fish oil capsules are significant but this differs for different products. Therefore, sustained research efforts are necessary in this area.

A study has been conducted on o/w emulsion, which included NE coated fish oil droplets. In the study a lipid which was solid or a partially solid at room temperature, milk protein coated lipid (ie, a nonoxidizable lipid) was used for fortification of FY. However, the method involved in making yogurt fortified with an oxidizable lipid (an LC PUFA or ester, plant oil, fish oil, or omega-3 fatty acids) includes different steps. These include adding yogurt culture to milk. Yogurt culture is an emulsion wherein core lipid comprises an oxidizable lipid. The mixture is then heat treated. And finally, the heat-treated mixture is fermented to produce yogurt fortified with oxidizable lipid (Chee et al., 2005). To date, not many food formulations fortified with omega-3 fatty acids have been reported in literature. However, constant research to overcome the constraints being encountered in this area of food fortification is being conducted.

## 11 Usefulness of NEs in Fortification of Food With Vitamins (A, D, and E)

Vitamins and minerals are added to a range of food products for various reasons. They are added to make up for the loss caused during processing and storage of food. They are also added to food to meet the nutritional needs of infants and the elderly. They can

also be added to food as a preventive measure in the case of specific consumers or for the groups which are at risk. Traditionally, higher levels of additions of vitamins and minerals than required in the end product had been resorted to make up for loss during processing and storage. But high overages can be avoided by using microencapsulated/NE forms.

Susceptibility of food to decay during processing and storage and its reaction with other components in the food system is often a challenge in fortification of food with vitamins and minerals. Iron, for example, may react with fatty acids in the fortified food, forming free radicals that induce oxidation. Other characteristics that may be affected are color, taste, odor, appearance, and alterations. These need to be avoided altogether because they affect consumer acceptability of the product. The stability of the fortifying agent is another issue. Vitamins and minerals are generally sensitive to such factors as temperature, moisture, pH, oxygen, air, and light. These should be controlled during processing and storage of fortified foods. Here vitamin C can be quoted as an example. It is extremely unstable under several conditions, especially in high heat and humidity. It has been reported in literature that over both short-term and long-term periods, the bioavailability of α-tocopherol of fortified NE cream cheese was significantly higher vis-à-vis fortified mayonnaise (Schneider et al., 2012). The bioavailability of α-tocopherol from fortified o/w NE is influenced by interface properties and the used food matrix. This in turn affects the kinetics of bioavailability profile (shown in Fig. 18.3). A technological characterization of the test foods has shown that particle size and boundary layer positively correlate with α-tocopherol bioavailability. However, there was no relationship between the amount of extractable fat and the bioavailability of α-tocopherol. In concluding it can be said that fortified food with high α-tocopherol bioavailabilities can be used to help people ingest adequate amounts of vitamin E. Fortified foods with NEs have been documented to have high α-tocopherol bioavailabilities that can be merchandised as functional foods with disease prevention properties. Nevertheless, these results need to be validated with additional kinetic studies.

NEs formed by spontaneous emulsification/low energy homogenization have been found to offer simple and inexpensive means of encapsulating vitamin D (Guttoff et al., 2015). The NEs developed have been reported to be useful as vitamin D delivery systems for utilization in functional food and beverage products. The type, quality, and the attendant conditions under which they are prepared determine the early size of NE droplet. Formation of NEs comprising droplets of size <200 nm have been reported in

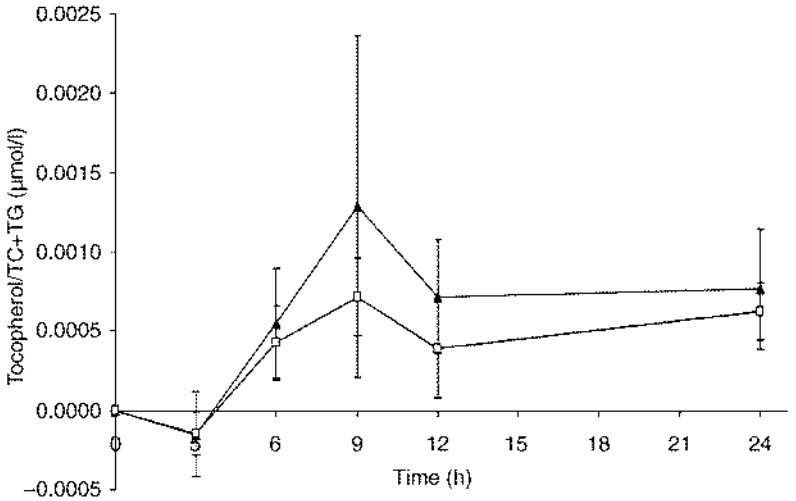



**Figure 18.3.** Mean serum α-tocopherol total cholesterol (TC) + Triglyceride (TG) concentration corrected to the baseline level vs time profiles after consumption of either cream cheese (Δ) or mayonnaise (□) fortified with 60 mg α-tocopherol of the short-term kinetic study, $n = 5$.

literature with surfactant-to-oil ratio of 1:1 and under specified stirring condition using a nonionic surfactant (Tween 80). The confirmation of the result was obtained with titration process. The thermal stability of the NEs can be improved by adding an anionic cosurfactant (SDS) prior to heating. The storage at ambient or to say immediate surrounding temperature kept these NEs stable materially for 1 month. At higher temperatures (>80°C), however, these were found to be vulnerable to droplet growth. Nevertheless, the same method had previously been used to encapsulate another oil-soluble vitamin (vitamin E).

It suggests that it may have more general applications for this purpose. But so far as the preparation method of NE products is concerned, the reciprocal action among the ingredients, ionic composition, environment in which the product is stored and their pH will be different for them. Therefore, to test the stability of vitamin D and the delivery systems under recognized conditions is essential so as to determine the satisfactoriness of the commercial product for marketing.

Recently, a vitamin A, D, and E complex of NE formulation (LaVita) for usage in drinking water or feeds of poultry and livestock has been developed (by Korea BNP). The contents of vitamin A, D, and E in these products were 50,000,000, 5,000,000, and 20,000 IU/L, respectively. The pharmacokinetics assessment of vitamin A, D, and E complex of NE formulation (LaVita), in

comparison to the general product, was performed in the male rat plasma by a single oral dose at 20 mL/kg body weight ($n$ = 3 per group). For NE formulation (LaVita), $C_{max}$ of vitamin A and E in plasma were much higher and the area under the curve (AUC) (Fig. 18.4) of vitamin A, D, and E were 14–63% higher, and the half-life of vitamin E was twofold longer than the general product. According to statistical analysis, each $C_{max}$ of vitamin A, D, and E was significantly higher than the general product (Lee et al., 2011).

Besides food fortification, NEs also find immense use in fabrication of edible films (Durrani et al., 2014). It is a known fact that fresh-cut apples have a low shelf life due to high vulnerability to oxidation in open air. Nowadays, edible coatings containing vitamin E NEs are being used to increase not only the shelf life of the fruit, but also to add to its nutritive values.

Four different edible coatings have been used containing vitamin-E NE. The coatings as have been used in different scenarios are discussed:

*Coating 1*: This type of coating contained a mixture of vitamin C (ascorbic acid), vitamin E (α-tocopherol), calcium chloride, and potassium sorbate. In this type of coating 1% vitamin C was added in calcium chloride and potassium sorbate of 0.25 and 0.1%, respectively. It was then added to the mixture as an antimicrobial agent. 0.125% of vitamin E NE, as a fortified element and as an antioxidant was also added to the mixture.

*Coating 2*: It was composed of two parts. The first part was prepared in the same way as in type 1 coating. 1% vitamin C, 0.25% calcium chloride, and 0.125% vitamin E NE were mixed together. The next part of the coating was prepared by mixing a polysaccharide-based coating of methyl cellulose containing potassium sorbate in it. It was prepared by dissolving powdered form of methyl cellulose in 200 mL mixture of water-ethyl alcohol containing three parts of water and one part of ethyl alcohol. The mixture was stirred for 10 min at 80°C. To obtain a shiny and transparent surface, ethyl alcohol was added to the mixture. 2% solution of propylene glycol was also added as a plasticizer in the formulation.

*Coating 3*: It was also composed of two parts. First part of coating was again the same as it was in type 2 coating. It was prepared by mixing 1% ascorbic acid (vitamin C), 0.25% calcium chloride, and 0.125% vitamin E NE. The other part of coating was composed of a coating of methyl cellulose-stearic acid, a polysaccharide coating, containing potassium sorbate in it.

*Coating 4*: This type of coating was again divided into two parts. In first part a mixture of 1% fresh lemon juice, 0.25% calcium chloride, and 0.125% vitamin E NE was prepared. In the next part methyl cellulose containing potassium sorbate was

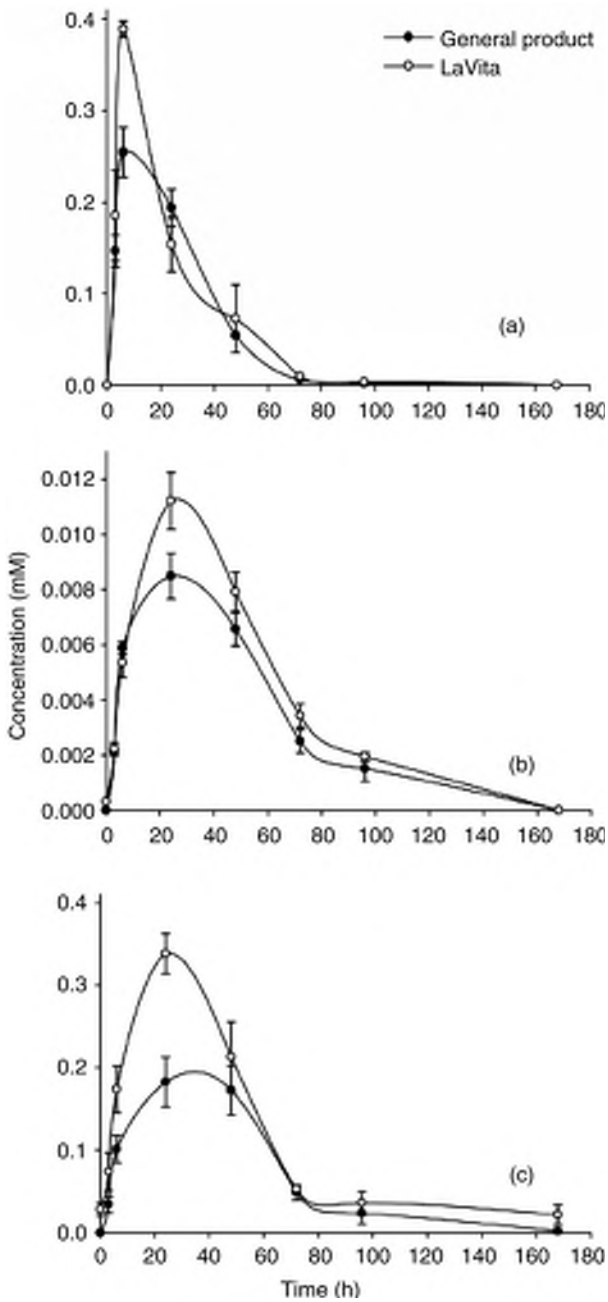



**Figure 18.4. Vitamin A, D, and E concentrations in rat plasma after a single oral administration of LaVita and general product.** (a) Vitamin A, (b) vitamin D, and (c) vitamin E.

prepared. Vitamin E NE was successfully prepared by EPI (emulsion phase inversion) method and the particles size distribution was checked by DLS (dynamic light scattering) method. Potassium dorbate and calcium chloride were added, respectively, as antimicrobial and antioxidant agents. In one coating, fresh lemon juice was used in place of ascorbic acid for comparison. To reduce respiration and water vapor permeability, methyl cellulose, and stearic acid were added in different ratios to all the four coatings. Prepared coatings were then applied on freshly cut apple pieces using dip method. Various characterization parameters were performed to analyze the quality of vitamin E fortified edible nanocoatings such as weight loss, titrateable acidity, total soluble solids, and total phenolics for 2 weeks. In addition, antimicrobial activity of the prepared edible coatings was done using LBA (luria bertani agar) culture media. All the coatings showed good results but the coating containing fresh lemon juice gave comparatively better results to prevent oxidation and resultant browning of apples.

## 12 Efficacy of NEs in Pharmaceuticals

NEs serve as the best possible medium for effective drug administration. They have the ability to protect the drugs from hydrolysis and enzymatic degradation. They can dissolve large quantities of hydrophobic drugs (Aboofazeli, 2010; Pires et al., 2009; Abhijit et al., 2015). NEs help in continuous and guarded discharge of drug over a period of time. They assist in adjusting the injectable dose during the drug administration period. There is no fear of sedimentation, creaming, and flocculation, either. Besides, it provides large surface area and free energy. These characteristics indicate the advantages NEs deliver over emulsions because the latter are made up of bigger sized particles making them inappropriate for oral administration or through injectable route. They are also placed in an advantageous position because of their large interfacial area. It definitely impacts the carriage of drug and release thereof. Their vast interfacial area positively influences the drug transport and delivery. NE-based carriers enable the drugs to target the intended sites. Because of these inherent advantages NEs are now being used as drug delivery vehicles for nasal, dermal, ocular delivery, and so on. To put it simply, NEs are nowadays being commonly used to deliver active pharmaceutical ingredients (API) in topical, oral, nasal, ophthalmic, or in injectable dose form (Cushing et al., 2012). Although a number of lipid-based NE pharmaceutical products have been promoted in the past for over two decades but there are fewer examples of o/w emulsion products.

## 12.1 NE and Acute Coronary Syndrome (ACS)

*Ascendia's EmulSol* technology (http://www.drug-dev.com/Main/Back-Issues/nanoemulsion-formulations-Nanoemulsion-Formulation-767.aspx) using a high-pressure homogenization process, produces stable and optically clear NE by selecting specific long-chain triglycerides in combination with an ionizable surfactant without the use of organic solvents and with minimal use of cosurfactants. The elimination of solvents from the formulation reduces injection site irritation and is more acceptable for pediatric products. Further, the minimization of surfactants improves the safety and chemical stability of the resulting NE formulation.

The patentee, *Ascendia* uses its EmulSol technology to prepare antithrombotic drug clopidogrel. Clopidogrel (Cushing et al., 2012; Gao et al., 2013; Huang and Gao, 2013), in oral form is prescribed for acute coronary syndrome (ACS), and also following recent myocardial infarction, stroke, or in established peripheral arterial disease. It was first approved in 1997, was codeveloped and comarketed (as Plavix®) by two corporations (MyersSquibb and Sanofi). Acute coronary syndrome (ACS) in particular occurs when the supply of blood to coronaries is cut off abruptly either partially or totally. Clopidogrel 300–600 mg is administered regularly for treatment when ACS develops in a patient (it is no recommendation for self-medication). But the problem is that clopidogrel is available as tablet formulation in strength of 75 and 3000 mg tablets. Such prescription is not idyllic in emergency settings. A number of tablets that need to be given is between 2 and 4. Further, oral drug takes time to absorb and become active. Though clopidogrel gets absorbed rapidly, it takes several hours before peak blood concentration and therapeutic effect are achieved. Further, the solubility of clopidogrel is a limitation to it. Solubility, physical form, and chemical properties of clopidogrel offer barriers to developing injectable forms of the drug. So rapidly acting injectable form is desirable in acute settings to effectively prevent blood clots and to reduce the risk of severe events.

Because the formulation contains no solvent, the risk of injection site pain is greatly reduced. And, even though the free-base is poorly soluble at plasma pH, when contained in the oil phase of the Ns, the clopidogrel drug substance becomes much more soluble as shown in Table 18.1.

## 12.2 NE as Drug Delivery System for Nose and Brain Targeting

Numerous problems are related to the issue of delivering drugs to the brain. The problem is all the more important in hydrophilic

## Table 18.1 Successful Formulation of Clopidogrel Free-Base in an NE

| Aqueous Conc. (mg/mL) | | Comments |
|---|---|---|
| Clopidogrel (free base) | 7 mg/mL @ pH 1 buffer | Highly pH dependent solubility |
| | 0.002 mg/mL @ pH 7.4 buffer (simulated plasma pH) | Solubility crashes at higher pH |
| ASD-002 NE | >200 mg/mL in oil phase | Formulation suitable |
| | >20 mg/mL loading in total volume of NEs | 300 mg dose can be delivered with 10–15 mL injection |

cases, including in cases with elevated molecular load. This phenomenon can be attributed to resistant character of endothelium because endothelium acts as an obstruction between blood and brain (Pires et al., 2009; Ugwoke et al., 2005; Pardridge, 1999; Clark et al., 2001). Nanocarriers hold great promise. This is because they shield bioactives from degradation while leveraging synergy between lymphoid and mucosae tissues. Because of the promises the nanobased delivery system hold they will generate cognizable results in carrying drugs to the brain in the treatment of illnesses associated with the central nervous system (Ugwoke et al., 2005; Candace and Pollock, 2005). This route is painless, noninvasive, and well tolerated. The sensory system described as olfactory, the first cranial nerve, has a crucial connect between the nose and the brain. As a result, the drugs loaded with NEs when administered directly in the nasal mucosa can help effectively manage conditions such as Alzheimer's disease, migraine, depression, schizophrenia, Parkinson's diseases, and meningitis can be treated (Mistry et al., 2009; Csaba et al., 2009). Nasal cavity, described as *either of the two cavities lying between the floor of the cranium and the roof of the mouth and extending from the face to the pharynx* provide the most effective setting for NE-based drug delivery as it is moderately permeable, has large number of immunoactive sites, and shows reduced enzyme activity (Pardridge, 1999). Preparation of NE containing risperidone for its delivery to the brain via the nose has been reported in literature (Kumar et al., 2009; Kumar and Pathak, 2009). It is inferred that this emulsion is more effective through the nasal rather than intravenous route.

