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An Introduction to Food Grade Nanoemulsions

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Nandita Dasgupta • Shivendu Ranjan

An Introduction to Food Grade Nanoemulsions

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We dedicate this book to our parents

Foreword

To the readers of this book:

In the last decades, nanoscience and nanotechnology have been considered as becoming increasingly important for the society, making significant, extremely useful, and often unforeseen contributions in different areas of our life. One of such contributions is the application of nanotechnology in the development of foods as wonderfully described in this book by Dr. Nandita Dasgupta and Dr. Shivendu Ranjan. The focus of the work is on various aspects of nanotechnology related with food nanoemulsions, or nano-foods: their fabrication, ingredients, food engineering, marketing, safety, etc. These nanoemulsions, or colloidal delivery systems, are efficient for encapsulating various functional agents, such as flavors, colors, antimicrobials, micronutrients, and nutraceuticals, to be delivered inside the human digestive system. As a researcher involved in the nanotechnology/nanoscience area for several years, I can say that the authors have made very significant and extremely important contribution in our understanding of how nanotechnology could be applied in the very important area of human alimentation and in food and beverage

industries. I am sure that this book will be of great interest and benefit for everybody involved in nanotechnology-/nanoscience-related research, from a student to a professor.

Sincerely,

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Research interests: Computational/theoretical chemistry, nanoscience/nanotechnology, catalysis, metal and semiconductor nanoparticles

Preface

Recently, food and beverage industries are more concerned with the consumption of colloidal delivery systems to encapsulate functional agents, such as micronutrients and nutraceuticals as well as flavors, colors, and antimicrobials. As colloidal delivery systems, emulsions – especially nanoemulsions – are of remarkable importance; since they can conveniently be formulated from food-grade ingredients using comparatively basic food engineering processes, which are homogenization, shearing, ultrasonication, and mixing. Nanoemulsion-based delivery systems have particular physicochemical features that contribute to the benefits for specific applications. Nanoemulsions (*droplet diameter in the range of* 100 nm) are thermodynamically stable systems that generally consist of water-oil system stabilized by the surfactant. Nanoemulsion, or colloidal delivery system, is efficient to encapsulate functional agents, such as flavors, colors, antimicrobials, micronutrients, and nutraceuticals (Fig. 1).

One of the significant applications of nanoemulsions is to integrate lipophilic active ingredients into aqueous-based systems (food or beverage) which require continuing transparency, such as some fortified aqueous drinks which may be water, sauces, and soft drinks. It can be noted that, on longer storage, a nanoemulsion-based system may undergo instability; Fig. 2 depicts different forms of stability in food-grade nanoemulsions. Food-grade nanoemulsions are fabricated from palatable ingredients and are being increasingly employed in food and beverage manufacture for efficient delivery, protection, and encapsulation of bioactive and functional components. Such bioactive and functional components are nutraceuticals, vitamins, flavors, biologically active lipids, and preservatives. Recently, nanoencapsulated nutraceuticals have been termed as nanoceuticals. The miniature droplet size of the nanoemulsified system implies that they have an ample of possible benefits over conventional emulsified systems which are, but not limited to, increased bioavailability, higher stability, high optical clarity, lesser droplet aggregation, lesser gravitational separation, and texture modulator as well as improved and efficient bioactivity such as antimicrobials and antioxidants.

An introduction for the applications of nanotechnology in the development of foods has been reviewed in Chap. 1, which provides the introduction on nanofoods. Further, being specific, Chap. 2 gives a brief introduction on food-grade

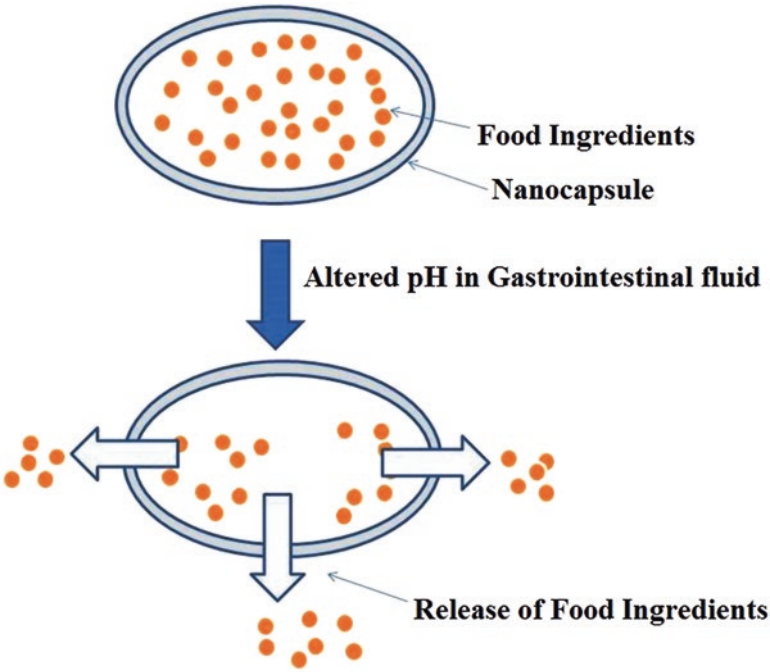

Fig. 1 Representative diagram to show the nano-encapsulation of active food compounds and pH-based release of active compounds. This may help in targeted delivery of active food ingredients and can lead to overcome many diseases (Chap. 1, Fig. 1.3)

nanoemulsion and its significance. Different techniques and methods for the fabrication of food-grade nanoemulsions have been discussed in chapter, while Chap. 4 elaborates on ingredients and components of food-grade nanoemulsions. Chapter 6 gives a critical review on the engineering aspects applied in the fabrication of food-grade nanoemulsions, and recently developed vitamin encapsulated nanosystems have been described in Chap. 7 which also discusses the challenges and opportunities of characterization of nanoemulsified systems. Market risks and opportunities of nanoemulsified foods have been discussed in Chap. 7, and packag-

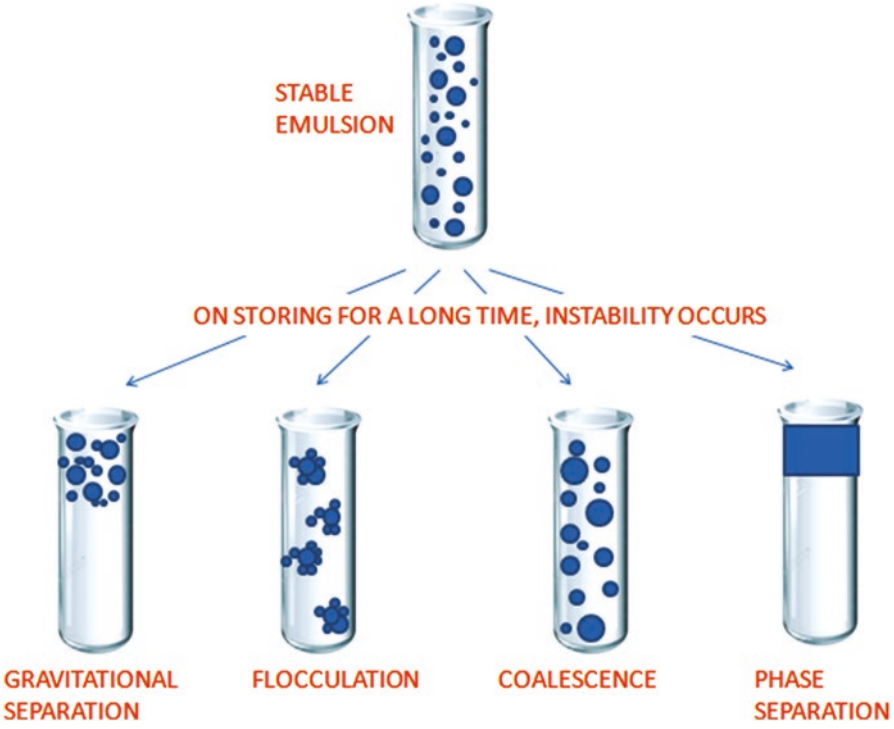

Fig. 2 Representation of commonly occurring physical instabilities in food emulsions (Chap. 2, Fig. 1)

ing techniques of nano-foods have been elaborated in Chap. 8. However, safety issues which include risk identification and risk management need not be ignored from the eyes of food engineers to deliver nano-foods to the society; this issue has been reviewed in Chap. 9.

Happy reading and wait for nano-foods in a store near you!

IIFPT, Thanjavur, Tamil Nadu, India Nandita Dasgupta
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Acknowledgments

Authors are dedicating this book to their parents and family members.

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This work would not have been possible without the support of their family members; authors appreciate their sacrifice, patience, and moral support throughout their research career.

Contents

About the Authors

Nandita Dasgupta has vast working experience in Micro/Nanoscience and is currently working at Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India. She has exposure of working at university, research institutes and industries including VIT University, Vellore, Tamil Nadu, India; CSIR-Central Food Technological Research Institute, Mysore, India; and Uttar Pradesh Drugs and Pharmaceutical Co. Ltd., Lucknow, India. Her areas of interest include Micro/Nanomaterial fabrication and its applications in various fields – medicine, food, environment, agriculture biomedical. She has published six edited books and one authored book with various international publishers. She has authored many chapters and also published many scientific articles in international peer-reviewed journals. She is the associate editor of *Environmental Chemistry Letters* – a Springer journal of 3.59 impact factor – and also serving as editorial board member and referee for reputed international peer-reviewed journals. She has received several awards and recognitions from different national and international organizations.

Shivendu Ranjan has extensive expertise in Micro/Nanotechnology and is currently working at Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India. His research interests are multidisciplinary and include: Micro/Nanobiotechnology, Nano-toxicology, Environmental Nanotechnology, Nanomedicine, and Nanoemulsions. He is the associate editor of *Environmental Chemistry Letters* – a Springer journal of 3.59 impact factor – and an editorial board member in *Biotechnology and Biotechnological Equipment*. He is serving as editorial board member and referee for reputed international peer-reviewed journals. He has published six edited books and one authored book with various international publishers. He has published many scientific articles in international peer-reviewed journals and has authored many book chapters as well as review articles. He has bagged several awards and recognitions from different national as well as international organizations.

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Chapter 1
Nanotechnology in Food Sector

Abstract Recently, applications of nanotechnology are in the lime light in all the sectors including food and nutrition. Food nanotechnology is becoming new frontiers of this century. The applications of nanotechnology in food and agriculture sector are comparatively more recent than the nano-drugs and nanopharmaceuticals. Smart and efficient delivery of active food components, protein bioseparation, nanoencapsulation of nutraceuticals are few of the emerging topics in food and agriculture nanotechnology. Other advancement in food and agriculture biotechnology is the nano-films and their applications in packaging also the smart materials for sensing properties make it more fit to be used in food and agriculture sector. Industrialists, scientists and researchers are focusing to meet their need with the help of nanotechnology e.g. high nutrition level, efficient nutritional delivery, more shelf life, good mechanical properties as well as longer shelf life and durability of food-products. In this review, we have summarized the applications of nanotechnology in food and nutraceuticals and also have identified the outstanding challenges to be overcome which further indicates future of this food nanotechnology. Here in this review, we have described (i) the recently launched marketed nano-foods; (ii) major applications of nanotechnology in food and its allied sectors (iii) challenges and opportunities in nano-food processing.

Keywords Nano-foods • Nano-food processing • Market • Nutritional delivery • Nano-antimicrobials

1.1 Introduction

Twenty-first century is witnessing a replacement scientific and industrial revolution as a consequence of the manipulation of matter at the nanometric level. This rising discipline (i.e. nanotechnology), that is expounded to the practices for planning, fabricating, measuring and manipulating matter at the nm scale, grows at a thoughtless pace and unique phenomena enable novel applications. Nanomaterials exhibit different chemical and physical properties, such as nano range size, size distribution, surface area and volume ratio, various surface properties, shape, chemical

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composition and agglomeration state, which are not apparent in bulk materials. Hence, it is not shocking that implementation of nanotechnology techniques in several industrial areas, such as cosmetics, medicine, food, construction materials, etc. have greatly grown in the last decade (Danie Kingsley et al. 2013; Perez-Esteve et al. 2013; Ranjan et al. 2014a; Dasgupta et al. 2015, 2016a; Jain et al. 2016; Shukla et al. 2017).

Nanotechnology applications in food sectors include: encapsulation and targeted delivery of particles, enhancing the flavor and sensory properties, adding antibacterial nanoparticles into food, increase shelf life of food, detecting contamination and improved food storage. Nanotechnology in food also results in change the nutritional functionality, eliminates different types of chemicals and pathogens from food (Chellaram et al. 2014). Nanotechnology has been results revolutionizing approach in food engineering food system from fabrication to processing, storage, and creation of new advanced materials, products, and applications. Currently, the market of nanotechnology food industry products has reached US$1 billion. The application of nanotechnology in the areas of food and food packaging is emerging rapidly (Ezhilarasi et al. 2013). Nanoparticles exhibit many features like high surface/volume ratio, ability of reassembling and self-reassembling, capacity of creating porous structure etc. give them potential for use in food industry (Perez-Esteve et al. 2013; Bernardes et al. 2014).

1.2 Marketed Products

The enormous potential of nanotechnology has attracted more than 400 companies to around the world, for apply concept of nanotechnology. Some of them are Kraft, Nestle, Unilever, Keystone, Heinz, Hershey, Aquanova and NutaLeaseetc and this number is expected to grow in coming years (Kaya-Celiker H 2012; Ranjan et al. 2014b). The movement of nanotechnology from lab scales to industrial scales and then to supermarket shelves and our kitchen shows the benefits and potential of nanotechnology in food industry. Taking the examples of Unilever and Nestle, Nanoemulsion based ice cream made by Unilever with various health benefits and without any compromise on taste; same way Nestle, has come up with a another product based on water-in-oil emulsion (10–500 nm) aiming at achieving faster and simpler thawing through the addition of polysorbates and other micelle forming substances (Silva et al. 2012).

Aquanova has formulated a nanocarrier system using nano range (30 nm) micelles, known as “Novasol” which is used to encapsulate active ingredients such as vitamins C and E and fatty acids, and claims that stability, effectiveness and bioavailability of these compounds can be improved by nanoscale carrier system. Aquanova in association with Zyme is offering omega 3 in 30–40 nm nanocapsules, which is 4000 times smaller than the existing product on the market (Silva et al. 2012; Ranjan et al. 2014b; Halliday 2015). Two main categories of NovaSolare: healthy functional compounds such as DL-α-tocopherol acetate; coenzyme Q10;

omega three fatty acids; and vitamins A, D, D3, E, and K and naturally present colorants as apocarotenal, β-carotene, curcumin, chlorophyll, sweet pepper extract, and lutein (Silva et al. 2012). Novasolalso can be used as a delivery system of hydrophobic substances meant for higher and quicker intestinal and dermal reabsorption including penetration of active ingredients. Aquanova also Claims for improved stability – thermal stability as well as pH stability – of standardized additive concentrations and encapsulated functional compounds (Silva et al. 2012).

NutraLease is working to increase the bioavailability of functional compounds such as lutein; lycopene; β carotene; vitamins A, D3, and E; Q10; phytosterols, and isoflavones which presents in various foods and in beverages. By implying low energy approach for production of nanoemulsion, improved encapsulation efficiency and increase its bioavailability of active and functional compounds in human body (Silva et al. 2012; Ranjan et al. 2014b; Halliday 2015). NutraLease nanoemulsion can also shield flavor compounds from processing conditions and enhance shelf life of various beverages. It is claimed that nanoemulsion can hold the flavor compounds and prevent their degradation by protecting them from unfavorable enzymatic reactions, oxidation reactions, temperature and hydrolysis, and they are thermodynamically more stable at a broad range of pH values (Silva et al. 2012). Another product brand known as nano-self-assembled structured liquids (NSSL) comes under the category of genetic food additives, which is composed of nanomicelles for encapsulation of nutraceutical compounds. NSSL is used for enhanced bioavailability which has application in nutraceutical field. It helps to release neutraceutical compounds into the membrane between the digestive system and the blood. There are many other products present in market which claim effective delivery and better bioavailability of food ingredients and drugs – details has been given in Table 1.1.

1.3 Nutrition and Food Processing

Nanotechnology in food development gives specific characteristics to food by altering the physiochemical properties and microscopic characteristics like texture, taste, other sensory properties, color, strength, processability, water solubility, stability during shelf life(thermal and pH stability), and oral bioavailability of functional compounds of natural food (Silva et al. 2012). Nanotechnology in food cultivation, production, processing and packaging or nano-additives in original food, results into nano-food (Sekhon 2010). The main objectives of nano-food are to improve the quality, safety and nutritional value of food and same time to reduce cost also. The functions of nanobiotechnology in nano-food processing are mainly focuses on the development of nanosized food ingredients and additives, and delivery systems for nutrients and supplements in the form of nutraceuticals. Various types of processing methods have been introduced such as nanoemulsions, surfactant micelles, emulsion layers, reverse micelles and functionally designed nanocapsules (Fig. 1.1) (Handford et al. 2014).