References appear in literature about formation of NE enclosing risperidone for release in brain via olfactory region (Kumar et al., 2009). It highlights the effectiveness of emulsion through nasal

route vis-à-vis when administered intravenously. In the development of vaccines, the delivery of drug through sensory system also finds relevance. Administration of antigen through nasal mucosa helps in achieving immunity. Risperidone, a benzisoxazole derivative, is an approved antipsychotic drug. It is usually available as tablet, oral liquid, and orally disintegrating tablet (Qizhi et al., 2004). But it has its deficiencies. One, due to extensive first pass metabolism it suffers rapid excretion and, therefore is poorly bioavailable. The other is its nontargeted delivery. This nontargeted delivery has its concerns. As a consequence there are side effects of the drug (http://www.drugs.com). Therefore, two issues need to be addressed: (1) improvement in the bioavailability of drug. Because the target site of risperidone is the brain, therefore a delivery vehicle is needed that prevents first pass metabolism, and (2) its permeability across the blood brain barrier, so that it helps in achieving the desired drug concentration at the site of action, that is, the brain. NEs are likely to help in targeted delivery, thus preventing the availability of drugs at a site other than the intended while reducing the side effects.

Risperidone NE and mucoadhesive NE have been characterized by such parameters as drug content, pH, percentage transmittance, globule size and zeta potential (Kumar et al., 2009). Following intranasal and intravenous administration biodistribution of risperidone NE, mucoadhesive NE, and risperidone solution (RS) was studied using the technetium label. The study developed a profile using optimized technetium-labeled (99mTc-labeled) risperidone formulations after intranasal (i.n.) and intravenous (i.v.) administration (Fig. 18.5). Gamma scintigraphy imaging of brain of rats showed that the brain/blood uptake ratio of 0.617, 0.754, 0.948, and 0.054 for RS (i.n.), risperidone NE (i.n.), mucoadhesive NE (i.n.), and risperidone NE (i.v.), respectively, at 0.5 h indicated direct nose to brain transport. The reported NEs formulation bypassed the blood–brain barrier. Studies conclusively demonstrated speedy transport of risperidone by mucoadhesive nanoemulsion (i.n.) vis-à-vis risperidone solution (i.n.), risperidone nanoemulsion (i.n.) and risperidone nanoemulsion (i.v.) into the rat brain (Rutvij et al., 2011). This was attributed to efficacious and direct nose to brain drug transport (direct transport percentage, DTP%) for mucoadhesive NEs.

## 12.3 NEs as Antimicrobial Agents

Another area of drug delivery where NEs find great applications is the fight against microbes. Successful studies have begun on the use of NEms as a prophylactic cure because of their antimicrobial activity (a human protective treatment) to protect people

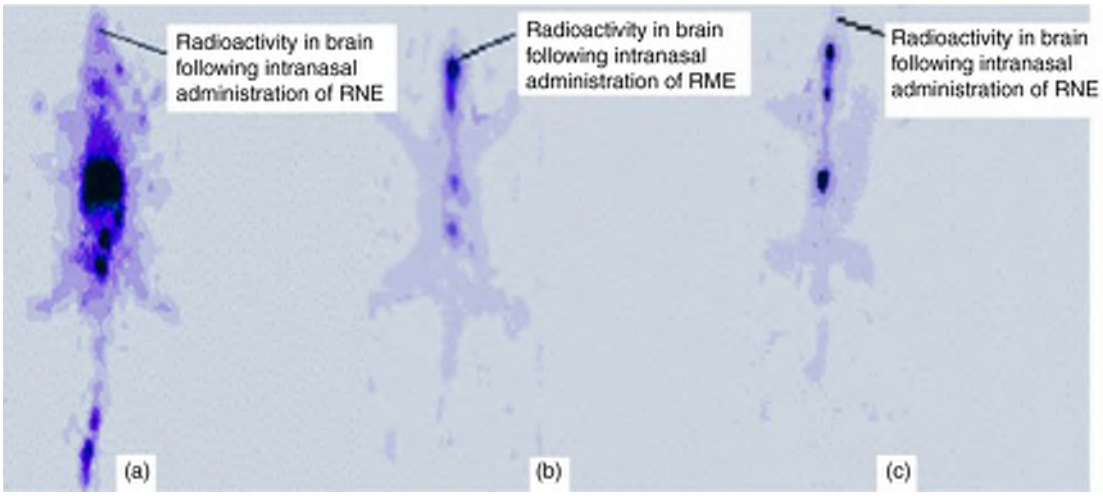



**Figure 18.5. Gamma scintigraphy images of rat (A/P view) showing the presence of radioactivity.** (a) Risperidone NE (i.v.), (b) mucoadhesive NE (i.n.), and (c) Risperidone NE (i.n.).

venerable to bioattack microbes or microorganisms, which are difficult to remove by washing hands. They include anthrax and Ebola. The US Army, during the end of the year 1999, tested a NEm effective against a large variety of organisms, so-to-say broad-spectrum, NEm on field for neutralization of anthrax microorganisms and then again in Mar. 2001 as a proxy for their chemical neutralization (Rutvij et al., 2011; Charles and Attama, 2011; Subhashis et al., 2011; Chime et al., 2014). The new technology that has evolved has been also employed to save limbs that develop gangrene or on wounds infected with clostridium botulinum. The basic theory behind these studies was that NE particles were thermodynamically driven. Therefore, they get pushed to fuse with organisms contained in lipid. The anionic charge on pathogen and the electrostatic attraction between the cationic charges of the emulsion enhances their ability to blend. The fusion of adequate number of nanoparticles with pathogens assist in the release of some of the energy shut within emulsion (http://nano.med.umich.edu/platforms/Antimicrobial-Nanoemulsion.html). It is this trapped energy and the actively involved constituents that weaken the pathogen lipid membrane leading to destruction of cells and their ultimate death. However, NEs, to be more effective in the case of spores, extra germination enhancers are required to be integrated with emulsion. The moment germination begins, the germination spores become venerable to antimicrobial activity of the NE (Fig. 18.6). One peculiar aspect of NE is that concentrations which exert selective toxicity on microbes

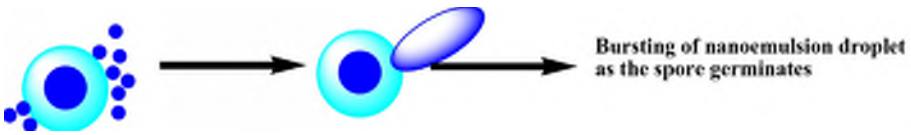



**Figure 18.6. Action mechanism of NE against spores.**

do not have any irritating effect on skin or mucus membrane. The level of detergent in each droplet is low. On acting in concert, these droplets generate enough energy and surfactant to damage microbes without any effect on healthy cells. As a result, NE manages to achieve topical antimicrobial activity equivalent to that attained previously by systemically administered antibiotics only.

NANOSTAT™ based on *proprietary* NE technology for decontamination is commercially marketed by Nanobio Corp. The decontaminating material is available in the form of foam, liquid, and spray for the treatment of viral and fungal skin (Charles and Attama, 2011; Hwang et al., 2013; Subhashis et al., 2011; Chime et al., 2014; Yashpal et al., 2013). They, however, cannot be injected into the bloodstream because they are known to lyse red cells.

Another problem that needs immediate focus these days is the development of resistance to antibiotics, posing serious threat to human population (Hwang et al., 2013). Bacteria have the ability to acquire the genetic elements efficiently and form biofilms. Breaking of these biofilms is difficult. NE technology has, however, come to the rescue of researchers for destruction of biofilms. In the recent past, *Acinetobacter baumannii* has emerged as a serious problematic pathogen due to the ever-increasing presence of antibiotic resistance. This has necessitated the development of novel, broad-spectrum antimicrobial therapeutic option. Antimicrobial NEs are emulsified mixtures of detergent, oil, and water (droplet size, 100–800 nm), which have broad antimicrobial activity against bacteria, enveloped viruses, and fungi. The screening of the antimicrobial activities of five NE preparations against four *A. baumannii* isolates have been carried out by Hwang et al. (2013). Briefly stated:

- NE 1 (N1) contained 2% (vol./vol.) Triton X-100, 2% (vol./vol.) tributyl phosphate, and 16% (vol./vol.) soybean oil;
- NE 2 (N2) contained 3% (vol./vol.) Tween 60, 3% (vol./vol.) soy sterol, 30% (vol./vol.) soybean oil, and 0.35% (wt./vol.) CPC;
- NE 3 (N3) contained 15% (vol./vol.) Tween 80, 3% (vol./vol.) ethyl oleate, and 6% (vol./vol.) octanol;
- NE 4 (N4) contained 8% (vol./vol.) Triton X-100, 8% (vol./vol.) tributyl phosphate, 64% (vol./vol.) soybean oil, and 50 mol/L EDTA;
- NE 5 (N5) contained 10% (vol./vol.) Triton X-100, 25% (vol./vol.) soybean oil, and 1% (wt./vol.) CPC.

Among them, N5, which contains 10% (vol./vol.) Triton X-100, 25% (vol./vol.) soybean oil, and 1% (wt./vol.) cetylpyridinium chloride (CPC) showed the best efficacy against *A. baumannii* in both its planktonic and biofilm forms and was selected for further study. The results demonstrated that while the killing of planktonic forms of *A. baumannii* was due to 1% CPC component of NE, the breakdown of biofilms was achieved via the emulsified oil and detergent fractions. It lays down a solid foundation for the utilization of NEs against the antibiotic-resistant forms of *A. baumannii*.

## 12.4 NE for Anticancer Drug Therapy

Taxol, a non-NE formulation is an anticancer ("antineoplastic" or "cytotoxic") chemotherapy drug. It is classified as "plant alkaloid," a "taxane," and an "antimicrotubule agent" and is used in the treatment of breast, ovarian, lung, bladder, prostate, melanoma, esophageal, as well as other types of solid tumor cancers. It is given as an injection or infusion into the vein. It is an irritant. If the medication escapes from the vein it can cause tissue damage. Anticancer agents the likes of paclitaxel can also be delivered with the help of NEs (Constantinides et al., 2000; Mattheolabakis et al., 2012). To be specific TOCOSOL paclitaxel is a cremophor-free o/w NE. For stabilization at a high drug load of 9–10 mg/mL, this NE contains a blend of two surfactants α-tocopherol-polyethylene glycol-1000-succinate and poloxamer and vitamin E as oil phase. With mean diameter of 60 and 150 nm, 99% particle size distribution gives nanodroplets a major advantage. Nanodroplets also enjoy an advantage because they can be filtered and sterilized at their manufacturing stage. Further, these nanodroplets also bear the feature of stability for 6.5 h in human plasma and also support a slower release of active drug as compared to cremophor paclitaxel. Research has been carried out where a variety of tumor cells were implanted in mice. Studies have established greater usefulness and pharmacokinetics of TOCOSOL paclitaxel vis-à-vis Taxol (Constantinides et al., 2000; Mattheolabakis et al., 2012). TOCOSOL paclitaxe utilizes the EPR effect (enhanced permeability retention) to improve plasma stability in cancer patients (Bogdanova et al., 2003). TOCOSOL paclitaxel, when administered at a dose of 175 mg/m$^2$ with a 15 min slow intravenous push, resulted in about a 50 and 75% decrease in whole-blood clearance and volume of circulation respectively, as compared to 3 h infusion of Taxol on similar parameters (Lissianskaya et al., 2004). Weekly TOCOSOL paclitaxel administration was further found to be effective and well tolerated in various efficacy studies conducted in ovarian cancer, nonsmall-cell lung cancer, and metastatic breast

## Table 18.2 Some Commercially Available NE Formulations

| Market Formulation | Therapeutic Indication | Manufacturer |
|---|---|---|
| Alprostadil palmitate | Vasodilator platelet inhibitor | Mitsubishi Pharmaceutical |
| AS03 | Adjuvant | Glaxosmithline |
| AS04 | Adjuvant | Glaxosmithline |
| Dexamethasone | Steroid | Mitsubishi Pharmaceutical |
| Flurbiprofen axetil | Nonsteriodal analgesic | Kaken Pharmaceuticals |
| MF59® | Immunologic adjuvant | Novartis |
| Palmitate alprostadil | Vasodilator platelet inhibitor | Mitsubishi Pharmaceutical |
| Propofol | Anesthetic | Astrazeneca |
| Vitamin A, D, E, K | Parenteral nutrition | Fresenius Kabi |

cancer (Constantinides et al., 2008; Dias et al., 2007; Moranhao et al., 1992). Clinical trial in patients to compare the efficacy of TOCOSOL paclitaxel and Taxol with metastatic breast cancer were still inconclusive. Encouraging preclinical results have, however, been reported with another NE formulation of paclitaxel (EmPAC) developed by Cornerstone Pharmaceuticals. It has been found to be superior to Taxol in a study conducted on animals in terms of its effect on cancer cells and tumors. A safe and secure cure for cancer is yet to be developed. However, for the present the scientists and researchers are passionately exploring options to find a stable cure for cancer.

Besides, the NEs have the capability to passively accumulate in tumors via the EPR effect. They are also capable of participating in the process of active uptake of tumors. This occurs via the process of receptor mediated endocytosis. Maranhao et al. (1993) have developed an NE LDE (low density apolipoprotein E) containing 40 mg cholesterol oleate, 20 mg egg lecithin, 1 mg triolein, 0.5 mg cholesterol (Raul et al., 1994; Debora et al., 2005; Maria et al., 2007; Harisa and Alanazi, 2014). It is manufactured without protein, however, when it is in contact with blood plasma, it acquires apolipoprotein E (apo E), which is also recognized by low-density lipoprotein receptors thus allowing endocytosis of the NE. LDE can, therefore be used as targeted antiplastic drug against cancer cells that overexpress low-density lipoproteins receptors (Harisa and Alanazi, 2014). It has also been demonstrated in patients that

after intravenous injections LDE can concentrate in cancer cells or solid tumors such as ovarian and breast cancers that overexpress these receptors.

Due to space constraints it is not possible to discuss more NE formulations on these pages. However, some more commercial examples of o/w NEs are listed in Table 18.2.

## 13 Conclusions

Applications and usages of NEs are all regulated, be it in food or pharmaceuticals either overtly or covertly. But they have proved to be versatile systems that can dissolve large quantities of hyrdrophobics (drug/nutraceuticals/vitamins, etc.) along with their mutual compatibility and have the ability to protect them from hydrolysis and enzymatic degradation. It makes them ideal vehicles for delivery of food and pharmaceuticals components because they prevent the food from oxidation/degradation by protecting them in surfactant coated droplets and ensure frequent and controlled release of drugs.

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# NANOEMULSIONS: AN EMERGING TECHNOLOGY IN THE FOOD INDUSTRY

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## 1 Introduction

Nanotechnology is an upcoming technology that finds widespread applications in engineering, chemical, pharmaceutical, and food industry (Huang et al., 2010). Within the food industry, it has experienced significant revolution over the past couple of years. Nanotechnology is defined as manipulation of matter within the size range of 1–100 nm. The potential applications of food nanotechnology involves modification of certain physical characteristics of food like texture, color, taste, sensory attributes, processability, and storage stability, leading to great number of new products (Silva et al., 2012). Such growth has been hastened by the potential of harnessing the large surface area to volume ratio of these nanomaterials to improve the aqueous solubility, bioavailability, improve sensory aspects of active ingredients (Acosta, 2009). Another promising area of nanotechnology within the food industry is the use of nanoemulsions as carriers for lipophilic bioactive components, drugs, flavoring agents, antioxidants, and preservatives (Graves and Mason, 2008).

Nanoemulsions are a subset of traditionally known colloidal dispersions and miniemulsions (Choi et al., 1985; Asua, 2002). Recently, nanonomenclature has been adopted owing to very small particles having mean radii between 1 and 100 nm (Fryd and Mason, 2012). Nanoemulsions tend to be transparent or only slightly turbid (similar to microemulsions). The

[†] This chapter is dedicated to the memory of Dr. Shishu Goindi.

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00019-2

relatively small particle size of nanoemulsions makes them highly stable to gravitational separation and droplet aggregation (McClements, 2005; Tadros et al., 2004). On the contrary, conventional emulsions have droplet size with mean radii between 100 nm and 100 μm. They are optically opaque and thermodynamically unstable owing to large globule size. They are more susceptible to creaming, cracking, gravitational separation, flocculation, and so on. The conventional emulsions used to be the mainstay of the food industry until few years ago. However, due to their innate instability and turbidity they are no longer the natural choice for the food and beverage industry. Various methods have been developed to prepare delivery systems of lipophilic functional compounds with particle diameters in the nanosize range (Fryd and Mason, 2012). Therefore, the focus area of research is to create nanoemulsions with long kinetic stability by controlling their composition, microstructure by adding stabilizers like emulsifiers, texture modifiers, weighting agents, and ripening retardants. However, like conventional emulsions, nanoemulsions may be of oil-in-water (o/w) or water-in-oil (w/o) types depending on whether the oil is dispersed as droplets in water, or vice versa. The internal structure and physical appearance of nanoemulsions in contrast with conventional emulsions is represented by Fig. 19.1. The prime concern is on colloidal dispersions suitable for encapsulating lipophilic components in aqueous environments, and so in this chapter we focused on o/w nanoemulsions that consist of small particles comprised of oil and surfactant molecules dispersed within an aqueous medium.

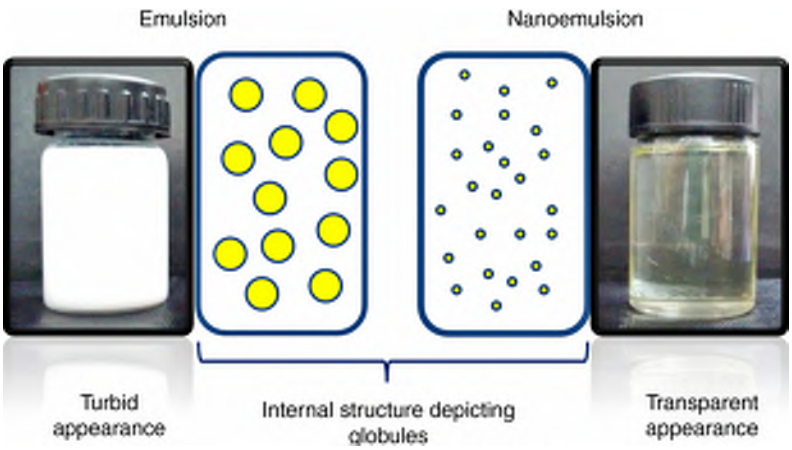


**Figure 19.1. Internal structure and physical appearance of emulsions and nanoemulsions.**

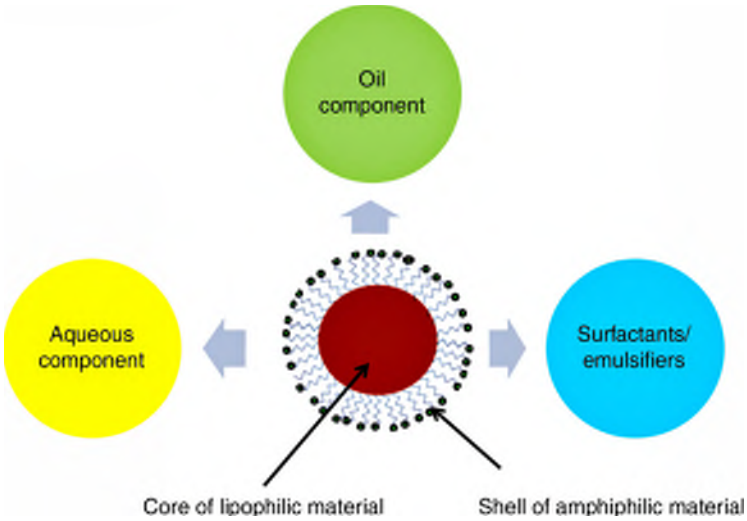


**Figure 19.2. Schematic representation of core-shell structure of particles in nanoemulsions along with different components of nanoemulsions.**

## 2 Composition of Nanoemulsions

An o/w nanoemulsion may be represented by core-shell-type architecture. The core and shell comprised of lipophilic component and amphiphilic surfactant, respectively, is represented by Fig. 19.2. The lipophilic material may be oils (acylglycerols) and other bioactive components. The surface active agents, phospholipids, proteins, polysaccharides or minerals, and so on, may form the amphiphilic shell. Nanoemulsions are composed chiefly of an oil component, an aqueous component, and a surfactant (emulsifier/stabilizer). Apart from these three main components, nanoemulsions consist of texture modifier, weighting agent, and ripening retardant (McClements and Rao, 2011).

### 2.1 Oil Component

The important physicochemical properties of the oil phase like water-solubility, interfacial tension, density, refractive index, phase behavior, viscosity, chemical stability, and so on, influences the stable formation and properties of nanoemulsions (Anton et al., 2007; Anton and Vandamme, 2009; Tadros et al., 2004; Wooster et al., 2008). Various nonpolar components like acylglycerols, free fatty acids, essential oils (EOs), flavor oils, waxes and fats, mineral oils, oil-soluble vitamins, and nutraceuticals (carotenoids, omega-3 fatty acids, curcumin, resveratrol, phytostanols,

## Table 19.1 List of Oils Used in Nanoemulsions

| Oil | Processing Technique | Functional Ingredient | References |
|---|---|---|---|
| MCT | HPH | β-Carotene | Yuan et al. (2008a,b) |
| MCT | HPH | Green tea extract | Kim et al. (2012) |
| MCT | Spontaneous emulsification | Vitamin E | Saberi et al. (2013) |
| MCT | HPH | 5-Demethyltangeretin | Zheng et al. (2014) |
| MCT | Spontaneous emulsification | Vitamin D | Guttoff et al. (2015) |
| Paraffin oil | Emulsion inversion point | — | Liu et al. (2006) |
| Sunflower oil | Ultrasound and HPH | — | Leong et al. (2009) |
| Olive oil, soyabean oil, sesame oil | Ultrasound | — | Wulff-Pérez et al. (2009) |
| Corn oil | Combined homogenization and solvent evaporation | — | Lee and McClements (2010) |
| Peanut oil | HPH | Resveratrol | Donsi et al. (2011) |
| Palm oil | HPH | Vitamin E | El Kinawy et al. (2012) |
| Grape seed and orange oil | Spontaneous emulsification | Resveratrol | Davidov-Pardo and McClements (2015) |
| D-Limonene | Ultrasound and microfluidization | — | Jafari et al. (2007) |
| Octanoic acid | Rotor/stator homogenization | — | Katagi et al. (2007) |
| Stearin rich milk fat | HPH | α-Tocopherol | Relkin et al. (2008) |
| Wheat bran | Ultrasonication | — | Rebolleda et al. (2015) |

coenzyme Q, and phytosterols) are potential candidates for being selected as oil components to form nanoemulsions. Mostly, triacylglycerol oils are used in the food industry due to their availability and low cost. Some of the oils having nutritive value like soybean oil, sesame oil, sunflower oil, olive oil, flaxseed oil, almond oil, and fish oils are widely used. These oils majorly contain long-chain triacylglycerols (LCT), though medium- (MCT) and short-chain triacylglycerols (SCT) are also being explored in the food industry (McClements and Rao, 2011). Table 19.1 lists the various ingredients used as oil phase in nanoemulsions.

### 2.2 Aqueous Component

Water is predominantly used as the aqueous phase along with other polar components like polyalcohols, saccharides, minerals,

proteins, acids, and bases. The physicochemical characteristics of the aqueous phase impact the stable formation of the nanoemulsion produced. The formation and stability of nanoemulsions can be achieved by optimizing the composition of aqueous phase.

## 2.3 Surfactants

A variety of stabilizers are added to nanoemulsions to improve their long-term stability as oil component and water component may break after some time. An emulsifier is amphiphilic in nature that helps in reducing the interfacial tension by adsorbing at droplet surfaces; facilitate their disruption, as well as protection against aggregation (Kralova and Sjoblom, 2009). Various environmental factors such as pH, ionic strength, temperature, and long-term storage affect the stability of a nanoemulsion, which indeed depends on the type of emulsifier used. Therefore, the selection of a suitable emulsifier or emulsifier combination is considered to be the vital factor for the optimization of a stable nanoemulsion. The most important types of emulsifiers used in the food industry are small molecule surfactants, phospholipids, proteins, and polysaccharides as enlisted in Table 19.2. Being natural ingredients, proteins and polysaccharides, which are also considered as safe in producing nanoemulsions. Generally surfactants are categorized into three classes possessing different electrical attributes: ionic, nonionic, and zwitterionic (McClements, 2005).

### *2.3.1 Ionic Surfactants*

Ionic surfactants are either cationic or anionic in nature. Generally anionic edible surfactants like citric acid esters of mono- and diglycerides of fatty acids (CITREM), diacetyl tartaric acid ester of mono- and diglycerides (DATEM), sodium caseinate, sodium dodecyl sulfate, and sodium lauryl sulfate offer more advantages in the food industry as compared to cationic ones like lauric arginate and β-lactoglobulin. But these have tendency to cause irritation at high concentration levels thus limiting their use in products requisites of high surfactant levels (Solé et al., 2006a,b; McClements and Rao, 2011).

### *2.3.2 Nonionic Surfactants*

These surfactants are the key ingredient in processing food grade nanoemulsions. Their major advantages include low toxicity potential, lack of irritation, and the ability to form stable nanoemulsions. Sorbitan monooleate, polyglycerol esters of fatty acids, sucrose monopalmitate, polyoxyethylene ether surfactants (eg, Brij 97), and ethoxylated sorbitan esters (eg, Tweens and Spans)

## Table 19.2 Emulsifiers Used in Nanoemulsions

| Nature of Emulsifier | Emulsifier/Surfactant | References |
|---|---|---|
| Anionic | Sodium dodecyl sulfate | McClements (2000) |
| Cationic | Dodecyltrimethylammonium bromide | McClements (2000) |
| | Modified starch | Jafari et al. (2007) |
| Nonionic | Polyethylene glycol (35) castor oil | Usón et al. (2004) |
| | Polyoxyethylene-4-lauryl ether | Izquierdo et al. (2004) |
| | Polyoxyethylene-6-lauryl ether | Izquierdo et al. (2005) |
| | Polyglycerol esters of fatty acids | Tan and Nakajima (2005b) |
| | Polyoxyethylene-660-12-hydroxy stearate | Anton et al. (2007) |
| | Sucrose fatty acid ester (L1695) and decaglycerol monolaurate (ML 750) | Yin et al. (2009) |
| | Span 20, 80 and Tween-20, 80 | Porras et al. (2008), Chang et al. (2013), Sari et al. (2015), Davidov-Pardo and McClements (2015), Rebolleda et al. (2015) |
| | Tween-40, 60 | Yuan et al. (2008a), El Kinawy et al. (2012) |
| Miscellaneous | Whey protein concentrate and whey protein hydrolysate | Chu et al. (2007) |
| | Gelatin | Ribeiro et al. (2008) |
| | Sodium caseinate | Yin et al. (2009) |
| | Whey protein isolate | Lee and McClements (2010) |
| | Lipoid S75-3® | Anton et al. (2007) |
| | Lipoid S75-3® | Anton et al. (2007) |
| | Soybean protein isolate, whey protein isolate, and β-lactoglobulin | He et al. (2011) |
| | PEG-30 castor oil/sorbitan oleate | Bernardi et al. (2011) |
| | β-Lactoglobulin | Zheng et al. (2014) |

are widely used nonionic surfactants in the food industry (Liu et al., 2006; Jafari et al., 2007; McClements and Rao, 2011).

### *2.3.3 Zwitterionic Surfactants*

Zwitterionic (amphoteric) surfactants have both cationic and anionic groups attached to the same molecule, and can have net charge depending on the pH of the solution. In foods, natural

phospholipids such as lecithin are most commonly used. Natural phospholipids are often blended with other surfactants to improve the formation and stability of nanoemulsions rather than using it alone (Trotta et al., 1996; de Morais et al., 2006; Hoeller et al., 2009). For instance, a blend of hydrophilic and lipophilic surfactants helps in the formation of nanoemulsions using low-energy and high-energy approaches. The mixed-emulsifier systems help in reducing instability due to particle aggregation after formation of nanoemulsions.

Cosurfactants are also required in the formation of nanoemulsions using low-energy methods. Cosurfactants are amphiphilic, surface active agents possessing hydrocarbon chain and a hydroxyl group. These do not form stable nanoemulsions due to the presence of a small polar head group. The underlying physicochemical mechanisms for the ability of cosurfactants to form nanoemulsions are fluidization of the interface, induction of interfacial curvature, optimization of the viscosity ratio of both phases, and reduction of the electrical repulsion at an interface (Gradzielski, 1998; Garti et al., 2001; Shafiq-un-Nabi et al., 2007; McClements and Rao, 2011).

## 2.4 Miscellaneous Ingredients

### *2.4.1 Functional Compounds*

A diverse range of lipophilic ingredients like bioactive lipids, flavors, antimicrobials, antioxidants can be encapsulated leading to increase of their bioactivity, desirability, and palatability (Chen et al., 2006; McClements et al., 2007; Shefer and Shefer, 2003; Ubbink and Krüger, 2006). The major lipophilic functional food compounds that are incorporated into nanoemulsions are divided into four categories: fatty acids (eg, omega-3 fatty acids), carotenoids (eg, β-carotene), antioxidants (eg, tocopherol), and phytosterols (eg, stigmasterol) (Silva et al., 2012).

### *2.4.2 Texture Modifier*

A texture modifier is defined as a substance that increases the viscosity of the aqueous phase (Imeson, 2010). The stability of commercial products is often achieved by adding texture modifiers, which act as a retardant of droplet movement, but they may also be incorporated to provide required textural attributes such as creaminess, richness, thickness, or gel strength. In the food industry, biopolymers such as polysaccharides (eg, starch, pectin, alginate, carrageenan, xanthan gum, guar gum) or proteins (eg, egg, milk, vegetable proteins) are the most widely used texture modifiers (McClements and Rao, 2011).

### 2.4.3 Weighting Agent

Creaming and sedimentation are the types of instability generally found in emulsions that can be prevented by adding a weighting agent. A weighting agent is a substance that is generally added to the oil droplets in o/w emulsions that match their density to the adjacent aqueous phase. It helps in reducing the driving force required for gravitational separation, thereby forming a stable emulsion. Also in nanoemulsions, the density of the oil droplets can be matched to the adjacent aqueous phase by coating them with thick dense layers, or by partially crystallizing the lipid core. Examples of most commonly used weighting agents in the food and beverage industries are brominated vegetable oil, sucrose-acetate isobutyrate, ester gum, and damar gum (McClements, 2005; McClements and Rao, 2011).

### 2.4.4 Ripening Retarder

Ripening retarder is a stability enhancer that is added to food grade nanoemulsions, which are prone to Ostwald ripening (OR). OR is a process in which small oil droplets diffuse through the intervening aqueous phase to form a large droplet. It mostly occurs in nanoemulsions containing oils that are susceptible to OR such as EOs, flavor oils, and SCTs. A ripening retardant is a highly hydrophobic material, which is incorporated into oil globules where it gets solubilized and retard OR as a result of generation of entropy of mixing effect that contravene the OR effect. Examples of ripening retarder include a long-chain triglyceride, mineral oil, or ester gum (Kabalnov et al., 1987; Sonneville-Aubrun et al., 2004; McClements and Rao, 2011).

### 2.4.5 Cosolvent/Solvents

Many processing techniques of nanoemulsions are dependent on use of solvents/cosolvents. Cosolvents are highly polar molecules, surface inactive agents but may modify the emulsifier properties such as surface-activity, partition coefficient, and their ability to form colloidal structures due to change in hydrophobic interactions present in aqueous phase. Most commonly used cosurfactants are short- and medium-chain alcohols and cosolvents are polyols like propylene glycol (PG), glycerol, and sorbitol (Yaghmur et al., 2002; Flanagan and Singh, 2006). Solvents like hexane, tetracane, and acetone are widely used in food industry. Though certain solvents are included in the US Food and Drug Administration's (FDA) generally recognized as safe (GRAS) list but their use is not well accepted by consumers. These solvents should be replaced by natural oils or MCT, and so on. Table 19.3 lists various solvents used in nanoemulsions.

**Table 19.3 Solvents Used in Nanoemulsion Processing With Corresponding Functional Ingredient**

| Solvent | Processing Technique | References |
|---|---|---|
| Hexadecane | PIT method | Izquierdo et al. (2001) |
| *n*-Decane | PIT method | Ee et al. (2008) |
| Isohexadecane | PIT method | Izquierdo et al. (2004) |
| *n*-Hexane | Emulsification–evaporation technique | Tan and Nakajima (2005a) |
| Tetradecane | PIT method | Rao and McClements (2010) |
| Ethyl acetate | Combined homogenization and solvent evaporation method | Lee and McClements (2010) |
| Acetone | Solvent displacement technique | Yin et al. (2009) |

## 3 Processing of Nanoemulsions

Nanoemulsions are fabricated using various approaches which can be broadly classified as high-energy and low-energy approaches depending on the principle involved (Tadros et al., 2004; Leong et al., 2009; Acosta, 2009). The size of the nanoemulsion droplets is governed by the approach used, the operating conditions, and the composition of the system (McClements, 2011).