Table 1.1 Some Nano food or nano-supplements present in market with applications

Products	Company	Details	Application	Reference
Canola Active Oil	Shemen Industries, Israel	Micelles (nanodrops) act as liquid carrier for the delivery of healthy components (such as vitamins, minerals and phytochemicals)	Deliver healthy components of foods, increase bioavailability and prevent degradation from digestive enzyme	The Project on Emerging Nanotechnology (2013)
Nanoceuticals™ Slim Shake Chocolate	RBC Life Sciences, USA	Pure cocca infused "Nanoclusters"	To enhance taste without added extra sugar and increase absorption	
Nanotea	Shenzhen Become Industry & Trade Co., Ltd.China	Nano-selenium rich tea	Nano-tea can release effectively all the excellent essences of the tea, thus boosting the absorption (absorbing viruses, free radicals, cholesterol and blood lipid) and annihilation of viruses through penetration so that a good supplement of selenium has been achieved.	
Maternal Water	La Posta del Aguila, Argentina	Mineral water (without any chemical treatment) with nano colloidal silver ions	Especially for baby and mom in the gestation period	
24Hr Microactive® CoQ10	Genceutic Naturals, USA	nanosized CoQ10(coenzyme q10) and ß-Cyclodextrin matrix	More stability against heat and light, matrix showed a sustained release and showed better bioavailability than ordinary CoQ10 by a factor of 3.7. In addition, Genceutic Natural's 24Hr Nano CoQ10 matrix has 40% more consistent absorption than ubiquinol, a metabolized form of CoQ10 often touted as having superior absorption.	
Anabolic Vitakic	Muscletech sports nutrition supplements, USA	The Nanomolecular Multi-action Rapid-Release Caplet has been infused with a precise portion of a musclebuilding ingredient, as well as a calculated dose of the multivitamin complex that has been nanoparticulatd to a size that's up to 8800 percent smaller than normal. [Note-This claim was found in the E-shop but not in the brand's website]	Rapid delivery of nutrients require in muscles development	

Bionic Joint Support™	Life Enhancement, USA	Nanospheres made up of liposomal phospholipids used to deliver glucosamine, chondroitin sulfate, hyaluronic acid and copper salyicilate	Increase bioavailability and actively carry is nutrients into your joint structures.	
MesoCopper®	Purest Colloids, Inc.USA	Nano-sized copper suspended in pure deionized water	Various health applications	
Lypo-Spheric Vitamin C	LivOn Labs, USA	Vitamin C encapsulated in liposome Nano-spheres® Liposome made from fat soluble phospholipids	Protect the vitamin C from destruction in the digestive system hence greater bioavailability	
NanO Bio-Sim	Nano Health SolutionsUSA	Diatomaceous earth (contains silica) is uniquely treated and then nanoized and combined with a small amount of sugar cane and kosher distilled vinegar.	Boost immune system and has anti-parasite activity	
naNO Vapor	Iovate Health Sciences Research, Inc.USA	Nano-sized key components used in muscle building biomedically engineered with exclusive Nano-Diffuse™ technology	Used as pre-workout supplement, can easily deliver to muscles, triggers rapid gains in muscle size and boost strength	
Nanoceuticals™ Artichoke Nanoclusters	RBC Life Sciences®, USA	NanoClusters, a nanosized powder added to nutritional supplements	Scavenge more free radicals, stimulate the source of energy, increase hydration, balance the body's pH, reduce lactic acid during exercise, reduce the surface tension of foods and supplements to increase wetness and absorption of nutrients	
Aquanova® Novasol®	Aquanova®, Germany	Nano-micelle of lipophilic or water insoluble substances	Micelle is proving to be an optimum carrier system of hydrophobic substances for a higher and faster intestinal and dermal resorption and penetration of active ingredients	
ASAP Health Max 30	American Biotech Labs, USA	Nano silver particles	Helps support immune system as fast as possible.	

(continued)

Table 1.1 (continued)

Products	Company	Details	Application	Reference
HydracelTM	RBC Life Sciences®, Inc.USA	NanoClusters colloids	Greater nutrient absorption from your food and supplements, Reduced surface tension makes water wetter, Toxins are eliminated more effectively	
NanoCurcuminoids™	Life Enhancement, USA	Curcuminoids encapsulated with Solid-lipid nanospheres	Enhance solubility and absorption, targeted delivery of curcuminoids therefore increased bioavailability	
NanoResveratrol™	Life Enhancement, USA	Lipid nanospheres encapsulate lipophilic Resveratrol	Enhance solubility and absorption, targeted delivery of curcuminoidstherefore increased bioavailability	
Nanotrim™	NanoNutra™ Labs, USA	All natural, nano-scaled ingredients	Improve cellular health and the burning of fat for energy	
Nitro-Tech Hardcore	Muscletech sports nutrition supplementsUSA	Protein nanoparticles prepared by NanomolecularHyperdispersion Technology	Hyperaccelerate muscle metabolism and protein synthesis to build muscle faster than any other protein can to increase muscles size	
NanosiliceoKapseln	Neosino, Germany	Nanosiliceo capsules with silicon	Support the cell structure and cell metabolism, regulate the hydration of the skin and support the elasticity of the connective tissue to promote the supply of nutrients to support healthy life functions	

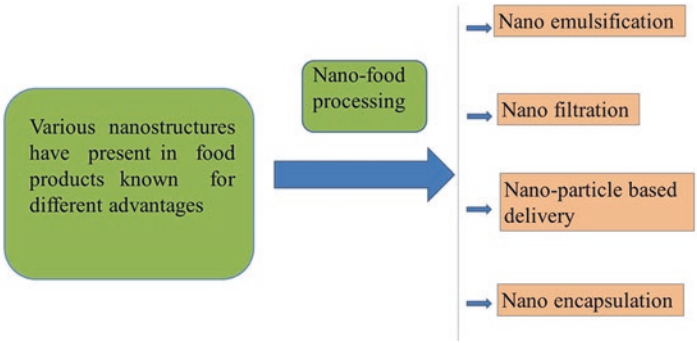

Fig. 1.1 Different forms processing methods used in development of functional nano-foods for specific functions. Various types of processing methods have been introduced such as nanoemulsions, surfactant micelles, emulsion layers, reverse micelles and functionally designed nanocapsules

To enhance the bioavailability of various functional ingredients such as vitamins, carotenoids, flavouring agents, prebiotics, antimicrobials, antioxidants, peptides and proteins, omega fatty acids, colorants and preservatives are incorporated into the delivery system instead of directly administrated in their pure form,(e.g. nanostructures) (Elliott 2002; Maddinedi et al. 2015; Nandita et al. 2015, 2016; Ranjan et al. 2015, 2016b, c; Babu Maddinedi et al. 2016; Dasgupta et al. 2016b; Janardan et al. 2016; Ranjan and Ramalingam 2016; Siripireddy et al. 2017; Tammina et al. 2017; Walia et al. 2017). The processing steps also degrade these active compounds which results into poor bioavailability. So for increasing the absorption of these functional food ingredients, nanoencapsulation and nanoemulsion of these compounds can be done. The inorganic compounds such as silicon dioxide (E551), magnesiumoxide (E530), titanium dioxide (E171), in their nano-structure form, have been used as oxygen and moisture barrier by coating the materials. Antibacterial activity of these compounds mainly of nanosilver also has been studied (Chaudhry and Groves 2010). Nanoclusters™ system of a variety of products has been launched in the market which increases the surface to volume ratio of the particles.

For example, slim shake chocolate with infused cocoa flavoured nano silica increases the absorption on taste buds to enhance the chocolate flavour. Another NanoCluster™, developed by RBC Life Sciences® Inc. (Irving, TX, USA), is used as delivery system for various ingredients in food products (Ranjan et al. 2014b).

The development of nanostructures for their uses in food products with different purposes such as (Chaudhry et al. 2008; Luykx et al. 2008; Weiss et al. 2008) (i) prevent the degradation of nutraceutical compounds during the manufacturing processes, storage and distribution and same time improve their stability, (ii) to enhance the sensory properties of food products and thereby develop new products with new consumer sensations such as develop new taste, texture, flavour enhancement, colour alteration and consistency. (iii) to improve the nutritional value of poorly soluble functional food ingredients (e.g. hydrophobic vitamins) by enhancing their

bioavailability, (iv) prevent the damages from oxygen and water by acting as barrier therefore increase in food shelf life (Leclercq et al. 2009), (v) to develop new food product with low fat, low carbohydrate, intended for low calories food product (e.g. mayonnaise, spreads and ice creams), (vi) to develop the control delivery systems for various food ingredients. Nanoencapsulation of active ingredients offers the controlled release of these substances at desired site in gastrointestinal space and prolong the retention time (Krasaekoopt et al. 2003; Medina et al. 2007). However, lipophilic ingredients such as phytosterols antioxidants and carotenoids can be easily solubilised in water by using nanotechnology (Chen et al. 2006a, b). Various compounds such as phytosterols, Lycopene and beta-carotenes are integrated with the nano-carriers, and are used in the production of healthy food products, mainly to prevent the deposition of cholesterol in our body (Gruère 2012; Ranjan et al. 2014a, b). The use of nanotechnology in the application of health added products gives a lot of health concern customers to industries. There are many nanoparticles of various metals and other compounds which have been created and accepted by Food and Drug Administration (FDA) and regarded as generally regarded as safe (GRAS) by FDA, example Lycopene nanoparticles of size 100 nm (US5968251). The health benefits from water dispersible lycopene nanostructure can be obtained when it used in soft drinks (Limpens et al. 2006). Another products with lycopene nanostructure aresuch as baking mixtures and blancmanges (Chaudhry and Groves 2010). Conclusively, it can be stated that the recent trends of nano-food focuses on encapsulation techniques, but at the same time it should have proper quality check and toxicity analysis before launching in the market.

Another type of ingredients such as ω-3 polyunsaturated fatty acids have very high nutritional value and very important for our body but these ingredients cannot be taken directly as in their pure form because these ingredients are very prone to degradation by processing steps. The stabilization and protection of ω-3 polyunsaturated fatty acids from the undesirable oxidation reactions can be done by using nanotechnology techniques (Ruxton et al. 2004; Lavie et al. 2009). Nanostructures of Casein micelles has been developed by Zimet et al. (2011) which have shown stability against oxidation of docosahexaenoic acid oxidation up to 37 days at 4 °C (Zimet et al. 2011). Bioral™ nanocochleate (a product launched by BioDelivery Sciences International) is an efficient delivery system for the nutrients. It composed of ~50 nm phosphatidylserine carrier, derived from soya beam. Along with the delivery this system also used for the protection of antioxidants and micronutrients from degradation during manufacturing process and storage (Chaudhry and Groves 2010). The low bioavailability of coenzyme Q10 (CoQ10) due to less absorption at desired site hence it cannot be reach at its therapeutic dose concentration (Maherani et al. 2012). The use of nanotechnology for CoQ10 compound have improved the therapeutic value and have shown great potential for use as first-line therapeutic agent for prophylaxis of consumers for better health (Ankola et al. 2007). The use of natural dipeptide antioxidants (e.g. L-carnosine) as biopreservatives have got some limits and cannot used directly in food as they are highly unstable. The interaction of peptide moiety with food components and proteolytic degradation make it highly unstable. The nanoencapsulation of these natural antioxidants by

nanoliposomes can overcome these limitations (Maherani et al. 2012). To deliver safe nano-foods nano-additives or stabilizers used in nano-foods should be of natural grade – which will ultimately support the delivery of safe nano-foods.

Wen and co-workers (2006) described the applications of liposomes in oral delivery of functional food ingredients like enzymes, antimicrobial compounds, proteins, and flavours (Wen et al. 2006). The entrapment of proteolytic enzymes in liposomes can reduce the time for the production process to half than normal processing time in cheese production (Mozafari et al. 2006), without compromising flavour and texture. The essential oil entrapment with in zein nanostructure permits the dispersion in water, thereby increasing their potential for use in food preservation as antimicrobial and antioxidant agents (Wu et al. 2012). A new technique of Self-assembly of the chitosan nanostructure with an edible polypeptide poly (γ-glutamic acid) has been developed by Tang et al. (2013), used in the manufacturing of food additives for drink, dietary supplements (Tang et al. 2013). Similarly this technique also can be used for the mask the odour and taste of the fish by entrappingthe fish oil into nanostructures (Chaudhry et al. 2008). Zein gelatine and casein nanoparticles have been synthesized for the delivery of hydrophobic bioactive compounds with distinct and ideal surface functionality and morphology and have huge potential for their uses in future for the delivery system (Chen et al. 2013; Ye and Harte 2013; Ye et al. 2014; Li et al. 2015).

The reduced requirement of stabilizers in nanoemulsions, enhances the quality of food since they protect ingredients against their breakdown and separation of food and thereby decrease requirement of fat (Cushen et al. 2012). Nanoemulsion products have the great taste and mouth feel and they are as creamy as normal food products. For instance the transportation of essential oils and flavour compounds (e.g. carvacrol and thymol) by zein nanostructure can be done (Sozer and Kokini 2009; Wu et al. 2012). Another objective of the nanostructure is to encapsulate the nutraceuticals compounds such as vitamins or to mask the unpleasant odour/flavour of α-lactalbumin nano-tube, which is extracted from milk protein (Graveland-Bikker and De Kruif 2006; Srinivas et al. 2010). Hence different type of nanostructures according to their origin can be used in food products and supplements for different purposes (Cushen et al. 2012).

1.4 Food Nanoadditives and Nanoingredients

The role of nanotechnology in food industries is very much important which enable them to developed food products with some value added quality. Nanotechnology in food development gives specific characteristics to food by altering the physiochemical properties and microscopic characteristics like color, texture, taste, strength, processability, water solubility, other sensory attributes, thermal stability, stability during shelf life, and oral bioavailability of functional compounds of natural food (Fig. 1.2) (Silva et al. 2012).

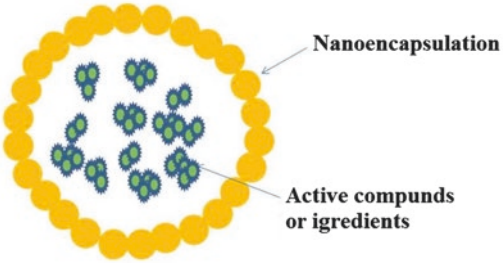

Fig. 1.2 Pictorial representation of food additives of food ingredients encapsulated in Nano-sized droplets or lipid nanoparticles. Nanotechnology in food development gives specific characteristics to food by altering the physiochemical properties and microscopic characteristics like color, texture, taste, strength, processability, water solubility, other sensory attributes, thermal stability, stability during shelf life, and oral bioavailability of functional compounds of natural food

The application of nanotechnology in the development of bioactive compounds with good bioavailability and increase their stability (by preventing degradation during processing and storage) and solubility. For instance the absorption and bioavailability of lycopene and resveratrol in body (Hsieh 2009) can be enhanced by using nanocristals of these compounds. Very high absorption has been shown in case of nanoparticulated nutrients which contains calcium nanoparticles containing calcium which show an excellent absorption rate (Jeon and Lee 2009). Nanoemulsions can be used to encapsulate functional food components, have great potential and received a great attention due to their ability to solubilize lipophilic molecules, e.g. coenzyme Q10, an oil-soluble vitamin-like substance; and enhance its stability and bioavailability (Yu et al. 2009a, b). Comparative study of authorized additives titanium dioxide (TiO2) or E-171 showed high visual transparency with high shielding against UV light when used at nanoscale crystals than microsize crystals (Latva-Nirva et al. 2009).

1.5 Nanoencapsulation of Nutrients and Their Delivery Mechanisms

Nano-encapsulation packing technique of substances in miniature which provides many benefits such as higher bioavailability, controlled release, prolonged residence time and high shelf-stability of active compounds. Similarly, nanocapsulation can reduce the quantity of active ingredients requires at particular site in human body due to efficient delivery.The protection of bioactive compounds such as protein, lipid vitamin, carbohydrate and antioxidants from unfavorable conditions, can be achieved using this technique for production of various functional foods with improved functionality, bioavailability and stability. Generally compound used in nanoencapsulation are not good for health, they should benon-toxic and biodegradable in the human body. There must be some toxicological safety evaluation which

declares whether a specific product is safe for human body or not. The proper regulation of testing of nanoparticles is currently not completely in practice and common toxicity tests may not be appropriate for nanoparticles hence new appropriate technique should be to test the nanoparticles. For the assurance of the effectiveness of the nanoencapsulated compound, that whether they are improving the specific characteristic of the compound or not, but few methods have been developed (Fig. 1.3) (Gutiérrez et al. 2013). One patented technique for nonoencapsulation has the ability to encapsulate bioactive and active compounds in neutraceutical products. These nanocapsule can be easily degrade at targeted tissues and deliver the active ingredients.Octenyl succinic anhydride-ε-polylysine has two role, first one is used as either emulsifiers or surfactants in the encapsulation of nutraceuticals compounds or drugs and second is the antimicrobial activity (Yu et al. 2009a, b).

Lipid based nano-encapsulates also used in food products/supplements includes nanoliposomes, nanocochleates and archaeosomes (at an experimental stage) (Mozafari et al. 2006; Ranjan et al. 2014b). Single and bilayer arrangements of liposomes have hydrophobic/hydrophilic interactions among lipid/water and lipid/lipid interfaces. They can easily entrap the water and lipid soluble materials and regulates their delivery and release due to their unique properties like small size and hydrophilic/hydrophobic in single compound (Mozafari et al. 2006, 2008). A very stable, efficient and precise delivery system is represented by "cochleates" – a phospholipid-divalent cation precipitate composed of naturally occurring materials, developed and patented by BioDelivery Sciences International Inc., Newark, NJ (Ranjan et al. 2014b).

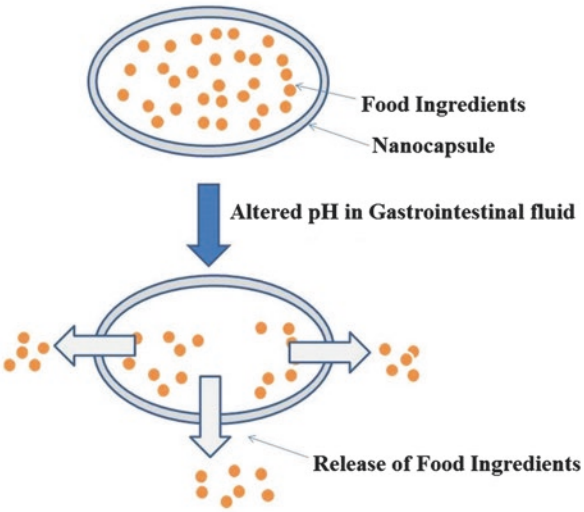

Fig. 1.3 Representative diagram to show the applications of nanotechnology in pH based release of active compounds. For the assurance of the effectiveness of the nanoencapsulated compound, that whether they are improving the specific characteristic of the compound or not, but few methods have been developed for targeted delivery of active food compunds

Nanocochleate is another system, has been developed to encapsulate the nutrient for their specific delivery. These structures are multi-layered and consist of large, continues and solid lipid bilayer sheet rolled up in spiral manner with little or no internal aqueous space (Mozafari et al. 2006). A soy based phospholipid of nanocochleates consist approx.75% weight of lipids such as phosphatidic acid, phosphatidyl inositol, phosphotidyl serine, dioleoylphosphatidyl serine, phosphatidyl glycerol and/or a mixture of one or more of these lipids with other lipids. Nonocoiled nature of nanocochleates stabilizes and protect various micronutrients by wrapping around them and improved their nutritional value of processed foods (Sekhon 2010). They fusion between outer layer of cochleate and cell membrane leads to the delivery of micronutrient they are carrying. Various type of bioactive material such as poor water soluble compounds, large hydrophilic molecules, protein and peptide drugs can be encapsulated with the nanocochleate structure (Moraru et al. 2003). Functional compounds such as nutraceuticals, antimicrobial and flavour can be encapsulated by the liposome (Were et al. 2003; Kalpana Sastry et al. 2013; Ranjan et al. 2014b), but the uses of non-food grade agents for the stability of structure in the preparation phase can be toxic for the cells and may left in cells after delivery (Mozafari et al. 2006). Lipid nanocarrier can entrap both lipid and water soluble vitamin which results into stability in different medium. It has been seen that nanoencapsulation of vitamin E into nanoliposomes with tea polyphenol (water soluble) give the good bioavailability (Ma et al. 2009). The combined nanoencapsulation of vitamin E with vitamin C has also has been done for the delivery of both vitamin together (Marsanasco et al. 2011). The major factors which influence the liposome's structure was incubation in buffer solution and stomach pH. The degradation of liposome at desire site influence the higher absorption of the bioactive compound and their greater bioavailability (Katouzian and Jafari 2016). Dietary phytoconstituents capsaicin loaded nanoliposome has been developed and evaluated against liver oxidative stress. It has been shown that liposome encapsulating capsaicin acts as a promising therapeutic agent in reducing liver oxidative stress produced by different stress factors (Fig. 1.4) (Giri et al. 2016).

The naturally occurring bioactive compounds in certain foods have physiological properties which can reduce the risk of various diseases including cancer. The compounds such as omega-3 and omega-6, probiotics, b-carotene, prebiotics, vitamins and minerals have been encapsulated by various method to enhance efficiency of these compounds their good bioavailability reflects gastrointestinal retention time extended due to better bio-adhesive capacity in the mucus that covers the intestinal epithelium (Gutiérrez et al. 2013; Bernardes et al. 2014). Nanocapsules have been developed to mask the unpleasant tuna fish oil taste and odour and to deliver it to stomach (Neethirajan and Jayas 2011). Various method of encapsulating bioactive compounds includes nanoemulsion, liposomes or nanocapsules etc. Nanoemulsion system can be of multi-phase, which corresponds to preparation of oil-in-water (O/W) emulsion means emulsions consist of oil droplets within an aqueous phase, improves solubility and bioavailability of bioactive compounds due to the reduction of incomplete dissolution of lipids and decreased droplets size (Yin et al. 2009), while liposomes are formulated with lipids and phospholipids (such as phosphatidyl

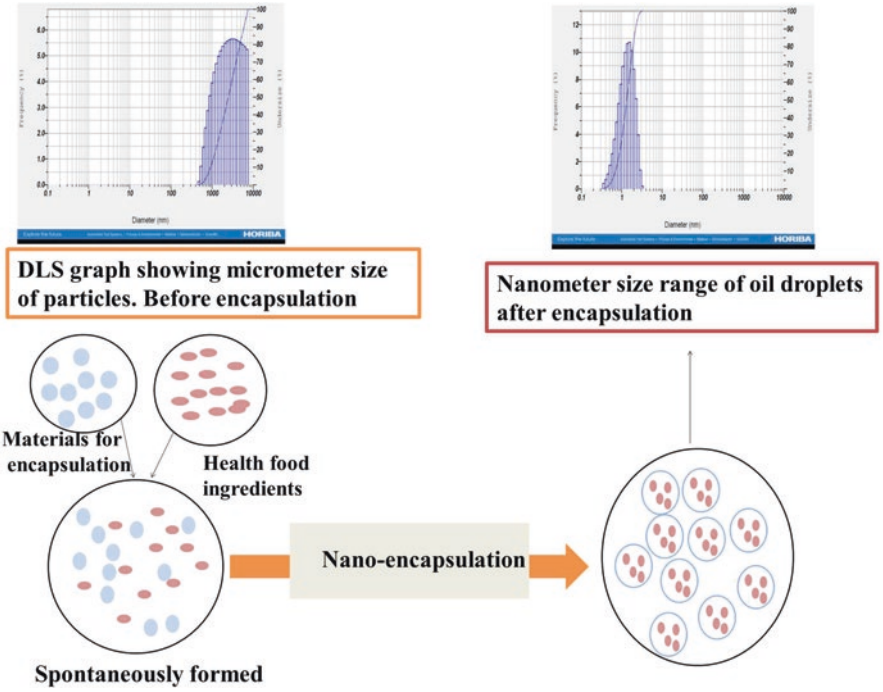

Fig. 1.4 Illustration for the basic mechanism and steps to be followed in nanoemulsion or nano-encapsulate fabrication. It has been depicted that the liposome encapsulating capsaicin acts as a promising therapeutic agent in reducing liver oxidative stress produced by different stress factors

choline) which can be in the form of single or multiple bilayers, providing protection to the compound (such as vitamins) encapsulated in their core (Soppimath et al. 2001). However, they are highly unstable in acidic pH conditions and therefore degraded rapidly in stomach, whereas liposome are thermodynamically unstable unlike nanoemulsions (Observatory Nano and Commission 2009).

Nanoencapsulated polymers can be formed by the molecule consistof regions (typically one hydrophobic and one hydrophilic monomer) with opposite affinities for an aqueous solvent. Some of the natural biopolymerslike albumin (protein), gelatin (protein) (Zwiorek et al. 2004), chitosan (saccharide), alginate (saccharide), collagen (protein) and the milk protein α-lactalbumin (Graveland-Bikker and De Kruif 2006) are examples of such nanoencapsulated polymer delivery systems. The ability of protein-based nano-encapsulates to bind easily with lipids, polysaccharides, or other biopolymers and form complexes, makes them interesting among all other nanostructures. They can also encapsulate the wide variety of nutrients like others (Chen et al. 2006a, b).

In addition, numerous copolymershave been developed to date, leading to the fabrication of nanocapsules, nanospheres, micelles, polymersomes (Letchford and Burt 2007). Although there are many nanostructures have been developed but a

fundamental understanding of interaction between polymer–polymer and polymer–nutraceuticalat the molecular level and their influence on functional properties of the delivery systems are essential to confirm the design of ideal nutraceutical carriers for use in the food industry.

Probiotics are generally referred as the mixture of bacterial species such as *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Bifido bacterium* spp. and present in dairy food like yoghurts, yoghurt-type fermented milk, cheese and puddings and fruit based drinks etc. Their viability in there foods products can be increased by nanoencapsulation. Nanoencapsulation is desirable to develop designer probiotic bacterial formulations that triggered their delivery to certain parts of the gastrointestinal tract where they interact with specific receptors (Kailasapathy and Rybka 1997; Vidhyalakshmi et al. 2009). The enhanced shelf life of probiotic organisms has been seen when nanoencapsulated with calcium alginate (Kailasapathy and Rybka 1997). Curcumin, a natural pigment present in turmeric and responsible for yellow colour has health benefits properties which can be enhanced more by encapsulation in nanoemulsions (Wang et al. 2009). The bioavailability of lycopene can be enhanced by fortifying nanoparticles of lycopene in tomato juice, pasta sauce, and jam (Auweter et al. 1999). Milk protein casein can act as a neutral nano-carrier and employed as a vehicle for delivering mineral nutrients such as vitamin D2 (Semo et al. 2007).

1.6 Efficient Antimicrobial Activity

The bioactive compounds (essential oils) are the natural antimicrobial agents and can be increased the shelf life of food products for some extent. But when the same bioactive compounds are used in nano range (nanoemulsion) form, they can exhibit increased antimicrobial activity as compared to the normal form. This increased antimicrobial activity of NEs is due to their very small droplet size (Ranjan et al. 2016a). Nanoemulsion of other various essential oils such as carvacrol, cinnamaldehyde and limonene have been shown antimicrobial activity against three different microorganisms *Escherichia coli*, *Saccharomyces cerevisiae*, and *Lactobacillus delbrueckii* (Ranjan et al. 2016a). On other hand, Silver has shown antimicrobial activity. Moreover its effect can be increased by combination with other antimicrobial substances such as extracts of grape fruit, purslane, or with essential oils (Kim 2006).

Silver acts differently from other antimicrobial and shows broad spectrum toxicity to numerous strains of bacteria, fungi, algae and it may to some extends for some virus. The mechanism of action of nanosilver on microbes is such that it brings structural changes in bacterial cell wall and nuclear cell wall due to its binding with tissue proteins and cause cell disruption and finally death. The binding of nanosilver to DNA and RNA can also cause the death by inhibiting bacterial replication (Rai et al. 2009). The use of the silver nanoparticles as sterilizer of freeze-dried foods (Fujime and Kubota 1985) has been seen in food companies. Other function of nano-silver is anti-ripening activity (Malshe and Malshe 2007).

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Chapter 2
Food Nanoemulsions: Stability, Benefits and Applications

Abstract Applied nanoscience has gained much attraction towards medical, pharmaceuticals, food and agriculture and also industries has received great attention from the scientific community. Sharp increase in consumers' demand for safer as well as healthier foods has given the opportunity to develop new products to encapsulate, protect and release the nutrition as well as active food compounds. Due to toxic effects of metal nano-particles, colloidal nanomaterials are more in use to fabricate food-grade nanomaterials – mainly nano-emulsions. Nanoemulsions found to be more efficiently bioactive than micro- or macro-emulsions. Nanoemulsion technology is well suitable for the stable and efficient encapsulation of active food component with increased preventive measure and improved bioavailability. Here in this review, (i) the stability factors, advantages and disadvantages of nanoemulsions have been discussed when used in food. (ii) the major applications of nanoemulsions in food have been discussed and also the recent researches, challenges and opportunities have been discussed.

Keywords Food-grade nanoemulsions • Delivery • Transport system • Texture • Stabilisy • Shelf life

2.1 Introduction

In the past few decades, intensive research interest has been directed for the use of nanotechnology in food processing industries because of the requirement to encapsulate, protect and release lipophilic bioactive component (Maddinedi et al. 2015; Babu Maddinedi et al. 2016; Dasgupta et al. 2016b, c, d; Janardan et al. 2016; Ranjan and Ramalingam 2016; Siripireddy et al. 2017; Tammina et al. 2017; Walia et al. 2017; Danie Kingsley et al. 2013; Ranjan et al. 2014, 2015, 2016a, b; Dasgupta et al. 2015, 2016a; Jain et al. 2016; Shukla et al. 2017).

By virtue of their fine droplet diameter, larger surface area to volume ratio, and novel physicochemical properties like thermo-dynamical variability and transparent appearance, nanoproducts such as nanocarriers, nanoemulsions, nanoliposomes etc. have been especially studied (Kong and Park 2011; Kong et al. 2011; Lam et al.

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2014; Jaiswal et al. 2015; Youssof et al. 2016). Nanoemulsions are thermodynamically stable, transparent (or translucent) dispersions of oil in water stabilized by an interfacial film of surfactant and co surfactant molecules having the droplet size less than 100 nm (Bali et al. 2011; Singh et al. 2013; Dasgupta et al. 2016b). Nanoemulsions have a number of advantages over conventional emulsions for certain applications due to their relatively small particle size. They scatter light weakly and so tend to be transparent or translucent (Anton et al. 2008). They have high stability to particle aggregation and gravitational separation so they don't undergo creaming, flocculation, coalescence and sedimentation (Mason et al. 2006). They also have unique rheological characteristics and can greatly increase the bioavailability of encapsulated lipophilic components (Walstra 1999; Ranjan et al. 2014; Dasgupta et al. 2015, 2016b).

Nanoemulsion is an emerging trend in food and pharmaceutical industries, used as an efficient delivery system for drugs and various lipophilic compounds. Nanoemulsions are kinetically stable liquid dispersions with its droplet size ranging from 50 to 500 nm (Lovelyn 2011). Due to its small droplet size, nanoemulsions are optically transparent systems. Nanoemulsions have an edge over conventional delivery systems as it shows high stability against coalescence or phase separation (Lovelyn 2011). It enhances the bioavailability and absorptive capacity of lipophilic compounds. Good understanding of the physiochemical properties of food nanoemulsions would give us an effective formulation technique to improve its application in food industries.

Nanoemulsions are bi-phasic systems constituting of an aqueous phase and an organic phase. Emulsions, by nature, are basically unstable and hence, addition of surfactants otherwise called emulsifiers is required which works by decreasing the surface tension between the two phases as surfactants bind both the phases together due to its amphiphilic nature. Surfactants are classified depending upon the hydrophilic "head groups" into: (a) ionic surfactants (b) non-ionic surfactants (c) amphoteric surfactants.

Hydrophilic-lipophilic balance can be used as a parameter for the selection of surfactants. Surfactants with low hydrophilic-lipophilic balance ranging from 3 to 6, are generally used for the synthesis of water-in-oil emulsions (w/o) and the ones with high hydrophilic-lipophilic balance (8–18) are used for the preparation of oil-in-water emulsions (o/w) (Setya et al. 2014). For instance, o/w emulsions are fabricated by allowing the oil (dispersed phase/organic phase) to disperse in the water (continuous phase/aqueous phase) and vice versa for w/o emulsions. O/W emulsions are formulated most commonly as they have more potential benefits within the commercial industry (McClements 2011). There are a wide variety of surfactants prevailing in the market that are utilized for nanoemulsion formulations. Surfactants used can be of different origin, from microbial, plant and synthetic background.

Formation of nanoemulsions is unspontaneous and thereby, requires external sheer to break down larger molecules into smaller ones (Lovelyn 2011). This can be achieved by two different approaches namely, high energy approach and low energy approach which has been discussed later in this thesis.

2.2 Nanoemulsions *Versus* Micro/Macro-Emulsions

Nanoemulsions are gaining great interest in the recent years for its tremendous properties. The main difference between conventional emulsion (particle diameter: 1–20 μm) and nanoemulsion (particle diameter: 10–500 nm) lies in the size of particles. They are considered superior over other conventional delivery methods for reasons like optical transparency, high stability, controlled release, resistance towards gravitational separation, coalescence, flocculation or phase separation (Fig. 2.1).

Nanoemulsions are more physically stable with no apparent coalescence and flocculation even when left undisturbed for a long time. Another reason for preferring nanoemulsions over micro/macroemulsions is that the smaller the particle size of the dispersed phase, the more stable the emulsion will be and the longer it will take to settle out of the emulsion. Most aroma molecules are soluble in fat phase. Therefore, the smaller the particle size of emulsion, the greater will be the surface area for aroma molecules to adsorb onto the fat surface.

Nanoemulsion preparation requires far lesser concentration of emulsifiers as compared to microemulsions (Tadros et al. 2004). Another interesting feature of

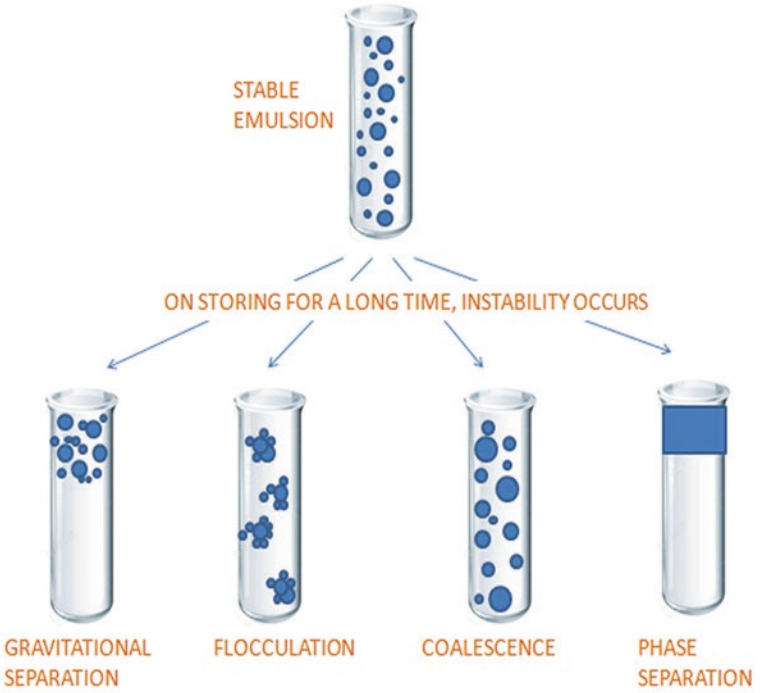

Fig. 2.1 Representation of commonly occurring physical instabilities in food emulsions. Long-term storage of nanoemulsions can cause instability that can be seen in many forms as depicted in this figure

nanoemulsion is that they are kinetically metastable *i.e.*, there is no change in the droplet size distribution even on diluting the emulsion.

Due to their nano-ranged droplet size as compared to conventional emulsions, they scatter light weakly and hence, appear optically transparent. This property gives them an edge over conventional emulsions which are opaque/turbid in nature as their droplets scatter light strongly (McClements 2011). This allows nanoemulsions to be incorporated in optically transparent products, widely in beverage industries and cosmetic industries. These reasons give nanoemulsions an edge over micro/macro emulsions.

Apart from these advantages, fabricating nanoemulsion for food based products is more of a random process. There is a need to optimize conditions for making a desired nanoemulsion. One has to consider several factors including the type of surfactant, oil, phase concentrations and the physical parameters to obtain a stable nanoemulsion. Even selection of proper oil and surfactant is also a confusing step. The ratios of phase concentration play a major role in determining long-term stability of nanoemulsion. The properties of oil will determine the matrix to encapsulate the active compound whereas the surfactants form an interfacial membrane around droplets of the dispersed phase which inhibits coalescence (Barradas et al. 2014). Moreover, the food material itself is subjected to a number of mechanical agitations in the process of manufacturing, handling and storage.

The worldwide sales of nano-foods, beverage and packaging sector increased from US$ 150 million in 2002 to US$ 860 million in 2004 and have reached nearly US$ 20.4 billion by 2010 (Helmut Kaiser Consultancy 2015a). Further it has been expected to exceed by three to five folds by 2040 (Helmut Kaiser Consultancy 2015b). According to the report, the existing applications are mainly for improved food packaging, with some applications of nutraceuticals delivery systems. The report estimated that by 2020 the overall market value would reach more than US$5.8 billion (Ranjan et al. 2014; Dasgupta et al. 2015). More than 200 companies/private sectors are actively involved in research and development to develop novel Nano-foods – lead by USA followed by Japan and China (Helmut Kaiser Consultancy 2015a, b). There is a major scope for growth in the nano-food sector mainly in developing countries. Many of the leading food industries including H.J. Heinz, Nestlé, Hershey, Unilever, Coca-Cola, and Kraft foods etc. are investing heavily in nano-food technology research and development.

Nanoemulsion technology has been applied to food processing industries for quite a long time including butter, cream, fruit beverages, soups, salad dressings, mayonnaise, salad cream, ice cream, spreads, margarine etc. However, research is now extended to diverse fields of food processing with nanoemulsion with more focus on increasing bioavailibilty of lipophillic compounds (Fig. 2.2). In this review we have discussed in details about the current research trends, future posbilities, challenges and opportunities in the field of – nanoemulsion fabrication and characterization, day to day – and market-applicability, rules and regulatory guidelines of globally accepted regulatory agencies. This review also have concluded about the benefits of nanoemulsion based products on human and animal health.