### 3.1 High-Energy Approaches

The high-energy approaches are the most versatile means of producing food grade nanoemulsions as they can be used with wide variety of oil phases like triacylglycerols, flavor oils, EOs, and emulsifiers like proteins, polysaccharides, phospholipids, and surfactants. The selected composition of the formulation, homogenizer type, the quantity of energy applied, homogenizer operating conditions, physiocochemical properties of each component are the most important factors while formulating nanoemulsions using the high energy approach (McClements, 2011; Silva et al., 2012). Droplet disruption and droplet coalescence are the two opposing forces operating within a homogenizer which govern the size of the droplet formed (Jafari et al., 2007). Only those devices which have the capacity to generate extremely intense disruptive forces are capable of forming tiny droplets in a nanoemulsion (McClements and Rao, 2011). The restoring forces that hold the droplets into spherical shapes must be exceeded by

the disruptive forces of the homogenizer to break them into smaller droplets (Schubert et al., 2003). The restorative forces are determined by the Laplace pressure:

$$\Delta P = \frac{\gamma}{2r}$$

where ($\gamma$) is interfacial tension and ($r$) is droplet radius. Thus, with a decrease in the size of the droplet it becomes increasingly difficult to break it further, which necessitates the use of high-pressure homogenizers (HPH). The high-energy approaches involve the use of mechanical devices, which generate intense disruptive forces that lead to the formation of tiny oil droplets by mixing and disrupting the oil phase and the aqueous phase, for example, high-pressure valve homogenizers (HPVH), microfluidizers, and ultrasonicators (Kentish et al., 2008; Lee and Norton, 2013).

### 3.1.1 High-Pressure Valve Homogenization

The use of these homogenizers is the most popular method of creating fine emulsion in the food industry because they can be used with a wide variety of different oils and emulsifiers (Schubert et al., 2003). The HPH supply the available energy in the shortest time and have the most homogenous flow to produce the smallest size (Solans et al., 2005). In the HPH, the mixture is pumped at a very high pressure (50–100 MPa) through a restrictive valve, which leads to the formation of very fine emulsion (Quintanilla-Carvajal et al., 2009; Silva et al., 2012). These homogenizers are most efficient in reducing the size of a preexisting coarse emulsion rather than formulating a nanoemulsion directly from separate oil and aqueous phase (McClements and Rao, 2011). A coarse emulsion is first prepared with the help of a high shear mixer and then it is fed into the homogenizer (Fig. 19.3a). The pump of the homogenizer on its backstroke pulls the coarse emulsion onto its chamber and with a forward stroke it forces it through a narrow valve at the end. Intense disruptive forces like turbulence, shear, cavitation, impingement, shear stress, pressure gradient, and extentional shear are experienced by the emulsion as it passes through the valve, which causes the larger droplets to break into smaller droplets (Floury et al., 2004). The droplet size decreases with an increase in homogenization pressure and emulsifier adsorption rate and a decrease in the interfacial tension. The droplet size decreases if the disperse-to-continuous phase viscosity ratio falls within a certain range ($0.05 < \eta_D/\eta_C < 5$) (Tadros et al., 2004; McClements and Rao, 2011).

High-speed rotor-stator devices such as the ultraturrax are also used to prepare nanoemulsions. The energy provided by devices

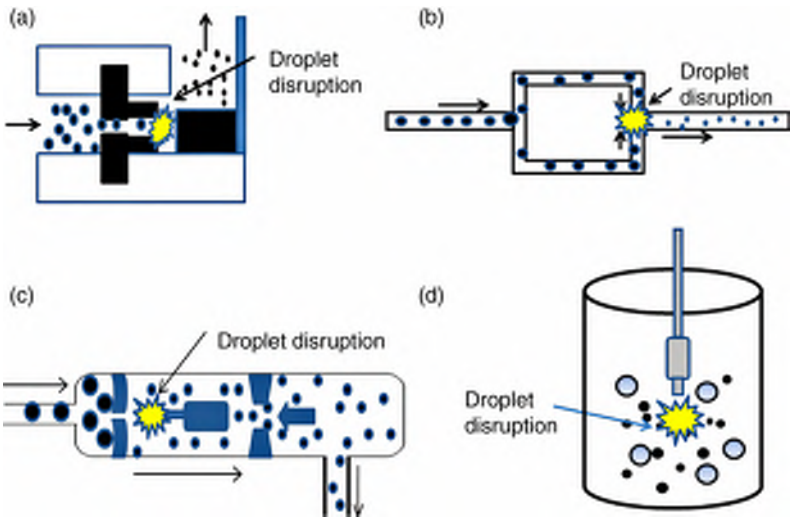


**Figure 19.3. Schematic representation of various devices based on high-energy approaches.** (a) High-pressure value homogenizer; (b) microfluidizer; (c) ultrasonic jet homogenizer; and (d) ultrasonic probe homogenizer.

generate heat leading to a decreased efficiency and such devices do not provide good dispersion in terms of droplet size when compared to other high energy approaches (Silva et al., 2012).

### *3.1.2 Ultrasonication*

Ultrasound technology has emerged to be an energy-efficient and promising technique for the generation of nanoscale emulsions. The mixture of oil phase, aqueous phase, and surfactant is subjected to high-energy sound waves creating emulsion droplets by cavitation. The frequency of these sound waves is >20 kHz (Jafari et al., 2007). It can be used to separately homogenize oil-and-water phase, or reduce the size of the droplets of preexisting coarse emulsions (McClements, 2011).

The droplet size obtained is governed by the design of the ultrasonic reaction chamber, intensity of ultrasonic waves, sonication time, and the product composition (viscosity of oil and aqueous phase, type, and amount of emulsifier) (Leong et al., 2009). For the commercial production of nanoemulsions large-scale continuous flow ultrasonic jet homogenizers are available. In such devices the sample to be homogenized is made to flow through a channel which contains an element that is capable of generating intense ultrasonic waves as represented by Fig. 19.3c (McClements, 2011). Generally bench-top probe sonicators are used at laboratory scales as represented by Fig. 19.3d. Ultrasound

is more versatile in terms of operation and cleaning and is considered a cost-effective method to generate the food grade nanoemulsions (Abismail et al., 1999).

### *3.1.3 Microfluidizers*

These devices are somewhat similar in design to HPVH because in these a coarse emulsion premix is forced through a narrow orifice at high pressure to promote droplet disruption. But the difference lies in the design of the channels through which the coarse emulsion is made to flow. It has an inlet chamber, which is made in such a way that it splits the coarse emulsion flowing through the channel into two streams. Then, in an interaction chamber these streams are made to impinge on each other at high velocity generating intense disruptive forces that break up droplets leading to the formation of a fine emulsion as represented by Fig. 19.3b (McClements and Rao, 2011; McClements, 2011). The size of the droplets obtained with HPH and microfluidizer is the same but it takes several passes for the HPH to arrive at the smallest size but in the case of microfluidizer, higher impinging jet creates high-shear stresses creating droplet deformation and breakup in the first pass. The main advantage of this method is the combination of extremely high-peak shear rates, high-volume throughput of nanoscale droplets, and reasonably uniform size distribution (Mason et al., 2006). However, microfluidizer is very expensive and has a high-equipment wear rate, which decreases the efficiency of the process (Leong et al., 2009).

## 3.2 Low-Energy Approaches

The basic principle involved in the formation of nanoemulsion by the low-energy approaches is the spontaneous formation of oil droplets in oil-water-emulsifier mixtures when their composition or environment is altered (Anton and Vandamme, 2009; Anton et al., 2008). It is carried out generally at constant temperature by changing the composition (Usón et al., 2004) or at constant composition by changing the temperature (Morales et al., 2003). Various methods employed are spontaneous emulsification, phase inversion temperature (PIT), phase inversion composition, and emulsion inversion point (Bouchemal et al., 2004).

The main drawback of the low-energy approach is the limited types of oils and emulsifiers that can be used. However, lower manufacturing costs, simple production methods, and ability to create smaller particle sizes are the advantages of low energy approaches (Walker et al., 2015).

### 3.2.1 Spontaneous Emulsification

The formation of nanoemulsions by the migration of surfactant and/or solvent molecules from the dispersed phase to the continuous phase without involving a change in the spontaneous curvature of the surfactant is called self-emulsification (Solans and Solé, 2012). The emulsions prepared by this method are usually referred to as self-emulsifying drug-delivery systems.

Since in this process it is necessary to use a relatively high concentration of synthetic surfactants to form nanoemulsions, it is unsuitable for many food applications due to regulatory, cost, or sensory reasons (McClements, 2011). The solvent removal might be a limitation in this technique (Solans et al., 2005).

### 3.2.2 Phase Inversion Temperature

This method was introduced by Shinoda and Saito (1968). The temperature at which an o/w emulsion transforms into a w/o emulsion or vice versa is known as the phase inversion temperature (PIT) (McClements and Rao, 2011). Temperature affects the molecular geometry or solubility of nonionic surfactants (Fryd and Mason, 2012; Silva et al., 2012; McClements, 2011). At PIT, nanoemulsion droplets suddenly break up upon rapid cooling. At low temperatures the surfactant monolayer forms oil-swollen micellar phases (o/w nanoemulsions) that may coexist with an excess oil phase due to positive spontaneous curvature. At high temperatures the spontaneous curvature becomes negative and water-swollen reverse micelles (w/o nanoemulsion) are formed which coexist with excess water phase. Whereas at intermediate temperature, the spontaneous curvature becomes almost zero and a bicontinuous phase microemulsion is formed where comparable amounts of water-and-oil phases coexist with both excess water-and-oil phases (Solans et al., 2005). The droplet size and the interfacial tension reaches a minimum at the PIT (Tadros et al., 2004).

At this temperature, the emulsions formed are highly unstable. Therefore, these are rapidly cooled or heated (by about 25–30°C), so that kinetically stable nanoemulsions can be formed which have a very small droplet size and a narrow size distribution (Solans et al., 2005; Izquierdo et al., 2004). The advantages associated with this process include the involvement of a relatively simple process, prevention of drug degradation during processing, low-energy requirement, and an easy industrial scale up (Anton et al., 2008; Silva et al., 2012).

### 3.2.3 Phase Inversion Composition

This approach is similar to the PIT technique but rather than changing the temperature the optimum curvature of

surfactant is changed by altering the composition of the system (McClements, 2011). The addition of salt would invert an o/w emulsion stabilized by an ionic surfactant to w/o emulsion. The salt ions screen the electrical charge on the surfactant head groups which in turn changes the packing parameter from $p < 1$ to $p > 1$ (Maestro et al., 2008). Similarly, a w/o emulsion containing high salt content can be phase inverted into o/w emulsion by diluting it with water so that the ionic strength is reduced below a critical level.

### 3.2.4 Membrane Emulsification

This method is unique in the sense that it employs a specially designed glass membrane like Shirasu porous glass (SPG) (made from Shirasu, ie, volcanic ash, boric acid, and calcium carbonate) as an emulsifying agent (Nakashima et al., 2000). During the process of membrane emulsification, emulsion droplets are dispersed in the immiscible continuous phase by passing the dispersed phase through a membrane. Either w/o or o/w emulsions may be produced depending on the hydrophobicity/hydrophilicity of the membrane. In order to detach the dispersed phase liquid drops from the membrane surface, this process employs shear at the surface of the membrane. In membrane emulsification, the continuous phase flow and the adsorption kinetics of the surfactant are the governing factors for the size of the droplets (Oh et al., 2011). This emulsion can be further formulated into a multiple emulsion by subjecting it to a secondary emulsification or convert the liquid phase into a solid phase by subjecting it to process/reaction (Vladisavljević and Williams, 2005). It utilizes less surfactant as compared to the high-energy processes and forms emulsions with a narrow size distribution range (Silva et al., 2012). The major drawback of this method is that the disperse phase flux through the membrane is low, which is problematic during the scale up (Sanguansri and Augustin, 2006).

### 3.2.5 Solvent Displacement

This approach is carried out by mixing water miscible organic solvent containing lipophilic functional compounds in an aqueous phase containing an emulsifier. The organic solvent rapidly diffuses into the aqueous phase forming nanoemulsions with a high yield of encapsulation. The organic solvent is removed from the nanoemulsion under reduced pressure. The drawback of this process is that it is limited only to water miscible solvents (Chu et al., 2007; Silva et al., 2012).

### 3.2.6 Emulsion Inversion Point/Catastrophic Inversion

This method is based on the finding that by changing the water volume fraction, the spontaneous radii of curvature of surfactant

is altered. Increasing amounts of water is added to a w/o emulsion, which has a high oil-to-water ratio under continuous stirring and constant temperature (McClements, 2011). Above a critical water content the emulsion reaches a catastrophic phase inversion point where the water droplet concentration is so high that the droplets are tightly packed together and the emulsion changes from a w/o to an o/w nanoemulsion system (Anton et al., 2008). The size of the droplets is controlled by variables such as stirring speed and rate of water addition (Thakur et al., 2008). The emulsifiers required in this method are limited to small molecule surfactants, which form flexible monolayers and are able to stabilize w/o emulsions for a short duration and o/w emulsion system for a long duration (Fernandez et al., 2004).

# 4 Properties of Nanoemulsions

## 4.1 Optical Properties

The optical properties of colloidal dispersions are characterized in terms of opacity and color. The opacity of nanoemulsions (clear or slightly turbid) is expressed by turbidity ($\tau$) and characterized by transmission measurements (McClements, 2005; McClements and Rao, 2011). The turbidity of nanoemulsions is directly proportional to refractive index contrast, particle concentration, and the color intensity is inversely proportional to degree of light scattering and turbidity. The mean particle size and narrow particle-size distribution influences the formation of optically transparent nanoemulsions. Wooster and coworkers reported that if the droplet size in a nanoemulsion is less than 80 nm the nanoemulsion formed will be optically transparent in nature (Wooster et al., 2008).

Food industry demands some products to be clear or slightly turbid like soft drinks, juices, jellies, desserts, sauces, dressings, and other products should be opaque like mayonnaise, sauces, creams, yogurts. Therefore, the optical properties influence and play a significant role in optimizing the final appearance of a nanoemulsion.

## 4.2 Rheological Properties

Depending on the composition and microstructure, foods containing lipid droplets may exhibit a wide variety of rheological characteristics ranging from viscous liquids, viscoelastic liquids, and viscoelastic solids, elastic solids to plastics (Quemada and Berli, 2002; Walstra, 2003; Genovese et al., 2007). Nanoemulsions owing to their relatively small droplet size exhibit considerably

different rheological properties than conventional emulsions. This property of nanoemulsions is utilized for modification of texture of food and other products.

The overall rheology of a nanoemulsion based food products is dependent on droplet characteristics. For the products requiring low viscosity like beverages, the droplets should not increase the overall viscosity. Also, the droplets help in thickening the system to form a gel network for highly viscous or gel-like food products such as dressings and desserts (McClements and Rao, 2011).

## 4.3 Physicochemical Stability of Nanoemulsions

Nanoemulsions are metastable systems that tend to break down over time through a variety of different physicochemical mechanisms. Physical instability includes gravitational separation, flocculation, coalescence, OR. Chemical instability means chemical degradation due to oxidation and hydrolysis (Dickinson, 1992; Friberg et al., 2004; McClements, 2005).

### *4.3.1 Gravitational Separation*

Gravitational separation is the instability that occurs due to difference in relative densities of the dispersed and continuous phases. Instability in emulsions can be either creaming or sedimentation. An o/w emulsion may be prone to sedimentation if it contains crystalline lipids or small oil droplets covered by a relatively thick and dense shell. Stokes' law governs the velocity that a particle moves upward in nanoemulsion due to gravitational separation:

$$\upsilon = \frac{-2gr^2(\rho_{\text{particle}} - \rho_0)}{9\eta_0}$$

where ($\upsilon$) is the creaming velocity, ($g$) is the acceleration due to gravity, ($r$) is the particle radius, ($\rho_{\text{particle}}$) is particle density, ($\rho_0$) is the continuous-phase density, and ($\eta_0$) is the continuous-phase viscosity. The particles would concentrate at either top or the bottom of a sample under the influence of gravitational forces alone. However, Brownian motion (forces) along with the thermal energy of the system help particle move inside the dispersion. The root mean square distance traveled by a particle inside dispersion due to Brownian motion is governed by:

$$\Delta = \sqrt{(2Dt)}$$

where ($t$) is the time and ($D$) is the translational diffusion coefficient of the particle (Walstra, 2003; McClements and Rao, 2011).

The movement of larger particles is dominated by gravity in emulsions, and the movement of smaller particles is dominated by Brownian motion in case of nanoemulsions. Hence, creaming in nanoemulsions may not take place when the particle diameter is below 70 nm due to the domination of Brownian motion effects.

### 4.3.2 Droplet Aggregation

The relatively small particle size of nanoemulsions provides better stability to particle aggregation such as flocculation or coalescence as compared to conventional emulsions (Tadros et al., 2004). The total colloidal interactions between two adjacent droplets in nanoemulsions are additions of the attractive interactions (van der Waals and hydrophobic) and repulsive interactions (electrostatic and stearic).

The magnitude of total colloidal interactions is directly proportional to droplet size (McClements, 2005). The nature of these colloidal interactions is affected by changes in bulk physicochemical properties of the various phases like dielectric constant, refractive index, particle radius, and charge on the particle, rheology, as well as hydrophobicity. The properties of the dispersion medium like pH, temperature, osmotic pressure, and ionic strength also affect the nature of colloidal interactions. The formulation of nanoemulsion delivery systems can be optimized to obtain better stability to droplet aggregation and gravitational separation (McClements and Rao, 2011).

### 4.3.3 Ostwald Ripening

OR is the process describing the increase in the mean size of the droplets over time in a nanoemulsion as a result of diffusion of oil molecules from small to large droplets through the intervening fluid (Kabalnov, 2001; McClements and Rao, 2011). The aqueous-solubility of an oil present within a spherical droplet increases with decrease in droplet size, leading to higher concentration of solubilized oil molecules in the aqueous phase surrounding a small droplet as compared to larger droplet (Kabalnov and Shchukin, 1992; McClements, 2005). As a result of this concentration gradient, the solubilized oil molecules tend to move from smaller droplets to larger droplets. As a result, the droplet size increases over time. In practice, the main factor determining the stability of a nanoemulsion to OR is the water-solubility of the oil phase.

LCTs (eg, corn, soy, sunflower, or fish oils) are relatively stable toward OR due to low water solubility. On the contrary, SCTs, flavor oils, and EOs are more prone to OR due to appreciable water solubility (Wooster et al., 2008; Li et al., 2009). OR can be avoided

by incorporation of a ripening inhibitor. A ripening inhibitor is a nonpolar molecule like a LCT, which is soluble in the oil phase but insoluble in the water phase. The mechanism of inhibition of OR is through generation of entropy of mixing that counterbalances the curvature effects.

For instance, the droplet size of orange o/w emulsion containing different concentration of corn oil as ripening inhibitor changes upon storage. Orange oil is more prone to OR owing to high solubility in water (fourfold). The addition of corn oil (10%) into the lipid phase inhibited the OR in orange o/w emulsion (McClements and Rao, 2011).

### *4.3.4 Chemical Stability*

The large specific surface area owing to the small size of the lipid droplets in o/w nanoemulsions impact the chemical degradation of any encapsulated components that occurs at oil–water interface, for example, oxidation or hydrolysis. A transparent nanoemulsion containing small droplets may be more prone to degradation than an opaque conventional emulsion if degradation is mediated by light (UV or visible). Hence, it is important to improve the chemical stability of labile components present in nanoemulsions and emulsions by incorporating antioxidants or sequestering agents.

## 5 Characterization Techniques of Nanoemulsions

The physicochemical properties of nanoemulsions include particle size, size distribution, zeta potential, and crystallinity. These properties can be studied by various techniques. Dynamic light scattering (DLS) helps in determining the hydrodynamic diameter and the zeta potential indicates stability of nanoemulsions. Transmission electron microscopy (TEM) is a technique capable of resolution of order of 0.2 nm and used to study morphology and structure of nanoemulsions. Scanning electron microscopy (SEM) is used to study the surface morphology, provides three-dimensional appearance of surface. Atomic force microscopy (AFM) is recently developed high resolution (0.1 nm) microscopic technique and assists in analyzing the structural and functional properties at submolecular levels. Differential scanning calorimetry (DSC) is a thermoanalytical technique used to detect phase transitions, including the melting of crystalline regions and analyzing the proportion of ice crystals in the nanoemulsions. Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR), X-ray diffraction (XRD), and small-angle-X-ray

scattering (SAXS) are the techniques used to study crystalline structure, chemical composition, and physical properties of materials (Silva et al., 2012).

## 6 Applications in the Food Industry

The potential use of nanoemulsions in the food industry has become a subject of much interest in past few years. With the general population getting more exposure regarding the potential health benefits of various functional foods in preventing ailments like cancer, diabetes, and atherosclerosis, the inclusion of these foods in daily diet regimen has become imperative. This enhanced consumption has made the segment of food nanotechnology to grow at the rate of 7% per year and is expected to reach a staggering $241.1 billion by 2019 (BCC research). The key areas where nanoemulsions have been successfully used in the food industry are illustrated by Fig. 19.4.


**Figure 19.4. Various applications of nanoemulsions in food industry.**

## Table 19.4 List of Functional Ingredients Encapsulated Into Nanoemulsion Systems

| Functional Ingredient | Category | References |
|---|---|---|
| Citral | Flavoring agent | Yang et al. (2011), Zhao et al. (2013) |
| D-Limonene | Flavoring agent | Lu et al. (2014) |
| Omega-3 fatty acids | Nutraceutical | Bromley (2013) |
| Oleoresin capsaicin | Nutraceutical | Choi et al. (2009) |
| Resveratrol | Nutraceutical | Davidov-Pardo and McClements (2015) |
| β-Carotene | Coloring agent | Liang et al. (2013), Yuan et al. (2008a), Tan and Nakajima (2005a,b), Chu et al. (2007), Yin et al. (2009), Mao et al. (2009) |
| Phenolic grape extract | Natural preserving agent | Spigno et al. (2013) |

## 6.1 Encapsulation of Functional Ingredients to Improve Stability

The various functional ingredients like flavors, colorants, nutraceuticals, and natural preserving agents are encapsulated into nanoemulsions to improve their stability. Generally flavors and colorants have aldehydic, ketonic, or ester bonds in their structures that are prone to oxidative degradation. Encapsulation within nanoemulsions will prevent them from oxidative or photolytic degradation, and hence extend the shelf life. Table 19.4 lists the various functional components encapsulated into nanoemulsions.

### *6.1.1 Encapsulation of Flavors*

Emulsions are widely used in beverage products as it is frequently necessary to incorporate water-insoluble flavors into an aqueous beverage. It is often desired that the beverage should be clear in appearance. However, the use of conventional emulsions results in aesthetically unappealing products due to resulting turbidity, thereby requiring the use of other additives like alcohol to achieve desirable clarity. This often results in regulatory restrictions in some countries (Zhang et al., 2016). One of the possible ways to address this issue includes encapsulation of flavors in nanoemulsions, which are inherently clear owing to droplet size of $<100$ nm. They can encapsulate the flavor and provide protection

against the effects of temperature, oxidation, hydrolysis, enzymatic reactions, and are thermodynamically stable over a wide pH range. Nanoemulsions are used as flavor delivery systems in food products like beverages, sauces, or dressings, and provide excellent shelf-life stability over a large temperature range for a series of months without significant deterioration, rancid odor, or bitter flavor.

Citral is α,β-unsaturated aldehyde with one additional double bond. Degradation of citral will lead to loss of the lemon-like aroma and the production of various off-flavor compounds, which limits its application in the food and cosmetic industries (Ueno et al., 2004). Yang and coworkers reported the utilization of o/w nanoemulsions to encapsulate citral. They also evaluated the effects of six different antioxidants (β-carotene, tanshinone, naringenin, tangeretin, black tea extract, and ascorbic acid) on citral's chemical stability under acidic condition (pH 3.0). Citral o/w nanoemulsions improved chemical stability of citral and reduce the production of many off-flavor compounds. In addition, the incorporation of the appropriate antioxidants (ie, β-carotene, tanshinone, and black tea extract) with citral together could further inhibit citral degradation as well as lipid oxidation (Yang et al., 2011). Also, Zhao and coworkers reported the effect of ubiquinol-10 ($Q_{10}H_2$), an antioxidant on citral stability and off-flavor formation in o/w nanoemulsions. The optimum concentration of $Q_{10}H_2$ was determined to be 0.10 wt% ($Q_{10}H_2$/citral ratio 1:1), which can effectively protect citral from chemical degradation and oxidation in the system (Zhao et al., 2013).

D-Limonene, an essential oil extracted from citrus fruits with chemopreventive activity, is prone to oxidation and loses its lemon-like flavor under normal storage conditions. Lu et al. (2014) reported the nanoemulsion of D-limonene in water system to protect oxidative degradation of D-limonene. The developed nanoemulsions were stable with particle size in the range of 20–50 nm (Lu et al., 2014).

### *6.1.2 Encapsulation of Colorants*

β-Carotene, used in the food industry, is a precursor of vitamin A, natural colorant, and an antioxidant. It has health-benefiting functions in the prevention of serious health disorders such as cancer, cardiovascular disease, macular degeneration. It is a labile compound and easily degraded by heat, light, and oxygen. Attempts are being made to increase the shelf stability of β-carotene toward various processing conditions.

Tan and Nakajima reported the preparation of β-carotene nanodispersions by emulsification–evaporation technique. The

physically stable β-carotene nanodispersions of volume-weighted mean diameter, ranging from 60 to 140 nm were prepared (Tan and Nakajima, 2005a). The same research group evaluated the effects of six different polyglycerol esters of fatty acids (PGEs) as nonionic emulsifiers on the physicochemical properties and stability of β-carotene nanoparticles in o/w dispersions produced by an emulsification–evaporation technique (Tan and Nakajima, 2005b).

Chu and research group prepared sodium caseinate (SC) stabilized β-carotene nanodispersions using emulsification–evaporation method. The nanodispersion showed a monomodal β-carotene particle size distribution with a mean particle size of 17 nm. Increasing the SC concentration decreased the mean particle size and improved the polydispersity of the nanodispersions (Chu et al., 2007).

Yuan et al. reported the preparation of β-carotene o/w nanoemulsions by HPH method. The developed β-carotene nanoemulsions had good physical stabilities, but chemically significant degradation of β-carotene occurred during storage (Yuan et al., 2008b).

Yin and coworkers reported the development of β-carotene nanodispersions using solvent displacement technique. The different emulsifiers studied were SC, Tween-20, decaglycerol monolaurate (ML750), and sucrose fatty acid ester (L1695). Among all the emulsifiers, the SC-stabilized β-carotene nanodispersions were the most stable against oxidation by free radicals. However, the stability of β-carotene in these systems was improved owing to the antioxidative activity of SC (Yin et al., 2009).

Mao and coworkers developed β-carotene o/w nanoemulsions using mixture of stabilizers. A 1:1 blend of Tween-20 and whey protein isolate improved the stability of β-carotene nanoemulsion, due to stabilizer-protein interaction (Mao et al., 2009).

β-carotene is encapsulated into nanoemulsions stabilized by modified starch and then spray-dried to powders after the emulsification process. The powders showed a good dissolution in water and the reconstituted emulsions had similar particle sizes with the fresh nanoemulsions. A 30-day storage test was carried out to investigate the effect of relative humidity (RH) on the storage stability of β-carotene powders at 25.0°C. The results showed that modified starches with lower film oxygen permeability had a higher retention of β-carotene during storage. The glass transition temperature of powder in different RH conditions also affected the rate of β-carotene degradation. Overall, these results provide useful information for choosing wall materials and storage conditions to protect nutraceuticals in delivery systems to improve their stability (Liang et al., 2013).

### 6.1.3 Encapsulation of Nutraceuticals

Nutraceuticals is the term used to describe the product derived from food sources with extra health benefits in addition to the basic nutritional value found in foods. Omega-3 fatty acids are a type of polyunsaturated fatty acid (PUFA) with a double bond (C═C) at the third carbon atom from the end of the carbon chain. They have long been investigated for their cardioprotective and antiinflammatory roles, which has led to their increased use as dietary supplements (Ross et al., 2007). However, their susceptibility to oxidation gives them an unpleasant taste. European Patent EP2563164A1 described a nanoemulsion formulation of omega-3 fatty acids with minimal unpleasant (fishy) odor and/or taste with enhanced stability (Bromley, 2013).

Choi et al. reported food nanoemulsion systems consisting of water and oleoresin capsaicin (OC), Tween- 80, PG, sucrose monostearate (SM), and their corresponding mixtures as food vehicles. The OC nanoemulsions prepared by ultrasonication using systems of OC/Tween-80/water, OC/Tween-80/water + PG, and OC/Tween-80/water + SM, resulted in particle sizes ranging from 15 to 100 nm. The OC nanoemulsions remain stable during storage (Choi et al., 2009).

Resveratrol is a natural polyphenol (stilbinoid) found at relatively high levels in grape skins, blueberries, raspberries, and so on. It has number of potential benefits like antioxidant, cardioprotective, neuroprotective, antiinflammatory, anticancer, and antiobesity effects (Neves et al., 2013). Recently, resveratrol nanoemulsions consisting of 10% oil phase (grape seed oil plus orange oil) and 10% surfactant (Tween-80) prepared using spontaneous emulsification method had been reported. The results of this study depicted that resveratrol could be encapsulated within nanoemulsion using low energy processing technique and protected against UV light induced isomerization and degradation (Davidov-Pardo and McClements, 2015).

### 6.1.4 Encapsulation of Natural Preserving Agents

A powdery phenolic extract from red-grape marc is a typical wine-making by-product. This extract offers potential applications as a natural low-cost antioxidant to improve quality and extend the shelf life of foods without the use of synthetic additives. Spigno et al. reported the encapsulation of a phenolic grape marc extract to enhance its lipid solubility and antioxidant efficiency for application as a natural preserving agent of hazelnut paste. Hazelnut paste is a food ingredient used in different processed foodstuff such as ice creams, confectionery and bakery products.

After processing into paste, hazelnuts become more prone to oxidation because of increased surface area exposed to oxygen and damaged structure that causes deterioration reactions to proceed faster. Encapsulation improved phenolic efficiency against lipid oxidation, by increasing extract dispersability in the paste and preserving the antioxidant activity, with the o/w nanoemulsion (Spigno et al., 2013).

There are number of commercially available technologies based on nanoemulsions in the food industry. NutraLease Ltd., a technology start-up company, develops carriers for nutraceuticals to be incorporated in food systems and cosmetics formulations through nanosized self-assembled structured liquids (NSSL) technology and self-assembled nanoemulsion technology. NSSL technology enhances the solubility of different compounds in water-based or oil-based media to improve their bioavailability. The nutraceuticals incorporated in the carriers include lycopene, β-carotene, lutein, phytosterols, CoQ10, lipoic acid, and docosahexanoic acid (DHA)/eicosapantenoic acid (EPA). The company focuses on fortifying foods and beverages (Silva et al., 2012; NutraLease, 2011).

Another example of nanotechnology in the food industry is provided by Aquanova, with their NovaSol beverage solution. The NovaSol portfolio is divided into bioactive functional compounds like $CoQ_{10}$, DL-α-tocopherol acetate, vitamins, omega-3 fatty acids and natural colorants like β-carotene, apocarotenal, chlorophyll, curcumin, lutein, and sweet pepper extract. Aquanova claims enhanced stability (both in terms of pH and temperature) of encapsulated functional compounds and standardized additive concentrations (AquaNova, 2011; Silva et al., 2012). Table 19.5 enlists the various commercially available nanotechnology-based food products.

## 6.2 Prevention of Food Spoilage/Antimicrobials

As the need for safer and effective preservatives is realized, there has been continuous search for natural preservatives that could substitute for synthetic preservatives in the food and juice industry. One class which has received greater attention includes nanoemulsions based on EOs. These EOs are explored as natural preservatives (antimicrobial) within the food industry as a substitute for synthetic chemical preservatives. Their antimicrobial activities against various food pathogenic microorganisms are already reported in literature. Some of the EOs with antimicrobial effects includes thyme (thymol), oregano (carvacrol), clove (eugenol), orange (limonene), and cinnamon (cinnamaldehyde).

# Table 19.5 List of Nanotechnology-Based Food Products in the Market

| Food Product Name | Company |
|---|---|
| NanoResveratrol™[a] | Life Enhancement, USA |
| Nutri-Nano™ CoQ-10 3.1x Softgels[a] | Solgar, USA |
| 24Hr Microactive® CoQ10[b] | Genceutic Naturals, USA |
| Hydracel™[b] | RBC Life Sciences®, Inc., USA |
| Nanoceuticals™ Slim Shake Chocolate[b] | RBC Life Sciences®, Inc., USA |
| MesoGold®[b], MesoPalladium™[b], MesoPlatinum®[b], MesoSilver®[b] | Purest Colloids, Inc., USA |
| ACZ Nano Advanced Cellular Zeolite[b] | Vitality Products Co., Inc, USA |
| NanoSlim Dietary Supplement[b] | NanoSlim, Canada |
| Nanotea[b] | Shenzhen Become Industry & Trade Co., Ltd., China |
| Maternal Water[b] | La Posta del Aguila, Argentina |
| Canola Active Oil[a] | Shemen, Israel |
| Fortified Fruit Juice[a] | High Vive, USA |
| Daily Vitamin Boost[a] | Jamba Juice Hawaii, USA |
| Colloidal silver[b] | Fairvital, Germany |
| Colloidal Silver Liquid[b] | Skybright Natural Health, New Zealand |
| Bionic Joint Support™[b] | Life Enhancement, USA |
| NovaSol® Q10[b] | Aquanova®, Germany |
| NovaSol® E[b] | Aquanova®, Germany |
| NovaSol® ADEK[b] | Aquanova®, Germany |
| NovaSol® Omega[b] | Aquanova®, Germany |
| NovaSol® Apo[b] | Aquanova®, Germany |
| NovaSol® D[b] | Aquanova®, Germany |
| NovaSol® βC[b] | Aquanova®, Germany |
| NovaSol® Curcumin[b] | Aquanova®, Germany |
| NovaSol® Lutein[b] | Aquanova®, Germany |
| NovaSol® CP[b] | Aquanova®, Germany |
| NovaSol® SP[b] | Aquanova®, Germany |

[a] McClements and Rao (2011).
[b] Nanotechproject (2006).