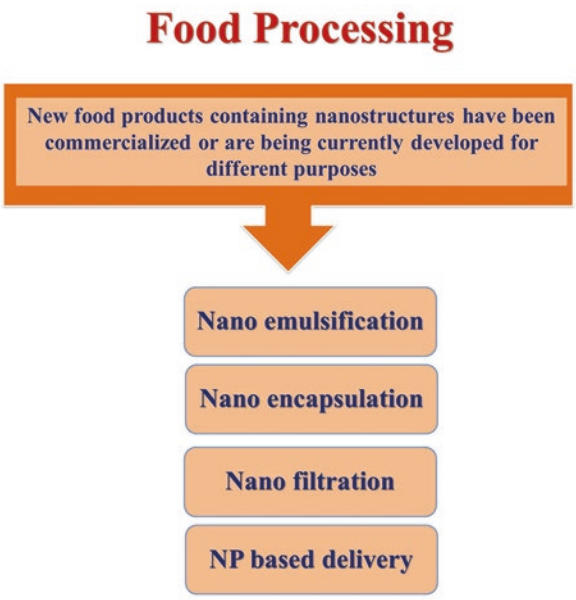

Fig. 2.2 Major types of nano-structures or processes which are being used to develop nano-foods to be launched in market (*Adopted from:* Dasgupta et al. 2016a). It can be noted that, research is now extended to diverse fields of food processing with nanoemulsion with more focus on increasing bioavailibilty of lipophillic compounds

2.3 Nano Emulsions in Food

Nanoemulsion provides a wide array of applications to be explored for food industries. It is now possible to encapsulate various lipohillic components for different end uses such as for increasing bioavailability, stability or fortification. A detailed description has been discussed regarding the significance of nanoemulsions with reference to application in food industries. The applications of nanoemulsions in food and drug has been discussed extensively in the book published by the researchers (Ezhilarasi et al. 2013; Anandharamakrishnan 2014; Ranjan et al. 2016c, d). For improving the solubility, stability, bioavailability, and efficient utility of many bioactive compounds edible/food-grade/drug-grade nanoemulsions establish one among the most promising and challenging systems. Using many novel technologies these improvised nanoemulsion's properties can be achieved which will be applicable for diverse industrial applications. Additionally, the main challenge to the researcher is to standardize suitable handling operations and facilities for the scaling-up to industrial level production to overcome its wide utilization. The major application other than different routes of drug delivery (for bio-efficacy of the bioactive compounds) is the improved bioactivity primarily antimicrobial activity.

Nanoemulsions consist of a lipid phase dispersed in an aqueous continuous phase, with each oil droplet being surrounded by a thin interfacial layer generally

consisting of emulsifier molecules. The application of nanotechnology in the food field may lead to the modification of numerous macro-scale food characteristics, such as coloring strength, taste, texture, shelf life stability and other sensory attributes, leading to a huge number of new products.

Reducing the size to nano-range considerably enhances the properties of bioactives like its solubility, stability, releasing capacity, targetability and prolonged residence time in the GI tract. Nanotechnology has been useful in agri-food field in many ways such as food processing, food additives, flavorings, food packaging, development of novel foods and pesticides (Handford et al. 2014). Usually, nanoemulsions are highly stable to gravitational separation as the relatively small particle size demonstrates Brownian motion that effectively dominates gravitational forces and thereby, particles resist to settle down demonstrating fairly good physical stability (Gadhave 2014).

Food companies have been using nanoemulsion in the development of food products since a long time to improve their properties like texture, colour, flavour, odour, solubility, bioavailability and shelf life. Titanium oxide is a widely used whitening agent to be added in cheese, yogurts and chocolates. In terms of food processing, silver nanoparticles have been incorporated into the food processing systems to prevent microbial contamination (Handford et al. 2014).

Nanotechnology can also improve the water solubility, thermal stability, and oral bioavailability of functional compounds. Functional compounds which claim to provide health benefits as they can help in prevention or treatment of diseases are already available in the marketplace in the form of capsules or tablets. It has become evident that these solutions may not sustain all the health benefits mainly because of their low bioavailability, which is particularly the case with lipophilic compounds. Improving the bioavailability of functional compounds is often related to enhancing its absorption in the gastro-intestinal tract. The advancement in nanotechnology offers several possible solutions for improving water solubility and bioavailability *e.g*., of lipophilic functional compounds such as nutraceuticals, drugs, flavors, antioxidants, and antimicrobial agents.

In regards to food packaging, nanoparticles can be incorporated (resulting into the concept of nanopackaging) by using antimicrobial compounds to prevent food pathogens and thus, increasing the shelf life of the food product. This has greatly reduced the amount of food wastage as food disposal would be minimized with increased shelf life of food. Besides this, nanosensors incorporated in packaging material helps in the determination of food spoilage and hence, would act as a quality control measure, preventing any compromise with human health.

2.3.1 Increased Bioavailability, Bioefficacy and Oral Route of Delivery

Nanoemulsion formulations shows several benefits for conventional oral administration/oral drug delivery (ODD) such as increased absorption, improved clinical potency, and decreased drug toxicity and therefore reported as ideal way for ODD

for drugs mainly for steroids, hormones, diuretic and antibiotics. Several pharmaceutical drugs with high potency and specific mechanism of action (mainly peptides and proteins based drugs) are difficult for ODD. ODD in conventional formulation (non- nanoemulsion based) causes 10% or lesser drug as therapeutically active. Because of these problems most of the protein and peptide based drugs are available for PDD, however, peptide drugs have an extremely short biological half life when administered parenterally, so require multiple dosing. Some of the examples for NE-based drugs for ODD which have replaced the existing drugs as mentioned below. A nanoemulsion formulation of cyclosporine, named Neoral® has been introduced to replace Sandimmune®, a crude oil-in-water emulsion of cyclosporine formulation. Neoral® is formulated with a finer dispersion, giving it a more rapid and predictable absorption and less inter and intra patient variability (Singh and Vingkar 2008; Ganta et al. 2010; Sun et al. 2012; Yu and Huang 2012; Dasgupta et al. 2014; Jaiswal et al. 2015).

Bioavailability is an important measure to ensure biological efficacy of the active food compounds as well as oral drugs. Several complex stages involved in the efficient bioavailability of nanoemulsions e.g. emancipation, absorption, distribution, metabolism, and elimination (Shah et al. 2010; Rein et al. 2013; Soltani et al. 2016). The bioavailability of lutein nanoemulsions with 150 nm of droplet diameter was analyzed on human subjects who consumed a lutein supplemented pill or a lutein nanoemulsion fortified to orange juice (Study 1 and 2 with dose range of 6 mg/day and 2 mg/day respectively for 1 week). In study 1, after consuming the 6 mg supplement and nanoemulsion the mean serum lutein concentrations increased by 104% and 167% respectively. Correspondingly, in study 2 the increased lutein serum concentrations by 37% and 75% was observed for the consumption of 2 mg lutein supplement and nanoemulsion respectively. Although there might be the lutein loss occurs nanoemulsion preparation still nanoemulsion consuming subjects showed more serum lutein concentrations corresponding to subjects receiving the lutein supplement from which it can be concluded that lutein nanoemulsion has significantly more bioavailability than the pills supplemented with lutein (Vishwanathan et al. 2009; Arunkumar et al. 2013; Uzun et al. 2016).

Nanoemulsions are the potential method to deliver bioactive compounds with more efficacies due to its capability to enhance bioactive solubilization and absorption in the gastrointestinal tract (GIT), due to the reason of surfactant-induced permeability changes. The fraction of nanoemulsions available for absorption determines its bioactivity, which is ultimately decided by measure of its specific biological affects (*in vitro* and *in vivo*). It can be said that, improving the bioavailability of bioactive food/drug compounds through nanoemulsions can finally improve their bioefficacy of nanoemulsions (Rein et al. 2013). The bioavailability of coenzyme Q10 nanoemulsion – with the droplet size of 60 nm – has been reported in an *in vivo* analysis to be 1.7 times higher bioactive than its crystalline form(Hatanaka et al. 2008). Likewise, nanoemulsion for antioxidant synergy formulation (ASF), containing delta-, alpha- and gamma-tocopherol has been formulated and analyzed through *in vivo* analysis for their influence on anti-inflammatory activity and bioavailability and has been reported for exhibited enhanced anti-inflammatory properties com-

pared with their suspensions. Additionally, nanoemulsions of gamma-tocopherol and delta-tocopherol showed a significant consequence – i.e. they have shown their activity with enhancement of 2.2- and 2.4-fold, respectively in comparison to the suspensions (Kuo et al. 2008). Similar to this study, curcumin nanoemulsions has been fabricated through high-speed homogenization (619 nm) and high-pressure homogenization (80 nm) to check enhanced anti-inflammatory activity and was found to show 43% and 85% inhibition, respectively, of mouse ear edema induced by 12- O-tetradecanoylphorbol-13-acetate (Wang et al. 2008). Recently, curcumin lipid nanoemulsions by a customized thin-film hydration method using hydrogenated l-α-phosphatidylcholine, soybean oil, and co-surfactants was fabricated with droplet size of 47–55 nm droplet size which has shown stability for 60 days at 4 °C with respect to particle size. The fabricated curcumin nanoemulsion exhibited more efficient anticancerous activity compared to curcumin solution in cell line based analysis using leukemic cell lines and B16F10 (Anuchapreeda et al. 2012). Likewise, with the target to enhanced antitumor activity and bioavailability, nanoemulsion has been fabricated using a natural flavonoid, fisetin (3,3′,4′,7-tetrahydroxyfl avone) and found to be stable at 4 °C for 30 days with droplet diameter of 153 nm. *In vivo* pharmacokinetic studies using mice model reported that the said nanoemulsion had 24-fold increased relative bioavailability than free fisetin when administered intraperitoneally. Interestingly, it exhibited antitumor activity in mice at lower doses (37 mg/kg) than free fisetin (223 mg/kg) (Ragelle et al. 2012).

Nanoemulsions-based systems are particularly suitable for encapsulating and delivering lipophilic bioactive components. A high-pressure homogenisation method was used to prepare lycopene-enriched nanoemulsions stabilized by globular proteins or non-ionic surfactants (Ribeiro et al. 2003; Bou et al. 2011). Recently, a high pressure homogenisation method was used to prepare β-carotene enriched nanoemulsions stabilized by small molecule surfactants (Tween 20 and decaglycerol monolaurate) and biopolymers (modified starch) (Yuan et al. 2008; Mao et al. 2009). Membrane homogenisation methods have been investigated as an alternative means of encapsulating carotenoids (astaxanthin) in nanoemulsions due to their ability to produce narrow particle size distributions, low energy requirements, and mild processing conditions (Henelyta et al. 2005). High-pressure homogenisation has also been investigated as a means of preparing lutein-enriched emulsions stabilized by proteins (Batista et al. 2006) and phospholipids (Losso et al. 2005). Nanoemulsions containing carotenoids have been prepared using combined homogenisation/solvent displacement and high pressure homogenization methods (Hélder et al. 2011). Commercially, colloidal dispersions containing β-carotene are typically stabilized against chemical degradation by adding antioxidants, reducing oxygen levels, and minimizing exposure to light and pro-oxidants. However, once a sealed product is opened and exposed to the atmosphere some of these protective measures may be lost. Qian et al., have developed β-carotene nanoemulsions with orange oil as the carrier oil phase and globular protein (β-lactoglobulin) as the emulsifier and found that β-Carotene degradation was considerably slower in β-lactoglobulin-stabilized nanoemulsions than in Tween 20-stabilised ones (Qian et al. 2012). Long-chain

omega-3 polyunsaturated fatty acids were nanoemulsified and have shown increased bioavailabilty than their bulk counterpart (Lane et al. 2014).

Curcumin nanoemulsions were made with organogel and Tween 20 (as emulsifier). The lipolysis profile revealed that the digestion of nanoemulsion was significantly faster and *in vivo* pharmacokinetics analysis confirmed that oral bioavailability of curcumin in the nanoemulsion increased by 9-fold compared with unformulated curcumin (Yu and Huang 2012). Coenzyme Q10, also known as ubiquinone, an important component of the electron transport chain, is a vitamin like substance and possesses antioxidant property. To test bioavailability of nanoemulsion with a standard commercial CoQ_{10} product, it was tested with different food grade oil and emulsifiers. It was demonstrated that emulsion with coconut oil (as oil phase), skim milk aqueous solution (as aqueous phase), and calcium stearoyl-2-lactate (as emulsifier) produced an optimal formulation (Thanatuksorn et al. 2009).

2.3.2 Controlled Release

Nanoemulsions are being used to incorporate and release of functional/bioactive compounds – nutraceuticals, vitamins, flavors, antimicrobials, drugs etc. – and this ability to incorporate and release depends on the composition, properties, and microstructure of the nanoemulsions as well as the molecular characteristics of the functional components (McClements 2011). This section discuss about the controlled release of lipophilic components – non-volatile with full and partial lipophilic characters and volatiles – through nano- encapsulation.

The concentration of volatile components (esp. aromas) is important in headspace – above the product – to determine the flavor perception and aroma. The equilibrium among the partition coefficient of air-water and oil-water determine the initial concentration of aromatic component in the head space. Lipid concentration has important consequences for flavor perception and the head-space concentration of non-polar volatile decreased with increase in lipid concentration (Walstra 1999). It can be noted that, the time taken to release such volatile is also greatly depends upon lipid concentration. This can be attributed by the fact that a low fat product gives an strong initial burst release of volatile while a high fat product gives a sustained release (Malone et al. 2003a, b; Sari et al. 2015). It can be concluded that by limiting the lipid concentration we can check the release profile of the volatiles. It the same time, the same may not be true for nanoemulsion based products as the size of the droplets is very small in case of nanoemulsion and rate of diffusion of flavor/volatile/aroma molecules is very fast (Weiss et al. 2008; Mao et al. 2009; Choudhury et al. 2014; Ma et al. 2016).

The Non-volatile bioactive compounds (with partial hydrophilic character) can be released by simple dilution. After the dilution of nanoemulsion, to balance the oil-water partition coefficient – some of the bioactive compound will move from oil droplet to the aqueous phase of system which may be considered as meachanism of release of such non-volatile (Walstra 1999; Boon et al. 2008; Maher et al. 2015).

Alternatively, the non-volatiles with higher oil-water partition coefficients (i.e. more lipophillic) may not move on dilution but digestion of lipid phase plays a mojor in teir release and the mixed system of micelles and liposomes can be formed. In the earlier case release rate greatly depends on rate of dilution while in the later case it depends on rate of enzyme digestibility of the nanoemulsion droplets. In comparision with conventional emulsions the nanoemulsions will be digested more ravidly since the nanoemulsion droplets have much higher oil-water interfacial area. Very few studies are there to deal with the behavior of nanoemulsions within GIT which have been reviewed earlier (Ganta et al. 2010; Donsì et al. 2012; Dasgupta et al. 2014, 2016a; Sari et al. 2015).

In case of non-volatiles (with more hydrophilic character), head-space concentration is of least interest while the release of non-volatiles in human body – aqueous phase of gastrointestinal tract (GIT) – is the main focus. There are number of trigger mechanisms which also control the extent and rate of release; some of them can be listed as – dilution, pH, ionic strength, temperature, enzyme activity, etc. It can be noted that, the increase in the concentration of bioactive encapsulated compound in aqueous phase of GIT or in some target organ determines its release efficiency as a function of time (McClements 2011; Jain et al. 2016). Interestingly, it can be noted that release profile of non-volatile bioactive compounds is entirely different in case of nano-emulsion as compared to conventional emulsions. It has been found that by the decrease in the droplet size the solubility of the non-volatile bioactive component increase by many folds which ultimately alters the oil-water partition coefficient of the encapsulated compound within the nanoemulsion and affects the bioavailability and release profile. It can be noted that the rate of release can be determined by relating the total time taken to diffuse of half of the bioactive components to the system. Additionally, it should be highlighted that for nanoemulsions with droplet size less than 100 nm – the release rate is too faster (Walstra 1999; McClements 2011; Sari et al. 2015) which can be slowed down by some extent by using highly impermeable and thick coating surrounding the lipid droplets. Interestingly, the droplets may be encapsulated in the hydrogel particles that increase the path length that compounds must diffuse and ultimately which increase the time taken to release the active component to slow down the rate of release (Malone et al. 2003a, b; Komaiko and McClements 2015).

2.3.3 Inhibits Oxidation

Various anti-oxidants (natural or chemical) are emulsified mainly for two reasons: to enhance the nutritive quality of the product or to prevent the food particles from oxidative damage and thus increasing the shelf life of the product. Lipid-based nanoencapsulation systems enhance the performance of antioxidants by improving their solubility and bioavailability, *in vitro* and *in vivo* stability, and preventing their unwanted interactions with other food components. Examples of lipid-based nanoencapsulation systems for the protection and delivery of foods and nutraceuticals are nanoliposomes, nanocochleates and archaeosomes (Mozafari et al. 2008; Maher et al. 2015).

A nanoliposome is an artificially-prepared vesicle composed of phosphatidylcholine – enriched phospholipids with the size range of a few nanometers. It may also contain mixed lipid chains with surfactant properties. They can be prepared by disrupting biological membranes of single cell organism and can also serve as encapsulating material (Torchilin 2012; Perche and Torchilin 2013). Nanocochleates are cylindrical microstructures consisting of a series of lipid bilayers with little or no internal aqueous space. They are stable phospholipid-cation precipitates. It is made up of naturally occurring materials like phosphatidylserine and calcium, so it's most unlikely for nanococleates to trigger immune response (Dwivedi et al. 2009; Hussain et al. 2012; Bhosale et al. 2013). Archaesomes are liposomes made from the polar lipids extracted from the membranes of *Archaea*. They have better physic-chemical stability such as thermal, pH, oxidative stress and against phospholipases and bile salts than conventional liposomes (Patel and Chen 2006; Tubesha and Ismail 2014; Li et al. 2015).

2.3.4 Stability

The most important aspect of nanoemulsion is to be dispersed uniformly in the solution. Although nanoemulsions have extreme Laplace pressures, of order 10–100 atm, the droplets can remain stable against Ostwald ripening, due to strong Brownian motion of the tiny droplets. Nanoemulsions are ideal for products in which gravitational creaming must be prevented to ensure a long shelf life (Graves et al. 2005; Li et al. 2015). If oil and water are mixed and small oil droplets are formed and dispersed throughout the water, eventually the droplets will coalesce to decrease the amount of energy in the system. However, if solid particles are added to the mixture, they will bind to the surface of the interface and prevent the droplets from coalescing, thus causing the emulsion to be more stable (Dickinson 2010; Nazarzadeh et al. 2013).