However, the hydrophobic nature of EOs limits their inclusion in the foods and often results in loss of antimicrobial efficacy leading to negative impact on quality of food (Sugumar et al., 2015). Recently the focus of using EOs as antimicrobials has shifted from conventional admixture to more unique nanoemulsion formulations. This way the integrity of EOs as antimicrobials is maintained and at the same time the quality of food is not impacted.

Oregano oil nanoemulsions were formulated with a food-grade emulsifier (Tween-80) and evaluated for their efficacy in inactivating the growth of foodborne bacteria on fresh lettuce. The nanoemulsions were effective and inhibitory effects on *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* were reported (Bhargava et al., 2015).

The high efficacy orange oil nanoemulsions as antimicrobial as compared to control in preventing the apple juice spoilage against *Saccharomyces cerevisiae* has been reported recently (Sugumar et al., 2015).

D-Limonene nanoemulsions showed a higher antimicrobial efficacy in juices inoculated with *Lactobacillus delbrueckii* with minimal alteration of the organoleptic properties of juices (Donsi et al., 2011). Nanoemulsions of lemon grass oil were used to provide antimicrobial coating on plums. The antimicrobial efficacy of coating was evaluated against *Salmonella typhimurium* and *Escherichia coli*. Coated plums were found to be more firm and showed extended shelf life in comparison to uncoated plums (Kim et al., 2013).

Nanoemulsions containing antimicrobial agents for the decontamination of food packaging equipment and for application to food surfaces had been reported (Shekhon, 2010). Some of the recent advancements pertaining to reducing spoilage of foods include innovative packaging incorporating EO as antimicrobials. Active packaging denotes a means of increasing the shelf life of foodstuffs without changing their fresh-like characteristics as thermal processing usually does. Nanoemulsions prepared from EOs obtained from clove bud (*Syzygium aromaticum*) and oregano (*Origanum vulgare*) had been successfully incorporated into edible films and provided antimicrobial activity against several foodborne pathogenic and spoilage microorganisms. Sliced bread showed enhanced shelf life against yeast and molds when packed in methylcellulose films containing nanoemulsions of clove bud and oregano. Edible films have the distinct advantage of controlling the release of antimicrobials in the food matrix (Otoni et al., 2014b). Pectin/papaya puree/cinnamaldehyde nanoemulsion edible films were reported to have improved in vitro antimicrobial properties against *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Otoni et al., 2014a).

Chang et al. reported the preparation of nanoemulsions (10 wt% total oil phase) by titration of a mixture of EO (carvacrol), carrier oil (MCT), and nonionic surfactant (Tween-80) into an aqueous solution with continuous stirring. The antimicrobial efficacy of the nanoemulsions increased as the carvacrol concentration increased. Hence, the carrier oil concentration must be carefully controlled to obtain good physical stability and antimicrobial efficacy (Chang et al., 2013).

Hamouda and coworkers developed w/o nanoemulsion (BCTP), in which the oil phase is made from soybean oil, tri-*n*-butyl phosphate, and Triton X-100. A liposome-like compound (P10) made of glycerol monostearate, refined soya sterols, soybean oil, peppermint oil, Tween-60, cetylpyridinium chloride, are added to form another nanoemulsion (BCTP 401). The average size of both nanoemulsions lies in the range of 400–800 nm. Both BCTP and BCTP 401 inactivated >90% of *Bacillus anthracis* spores and were also sporicidal against three other *Bacillus* species. These nanoemulsions were uniform, stable, nonirritant, and nontoxic over other available sporicidal agents (Hamouda et al., 1999).

Hamouda et al. reported the biocidal activity of a novel nonionic surfactant nanoemulsion (8N8). It was effective as bactericidal, virucidal, and fungistatic agent. The rapid and nonspecific inactivation of vegetative bacteria and enveloped viruses, in addition to its fungistatic activity and low toxicity in experimental animals, makes 8N8 a potential candidate for use as a topical biocidal agent (Hamouda et al., 2001).

## 6.3 Enhancement of Bioavailability

The term bioavailability refers to the fraction of a dose that is available at the site of action in the body (Acosta, 2009). The formulation of different bioactive agents as nanoemulsions results in increase in uptake, absorption, and hence bioavailability. The various mechanisms by which the enhancement of bioavailability takes place includes rate of release (due to the large surface area), the increase in the residence time due to the small size of the nanodroplets (entrapment in the mucus layer), or the direct uptake of the nanodroplet through paracellular or transcellular route. Also, the increase in water-solubility of highly lipophilic components due to decrease in droplet size leads to improvement of bioavailability. Fig. 19.5 illustrates the various mechanisms of bioavailability enhancement. However, the relative significance of these different mechanisms for nanoemulsions with versatile droplet size, composition, as well as surface characteristics is not fully understood. Nanoemulsions have also been shown to improve the

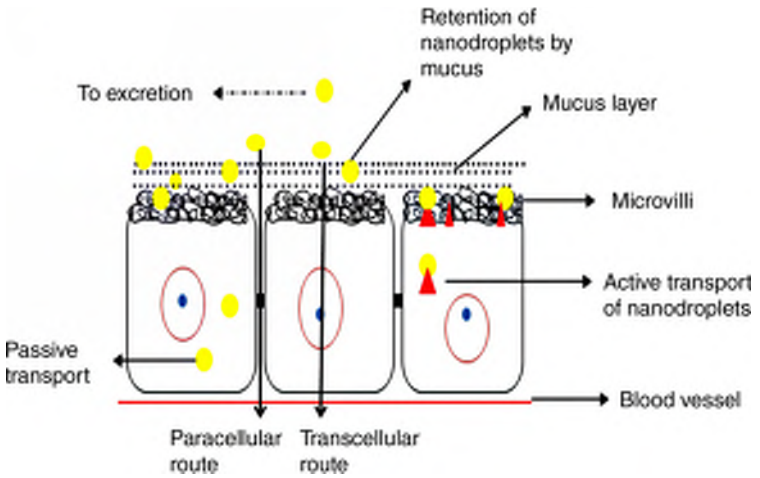


**Figure 19.5. Representation of different mechanisms involved in bioavailability enhancement by nanoemulsion droplets.**

bioavailability of various lipophilic nutraceuticals like vitamin E, vitamin D, rutin omega-3 fatty acids, tocopherols (Hatanaka et al., 2008; Ozaki et al., 2010). Targeted delivery of nutraceuticals to various sites in the human body, with improved efficacy that can be achieved by nanoemulsion technology (Vishwanathan et al., 2009; Huang et al., 2010). Table 19.6 enlists the nutraceuticals with bioavailability enhancement by nanoemulsion technology.

Curcumin is the most active and least stable bioactive component of turmeric (*Curcuma longa*) plant. The erratic oral availability of curcumin can be increased by incorporating it within nanoemulsions. High-speed and high-pressure homogenized o/w emulsions using MCT as oil and Tween-20 as emulsifier, with mean droplet sizes ranging from 79.5 to 618.6 nm had been reported. There was significant inhibition in mouse ear edema after oral administration of 1% curcumin o/w nanoemulsions (Wang et al., 2008; Huang et al., 2010).

Donsi et al. (2011) reported the fabrication of nanoemulsions of two different bioactive compounds, resveratrol (grape fruit extract), and curcumin, with improved antioxidant and/or antimicrobial activities. The encapsulation of resveratrol (0.01 wt%) in peanut oil-based nanoemulsions improved its stability, as shown by the significant reduction of the chemical degradation of *trans*-resveratrol to *cis*-resveratrol. Curcumin (0.1 wt%) was encapsulated in solid lipid (stearic acid) nanoemulsions that trapped the compound in a solid matrix, which contributed to improve its solubility in aqueous systems and to avoid the recrystallization and settling of the bioactive compound over time.

## Table 19.6 List of Various Nutraceuticals With Bioavailability Enhancement by Nanoemulsion Technology

| Nutraceutical | Health Benefit | References |
|---|---|---|
| Curcumin | Antioxidant, antiinflammatory, anticancer, and chemopreventive | Wang et al. (2008), Huang et al. (2010), Donsi et al. (2011), Sari et al. (2015) |
| $CoQ_{10}$ | Anticarcinogenic | Hatanaka et al. (2008) |
| α-Tocopherol | Antioxidant | Hatanaka et al. (2010) |
| Resveratrol | Antioxidant | Donsi et al. (2011) |
| Green tea extract | Antioxidant and hypolipidemic agent | Kim et al. (2012) |
| 5-Demethyltangeretin | Anticarcinogenic, antiinflammatory, antiviral, antioxidant, antithrombogenic, and antiatherogenic | Zheng et al. (2014) |
| Rutin | Antiinflammatory, antioxidant | Macedo et al. (2014) |
| Ellagic acid | Anticancer, antioxidant | Avachat and Patel (2014) |
| Lycopene | Anticancer, cardioprotective | Ha et al. (2015) |

Recently, Sari and coworkers reported the encapsulation of curcumin in MCT oil droplets of nanoemulsion prepared by ultrasonification using whey protein concentrate-70 and Tween-80 as emulsifiers with an encapsulation efficiency of 90.56 ± 0.47%. The prepared nanoemulsion has particles of average diameter 141.6 ± 15.4 nm and zeta potential of 6.9 ± 0.2 mV. In vitro release kinetics of curcumin from nanoemulsion by simulated gastrointestinal studies showed that the curcumin nanoemulsion was relatively resistant to pepsin digestion but pancreatin causes release of curcumin from nanoemulsion. The slow release of curcumin from the nanoemulsion was supposed to increase bioavailability. The prepared nanoemulsion was stable to pasteurization, different ionic strengths (0.1–1 M) and pH ranging from 3.0 to 7.0. Hence, nanoencapsulation of highly lipophilic and unstable compounds is an effective platform to increase the hydrophilicity, bioaccessibility, and to protect them from degradation (Sari et al., 2015).

$CoQ_{10}$ is a lipophilic naturally occurring, GRAS certified, chemical acting as vital intermediate of electron transport chain in the mitochondria. $CoQ_{10}$ finds wide applications as food supplements and in the cosmetic industry. Also it has been

reported to be used for chronic heart failure. However, the water solubility of $CoQ_{10}$ is poor leading to low and variable bioavailability depending on food intake. Hatanaka et al. (2008) reported the preparation of nanoemulsion $CoQ_{10}$ complex with submicron diameter and improved oral bioavailability (1.7 times higher AUC and $C_{max}$) in rats.

Vitamin E is an essential nutrient derived from various crops, such as barley, wheat, and soybean. It has been widely used as functional food, food additives, and in cosmetics and pharmaceuticals as an antioxidant. Hatanaka and coworkers also developed a novel nanoemulsion formulation of α-tocopherol (α-TC) with enhanced oral bioavailability and pharmacological effects. Three nanoemulsion formulations of α-TC at different loading amounts (10, 30, and 50%) were prepared by a mechanochemical method. After oral administration of 10% α-TC loaded nanoemulsion formulation (30 mg α-TC/kg) in rats, higher α-TC exposure was observed with a 2.6-fold increase of bioavailability as compared to the control mixture of oil and α-TC. Hence, nanoemulsion approach might be efficacious to improve the oral bioavailability and antioxidative activities of α-TC (Hatanaka et al., 2010).

Green tea has been considered a healthy beverage since ancient times and recommended for headaches, body aches, pains, digestion, depression, detoxification, and as an energizer. A major component of green tea, (−)-epigallocatechin-3-gallate (EGCG), has several cellular and molecular effects related to the health-promoting actions of tea catechins. Kim et al. reported the nanoemulsification of green tea extract (GTE) with improved bioavailability and enhanced intestinal uptake. The antioxidant and hypolipidemic effects of nanoemulsified green tea extract (NGTE) and GTE were studied in mice. Both GTE and NGTE showed similar composition/concentration of total catechins and comparable antioxidant effects. However, the NGTE group showed greater hypocholesterolemic effects. These results suggest that nanoemulsification significantly increased hypocholesterolemic effects of GTE in vivo due to increased bioavailability (Kim et al., 2012).

5-Demethyltangeretin (5DT), a citrus flavonoid, has shown promising anticancer effects. However, its application as nutraceutical is limited owing to its low water solubility and poor oral bioavailability. The delivery systems based on nanoemulsion as carriers had been reported to improve the bioavailability of 5DT and its uptake by intestinal cancer cells. Such type of delivery system improved the applicability of 5DT in food and beverage industry (Zheng et al., 2014).

Rutin is a polyphenolic compound found in citrus fruits. It has diverse pharmacological activities including antiallergic,

antiinflammatory, vasoactive, antitumor, antibacterial, antiviral, hypolipidimic. Macedo and coworkers developed rutin-loaded nanoemulsion by HPH technique and determine the release profile of the drug in vitro. Drug release was characterized by an initial burst, which decreased over the time, showing a sustained release profile. After 24 h, nearly 65% of rutin had been released from nanoemulsion. The developed system proved to be stable and suitable to encapsulating poorly water-soluble drugs (Macedo et al., 2014).

Ellagic acid (EA), a plant polyphenol known for its wide range of health benefits has limited use due to its low oral bioavailability. Recently, a new self-nanoemulsifying drug delivery system (SNEDDS), based on the phospholipid complex technique to improve the oral bioavailability of ellagic acid had been reported. The in vitro drug release from SNEDDS was found to be higher compared to EA suspension and complex, while ex vivo studies showed increased permeation from SNEDDS compared to EA suspension. Moreover, SNEDDS overcome the food effect which was shown by EA suspension. Thus, SNEDDS were found to be influential in improving the release performance of EA, indicating their potential to improve the oral bioavailability of EA (Avachat and Patel, 2015).

Very recently lycopene nanoemulsions to protect the antioxidant activity and improve the bioaccessibility of lycopene-enriched tomato extract (containing 6% of lycopene) by an emulsification–evaporation method had been reported in literature. Lycopene nanoemulsions exhibited higher antiradical efficiency and antioxidant activity. Interestingly, nanoemulsions with droplets smaller than 100 nm showed the highest in vitro bioaccessibility, which could be interpreted as evidence of nanoemulsification enhancing the in vitro bioaccessibility of lycopene (Ha et al., 2015).

## 6.4 Texture Modification

Nanoemulsions are also used to modify the texture of food products due to their different rheological attributes than conventional emulsions. The gelation of nanoemulsions is possible at a reduced concentration of oil phase as compared to conventional emulsions for producing highly viscous (gel-like) low-fat products. This attribute could be useful in making sauces, dressings, mayonnaise, and so on. Some food industries are utilizing nanotechnology application to their food products. Unilever reduced the fat content in ice cream from 16% to 1% through the use of nanoemulsion technology to make healthy ice cream (Silva et al., 2012).

## 7 Conclusions

Nanoemulsions offer a range of desirable physical properties and chemical compositions that can provide significant advantages over microscale emulsions. They have widespread applications in food, beverage, and pharmaceutical industries as delivery systems. Nanoemulsion-based products are optically transparent along with an increase in bioavailability of an active component. As a general recommendation, when applied to the food industry nanotechnology needs to be affordable, simple to use, and with readily perceived advantages in order to be a real alternative to the standard methodologies. There are various challenges like limited food grade stabilizers or other ingredients available. The food industry would like to prepare nanoemulsions from legally acceptable, label-friendly, and economically viable ingredients. Last and most important is the toxicological concerns due to the nanosize of the droplets that could alter the normal function of gastrointestinal tract. The lymphatic system via the portal vein helps in transportation of nanodroplets out of the epithelial cells. The circulating nanodroplets may then be metabolized, excreted, or accumulate within certain tissues. Due to high surface area the particles will accumulate and cause toxic effects for prolonged periods of time. In a nut shell, the nanotechnology revolution that hit the food industry is expanding and nanotechnology-derived food products will be available increasingly to consumers worldwide in the coming years.

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# 20

# SCIENTOMETRIC OVERVIEW REGARDING NANOEMULSIONS USED IN THE FOOD INDUSTRY

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## 1 Overview

### 1.1 Issues

The field of food science and engineering has been one of the most dynamic research fields in recent years with more than 750,000 papers as indexed by the Science Citation Index-Expanded (SCIE) as of December 2015 (eg, Brand-Williams et al., 1995; Fontana et al., 2010; Godfray et al., 2010; Halaas et al., 1995; Holick et al., 2011; Mata et al., 2010; Mead et al., 1999; Scallan et al., 2011; Vansoest et al., 1991; Zhang et al., 1994).

Similarly, the field of nanomaterials and nanoprocesses has been one of the most dynamic research fields in recent years with more than 1,000,000 papers as indexed by the SCIE as of December 2015 (eg, Atwater and Polman, 2010; Bae et al., 2010; Dreyer et al., 2010; Geim and Novoselov, 2007; Iijima, 1991; Novoselov et al., 2004; Oregan and Gratzel, 1991; Yella et al., 2011).