Various properties of the solid particle affect the stability of the emulsion. The angle of the particle's contact with the surface of the droplet is a characteristic of the hydrophobicity. If the contact angle of the particle to the interface is low, the particle will be mostly wetted by the droplet and therefore will not be likely to prevent coalescence of the droplets. (Wetting is the ability of a liquid to maintain contact with a solid surface, resulting from intermolecular interactions when the two are brought together.) The stabilization energy of an emulsion is given by

$$\Delta E = \pi r^2 \gamma_{OW} \left(1 - \left|\cos\theta_{OW}\right|\right)^2$$

where, r is the particle radius, γ_{OW} is the interfacial tension, and θ_{OW} is the contact angle. When the contact angle is approximately 90°, the energy required to stabilize the system is at its minimum (Velikov and Velev 2011; Ozturk et al. 2015).

Nanotechnology has major role to provide a stable emulsion with more shelf life of the product. In this section we have discussed the role of nanotechnology and the factors to be measured/checked to get more stabilized emulsion phase. This is the most

common form of physical instability in nanoemulsions which takes place because of number of causes – creaming and sedimentation which depends on the relative densities of the surrounding aqueous phase and the oil droplets. Creaming is an upward movement of droplets with the condition when the droplets have lower densities than the aqueous phase. Generally, oil phases used in nanoemulsions have lower densities than water which leads to the major factor for creaming (McClements and Rao 2011; McClements and Xiao 2012; McClements 2013; Tabibiazar et al. 2015). Further creaming may leads to ringing i.e. growth of visible ring of oil on the top of the product. The reason behind ringing may be attributed as the large diameter of the droplet in the initial emulsions.or droplet growth during the storage. Alternatively, sedimentation will take place when the droplets have a higher density than the aqueous phase and is a downwards movement of droplets. More elaborately, it can be stated that when NE contains excess amount of weighing agent within the oil phase then sedimentation will be more prominent. Another major factor reason behind the sedimentation is that if the nanoemulsions have smaller radius of oil droplets which will be covered by thick and dense interfacial layers (McClements and Xiao 2012; McClements 2013; Choudhury et al. 2014; Guttoff et al. 2015; Li et al. 2015). To decrease the upward and downward movement of oil droplets it has been suggested to keep smaller diameter of droplet size, lesser the density contrast and more viscous aqueous phase.

Apart from upward and downward movement of droplet which ultimately depends on the relative density of oil phase and water phase, Brownian motion is also one of the major factors for nanoemulsion stability. Brownian motion is defined as the random movement of the particle throughout the system irrespective of gravitational pull. It can be noted that when the droplet size is lesser than 100 nm then Brownian motion is more prominent while for more than 100 nm gravitational pull dominates (McClements 2011; McClements and Rao 2011). Practically it can be stated that lesser the droplet diameter, lesser the gravitational movement more will be the Brownian movement which leads to more stability. Additionally, the volume density of the shell layer may be increase which leads to increase in the overall particle density and supports Brownian motion to give more stability. This plays a major role to increase the stability for the nanoemulsion with smaller droplet diameter. Also, it has been suggested to use density matched droplets which can be controlled by the thickness of the adsorbed emulsifier layer and the core size of oil (Piorkowski and McClements 2013). All the above approaches discuss the role of nanoemulsions to increase the stability by decreasing the gravitational separation.

2.3.5 Antimicrobial

Many antimicrobial agents, whether natural or chemical have been emulsified and evaluated. Emulsions are considered as antimicrobial agents on the basis that bacteria cannot survive in pure fat or oil and that water is necessary for their growth and reproduction. Possible mechanism of action can be that these discrete droplets may selectively fuse with bacterial cell walls or viral envelopes destabilizing the

organism's lipid envelope and initiate disruption of pathogens (Al-Adham et al. 2000; Vijayalakshmi et al. 2014; Dasgupta et al. 2016b; Ma et al. 2016). Nanoemulsions have broad spectrum and non- specific antimicrobial activity. Thus, it is likely that nanoemulsion would not result in the development of resistant strains. Numerous reports on the bactericidal activity of nanoemulsions against clinical pathogens such as *Haemophilus influenzae*, *Bacillus cereus*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, and *Vibrio cholerae* and against food borne pathogens such as *Staphylococcus aureus*, *P. aeruginosa* and *Listeria monocytogenes* have been documented (Al-Adham et al. 2000; Fellows et al. 2001; Joe et al. 2012a; Neeru et al. 2014; Vijayalakshmi et al. 2014; Sugumar et al. 2015; Dasgupta et al. 2016b).

Sporicidal and antifungal activity of nanoemulsion has also been evaluated. Surfactin based sunflower nanoemulsion was found efficient in killing the Bacillus spores. It was observed that spore coat and cortex was disrupted with disintegration of the core contents by nanoemulsion treatment. They further reported that the initiation of germination could be inhibited by the action of nanoemulsion (Hamouda et al. 1999; Landry et al. 2014). The antifungal activity of selected nanoemulsion have showed highly effecient against selected food associated fungal pathogens such as *C. albicans* and filamentous fungi including *Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum, Aspergillus fumigatus and Fusarium oxysporum*. This inhibitory effect is due to the fact that nanoemulsions can act on fungi by disturbing fungal hyphae and spores and thereby resulting in reduced viability (Ciotti 2008; Joe et al. 2012a; Shivendu et al. 2016a, b).

Research studies have well established the use of bioactive compounds (essential oils) as natural antimicrobial agent which can further be used to increase the shelf life of the food, which have been described further in this section. Interestingly, the same bioactive compound in nanoemulsion form exhibit enhanced antimicrobial activity than the conventional form due to minute droplet size. The mechanism of action can be explained as, the small nanoemulsion droplets fuse selectively with bacterial cell membranes or viral envelops and thus destabilizing the lipid shroud and initiating their disruption (Baker et al. 2003; Baugh et al. 2006; Salvia-Trujillo et al. 2013; Dasgupta et al. 2016b). Corresponding to the same mechanism of action, nanoemulsion may potentially enhance the passive cellular absorption mechanisms, finally reducing the mass transfer resistances and increasing the antimicrobial activity (Donsì et al. 2012; Vijayalakshmi et al. 2014; Sugumar et al. 2015). The antimicrobial activity was investigated for nanoemulsions prepared with various essential oils (carvacrol cinnamaldehyde and limonene) against three different microorganisms (*Escherichia coli*, *Saccharomyces cerevisiae* and *Lactobacillus delbrueckii*). Different emulsifiers (a combination of Tween 20 with glycerol monooleate, lecithin, sugar ester and pea proteins) was used to stabilize the nanoemulsion of mean droplet size of about 100–200 nm. Nanoemulsions stabilized withcombination of Tween 20 and glycerol monooleate or sugar esters exhibited higher bactericidal activity over short time scales (2 h) whereas nanoemulsions stabilized with pea protein or lecithin exhibited bactericidal activity over a longer time scale (24 h). The concentration of bioactive compounds in the aquous phase correlates the emulsifier capability as well as antimicrobial activity (Donsì et al. 2012). Further as extension of the investigation,

they have investigated the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of nanoemulsions fabricated with terpene mixture and d-limonene against similar classes of microorganisms and it was reported that the antimicrobial activity is dependent on emulsion droplet size as well as with the class of microorganism. The application of the most proficient antimicrobial nanoemulsions was tested in pear and orange juices (Donsì et al. 2011).

Recently, nanoemulsions have been fabricated using selected cooking oils such as sesame oils, castor, sunflower, groundnut and coconut using surfactin (a biosurfectant) i.e. surfactin-based sunflower oil nanoemulsion (SSON) was with particle size of 72 nm, highly transparent and survived thermodynamic stability tests with low viscosity, surface tension, and density and has shown the highest antibacterial activity against *Salmonella typhi*, followed by *Listeria monocytogenes*, and *Staphylococcus aureus.* Additionally, it exhibited highest antifungal activity against *Rhizopus nigricans*, followed by *Aspergillus niger* and *Penicillium* sp. and a greater sporicidal activity against *Bacillus cereus* and *Bacillus circulans*. SSON was also analyzed for having antimicrobial activity over several food products (raw chicken, apple juice, milk, and mixed vegetables) showed a significant reduction in the bacterial and fungal populations on these foods (Joe et al. 2012a). Further, the influence of SSON was analyzed on the chemical, proximal, microbiological, and sensory qualities of Indo-Pacific king mackerel (*Scomberomorus guttatus*) steaks stored at 20 °C over a time period of 72 h and found that the treatment with the SSON decreased the values of chemical spoilage indicators during the storage period, and organoleptic evaluation exhibited an expansion of shelf life up to 48 h compared with antibiotic-treated samples and control (Joe et al. 2012b). Similar to this, antimicrobial activity of eucalyptus oil nanoemulsion (with droplet size of 16 nm was studied against three different microorganisms – *B. cereus*, *S. aureus*, and *E. coli*(Sugumar et al. 2013). Even at 10 folds of dilution, the nanoemulsion was showing 100% bactericidal activity. Likewise, Karthikeyan et al. have investigated the MIC and MBC of soybean oil nanoemulsion with droplet size of 308 nm against *Candida albicans*, *Lactobacillus casei, Streptococcus mutans*, *Actinomyces viscosus*, and mixed culture. However, higher MIC was observed for *S. mutans* and *A. Viscosus* (Karthikeyan et al. 2011; Ramalingam et al. 2012).

2.3.6 Inhibits Flavor Loss

It is difficult to keep the active flavor in foods during storage conditions. The quality and strength of the flavor of food products decreases due to physical and chemical processes (De Roos and Mansencal 2003; Komaiko and McClements 2015). Physical processes include diffusion of flavor compounds out of the food matrix or physical binding of flavor compounds by components of the food while chemical processes comprise the degradation of flavor compounds by oxidation, hydrolysis or chemical reactions with the food matrix (Winkel 2009; Jafari et al. 2013; Dasgupta et al. 2016a). Furthermore, transparent additives are preferred in

commercial application, such as food and beverages (Soottitantawat et al. 2003). Nanoemulsions can be used to incorporate non-polar functional components into food and beverage products without any change in its original colour (Chen et al. 2006; Hailong and Huang 2013).

D-limonene is used in food manufacturing and some medicines as a flavoring to mask the bitter taste of alkaloids. It has emulsified by both high energy and low energy approaches. By high energy approach, nanoemulsions are prepared by microfluidization (Ziani et al. 2012; Tang et al. 2013; Lee et al. 2014), homogenization (Jafari et al. 2013; Abbas et al. 2015) and ultrasonification (Li and Chiang 2012); with low energy approach by Catastrophic phase inversion (CPI) method. It is the process whereby an oil-in-water system (O/W) inverts into a water-in-oil system (W/O) and *vice versa* (Yanan et al. 2013).

Another approach to prevent flavor loss is to encapsulate with edible coatings. Edible films could be applied to food as active packaging, with the aim of gradually releasing aroma compounds with time and thus of maintaining the characteristic flavour of food product (Embuscado and Huber 2009; Piorkowski and McClements 2013). It can be noted that different types of packaging has been detailed reviewed earlier and the summary of the figure has been interpreted for more understanding (Fig. 2.3). Different food grade materials are used to form edible packaging with the most common being protein (wheat gluten, whey protein isolate, caseinate, soy

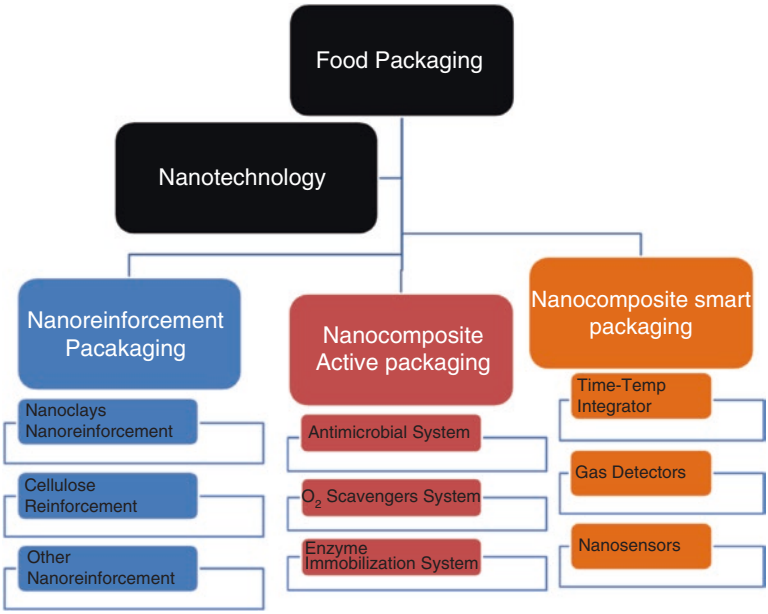

Fig. 2.3 Pictorial representation to sumamrize the different types and sub-types of nano-food-packaging being used along with their different applications. It can be noted that different types of packaging has been detailed reviewed earlier and the summary of the figure has been interpreted here for better understanding (*Courtesy:* Ranjan et al. 2014)

protein) and polysaccharides (starch, carrageenan, alginate etc.). Research is going on to encapsulate active molecules such as aroma compounds to form emulsified films (Karbowiak et al. 2006). Marcuzzo et al., have encapsulated aromatic compounds carrageenans films that were able to retain volatile compounds during film-process formation and released aroma gradually with time (Marcuzzo et al. 2010). For flavor retention, Dardelle et al., used dried yeast cells to encapsulate flavor compounds, which released its active molecule only under two conditions: at very high temperatures (about 243 °C) or above 0.7 of water activity (Dardelle et al. 2007). The active molecules are stabilised in a hydrophobic environment delimited by the inner phospholipid bi-layer.

2.3.7 To Provide Optically Transparent System

Incorporating hydrophobic bioactive compounds – antioxidants, antimicrobials, flavors, nutraceuticals and preservatives – into transparetnt and translucent products is the major challenge of nanoemulsions in foods (Graves and Mason 2008; Lee et al. 2011; Yang et al. 2012). To fabricate it is very essential that the droplet radius should be lesser than wavelength of the light so that light scattering can be avoided to provide more transparency. Additionally, it is a challenge to maintain the same size throughout the storage. Mie theory states the dependence of transparency on specific turbidity and particle size/size distribution. The specific turbidity is defined as the calculated turbidity divided by the droplet concentration expressed as a volume percentage. To fabricate the transparent nanoemulsions it has been suggested to keep smaller droplet radius and in more dilution. A detailed description regarding the transparency and specific turbidity against different sizes has been reviewed earlier (McClements 2011) and have stated that in case of monodisperse nanoemulsions, the predicted specific turbidity remained relatively low when the droplet radius was less than about 40 nm and the same is found to be higher for larger sized nanoemulsions. The dependence of transparency of nanoemulsions on mono- and polydispersity is still in theoretical stage and in question and was agreed in an experiment which agreed the Mie theory and reported more transparency for smaller droplet sized nanoemulsions (Wooster et al. 2008). Notably, the above conditions can be achieved by smaller mean radius and narrow size distribution i.e. higher monodisversity of nanoemulsions. The turbidity increases with the increase in droplet radius (Leong et al. 2009a, b; Ghosh et al. 2013a). Additionally, it has been found that high-energy approach is suitable for fabrication of transparent nanoemulsions (high-energy approach has been described in detail later in this review). It is a necessary need in front of research community to optimize several conditions for nanoemulsions fabrication with transparency. The conditions to be optimized are as – system composition (e.g., type and concentration of oil, surfactant and polymers), physicochemical properties of the component phases (e.g., viscosities and interfacial tensions of continuous and disperse phases) and homogenization conditions (e.g., energy intensity and duration). Notably, transparent nanoemulsions can also be fabricated by low

energy approach in our recent research (Dasgupta et al. 2016b; Jain et al. 2016) and earlier similar transparency was achieved by low energy approach.

2.3.8 Texture Modification

Because of the small droplet size, nanoemulsions may behave differently in terms of its rheological properties irrespective of the same oil have been used in nanoemulsions and conventional emulsions. This has widened the novel application of nanoemulsions to modify the texture of the end food products. Broad aspect is there for nanoemulsions to exhibit rheological characteristics viscoelastic liquids, viscous liquids, elastic solids, or viscoelastic solids, plastics – based on the composition, structure, droplet size and interaction (Lee 2004; Berli et al. 2005; Genovese et al. 2007; Calero et al. 2011; McClements and Rao 2011). Many reviews have summarized the application on nanoemulsions for texture modification (Gillies and Prestidge 2004; Izquierdo et al. 2004; Sonneville-Aubrun et al. 2004; Tadros et al. 2004; Mason et al. 2006; Tadros 2013) and interestingly the mathematical equation in terms of viscosity to justify the nanoemulsions as texture modifier has been described earlier. Normally, increasing the droplet concentration leads to increase in viscosity of the nanoemulsions; initially there will be gradual increase in nanoemulsion viscosity followed by sharp increase (McClements 2011; Piorkowski and McClements 2013). Notably in the region where the close packing (around and above nanoemulsion droplet) the nanoemulsion exhibits visco-elasticity and plasticity i.e. solid like characteristics (Fryd and Mason 2012; Mat Hadzir et al. 2013; Nazarzadeh et al. 2013). It can be noted that since the presence of the interfacial layer greatly contributes to their effective volume fraction of the nanoemulsions; so, for nanoemulsions with smaller droplet diameter will show steep increase in viscosity even at lower oil concentration. In another word it can stated that conventional emulsions need more oil concentration for steep increase in viscosity because of the above mentioned reason. For nanoemulsions – hydrocarbon O/W nanoemulsions and silicone O/W nanoemulsions stabilized by SDS – it was found to be liquid-like rheology at relatively larger droplet size and gel-like rheology for smaller. Notably in the recent era of nanoemulsion based research there is vast increase in manipulating the rheology of existing emulsions found using different techniques (Fryd and Mason 2014; Komaiko and McClements 2014; Scheffold et al. 2014; Zhu and Mason 2014; Hu et al. 2015; Kwon et al. 2015; Park et al. 2015). Considering the above techniques for the applications in food science; the end products will be having higher oil/fat concentration. While in recent era low fat concentration foods are the of main interest. To use the nanoemulsions with high viscosity in foods – there is a need to develop techniques to develop such nanoemulsions with lower oil/fat content and higher viscosity. Additionally, to increase the viscosity several binders/fillers may be used e.g. gelating or edible agar.