Furthermore, the field of emulsions has been another field of dynamic research fields in recent years with more than 290,000 papers as indexed by the SCIE as of December 2015 (eg, Beck et al., 1992; Compton and Nguyen, 2010; Flemming and Wingender, 2010; Kresge et al., 1992; Oregan and Gratzel, 1991; Talapin et al., 2010).

At the intersection of these research fields, a new specialized research field on the nanomulsions related to foods has emerged in recent years with more than 1700 papers (eg, Bucak et al., 2003; Dickinson, 2009, 2010; Huang et al., 2010; Livney, 2010; McClements and Li, 2010; McClements and Rao, 2011; Weiss et al., 2006).

There have been many scientometric studies relating to the research in food science and technology (eg, Alfaraz and

**Emulsions.** http://dx.doi.org/10.1016/B978-0-12-804306-6.00020-9

Calvino, 2004; Sastry et al., 2010; van Raan and van Leeuwen, 2002; Zhou et al., 2013) and relating to the research in nanomaterials and nanoprocesses (eg, Hullmann and Meyer, 2003; Porter et al. 2008; Rafols and Meyer, 2010; Schummer, 2004). Additionally, there have been limited number of papers on the scientometrics of emulsions (eg, Bailon-Moreno et al., 2005; Barcikowski et al., 2009).

However, there have been no scientometric studies on the nanoemulsions related to foods as indexed by the SCIE as of December 2015 as in the earlier mentioned and other research fields (eg, Baltussen and Kindler, 2004a,b; Dubin et al., 1993; Gehanno et al., 2007; Konur, 2011, 2012a,b,c,d,e,f,g,h,i,j,k,l,m,n,o, 2013, 2014, 2015a,b,c,d,e,f,g,h,i,j,k,l,m, 2016a,b,c,d,e,f,g,h,i,j,k; Paladugu et al., 2002; Wrigley and Matthews, 1986).

As North's New Institutional Theory suggests, it is important to have up-to-date information about the current public policy issues to develop a set of viable solutions to satisfy the needs of all the key stakeholders relating to the nanoemulsions in the food industry (Konur, 2000, 2002a,b,c, 2006a,b, 2007a,b, 2012c,d; North, 1994).

Therefore, the brief information on a selected set of 25 citation classics in the field of nanoemulsions in the food industry are presented following a brief scientometric overview of the research in the food science and engineering, nanomaterials and nanoprocesses, and emulsions to inform the key stakeholders as the first-ever study of its kind.

## 1.2 Methodology

The search for the relevant materials was carried in two major steps using the v.5.20 of the Web of Science in December 2015.

In the first step, the broad search was carried out for the underlying research fields of nanomaterials and nanoprocesses, foods, and emulsions. The relevant keyword sets were "TS = (*nano* or graphene*) OR WC = (nano*)," "TS = (food*) or WC = (food*)," and "TS = (*emulsion* or *colloid* or *emulsifier* or *surfactant* or *emulsification* or *emulsified* or *emulsive*)" for the research fields of nanomaterials and nanoprocesses, foods, and emulsions, respectively.

The key bibliometric data was extracted from these search activities focusing on the reviews and articles as the final set of references.

The located highly cited 25 papers were arranged in the order of the decreasing number of citations. The summary information about the located citation classics are presented in the order of the decreasing number of citations for the arranged topical areas, respectively.

The information relating to the document type, affiliation of the authors, the number and gender of the authors, the country of the authors, the journal where the paper was published, the subject area of the journal where the paper was indexed, the concise topic of the paper, and total number of citations received for the paper for the Web of Science databases were given in tables for each paper.

## 1.3 The Food Research in General—Overview

Using the keywords related to food science and engineering, 879,096 references were located; 769,934 of these references were articles and reviews. Meeting abstracts, notes, news items, and editorial materials formed the remaining part of the sample among other items.

The most prolific three authors, Wang, Y., Zhang, Y., and Wang, J., produced 1,019, 969, and 881 papers, respectively. The list of the most-prolific authors was dominated by the European, Chinese, and the US authors.

The most prolific country in terms of the number of publications was the United States with 206,438 papers forming 26.8% of the sample. Japan, England, Germany, and China followed the United States with 7.2, 5.8, 5.5, and 5.4% of the sample, respectively. Europe dominated the most prolific country list.

English was the dominant language of scientific communication in this field comprising 92.7% of the sample. German, Japanese, and French were the other major languages.

The most prolific national or regional institutions were US Department of Agriculture, University of California system, Institut National de la Recherche Agronomique, Consejo Superior de Investigaciones Cientificas, and Centre National de la Recherche Scientifique with 18,568, 15,766, 11,338, 10,420, and 8,034 citations, respectively.

Similarly, the most prolific single institutions were University of California Davis, Wageningen University Research Center, Cornell University, University of North Carolina, and Harvard University with 6,824, 6,547, 6,144, 5,957, and 5,880 citations, respectively. The US institutions dominated the most-prolific institution list.

Like the nanoresearch, the research in this field has boomed after 1990 comprising 91.1% of the sample. There was a general increasing trend in the number of papers over time starting with 7,284 papers in 1980 and making a peak with 50,985 papers in 2014. There was a significant rise in 1991, possibly due to the inclusion of the abstracts in the abstract pages of the indices for the first time.

The most prolific journals in terms of the number of publications were *Journal of Agricultural and Food Chemistry*, *Food Chemistry*, *Journal of Dairy Science*, *Journal of Food Science*, and *Bioscience Biotechnology and Biochemistry* with 31,987, 18,169, 16,217, 13,068, and 10,449 citations, respectively. It is notable that the journals related to the food science and engineering dominated the top journal list.

The most prolific subject categories in terms of the number of publications was *Food Science Technology*, *Chemistry Applied*, *Nutrition Dietetics*, *Biotechnology Applied Microbiology*, and *Agriculture Multidisciplinary* with 374,233, 108,027, 73,454, 57,721, and 48,790 citations, respectively. These findings justify the keyword search besides the subject category search in this field as only 48.6% of the papers were indexed under the Food Science and Technology subject category.

The most-cited papers in this field were dominated by the applications of food in a number of areas. For example, Vansoest et al. (1991) discuss the animal nutrition with 8686 citations. This top paper was followed by Zhang et al. (1994), Mead et al. (1999), Halaas et al. (1995), and Brand-Williams et al. (1995), focusing on obesity, food-related illnesses, plasma-proteins, and antioxidant activities, respectively.

There was a similar trend for the hottest papers published in the 2010s. For example, the hottest paper with 1610 citations was related to the food-borne illness (Scallan et al., 2011). Holick et al. (2011), Godfray et al. (2010), Mata et al. (2010), and Fontana et al. (2010) followed the hottest paper focusing on vitamin D deficiency, food security, microalgae, and healthy life span, respectively.

## 1.4 The Nanoresearch in General—Overview

Using the keywords related to nanomaterials and nanoprocesses, 1,113,350 references were located; 1,050,286 of these references were articles and reviews. Meeting abstracts, notes, letters, and editorial materials formed the remaining part of the sample among other items.

The most prolific three authors, Zhang, Y., Wang, Y., Liu, Y., produced 5499, 5479, and 5262 papers, respectively. The list of the most-prolific authors was dominated by the Chinese and the US authors.

The most prolific country in terms of the number of publications was China with 252,052 papers forming 24.0% of the sample. The United States closely followed China with 21,697 papers forming 21.7% of the papers. Japan, Germany, South Korea, India, and France were the other most prolific countries with 8.0, 7.6, 5.9, and

5.3% of the papers, respectively. It is surprising that China surpassed the United States in this field.

English was the dominant language of scientific communication in this field comprising 98.7% of the sample.

The most prolific national or regional institutions were Chinese Academy of Sciences, Centre National de la Recherche Scientifique, US Department of Energy, Russian Academy of Sciences, and University of California system with 50,263, 35,706, 24,926, 23,656, and 23,580 citations, respectively.

On the other hand, the most prolific single institutions were Tsinghua University, Indian Institute of Technology, Zhejiang University, National University of Singapore, and Nanyang Technological University with 10,212, 9,585, 8,267, 8,148, and 8,110 citations.

The nanomaterial research has boomed after 1990 comprising 99.8% of the sample with the seminal paper on the nanomaterials by Iijima (1991). There was a general increasing trend in the number of papers over time starting with 181 papers in 1980 and making a peak with 122,750 papers in 2014.

The research in 1980s, 1990s, 2000s, and 2010s formed 0.2, 5.8, 35.0, and 58.5% of the sample, respectively, with a significant rise (nearly four times rise) in 1991, possibly due to the inclusion of the abstracts in the abstract pages of the indices for the first time.

The most prolific journals in terms of the number of publications were *Applied Physics Letters*, *Physical Review B*, *Journal of Applied Physics*, *Journal of Physical Chemistry C*, and *RSC Advances* with 25,611, 23,603, 17,609, 16,264, and 13,394 citations, respectively.

The most prolific subject categories were materials science multidisciplinary, physics applied, chemistry physical, chemistry multidisciplinary, and nanoscience nanotechnology with 299,115, 215,179, 200,860, 165,115, and 151,115 citations, respectively.

Perhaps the most important finding from these data is that the only 14.4% of the papers were indexed under the subject category of nanoscience and nanotechnology, providing justification for the development of the search strategy outside this subject category through the keyword search.

The most-cited papers in nanomaterials were dominated by the carbon nanotubes, graphene, and solar nanomaterials. For example, Iijima (1991) discusses the carbon nanotubes in a seminal paper with 24,878 citations. This top paper was followed by Novoselov et al. (2004), Oregan and Gratzel (1991), and Geim and Novoselov (2007) focused on graphene, solar-cells, and graphene with 19,465, 15,165, and 14,201 citations, respectively.

There was a similar trend for the hottest papers in nanomaterials published in 2010s. For example, the hottest paper with 3031

citations was related to the solar cells (Yella et al., 2011). Dreyer et al., (2010), Atwater and Polman (2010), and Bae et al. (2010) followed the hottest paper focusing on graphene oxide, plasmonics, and graphene films with 2949, 2886, and 2773 citations, respectively.

## 1.5 The Emulsions Research in General

Using the keywords related to emulsions, 315,383 references were located; 294,479 of these references were articles and reviews. Meeting abstracts, notes, letters, and editorial materials formed the remaining part of the sample among other items.

The most prolific three authors, Zhang, Y., Wang, Y., and Li, Y., produced 816, 808, and 796 papers, respectively. The list of the most-prolific authors was dominated by the Chinese and the US authors.

The most prolific country in terms of the number of publications was the United States with 65,844 papers forming 22.4% of the sample. China, Japan, and Germany followed the United States with 14.5, 8.1, and 7.5% of the sample, respectively.

English was the dominant language of scientific communication in this field with 96.1% of the papers.

The most prolific national or regional institutions were Centre National de la Recherche Scientifique, Chinese Academy of Sciences, University of California system, Russian Academy of Sciences, and US Department of Energy with 9543, 7481, 5816, 3545, and 3510 citations, respectively.

On the other hand, the most prolific single institutions were Indian Institute of Technology, Lund University, Pierre Marie Curie University Paris 6, Harvard University, and Kyoto University with 1989, 1793, 1726, 1695, and 1614 citations, respectively.

Like the nanomaterials research booming after 1991, the emulsions research has boomed after the 1980s comprising 96% of the sample. There was a general increasing trend in the number of papers over time starting with just 843 papers in 1980 and making a peak with 19,933 papers in 2014.

The most prolific journals in terms of the number of publications were *Langmuir*, *Journal of Colloid and Interface Science*, *Colloids and Surfaces A Physicochemical and Engineering Aspects*, *Journal of Physical Chemistry B*, and *Journal of Applied Polymer Science* with 11,503, 7,910, 6,035, 4,074, and 2,785 citations, respectively.

The most prolific subject categories in terms of the number of publications were chemistry physical, materials science multidisciplinary, chemistry multidisciplinary, polymer science, and engineering chemical with 71,092, 45,952, 43,253, 24,979, and 20,886 citations, respectively.

The most-cited papers in this field were dominated by the applications of the emulsions. For example, Oregan and Gratzel (1991) discuss the colloidal $TiO_2$ films-based solar cells with 15,168 citations. This top paper was followed by Kresge et al. (1992) and Beck et al. (1992) focused on silver mesoporous molecular-sieves prepared with liquid-crystal templates with 11,800 and 8,211 citations, respectively.

There was a similar trend for the hottest papers published in the 2010s. For example, the hottest paper with 1329 citations was related to the colloidal nanocrystals for electronic and optoelectronic applications (Talapin et al., 2010). Flemming and Wingender (2010) and Compton and Nguyen (2010) followed the hottest paper focusing on the biofilm matrix and graphene oxide with 1081 and 676 citations, respectively.

## 1.6 The Research on the Nanoemulsions in the Food Industry in General—an Overview

Using the keywords related to nanoemulsions in the food industry, 1749 references were located. Only 171 of these papers were reviews. This finding suggests that this field has been a specialized field of research with a relatively small sample size and with a specific set of shareholders such as authors, institutions, and countries.

The most prolific three authors, McClements, D.J., Weiss, J., and Decker, E.A., produced 130, 32, and 25 papers, respectively. The list of the most-prolific authors was dominated by the European, Chinese, and the US authors.

The most prolific country in terms of the number of publications was the United States with 404 papers forming 23.1% of the sample. China, Iran, and England followed the United States with 20.5, 5.4, and 5.2% of the sample, respectively. It seems that China competes strongly with the most prolific country in terms of the number of publications in this field. However, Europe dominated the most prolific country list.

English was the dominant language of scientific communication in this field with 98.7%.

The most prolific single institution was the University of Massachusetts Amherst with 136 papers. Jiangnan University, King Abdulaziz University, and Universiti Putra Malaysia followed the top institution with 43, 38, and 37 papers, respectively.

On the other hand, the University of Massachusetts system, University of California system, Consejo Superior de Investigaciones Cientificas, and University of Tennessee system were the most prolific national or regional institutions with 136, 34, 27, and 24 papers, respectively.

Like the general nanomaterials research booming after 1991, the research in this field has boomed only in the 2010s comprising 81% of the sample. Additionally, a further 18% of the research papers were published in the 2000s with a further 1% published in the 1990s. These findings suggest that the research in this field has been relatively recent and more importantly will continue to gain importance in the near future.

The most prolific journal in terms of the number of publications was *Food Hydrocolloids* publishing 139 papers. *Journal of Agricultural and Food Chemistry*, *Analytical Methods*, and *Food Chemistry* followed the top journal with 123, 113, and 94 papers, respectively. It is notable that the top journal list was dominated by the journals related to foods and to a lesser extent by the journals related to colloids.

The most prolific subject category in terms of the number of publications was food science technology with 1002 papers forming 57.3% of the sample. Chemistry applied, chemistry analytical, and chemistry physical followed the top subject category with 27.0, 13.2, and 11.3% of the sample, respectively. Additionally, there were 107 papers published in the subject category of nanoscience nanotechnology.

The most-cited papers in this field were dominated by the reviews and discussion papers. For example, Dickinson (2009) discusses the hydrocolloids. This top paper was followed by Dickinson (2010), Livney (2010), Bucak et al. (2003), and Weiss et al. (2006) focusing on food emulsions, milk proteins, protein separations, and food nanotechnology, respectively.

There was a similar trend for the hottest papers published in the 2010s. For example, the hottest paper was related to the food emulsions (Dickinson, 2010). Livney (2010), McClements and Li (2010), McClements and Rao (2011), and Huang et al. (2010) were the other hottest top papers relating to milk proteins, structured emulsion-based delivery systems, food-grade nanoemulsions, and nutraceuticals, respectively.

# 2 The Nanoemulsions in the Food Industry

## 2.1 Overview

The research on the nanoemulsions in the food industry has been one of the most dynamic research areas in recent years with 25 citation classics. These citation classics were located and the key emerging issues from these papers are presented later under two main headings in the decreasing order of the number of citations (Tables 20.1 and 20.2).