2.3.9 Some Other Applications

Other than increasing bioavailability (Choudhury et al. 2014) the bactericidal (Ghosh et al. 2013a, b; Sugumar et al. 2014), antimicrobial (Ramalingam et al. 2012), antihelminthc (Karthikeyan et al. 2011), insecticidal (Pant et al. 2014) properties of nanoemulsion gave it importance to be used in agriculture – mainly to increase the shelf life of agricultural products and WQM (Ranjan et al. 2014; Dasgupta et al. 2015). Chaw et al. have formulated a nanoemulsion with the insecticidal and pesticidal activity. The green nanoemulsion – laden glyphosate isopropylamine – formulated were able to suppress creeping foxglove (*A. gangetica*), slender button weed (*D. ocimifolia*) and buffalo grass (*P*. conjugatum). This initial discovery could be the platform for developing better penetration of agrochemical formulations in the future (Jiang et al. 2013). Similarly they have used the same nanoemulsion with slight modification as a herbicide and have concluded that it is having controlling ability for *Eleusine indica* (Lim et al. 2012). To understand the activity of any nanoemulsion it is mandatory to understand the synthesis procedures (described below) and its bioactive component also to get better bioactivity the bioactive components retention should be more while formulating nanoemulsion. (Joseph and Bunjes 2014).

2.4 Applications of Nanoemulsion in Cosmetics

In Cosmetics, Nanoemulsions are gaining importance as potential and efficient vehicles for the controlled and effective delivery of active ingredients into the skin. Their properties of low viscosity and optical transparency make them suitable for their application in cosmetic industry (Solans et al. 2005). Due to their small size, nanoemulsions give pleasant feel to the skin which makes their use in cosmetics more appealing (Wu and Guy 2009). They have been accepted as essential constituents of cosmetics and possess additional qualities of preventing the harmful effects of sedimentation, creaming, flocculation or coalescence, which are usually observed with macro-emulsions (Chime et al. 2014). For this reason, nanoemulsions are widely used in cosmetic products such as body creams, bathing oils, sunscreen lotions and many more.

2.5 Applications of Nanoemulsion in Pharmaceuticals

Nanoemulsions have found their purpose in several applications owing to their small droplet size, high kinetic and thermal stability and optical transparency, as compared to conventional emulsions. It is known that some anti-cancer drugs with high therapeutic value are not much orally bioavailable due to the

gastro-intestinal drug barrier. Addressing this issue, a study had been conducted by (Mei et al. 2013) which shows that the nanoemulsion with eugenol as an oil ingredient was successful in enhancing the bioavailability of colchicine drug to almost twice the extent achieved by free colchine solution. This is mainly due to the fact that eugenol being a part of nanoemulsion is useful in increasing the absorption of colchicine in intestine.

Pharmaceutical nanotechnology has been developed to address the problem of low bioavailability of oral drug administration by using nanotechnology solutions such as nanoemulsions, nanoparticles, micelles and solid lipid nanoparticles (Mei et al. 2013). Thus, nanoemulsion is an interesting approach for safe and effective delivery of lipophilic drugs with poor oral bioavailability by increasing the efficiency of oral administration.

Nanoemulsions have also been reported for the delivery of herbal drugs as encapsulation of plant bioactives would greatly reduce its degradation while increasing its bioavailability. This has been proved by the results obtained by (Shen et al. 2011) where major enhancement in the intestinal absorption of colchicine was observed when encapsulated using nanoemulsion formulation.

2.6 Advantages of Nanoemulsion

- Low cost of production: Synthesis of nanoemulsion requires mere oil phase, emulsifier and aqueous phase and techniques to breakdown droplets to smaller sizes. Low-energy approach provides an option to avoid usage of high-tech equipments as required in high-energy approaches and thus, brings down the cost of production significantly.
- Suitability for various routes of administration: Different routes can be utilized for administration of naomemulsions such as intranasal administration, parenteral administration, intravenous administration or oral administration as described in Table 2.1 (Solans et al. 2005). The major advantage of nanoemulsions is its small droplet size and large surface area of the emulsion system which allows easy penetration of active ingredients through the skin. This property has paved the way of nanoemulsion into cosmetic and pharmaceutical industries where they are widely used in lotions and ointments.
- High drug/nutrient loading capacity: Due to the smaller particle size, nanoemulsions provide large surface area for loading great amount of drugs and nutrients.
- Delivery of poorly water-soluble ingredients: Emulsions prove to be an effective way to solubilise lipophilic compounds and thus, enhances its bioavailability by increasing its rate of absorption (Guttoff et al. 2015).
- High encapsulation efficiency: Emulsions are successful in encapsulating lipophilic compounds and protects them from unfavourable environmental conditions.

Table 2.1 A brief description of commonly used nanoemulsion delivery routes

	Applications	Advantages	Disadvantages	References
Intranasal	As nasal mucosa provides a direct connection between the nose and brain, this route can be used to target drugs to the brain for treating diseases related to Central nervous system (CNS) such as Alzheimer's disease, depression *etc.*	Direct entry of drugs to target site	High care has to be maintained	Westin et al. (2006) and Lovelyn (2011)
		Painless		
	Development of vaccines can also be achieved			
Transdermal	Nano-sized emulsions penetrate easily through skin pores to systemic circulation. This route has been widely explored to treat anxiety, depression *etc.*	Self administration is possible	At times, skin act as a barrier for effective penetration of ingredients	Lovelyn (2011), (2016)
		Controlled drug delivery		
		Drug delivery can be stopped by just removing the transdermal patch.		
		Absence of Gastro-intestinal complications		
Parenteral	Researchers have already developed an intravenous delivery system for the delivery of nanoemulsions, which showed favourable in vitro results	Most common and effective route of administration	Painful method.	Lovelyn (2011)
			Requires proper training for administration	
		High bioavailability	Risk of infections	
		Rapid method		
Oral	Nanoemulsions have been widely utilized via oral routes, for instance, in health supplements and emulsified food products	Painless	Low bioavailability	(2016), Lovelyn (2011)
		Ease of use	Chances of gastro-intestinal complications	
			Slow onset of actions	

- High anti-oxidant and anti-microbial property: Many researchers have formulated nanoemulsions using various types of oil possessing high anti-oxidant and anti-microbial property and thus, enhancing characteristic properties of encapsulated compounds. Nano-emulsions could enhance the stability of chemically unstable compounds by protecting them from oxidative degradation and degradation by light (Photo-degradation). The enhanced anti-microbial property increases the shelf life of food products. Nanoemulsion formulation protects drugs/nutrients that are highly susceptible to hydrolysis and oxidation.
- Prevention of bad odour or taste: Nanoemulsions help in encapsulating compounds with undesirable odour or taste, making them acceptable to be utilized. This could also be applied in perfumes for delivery of fragrant in many personal care products.
- High stability to gravitational separation and flocculation or coalescence: Due to the small size of nanoemulsions, its molecules follow Brownian motion which is sufficient to overcome gravitational force and thus, exhibit high resistance to physical instability (Chime et al. 2014).
- Suitable alternative: It acts as an efficient alternative delivery system for liposomes and other vesicle system which have proved to be less stable (Tadros et al. 2004).

2.7 Disadvantages of Nanoemulsion

- Lack of proper understanding regarding the formation of nanoemulsions: Accurate information of the amount of emulsifiers required is still lacking. Care must be taken on selecting emulsifiers which are necessary to stabilize nanodroplets as they may get toxic if used at a high concentration. Also, nanoemulsion stability is easily influenced by environmental parameters such as temperature and ph and this makes it highly essential to control emulsifying conditions.
- High concentration of emulsifiers: Emulsifiers are required in adequate amount to keep the emulsions stable. Large concentration of emulsifiers used for emulsion preparation can prove to be highly toxic causing severe damage to human body, if ingested and to environment, when disposed (McClements 2011). To further prove the possibility of toxicity of nanoemulsions because of the existence of emulsifiers, researchers have tested the toxicity of nanoemulsions in Caco-2 cells (He et al. 2011). Many other studies have been performed to analyze nanoemulsion toxicity on cell lines, animal models and microbes.
- High-tech equipments: Synthesis of nanoemulsions by high-energy approach requires usage of high-tech expensive equipments which may raise the cost of production.

- Lack of stability: If emulsions are not prepared using the most stable emulsifying conditions, instability in the form of flocculation or coalescence often take place during storage. Stability may get affected by variations in temperature and ph (Setya et al. 2014). Therefore, accurate emulsifying conditions are to be maintained while formulating emulsions.
- Storage problem of nanoemulsified industrial products: Long-term storage of nanoemulsions can cause instability that can be seen in many forms as shown in Fig. 2.1.
- Unknown fate of ingested nanoemulsions: There are unknown risks associated with oral intake of nanoemulsions including their ability to interact with bioactive compound and to change its biological fate within the gastrointestinal tract (McClements 2011).

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Chapter 3
Fabrication of Nanoemulsion: A Brief Review

Abstract Nanoemulsion is the best way to develop nano-vehicle for the protected, and efficient delivery of encapsulated vitamins, flavors, nutraceuticals, active molecules, and preservatives. Increased bioavailability, weaker light scattering gives transparency, texture modulator, higher stability, and protection of encapsulated molecules are few of the major advantages of nanoemulsions than conventional emulsions. Whereas, few risks also associated with nanoemulsions which might be solved, the risks are as their ability to change the biological fate of active compounds during their route of delivery or the toxicity of the components used in the fabrication. Understanding the theories behind fabrication of nanoemulsions is as important as as understanding the fabrication methodology. Here in this review, (i) different theories behind fabrication of nano-emulsions have been discussed in brief for better understanding of the authors; (ii) irrespective of listing out different nanoemulsion fabrication methodologies, the principle of those fabrication method have been discussed for better understanding; (iii) followed by recent examples from each fabrication methods; (iv) including the opportunities and future implications of those have been discussed.

Keywords Nano-emulsion fabrications • High energy approaches • Low energy approchaes • Theories of nanoemulsion fabrication

3.1 Theories Behind Nanoemulsion Fabrication

With the focus to fabricate stable nanoemulsions it is necessary to understand the theories of nanoemulsion formulation. Several scientific articles have summarized the theories for nanoemulsions formulation; the readers are advised to refer the following literature (Schuster 1996; Bali et al. 2008; Rajpoot et al. 2011). This section provides an overview of the theories of nanoemulsion formulation and also summarizes some recent works based on each theory.

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3.1.1 Thermodynamic Theory

This theory states the de dependence of formation of oil droplets form a bulk oil phase on interfacial surface area (A), interfacial energy ($\gamma\Delta A$) and the entropy of the system ($T\Delta S$). During the top down approach of decreasing diameter of oil droplets the interfacial area and interfacial energy increases consequently. The expression is given by the equation:

$$\Delta G_f = \gamma\Delta A - T\Delta S$$

where G_f is the free energy of nanoemulsion formulation, γ is the surface tension of the oil-water interface and T is the temperature. It can be noted that during the fabrication of nanoemulsions there will be very large change in ΔA; but at the same time γ remains positive all the time. According to the large change in ΔA it can be believed that the positive free energy might come which will be against the fabrication of stable nanoemulsions. It can be noted that γ remains very small and can be easily offset by the entropic components. Interestingly, the entropic components will be dominant because of the large change in entropy found during the formation of several droplets with smaller diameter. Thus the negative free energy of nanoemulsion fabrication can be obtained and hence it can be stated that the process of nanoemulsion fabrication is spontaneous and the resulting end product – nanoemulsion – is thermodynamically stable. Several researchers have evaluated the thermodynamic stability of food grade nanoemulsions which supports the above statement: thermodynamical stability of paparika oil nanoemulsion was evaluated by Pascual-Pineda and co-workers (Pascual-Pineda et al. 2015); the thermodynamic signature was evaluated by Fotticchia and co-workers for Nano-emulsion formation using isothermal titration calorimetry (Fotticchia et al. 2014); impact of alcohol on thermodynamic stability of protein-stabilized nanoemulsions was evaluated for efficient use in food sector (Zeeb et al. 2014, 2015).

3.1.2 Mixed Film Theory

This theory describes the formulation of nanoemulsions and formation of interfacial film and ultra low interfacial tension. It mainly describe the formulation of nanoemulsion by low energy approach where spontaneous formation of nanoemulsion droplets takes place. This spontaneous formation of droplet is considered because of formation of complex film by the surfactant and/or co-surfectants at oil-water interface which ultimately reduces the oil-water interface tension to very low values – near zero to negative values. It has to be assumed in mixed film theory that the nanoemulsion is liquid or duplex in nature i.e. having different characteristics at water and oil faces. The two dimentional spreading pressure (π) thus generated influence the interfacial tension (γ_i) which can be denoted by following equation:

$$\gamma_i = \gamma_{o/w} - \pi_i$$

where, $\gamma_{o/w}$ is oil-water interfacial tension without the presence of film. It can be noted that the use of large amount of surfactants and/or co-surfactants results to increase the spreading pressure (π) which may become larger than $\gamma_{o/w}$ resulting in the negative interfacial tension and finally provides the energy to increase the interfacial area. The increase in interfacial area ultimately causes reduction in droplet size. Recently using mixed theory, the food grade stable mustard oil-water nanoemulsion with fortification of vitamin E acetate has been developed using Tween as the surfactant (Nandita et al. 2016). Similarly, several stable food grade nanoemulsions have been developed e.g. d-limonene organogel-based nanoemulsion (Zahi et al. 2014); vitamin E-enriched nanoemulsion using co-surfactant (Saberi et al. 2013a, 2014a); Polyunsaturated (ω-3) based nanoemulsion (Gulotta et al. 2014); D-limonene Oil-in-Water Nanoemulsion (Yang et al. 2014).

3.1.3 Solubilization Theory

This theory describes the stability and solubility of nanoemulsion and considered it to be thermodynamically stable in monophasic solution e.g. oil-swollen (oil-in-water) or water-swollen (water-in-oil) nanoemulsions. The solubility difference between the normal micelle and nano-micelle can be described through this theory. It describes the relationship between the oil-in-water nanoemulsion and isotropic micellar region. The solubility of oil in normal micelle was found to be smaller and the concentration of all the aqueous concentration was found to be critical. The solubility of micelle at nano-sized droplet increases with respect to hydrocarbons. Additionally at nano-sized nano-micellar structure the formation of a large number of intermediate structures of low curvatures will not be there (Kumar et al. 2008; Rajpoot et al. 2011). Many researchers have fabricate stable hydrocarbon O/W nanoemulsions and O/W nanoemulsions stabilized by SDS in which this theory can be validated. This theory could also be justified to describe the rheology difference of nanoemulsions based on the droplet size – as described in earlier sections (Fryd and Mason 2014; Komaiko and McClements 2014; Scheffold et al. 2014; Zhu and Mason 2014; Hu et al. 2015; Kwon et al. 2015; Park et al. 2015).

3.2 Nanoemulsions Fabrication

Nano-emulsions are unstable in nature and thus it requires energy input either by mechanical devices or chemical components to be in equilibrium state. Apart, from composition, nanoemulsion formation also depends upon preparation variables such as emulsifying path, agitation, emulsification time and energy input – Fig. 3.1

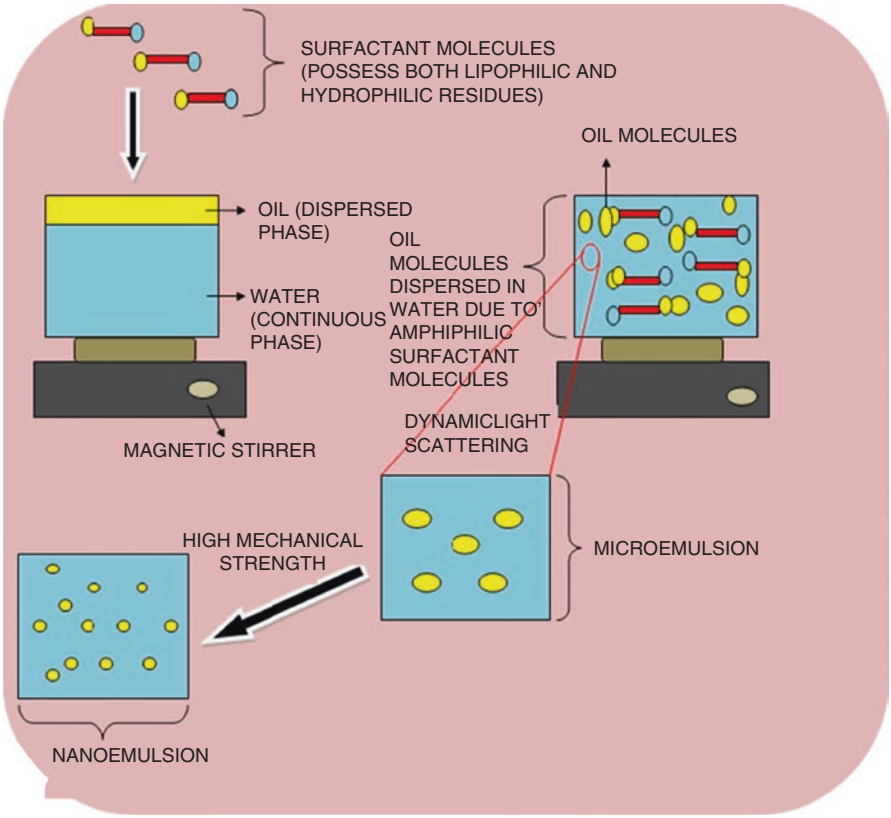

Fig. 3.1 Schematic representation of the role of surfactants – consisting of lipophilic residues, yellow in color and hydrophilic residues, blue in color – in the formation of emulsions using a magnetic stirrer. It can be noted that lipophilic residues get attached to the oil molecules and hydrophobic residues to the water molecules thus, breaking the surface tension between the oil (dispersed phase) and the water (continuous phase) and further resulting in the formation of emulsion. High-energy techniques are used to breakdown the size of emulsion particles to nano-range which can be observed using dynamic light scattering (*Courtesy*: Walia et al. 2017)

depicts the fabrication of nanoemulsions and effects of surfactants. Nanoemulsions can be produced using a variety of methods, which are classified as either high-energy or low energy approaches (Acosta 2009). In high energy approaches, intense energy is used to form small droplets from two immiscible oil and water phase. This high energy can be from homogenizer, microfluidizer, or sonicator (Gutiérrez et al. 2013; Sugumar et al. 2015), while in low energy approaches, the environmental conditions are altered, for spontaneous generation of nanoemulsions (Bouchemal et al. 2004; Ostertag et al. 2012; Saberi et al. 2013a; Nandita et al. 2016).

High energy approaches utilize strong caustic forces, usually generated by mechanical devices to break bigger droplets down to smaller droplets. On the other hand, low energy approach relies on spontaneous formation of oil droplets, achieved

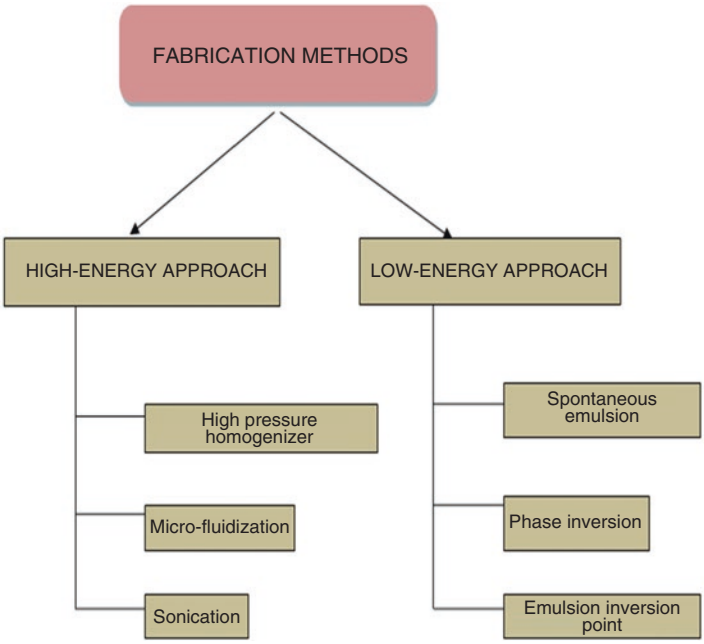

Fig. 3.2 Categorization of methods of fabricating nanoemulsion. Briefly, high energy includes sonication, high pressure homogenizer and micro-fluidozer and low energy approach includes spontaneous emulsification, phase inversion and emulsion inversion point

by altering emulsifying conditions (surfactant, oil and water concentration and temperature). Different types of high as well as low energy approaches of nanoemulsion fabrication have been enlisted in Fig. 3.2.

3.2.1 High Energy Approach

As the name suggests, high energy approach makes use of mechanical strength to reduce the droplet size to be dispersed evenly into the continuous phase by using mechanical devices such as homogenizers, sonicators or microfluidizer.

3.2.1.1 High Pressure Homogenizer

It is the most commonly used method for nanoemulsion preparation. In this method, a mixture of oil, water and surfactant is passed through a valve under high pressure. It works by allowing the emulsion to pass through a small orifice at sufficiently high pressure (500–5000 psi). Higher degree of cavitation can be caused by further reducing the gap size which increases the pressure drop. High pressure homogenization is

a highly efficient method, available for both lab-scale and industrial-scale processing (Setya et al. 2014). Cavitation and turbulence are the two main theories that explain the conversion of larger droplets into the smaller ones. Droplets are split apart by the generated eddie currents and thus, resulting in the reduction of droplet size. This method is highly successful in producing nano-range droplet size provided there is adequate surfactant concentration to keep the emulsion from coalescence. It has been researched that with increased pressure or increased number of passes of emulsion through the valve, droplet size decreases significantly (McClements 2011). The only downside of this method is that it may degrade the components of emulsion system as it operates at extremely high pressure and temperature.

Using this approach, researchers have fabricated vitamin E based nanoemulsion using natural surfactants namely, quillaja saponin and lecithin. The mean particle diameter was found to be 130 nm for quillaja and 120 nm for lecithin (Ozturk et al. 2014). This proves how efficiently nano-ranged particles can be formed using high pressure homogenizer.

As the mixture passes through the valve it undergoes, intensive disruptive forces that causes small oil droplets to be uniformly dispersed in aqueous phase. Generally, nanoemulsions formed by homogenizer are in the pressure range between 50 MPa and 100 MPa. However, pressure as high as 350 MPa have also been reported (Solans et al. 2005). Different nozzle designs are also available to increase the efficiency of droplet dispersion. It has been documented by Walstra in 1999 that logarithm of homogenization pressure (P) is directly proportional to logarithm of droplet diameter (d) with the constant of proportionality depending upon homogenizer type (Walstra 1999):

$$\log d \propto \log P$$

The droplet size can be reduced by increasing the applied pressure, number of cycles through the homogenizer and also the viscosity of the emulsion mixture passed through the homogenizer. In recent days several edible nanoemulsions have been fabricated for efficient delivery or more shelf life or more efficient other activities. Recently fabricated nanoemulsions by such method are as: d-limonene organogel-based nanoemulsion (Zahi et al. 2014); chemically stabile thymoquinone-rich nanoemulsion (Tubesha and Ismail 2014); alcohol assisted protein-stabilized nanoemulsions (Zeeb et al. 2014, 2015); natural bio polymer – Whey Protein Isolate and Gum Arabic (Ozturk et al. 2015); Quillaja saponin and lecithin (Ozturk et al. 2014) – based vitamin E nanoemulsion for efficient delivery; essential oil based food grade nanoemulsions (Salvia-Trujillo et al. 2015a).

3.2.1.2 Ultrasonic Homogenizer

Sonication technique makes use of high ultrasonic waves (frequency > 20 kHz) for the production of nano-ranged emulsions. This method is frequently used for small-scale production of nanoemulsions, majorly in research laboratories. Production of

emulsions had been one of the foremost applications of ultrasound devices. After this advancement, several different types of ultrasonic machines have been designed and developed to make emulsions. Cavitation is the basic phenomenon of this process. A sonicator probe, consisting of a piezoelectric crystal required for the conversion of electric waves into pressure waves, is made to contact with the dispersion emulsion system to generate mechanical vibration and cavitation that causes breaking down of larger droplet molecules to smaller sizes (Gadhave 2014). The droplet size tends to decrease with increasing sonication time and surfactant concentration.

It uses high frequency sound waves as the energy input. This method is faster than homogenization for formulating stable nanoemulsion with very small droplet diameter and low polydispersity. A sonotrode is used to generate a high shear force (mechanical vibrations) followed by the formation of acoustic cavitation that break the oil and water phases into small droplets. Droplet size can be optimized by controlling the process parameters such as oil concentration, emulsifier concentration, mixing ratio of oil and surfactant, viscosity of continuous phase, emulsification time and energy input (Ghosh et al. 2013). However, this method has two drawbacks; firstly it is preferred for low viscosity fluids rather than more viscous systems and secondly it is not efficient for industrial-scale applications (Hélder et al. 2011; Silva et al. 2012). Recently fabricated edible nanoemulsions by ultrasonic approach are as: fabrication of dextrin nanoparticles *via* emulsion cross-linking technique with future application in food and drug (Manchun et al. 2014); curcumin nanoemulsions stabilized by modified starch (Abbas et al. 2014, 2015); Walnut Oil Nanoemulsions (Homayoonfal et al. 2014); vitamin E-enriched nanoemulsions (Saberi et al. 2014b); lemongrass oil-loaded nanoemulsions (Salvia-Trujillo et al. 2014); emodin loaded nanoemulsion (Shi et al. 2015); albumin-stabilized nanoemulsion (Tabibiazar et al. 2015); wheat bran oil-in-water nanoemulsions (Rebolleda et al. 2015).

3.2.1.3 Microfluidizer

In this method, high pressure is used to force the mixture of water, oil, and surfactant through a valve in homogenizer or microfluidizer. There will be difference in channel design for microfluidizer and homogenizer. In case of microfluidizer, different streams of mixture with high velocity is made to collide with each other at 180° angle. This technique helps to achieve high turbulent shear force induced by high pressure with cavitation and exceptionally nanoemulsions with fine droplet size can be fabricated. Comparatively, microfluidizer has been studied with conventional emulsifying devices and found to give better droplet size distribution (Strawbridge et al. 1995). In a study it has been observed that, in comparision to ultrasound method microfuidization is an inferior method to fabricate nanoemulsion because it is unfavourable in many circumstances e.g. high pressure with long emulsification time which leads to re-coalescence of emulsion droplets and increase in the emulsion droplet size (Tang et al. 2013). Microfluidizers are quite similar to high pressure homogenizer in design as both make emulsions to pass through a narrow opening at high pressure. This technique operates through high pressure

displacement pump at high pressure (500–20,000 psi) (Setya et al. 2014). Nevertheless, there is a slight difference as the opening channel of this device causes the emulsion to split into two separate chambers, after which they collide in the interacting chamber at the distal end. Coarse emulsions are allowed to pass through the interaction time until desired droplet size is acquired. The studies have shown that droplet size decreases with increased surfactant concentration in the emulsifying system (McClements 2011).

Kamaiko and team conducted a study showing the fabrication of nanoemulsion to encapsulate fish oil using microfluidization technique, further demonstrating the advantages of using natural surfactants like sunflower phospholipids over synthetic surfactants. This study concluded that sunflower phospholipids can be effectively used as an emulsifier for delivering omega fatty acids into various food and beverage products (Komaiko et al. 2016). Recently a comparative analysis has been done to study efficiency of a Microfluidizer to a high pressure valve homogeniser (HPH) for the production of oil continuous emulsions by investigating the effect of pressure on droplet size. The results obtained show that the Microfluidizer and HPH have similar emulsification efficiency, giving droplets of ~60 nm diameter at a pressure of 50 MPa (Lee et al. 2014). Recently many edible nanoemulsions have been developed using microfluidzation, few of the recent examples are listed below: biopolymers (Salvia-Trujillo et al. 2015a) based nanoemulsions with edible coating (Salvia-Trujillo et al. 2015b; Zhang et al. 2015); essential-oil-loaded nanoemulsions with high antimicrobial effects (Acevedo-Fani et al. 2015); nanoemulsions stabilized by food grade biopolymers; β-carotene nanoemulsions (Jo and Kwon 2014); trans-cinnamaldehyde nanoemulsions in water melon juice to improve the shelf life (Jo et al. 2015).

3.2.2 Low Energy Approach

In this approach, the mixture is subjected to changes in either composition or environment such that spontaneous formation of emulsion takes place. However, relatively large amount of synthetic surfactants are required to produce stable nanoemulsions, this limits its application for food industries. Nevertheless, studies have shown that low-energy methods are often more efficient in producing small droplet sizes than high-energy ones (Saberi et al. 2013a).

3.2.2.1 Spontaneous Emulsification Method

To generate spontaneous formation of nanoemulsions rapid diffusion of the surfactant/solvent from the oil phase into the aqueous phase takes place. In this method, an organic phase – surfactant and/or a water-miscible solvent, containing oil – is poured into an aqueous phase – containing hydrophilic surfactant and water. Using the spontaneous method, the nanoemulsion can be formulated using simple stirring at room temperature rather than using expensive equipments e.g. homogenizer

(Saberi et al. 2013a). Number of forces are responsible for spontaneous emulsification such as negative interfacial tension, interfacial turbulence, stranding and stranding, phase inversion, formation and swelling of water/surfactant aggregates etc. (Santana-Solano et al. 2012) which is responsible for oxidative stability of spontaneous nanoemulsions (Walker et al. 2015).

This method can be used as an alternative to ultrasonication and high pressure homogenization as production of nanoemulsions through this method does not require any mechanical devices (Guttoff et al. 2015). In this method, nanoemulsions are spontaneously formed on mixing two liquids at a particular temperature and concentration. Spontaneous formation of nanoemuison droplets occur on the addition of water (aqueous phase) into the mixture of oil and surfactant with gentle stirring (Komaiko and McClements 2016).

The spontaneous emulsification method is widely accepted within the pharmaceutical industry, where it satisfies the requirement of formulating drug delivery systems to encapsulate bioactive compounds and lipophilic drugs. In food industry, the major drawback of this method is that it requires great amount of surfactants for the emulsion synthesis which may give rise to toxicological concerns. However, its applications outrun the drawbacks related to it as this method is frequently used for encapsulation of lipophilic compounds to enhance their bioavailability.

In a recent study, vitamin E nanoemulsion has been prepared using tween 80 surfactant by spontaneous emulsification method, producing mean diameter particle size of 50 to 100 nm. They concluded that with increasing concentration of glycerol, the amount of surfactant required to fabricate vitamin E nanoemulsion could be reduced. Also, turbidity and particle size of nanoemulsions could be minimized (Saberi et al. 2013b). This result gives an insight of using a cosolvent (glycerol) to prevent/reduce toxicity issues which may arise from usage of large surfactant amounts.

3.2.2.2 Phase Inversion Method

In this method, changing the amount of the surfactant plays a major role with changing temperature. In case of non-ionic surfactant, transition from an oil-in water (O/W) emulsion occurs due to changing the temperature to a desired level i.e. from low temperatures to a water-in-oil (W/O) nanoemulsion to higher temperatures known as transitional phase inversion. As the temperature increases from the room temperature to attain a minimal surface tension formation of fine dispersed oil droplets and this method referred as the phase inversion temperature method (Fernandez et al. 2004). It can be noted that apart from temperature many other parameters e.g. pH value, salt concentration is also responsible for phase inversion but varying these may change the sensory quality of food. At constant temperature, if the composition of the two phases is varied to form nanoemulsion then the process is called emulsion inversion point. Phase inversion method is considered as to give nanoemulsions with irreversibility and kinetically stability. Because of stability and non-degradability of active molecules this method is commonly used for active molecule encapsulation in food and drug industries. Additionally, this method allows an easy scale-up possibilities in less expenses (Anton et al. 2008).

The phase inversion temperature is the most widely used technique in the industry to fabricate nano-emulsions. Phase transition is brought by two approaches: either by keeping the temperature constant and altering the composition or vice versa (Setya et al. 2014). This method depends on the variations in solubility of non-ionic surfactants with temperature or changes in the optimum curvature. At lower temperatures, non-ionic surfactant's head group tends to be more hydrophilic and thereby, shows higher solubility in water. However, with increasing temperature non-ionic surfactants become lipophilic due to dehydration of the polyoxyethylene chains. Hence, the temperature at which the biphasic emulsion system changes from o/w to w/o emulsion is called Phase Inversion Temperature. On the other side, Phase Inversion Composition method is slightly different than Phase Inversion Temperature method as it involves the alteration in optimum curvature of the surfactant by changing the composition of the system (McClements 2011).

A study that used emulsion phase inversion method to fabricate vitamin E nanoemulsion showed the effect of long-term storage and environmental stress on the stability of nanoemulsion with results showing significant vitamin E degradation due to heat (Hategekimana et al. 2015). This proves that vitamins are sensitive to environmental stress and therefore, vitamin based emulsions need to be prepared by maintaining accurate emulsifying conditions.

3.2.2.3 Membrane Emulsification

With the requirement to get nanoemulsions of narrow size distribution range – précised with less standard deviation – and lesser surface tention, membrane emulsification method is used. This method is widely reported for liposomal nanoemulsions production and also for polymeric and lipidic nanoparticles fabrication (Jaafar-Maalej et al. 2011). Throughout the process, continuous phase is passed forcibly through porous membrane which allows the droplets to grow at pores and detach at certain size. Interestingly, the droplet size can be maintained depending upon the force acting on the droplets. Additionally, following factors are responsible to control droplet size – physical parameters (flow pressure, temperature, pH, presence of ions), membrane property (hydrophobicity, pore size, charge, and permeability) or properties of phases used (Laouini et al. 2012).

3.2.2.4 Solvent Displacement

This method is normally used for encapsulating organic pigments like β-carotene. In this method, nanoemulsions can be fabricated by pouring the organic phase (oil dissolved in a solvent like acetone or ethanol) into aqueous phase with surfactants. Many factors should be considered to select the solvent e.g. boiling point, water miscibility, toxicity, safety and legal status. Additionally, the organic phase should be soluble in solvent but only partially miscible with aqueous phase. Spontaneous emulsification occurs in solvent displacement method due to diffusion of organic

Table 3.1 Examples for recently developed nanoemulsions using different fabrication methods

Method	Encapsulating compound	Surfactant	References
High pressure homogenizer	Vitamin E	Natural surfactants: Quillaja saponin and lecithin	Ozturk et al. (2014)
Microfluidizer	Fish oil	Natural surfactant: Sunflower phospholipids	Komaiko et al. (2016)
Ultrasonication	Nigella sativa L. Essential oil	Synthetic surfactant: Polysorbate 80	Periasamy et al. (2016)
Spontaneous emulsification	Vitamin E	Medium chain triglyceride (MCT)	Saberi et al. (2013b)
Phase inversion	Vitamin E	Medium chain triglyceride (MCT) and orange oil	Mayer et al. (2013)
Emulsion inversion point	Allyl isothiocyanate (AITC)	Tween 80 and span 80	Li et al. (2015)

solvent into the aqueous phase, which ultimately be removed later by vacuum evaporation. When this emulsion is diluted with water then the organic solvent moves from the oil droplets into the aqueous phase, which causes the emulsions to shrink in size. The method requires additional effort for removal of the solvent (Lee and McClements 2010; Zuidam and Nedovic 2010). Few of the recently developed nanoemulsions using different fabrication methods have been enlisted in Table 3.1.

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Chapter 4
Ingredients and Components of Nanoemulsions

Abstract For formulating a food grade nanoemulsion, a food technologist not only has to check for the interacting forces between the oil, water and surfactant but also for various ingredients present in a food product such as preservatives, gelling agents, chelating agents, flavours, colorants etc. The molecular and functional aspect of each ingredient plays an important role in determining the stability and organoleptic property of a food product. However, most of the food products developed is due to mixing of ingredients based upon previous knowledge rather than the interaction studies of different components. Nano-encapsulation systems for active compounds often need a higher concentration of surfactant, which may lead to toxicity and irritancy problems. Consequently, judicious and logical selection of ingredients along with their optimum concentration is required, which has been focussed discussed in this review. Optimum selection as well as concentration would aid in better fabrication with desirable outcome.

The use of nanoemulsions in nutraceutical delivery has been reviewed, and it was noted that almost all the studies have not been very systematic with regard to selection of cosurfactants and surfactants. The main objective of this review is to provide a systematic understanding for the proper selection of oils, cosurfactants, surfactants for fabricating nanoemulsions.

Here in this review, (i) different ingredients for developing food-grade nanoemulsions have been described; (ii) recent research trends, challanges and opportunities have been discussed in each of the major sections; (iii) the importance and activity of the major ingredients have been discussed in detail.

Keywords Food-Grade Ingredients • Nano-Emulsions • Properties • Oil Phase • Surfactants

4.1 Introduction

A number of food ingredients/additives have been emulsified which is readily available in the market. Depending upon the parameter to be incorporated into the food material such as food fortification or enhancing stability, appearance, taste or

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texture, various types of active constituents have been employed such as vitamins, minerals, flavorings, acidulants, preservatives, colorings and antioxidants. Proteins, fat and sugar molecules are also emulsified and added to food materials for increased uptake and absorption or improved bioavailability or additives such as benzoic acid, citric acid, ascorbic acid, vitamins A and E, isoflavones, beta-carotene, lutein, omega-3 fatty acids, and coenzyme-Q10 (Ostertag et al. 2012). Different constituents to be emulsified are discussed further in each section.