# Table 20.1 The Citation Classics in Nanoemulsions in the Food Industry—Reviews and Discussion Papers

| No. | Author(s) | Year | Doc. | Affiliation | Country | No. of Auths. | M/F | Journal | Subject Area | Topic | No. of Citations |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Dickinson | 09 | A | Univ. Leeds | England | 1 | M | Food Hydrocolloids | Chem. Appl., Food Sci. Technol. | Hydrocolloids | 215 |
| 2 | Dickinson | 10 | R | Univ. Leeds | England | 1 | M | Curr. Opin. Colloid Interface Sci. | Chem. Phys. | Food emulsions | 210 |
| 3 | Livney | 10 | R | Technion Israel Inst. Technol. | Israel | 1 | M | Curr. Opin. Colloid Interface Sci. | Chem. Phys. | Milk proteins | 207 |
| 4 | Weiss et al. | 06 | R | Univ. Massachusetts, Amherst, Rutgers State Univ. | United States | 3 | M | J. Food Sci. | Food Sci. Technol. | Functional materials | 196 |
| 5 | McClements and Li | 10 | R | Univ. Massachusetts | United States | 2 | M | Adv. Colloid Interface Sci. | Chem. Phys. | Nanomulsion-based delivery systems | 162 |
| 6 | Dickinson | 08 | R | Univ. Leeds | England | 1 | M | Soft Matter | Chem. Phys., Mats. Sci. Mult., Phys. Mult., Polym. Sci. | Food emulsions | 159 |
| 7 | McClements and Rao | 11 | R | Univ. Massachusetts, Amherst | United States | 2 | M | Crit. Rev. Food Sci. Nutr. | Food Sci. Technol., Nutr. Diet. | Nanoemulsions | 144 |
| 8 | Jafari et al. | 08 | R | Gorgan Univ. Agr. Sci. & Nat. Resources, James Cook Univ. +1 | Italy, United States | 4 | M | Dry. Technol. | Eng. Chem., Eng. Mech. | Encapsulation efficiency | 142 |

(*Continued*)

# Table 20.1 The Citation Classics in Nanoemulsions in the Food Industry—Reviews and Discussion Papers (*cont.*)

| No. | Author(s) | Year | Doc. | Affiliation | Country | No. of Auths. | M/F | Journal | Subject Area | Topic | No. of Citations |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | Jafari et al. | 08 | R | Gorgan Univ. Agr. Sci. & Nat. Resources, James Cook Univ. +1 | Italy, United States | 4 | M | Food Hydrocolloids | Chem. Appl., Food Sci. Technol. | High-energy emulsification | 141 |
| 10 | Huang et al. | 10 | R | Rutgers State Univ. | United States | 3 | M | J. Food Sci. | Food Sci. Technol. | Nutraceuticals | 139 |
| 11 | McClements | 11 | R | Univ. Massachusetts | United States | 1 | M | Soft Matter | Chem. Phys., Mats. Sci. Mult., Phys. Mult., Polym. Sci. | Nanoemulsions | 132 |
| 12 | Augustin and Hemar | 09 | R | CSIRO | Australia | 2 | F | Chem. Soc. Rev. | Chem. Mult. | Encapsulation of food ingredients | 132 |
| 13 | Yaghmur and Glatter | 09 | R | Graz Univ. | Austria | 2 | M | Adv. Colloid Interface Sci. | Chem. Phys. | Aqueous nanodispersions | 129 |
| 14 | Murray and Ettelaie | 04 | R | Univ. Leeds | England | 2 | M | Curr. Opin. Colloid Interface Sci. | Chem. Phys. | Foam stability | 125 |
| 15 | McClements | 12 | A | Univ. Massachusetts | United States | 1 | M | Soft Matter | Chem. Phys., Mats. Sci. Mult., Phys. Mult., Polym. Sci. | Foam stability | 125 |
| 16 | Weiss et al. | 08 | A | Univ. Massachusetts, Univ. Iceland | United States, Iceland | 6 | M | Food Biophys. | Food Sci. Tech. | Solid lipid nanoparticles | 106 |
| 17 | Sagalowicz and Leser | 10 | R | Nestle Res. Ctr. | Switzerland | 2 | M | Curr. Opin. Colloid Interface Sci. | Chem. Phys. | Delivery systems | 105 |

*A*, Article; *R*, review; *M*, male; *F*, female.

# Table 20.2 The Citation Classics in Nanoemulsions in the Food Industry—Experimental Papers

| No. | Author(s) | Year | Doc. | Affiliation | Country | No. of Authors | M/F | Journal | Subject Area | Topic | No. of Citations |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Bucak et al. | 03 | A | MIT | United States | 3 | F | Biotechnol. Prog. | Biot. Appl. Microb., Food Sci. Technol. | Protein separations | 206 |
| 2 | Tan and Nakajima | 05 | A | Univ. Pertanian Malaysia +1 | Malaysia, Japan | 2 | M | Food Chem. | Chem. Appl., Food Sci. Technol., Nutr. Diet. | Nanodispersions | 133 |
| 3 | Qian and McClements | 11 | A | Univ. Massachusetts | United States | 2 | F | Food Hydrocolloids | Chem. Appl., Food Sci. Technol. | Nanoemulsions | 128 |
| 4 | Kentish et al. | 08 | A | Univ. Melbourne +1 | Australia | | F | Innov. Food Sci. Emerg. Technol. | Food Sci. Technol. | Nanoemulsion preparation | 122 |
| 5 | Yuan et al. | 08 | A | China Agr. Univ., Charles Sturt Univ. | China, Australia | 4 | M | Food Res. Int. | Food Sci. Technol. | Nanoemulsions | 120 |
| 6 | Leong et al. | 09 | A | Univ. Melbourne +1 | Australia | 4 | M | Ultrason. Sonochem. | Acoust., Chem. Mult. | Ultrasonic emulsification | 111 |
| 7 | Jafari et al. | 07 | A | Univ. Queensland, James Cook Univ. +1 | Australia, Iran | 3 | M | J. Food Eng. | Eng. Chem., Food Sci. Technol. | Nanoemulsions | 107 |
| 8 | Wang et al. | 08 | A | Rutgers State Univ. | United States | 6 | M | Food Chem. | Chem. Appl., Food Sci. Technol., Nutr. Diet. | o/w nanoemulsions | 104 |

*A*, Article; *R*, review; *M*, male; *F*, female.

McClements, D.J., was the most prolific authors with seven papers. The other most prolific authors with three papers were Bhandari B, Dickinson, E., He, Y.H., and Jafari, S.M. In total, 51 authors contributed to these 25 citation classics in this field.

The United States was the most prolific country with 10 papers. Australia, England, and Iran were the other most prolific countries with 7, 4, and 2 papers, respectively. In total, 12 countries contributed to these 25 citation classics in this field.

The most prolific institutions were University of Massachusetts Amherst, University of Leeds, James Cook University, Rutgers University, and University of Queensland with 7, 4, 3, 3, and 3 citations, respectively. In total 20 institutions contributed to these citation classics.

The most prolific publication years were 2008, 2010, 2009, and 2011 with 7, 5, 4, and 3 papers, respectively. Thus, it is significant to note that these citation classics have been relatively recent.

The most prolific journals were *Current Opinion in Colloid Interface Science*, *Food Hydrocolloids*, *Soft Matter*, *Advances in Colloid and Interface Science*, *Food Chemistry*, and *Journal of Food Science* with 4, 3, 3, 2, 2, and 2 citations, respectively. Thus, journals relating to colloids and food science were most prolific. In total, 15 journals published these citation classics.

The most prolific subject categories were food science technology, chemistry physical, chemistry applied, materials science multidisciplinary, nutrition dietetics, and physics multidisciplinary with 13, 9, 5, 3, 3, and 3 citations, respectively.

The citation impact of these classical papers was significant with 145 citations per paper and H-index of 25. The number of citations ranged from 104 to 214 for the Web of Science database.

There was a significant gender deficit among these papers as there were only 4 papers with a female first author out of 25 papers.

Seventeen of these classical papers were reviews and discussion papers in this field while 8 of them were experimental papers; 9 of the most prolific 10 papers were reviews and discussion papers.

In the following part, the brief information on the most cited papers will be provided in two major topical parts: reviews and discussion papers and experimental papers.

## 2.2 The Most-Cited Papers in Nanoemulsions in the Food Industry: Reviews and Discussion Papers

Dickinson (2009) discusses hydrocolloids in a paper originating from England with 215 citations. He considers the criteria for effectiveness in protecting newly formed droplets against

flocculation and coalescence together with the requirements to maintain long-term stability such as creaming. He compares the physicochemical characteristics of hydrocolloid emulsifying agents and other kinds of food emulsifying agents such as surfactants, proteins, and nanoparticles. He notes that interfacial interactions between protein and polysaccharide may occur through covalent bonding or electrostatic bonding.

Dickinson (2010) discuss food emulsions and foams by nanoparticles in a review paper originating from England with 210 citations. He notes that the key issues are the molecular factors controlling particle wettability and adsorption, the structural and mechanical properties of particle-laden liquid interfaces, and the stabilization mechanisms of particle-coated droplets and bubbles.

Livney (2010) discusses milk proteins as vehicles for bioactives in a review paper originating from Israel with 207 citations. He focuses on harnessing the casein micelle for delivering hydrophobic bioactives, discovering unique nanotubes based on enzymatic hydrolysis of α-la, introduction of novel encapsulation techniques based on cold-set gelation for delivering heat-sensitive bioactives including probiotics. He further discusses Maillard reaction based conjugates of milk proteins and polysaccharides for encapsulating bioactives and introduction of β-Ig-pectin nanocomplexes for delivery of hydrophobic nutraceuticals in clear acid beverages.

Weiss et al. (2006) discuss functional materials in food nanotechnology with a focus on the nanoemulsions in a review paper originating from the United States with 196 citations.

McClements and Li (2010) discuss structured emulsion-based delivery systems in a review paper originating from the United States with 162 citations. They focus on the conventional emulsions, nanoemulsions, multilayer emulsions, solid lipid particles, and filled hydrogel particles. They note that each of these delivery systems can be produced from food-grade ingredients such as lipids, proteins, and surfactants, using relatively simple processing operations such as mixing and homogenizing.

Dickinson (2008) discusses the interfacial structure and stability of food emulsions in a review paper originating from England with 159 citations. He notes that Maillard-type protein-polysaccharide conjugates have excellent emulsifying and steric stabilizing properties. The structure and stabilizing properties of the mixed protein-polysaccharide layer depends on the sequence of adsorption of the biopolymers to the interface.

McClements and Rao (2011) discuss the nanoemulsions in a review paper originating from the United States with 144 citations. They highlight that the small size of the particles in nanoemulsions offer a number of potential advantages such as higher

stability to droplet aggregation and gravitational separation, and high optical clarity. However, they caution that there may also be some risks associated with the oral ingestion of nanoemulsions, such as the potential toxicity of some of the components used in their fabrication.

Jafari et al. (2008a) discuss microencapsulation efficiency of food flavors and oils in a paper originating from Italy and the United States with 142 citations. They note that the properties of wall and core materials and the prepared emulsion along with the drying process conditions affect the efficiency and retention of core compounds.

Jafari et al. (2008b) discuss recoalescence of emulsion droplets in a review paper originating from Italy and Australia with 141 citations. They note that emulsion droplet size plays a key role in many emulsion properties such as stability, color, appearance, texture, and rheology. They cite low adsorption rate of the surface-active agent, low residence time of the emulsion in the emulsification zone, high rate of coalescence frequency, and extreme amount of energy density for overprocessing.

Huang et al. (2010) discuss bioavailability and delivery of nutraceuticals in a review paper originating from the United States with 129 citations. They note that curcumin nanoemulsions show 85% inhibition of TPA-induced mouse ear inflammation as well as the inhibition of cyclin D1 expression, while dibenzoylmethane (DBM) nanoemulsion shows about threefold increase in oral bioavailability compared to the conventional DBM emulsion. They further note that biopolymer micelles show significantly improved water solubility/dispersibility and in vitro anticancer activity of phytochemicals.

McClements (2011) discuss the nanoemulsions in a review paper originating from the United States with 132 citations. He highlights the potential advantages of using these nanoemulsions such as increasing the bioavailability of lipophilic substances and scattering light weakly. He then cautions about the risks associated with the oral ingestion of these nanoemulsions, such as the potential toxicity of some of the components used in their fabrication.

Augustin and Hemar (2009) discuss nano- and microstructured assemblies for encapsulation of food ingredients in a review paper originating from Australia with 132 citations. They focus on the impact of the choice of materials, formulation, and process on the structure of encapsulated ingredients and the release of the core.

Yaghmur and Glatter (2009) discuss characterization and the utilization of aqueous nanodispersions in a review paper

originating from Austria with 129 citations. They highlight the formation of emulsified microemulsions (EMEs) and the modulation of the internal nanostructure, exploring how variations in temperature, oil content, and lipid composition significantly affect the confined nanostructures. They note using the tilt-angle cryo-TEM (transmission electron microscopy) method or cryo-field emission scanning electron microscopy (cryo-FESEM) for observing the three-dimensional morphology of nonlamellar liquid-crystalline nanostructured particles.

Murray and Ettelaie (2004) discuss effects of nanoparticles as stabilizers of foams in a review paper originating from England with 125 citations. They distinguish between adsorbing and nonadsorbing nanoparticles and note that both particles give rise to bubble stability over and above that predicted by conventional explanations of the behavior of low-molecular-weight or macromolecular surfactants.

McClements (2012) discusses nanoemulsions in a paper originating from the United States with 124 citations. He notes that the small size of the particles in these kinds of delivery systems result in a number of potential benefits such as enhanced long-term stability, high optical clarity, and increased bioavailability. He distinguishes microemulsions and nanoemulsions where a microemulsion is thermodynamically stable, whereas a nanoemulsion is not. He cautions that the methods used to fabricate them, the strategies used to stabilize them, and the approaches used to design their functional attributes differ.

Weiss et al. (2008) discuss solid lipid nanoparticles (SLNPs) as delivery systems in a paper originating from Iceland and the United States with 106 citations. They note that specific crystal structures can be designed in SLNPs by using specific surfactant mixtures. They further note that microphase separations of the bioactive compound from the solidifying lipid matrix can be prevented resulting in an even dispersion of the encapsulated compound in the solid matrix, thereby improving chemical and physical stability of the bioactive.

Sagalowicz and Leser (2010) discuss the delivery systems for liquid food products in a review paper originating from Switzerland with 105 citations. They assert that the remaining technical challenges to solve in the future are most of the available delivery systems for aqueous products do not yet allow to significantly stabilize degradation sensitive “encapsulated” active ingredients against, for example, oxidation. They cite that the encapsulation capacity of some delivery systems is still quite poor and off-taste generation is possible above certain concentrations of added delivery systems as further challenges.

## 2.3 The Most-Cited Papers in Nanoemulsions in the Food Industry: Experimental Papers

Bucak et al. (2003) discuss protein separations using coated colloidal magnetic nanoparticles in a paper originating from the United States with 206 citations. They show that these phospholipid-coated nanoparticles are effective ion exchange media for the recovery and separation of proteins from protein mixtures. They find that these particles have high adsorptive capacities and exhibit none of the diffusional resistances offered by conventional porous ion exchange media.

Tan and Nakajima (2005) discuss β-carotene nanodispersions in a paper originating from Japan and Malaysia with 133 citations. They find that homogenization pressure and cycle had significant effects on various particle size parameters as they observe a volume-weighted mean diameter of β-carotene nanoparticles, ranging from 60 to 140 nm.

Qian and McClements (2011) discuss the formation of nanoemulsions in a paper originating from the United States with 128 citations. They find that the mean particle diameter decreased with increasing homogenization pressure and number of passes and the minimum droplet diameter was a function of emulsifier type and concentration. They further find that small-molecule surfactants formed smaller droplets than proteins.

Kentish et al. (2008) discuss the use of ultrasonics for nanoemulsion preparation in a paper originating from Australia with 122 citations. They obtain emulsions with a mean droplet size as low as 135 nm using a mixture of flaxseed oil and water in the presence of Tween-40 surfactant. They note that these findings are comparable to those for emulsions prepared with a microfluidizer operated at 100 MPa.

Yuan et al. (2008) discuss char β-carotene nanoemulsions in a paper originating from China and Australia with 120 citations. They find that the mean diameters of the dispersed particles were small and the size distribution was unimodal. They further find that the nanoemulsions produced with Tween-20 had the smallest particle sizes and narrowest size distribution.

Leong et al. (2009) discuss ultrasonic emulsification in a paper originating from Australia with 111 citations. They create remarkably small transparent oil/water nanoemulsions with average diameters as low as 40 nm from sunflower oil using ultrasound or high shear homogenization and a well-optimized surfactant/cosurfactant/oil system. They obtain the minimum droplet size of 40 nm only when both droplet deformability and the applied shear were optimal.

Jafari et al. (2007) discuss the production of nanoemulsions by microfluidization and ultrasonication in a paper originating from

Iran and Australia with 108 citations. They find that microfluidization was an efficient emulsification technique producing small emulsion droplets with narrow distributions compared with conventional emulsifying devices. However, increasing the microfluidization energy input beyond moderate pressures and cycles lead to overprocessing of emulsion droplets due to recoalescence. For ultrasound emulsification, increasing the energy input through improving sonication time helped to reduce emulsion size with minimum recoalescence of new droplets.

Wang et al. (2008) discuss antiinflammation activity of curcumin through oil/water (o/w) nanoemulsions in a paper originating from the United States with 104 citations. They obtain the enhanced antiinflammation activity of curcumin encapsulated in o/w emulsions by the mouse ear inflammation model. There is a 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.

## 3 Conclusions

The field of the nanoemulsions related to food science and engineering has emerged as a distinct research area in recent years building on the underlying research areas of food science and engineering, nanomaterials and nanoprocesses, and emulsions. Thus, this new research field shows the strong signs of interdisciplinarity booming in the 2010s.

The majority of these papers were reviews and discussion papers (17 papers) while 8 papers were experimental papers.

These citation classics deal with the important health and nutrition-related research issues with strong public policy implications.

The citation classics presented in this paper were helpful in highlighting important papers influencing the development of the research field as well as in determining the key research areas in this field.

Further research is recommended for the detailed studies including scientometric studies and citation classic studies in combination with the corresponding studies in the underlying research fields.

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