A nanoemulsion mainly consists of three components – oil, water and surfactant. A correct mix of these components determines the stability and property of emulsion. Edible oil can be derived from a number of sources including plant, animal, fish and nuts. Though over consumption of some of the oils is associated with diseases such as diabetes, obesity and cardiovascular diseases. Thus, a food scientist should focus on various properties of oil such as nutritional profile, flavor profile, crystallization behavior, oxidative stability and oil quality (Walstra 1999; Mizrahi et al. 2014; Xue and Zhong 2014). The ratio of oil and water is very important for the fabrication of nanoemulsion. The amount and unique properties of water greatly influences the organoleptic function of a food product. The crystallization of water also has a significant effect in food nanoemulsions. Smaller water crystals in emulsions, such as in ice cream, gives its characteristic texture and taste while larger crystals gives a rough texture. Manipulation of these components has to be done according to the food product to be developed (Chee et al. 2007; Soukoulis et al. 2014). Emulsifiers/surfactants provide stability to nanoemulsion by forming an interfacial film between the oil droplet and the water phase. It prevents from flocculation and/or coalescence even when the food product is subjected to different mechanical pressure during manufacturing, processing or storage. Various types of surfactants are used in food industries which have been approved by FDA but no single surfactant can be used for all food products. Thus, based upon the molecular characteristics a surfactant is chosen to give the desired effect. Further these components have been described in detail along with mechanism of actions and criterion affecting their selection.

4.2 Oil Phase

There are many oil phage components used in fabrication of food grade nanoemulsions such as cloud oil, flavour oil, nutraceutical lipids and fat soluble colorants. Each of them have been described below.

4.2.1 Flavour Oils

It contain characteristic aroma profiles with volatile constituents and are generally used as flavoring agents in the food and beverage industries e.g. lemon, orange, and peppermint oils. Additionally, it has been suggested to use the medicinal properties

(medicinal flavor oils) of plants to develop health beneficial food or nutraceuticals – may termed as Nano-*Ayurveda*. Composition of natural flavor oils greatly depends the extraction procedure used to isolate them, their biological origin, and any ensuing processing steps (Sawamura 2010; Xenakis et al. 2010). Additionally, the differences inisolation and processing procedures used causes variations in forms/folds of the flavor oils e.g. flavor oil extracted from cold processing are termed as single-fold oils and further the use of different steps mainly distillations determines the folds of it. The fold of the flavor oils determines the chemical composition and physicochemical properties which ultimately has similar effects on nanoemulsions and its activities and other properties (Lang and Buchbauer 2012; Baldissera et al. 2013). Notably, it has been established in our laboratory that higher fold oils are suitable to develop nanoemulsions (Nandita et al. 2016) and similarly it can be supported by the statement that lower fold oil is suitable for microemulsion fabrication (Rao and McClements 2011; Jeirani et al. 2013). The higher fold oils were more suitable for forming nanoemulsions than the lower fold oils, presumably because they contained constituents that more easily fit into micelle structures at nano-level.

4.2.2 Cloud Oil

The cloud oil is mainly composed of non-flavor oils *e.g.* terpene oils or triacylglycerol. Triacylglycerol oils are usually derived from natural resources as vegetable, corn, canola and sunflower oils, while terpene oils are typically extracted and purified by distillation of naturally occurring flavor oils. Nanoemulsion fabricated using cloud oils are known as cloud nanoemulsion. Because of very low water solubility these oils are stable at Ostwald ripening – unlike flavor oil. Normally, they do not participate directly to increase the flavor properties of nanoemulsions, instead they obliquely manipulate nanoemulsions' flavor because of their abilities to detach the flavor molecules among the oil, water, and headspace regions (Vijayalakshmi et al. 2014). The main application of cloud oil is to give the nanoemulsion based end product a desirable cloudy/turbid manifestation by strong scattering of lights with droplets. Experimentally it has been found that the best cloudy appearance can be obtained with the droplet size range of around 200e500 nm where efficient light scattering occurs. Additionally, from commercial point view the physical and chemical stability of the droplet size ranges should be maintained during processing, transport, storage and handling. It can be noted that cloudy oils are rich in unsaturated fatty acid and once the oxidation of unsaturated lipids starts then it produces rancid off-flavors and ultimately affects the flavor profile of nanoemulsion based products (Walstra 1999; McClements et al. 2009). Cloud oil physicochemical properties – mainly density, melting conditions and viscosity – has a critical role in fabrication and constancy of cloud oil nanoemulsions. The cloud oil density has influence on long-standing stability of nanoemulsions as it decides the creaming velocity of the nano sized droplets. Its melting characteristics determine the partial crystallinity of cloud oil droplets and finally can be vulnerable for droplet aggregation and phase separation of

nanoemulsions. Its viscosity will control the ability to fabricate small size ranged droplets throughout the homogenization process, the higher viscosities generally leads to large sized droplets (Dickinson 2010). These physicochemical properties of cloud oil plays negative role for those nanoemulsions which needs refrigerator for prolonged period and it is advised to use the oil phase that remains liquid and refrigerator temperature to avoid crystal formation and unwanted agglomeration.

4.2.3 Nutraceutical Lipids

The nutraceutical lipids – polyunsaturated fats, carotenoids, fat-soluble vitamins, fat soluble antioxidants, phytosterols and fat soluble colorants – has attracted the food market to be incorporated in commercial nano-foods. Either they are incorporated by homogenizing and/or encapsulated into the foods. Since, the nutraceutical lipids are prone to several factors for its chemical or physical degradation, so, using the same in commercial nano-food is still a major challenge. It can be noted that specific lipid nutraceutical has specific confronts which ultimately depends on their physicochemical properties – chemical stability solubility, melting point and oil-water partition coefficient (McClements et al. 2009; McClements and Li 2010). Henceforth, the nanoemulsions should be fabricated with extreme care and the fabrication protocol should be selected wisely to maintain the specific physicochemical properties of particular nutraceutical lipids used in the nanoemulsions.

4.2.4 Polyunsaturated Fats

The potential health benefits of polyunsaturated fatty acids (PUFAs) is the major driving force to grow the interest of their use in food and beverage sector esp. ω-3 fatty acid. (Rubio-Rodríguez et al. 2010; Ryckebosch et al. 2012; Filipović et al. 2015; Sánchez-Salcedo et al. 2016) ω-3 fatty acids are used in foods because of its potential medicinal properties against several diseases e.g. poor infant development cardiovascular disease, mental disorders, and immune response disorders (Gow and Hibbeln 2014; Hou et al. 2015; Nestel et al. 2015; Vauzour et al. 2015). These properties has attracted the food manufacturers to incorporate such PUFAs and fortify the nanoemulsions based foods since at nano-level droplet size ranges will provide efficient delivery and activities. It can be noted that PUFAs are highly vulnerable for oxidative degradation and ultimately causes problems in long term storage, additionally, they are extremely susceptible at smaller size range of nano range (van de Rest and de Groot 2014). It is advised to follow the preventive measures and the protocol to have the long term stability of PUFA based nanoemulsions which includes deactivation of pro-oxidants i.e. oxygen and transition metals, controlling quality of the initial ingredient, interfacial engineering and addition of anti-oxidants to have stable nanoemulsions.

4.3 Carotenoids

They are the lipophilic compounds which are used as colorants to provide colors esp. red, yellow and orange. Many of the researches have claimed their health benefits. Oxygen containing carotenoids called as xanthophylls – e.g. luteis and zeaxanthine – have been claimed to have properties to decrease and age related cataract and macular degeneration. Similarly, carotenoids without oxygen known as carotenes – e.g. β-carotene and lycopene – have potential to decrease the risk for prostrate cancer. These are few of the properties for which carotenoids have the growing interest to use in food and beverages and in recent days the interest is to use them in nanoemulsions (Bou et al. 2011; Arunkumar et al. 2013; Jo and Kwon 2014; Uzun et al. 2016).

However the chemical degradation of carotenoids is the major challenge for scientists to use it as food ingredients because carotenoids are critically stable in their natural environment but once they will be isolated they become highly unstable, becoming vulnerable to oxygen, pH, light and temperature (Goswami et al. 2015). As a result, distribution and use of carotenoids as a food ingredient may result in their faster degradation (Davidov-Pardo et al. 2016). It can be noted that while degradation the double bond of the carotenoid molecules will get lost which can be avoided by isomerizing carotenoid molecules to the cis configuraation (Liu et al. 2016). Other than protecting the carotenoids from degradation, the additional benefit of isomerization is that cis form of carotenoids are suggested to be more bioactive and increased bioavailability (Srivastava and Srivastava 2015). However, an additional major challenge for carotenoids to be used as food ingredients is that either in their natural or isomerized form the higher melting point for carotenoids will keep them in crystalline form at food storage and even at body temperature. These challenges should be avoided for successful applications of carotenoids to be used in nanoemulsion based foods (Yi et al. 2016).

4.4 Vitamins

To improve the nutritional value of nanoemulsion based foods, there has been increased interest to incorporate vitamins (mainly A, D, E and K) and nutraceuticals (e.g. flavonoids, curcumin, sterols *etc.*) (Hormann and Zimmer 2016). Vitamin E is found in several forms in which α-tocopherol is the most bio-active form which is widely used in cosmetics, pharma and food industries (Campardelli and Reverchon 2015). Antioxidant activity of vitamin E is it's majorly known apart from the other activities claimed for – reducing diabetes, cancer, cardiovascular diseases etc. (Li et al. 2015). For these reasons, there has been interest in fortifying many foods and beverages with Vitamin E. Vitamins are being emulsified to increase its bioavailability in organisms. However, most of the products of nanoemulsified vitamins are in cosmetics industry rather in food industry. There are citations for almost all of the vitamins but vitamin E

is the most commonly nanoemulsified. Stable nanoemulsions loaded with Vitamin-E were prepared using a water soluble cosolvent- glycerol. They were formed by titration of a mixture of vitamin E acetate, carrier oil and non-ionic surfactant (Tween 80) into an aqueous glycerol solution with continuous mixing (Saberi et al. 2013; Ozturk et al. 2015; Nandita et al. 2016). In another approach, vitamin E nanoemulsion was developed by canola oil by wash out method. In this method, surfactants and vitamin E were dissolved in the oil phase. The water and oil phases were separately preheated. Then the water phase is continuously added into the oil phase containing the surfactant and the active ingredient. The emulsification temperature was set at 74 °C with constant stirring (Morais and Burgess 2014; Nandita et al. 2016; Zheng et al. 2016).

Khalid et al. have developed a nanoemulsion of l-ascorbic acid with food grade emulsifier and soyabean oil by homogenization method. They obtained droplet diameter of 2.0–3.0 μm which was stable for more than 30 days (Khalid et al. 2013a, 2013b). Ziani et al., have fabricated nanoemulsion from vitamin D, E and lemon oil, an approach to incorporate vitamins with flavor (Ziani et al. 2012). A patent has been filed – US20130189316 A1 – for nanoemulsion of Vitamin K that can therapeutically replace Phytonadione Injectable Emulsion (Vitamin K in aqueous solution with a strong detergent to solubilise it). The drug sometimes causes hypersensitive reaction if injected intravenously or intramuscularly (Andrew 2013).

Vitamin E is becoming lost during processing, utilization and storage of the commercial food and beverage products – oxidation is the major cause for vitamin E instability (Cheng et al. 2016). To achieve the higher oxidative stability, vitamin E acetate has been advised to use in commercial food and beverages rather than vitamin E. It can be noted that in the gastrointestinal tract action of pancreatic esterase cause the breaking of vitamin E acetate to vitamin E (Mayer et al. 2013; Yang and McClements 2013). The higher lipophilic behaviours of vitamin E restrict its direct dispersion into aqueous solutions (Fuchs-Godec and Zerjav 2015). To have its efficient deliver it should be transported using colloidal system for which number of previous studies have proved the efficient emulsion based delivery system e.g. emulsions (Gonnet et al. 2010), microemulsions (Zhou et al. 2015) and nanoemulsions (Mehmood 2015). It has also been suggested that the increased bioavailability of vitamin E can be achieved by using colloidal delivery system rather than its bulk form (Saberi et al. 2015). Recently, Nandita et al., have fabricated the food grade nanoemulsion with increased encapsulation efficiency and have established a hplc based method to analyze and quantify the same (Nandita et al. 2016). Further research is required to increase the encapsulation efficiency and the delivery of vitamin E by altering the colloidal solutions.

4.5 Antioxidants, Flavors and Colorants

They are commonly known as lipophilic antioxidants and are used mainly in oil-in-water nanoemulsions to slow down the oxidative degradation of sensitive nutraceuticals which finally helps to increase the shelf life of nanoemulsion based foods. It

can be noted that the chemical degradation of above discussed sensitive nutraceuticals – mainly PUFAs and carotenoids – can be avoided using lipophilic antioxidants (McClements 2016; Sotomayor-Gerding et al. 2016). Additionally, these fat soluble antioxidants are used in food for health benefits to make food items with antioxidants properties. Few of the active lipophilic antioxidants used at industrial level are – ascorbyl palmitate, rosemary extracts, gallic acid, tert-butyl hydroquinone (TBHQ), butylated hydroxy toluene (BHT), and butylated hydroxy anisole (BHA), and alpha-tocopherols (Budilarto and Kamal-Eldin 2015; Mohammadi et al. 2016). It has been experimentally proved that these are the most active fat soluble antioxidants and one molecule of these has ability to scavenge two free radicals before it will become inactive. Additionally, the lag phase of oxidation reaction can be increased using these antioxidants. It can be noted that the use of antioxidant in nanoemulsions is not much explored and is still in naïve stage.

There are many lipophilic colorants are naturally available – such as paparika, lycopene, β-carotene and other carotenoids. Because of availability from natural sources they can be used as pigments or colorants in food (Meléndez-Martínez et al. 2015; Kiokias et al. 2016). Additionally, many of these colorants have medicinal properties – e.g. carotenoids as discussed above – and thus acts as nutraceuticals too. Most of these lipophilic colorants – carotenoids discussed earlier – from natural sources are extremely unstable and could chemically degrade which leads to rapid color fading during storage (Qian et al. 2012). In order to use these lipophilic natural colorats in nanoemulsions, it is necessary to understand the mechanism of their chemical degradation for each type of colorants. Moreover, it has been also suggested to understand the major factors responsible for chemical degradation (e.g. oxygen, pH, pro-oxidants, light etc.) to plan an efficient delivery system ad to make sure the exact shelf life of the product.

Products with high antioxidant activity tend to acquire bitter taste which reduces their consumer acceptability. Debittering techniques for food industries involves removal of hydrophobic peptides by chromatography, absorption of bitter peptides on activated carbon or selective extraction with alcohols. These techniques could result in a loss of bioactivity, as the majority of bioactive amino acids and peptides are hydrophobic in nature. Micro- and nanoencapsulation of polyphenolic compounds, such as chloroquine phosphate and trimebutine can help to reduce their off-flavours, astringency, bitterness, smell and increased their loading efficiency and bioavailability (Adjonu et al. 2014).

Carotenoids are organic pigments naturally synthesized by microorganisms and plants. They are used in food industries for both coloring and flavoring purpose. Apart from that, it has also been used for its health benefits. Carotenoids are insoluble in water and only slightly soluble in oil at room temperature, which greatly limits their applications. Thus, emulsification is a promising approach to increase its bioavailability. Many types of carotenoids have been emulsified and one of the carotenoid – lycopene is being commercially available also. Nanoparticulate carotenoid lycopene (LycoVit- brand name) is manufactured by BASF Company. A European company which has claimed the product to be used as food supplement or

food fortifying agent. This product has been classified as GRAS (generally regarded as safe) by the US FDA – BASF US Patent US5968251 (Gutiérrez et al. 2013).

β-carotene has been emulsified by various methods such as using lipid carrier/liposomes (Pardeike et al. 2009), using casein micelles (Dalgleish 2011) or by β-lactoglobulin complexes (Ron et al. 2010). Other carotenoids being emulsified include lycopene, lutein and astaxanthin. Lycopene emulsions have been prepared by used high pressure homogenizer and evaluated the thermal stability of lycopene in emulsion systems (Ax et al. 2003; Boon et al. 2008). Astaxanthin was also prepared by high pressure homogenization with mean diameter of the dispersed ranged from 160 to 190 nm (Kim et al. 2012). Nanoencapsulated lutein is developed using chitosan to improve its bioavailability. It was observed that lutein absorption was higher from nanoencapsules than mixed micelles (Arunkumar et al. 2013).

4.6 Plant Essential Oil and Active Compounds

Plant oils are mainly used as oil phase for making food grade nanoemulsions rather than encapsulating it. The detailed application of plant essential oil has been summarized in Table 4.1, aadditionally in the text – detailed information about the bioactive compounds have been provided – mainly phytosterols and phytostanols. These are the plant derived lipid which shows bioactivity (Otoni et al. 2014; Bhargava et al. 2015; Topuz et al. 2016). It has potential to inhibit the dietary cholesterol because of its capability to reduce the low density cholesterol as well as total cholesterol (Nicolosi and Wilson 2015). This property of phytosterol and phytostanols is the major cause for the increased interest to use plant oils in food and beverages particularly in nanoemulsion based foods they may act as a potential nutraceutical with potential medicinal properties. It has been experimentally established fact that 1.6 g phytosterols per day intake is able to reduce the LDL by 10% (Augustin and Sanguansri 2014). The intestinal absorption of phytosterols is very low and so dietary phytosterols do not have adverse effects on health.

The following properties of phytosterols and phytostanols – tendency to form crystals, low-oil solubility, low-water solubility and high melting point – are the main hurdles to incorporate phytosterols and phytostanols in nanoemulsified food products. It can be noted that, they are also susceptible to oxidative degradation Esterification of phytosterols with polyunsaturated fatty acids can be done to overcome these hurdles. The esterified esterification phytosterols will be digested in gastrointestinal tract and produces free fatty acids and phytosterols (Smoliga and Blanchard 2014; McClements 2015a; McClements et al. 2015; Yao et al. 2015; Zou et al. 2015). Although, the phytosterols are susceptible for oxidative degradation but it is unclear till date that whether their oxidized form will show the same bioactivity or will show toxicity. To overcome this, further encapsulation of phytosterols have been suggested.

Table 4.1 Recent research updates on plant oil based nano-emulsions or nano-encapsulation for achieving different activities

Plant oil	Method of production	Activity assessed	References
Rice bran oil	Emulsion phase inversion point	Stability, irritation potential and moisturizing activity	Bernardi et al. (2011) And Alfaro et al. (2015)
Eucalyptus oil	Ultrasonic emulsification	Antibacterial and wound healing	Sugumar et al. (2013, 2014, 2015) and Pant et al. (2014)
Peppermint oil	High pressure homogenization	Stability and antimicrobial	Liang et al. (2012), Bhargava et al. (2015) And Chen et al. (2015)
Palm oil	Commercially available nanoemulsion (Lipofundin) microfluidization	Plasma cholesterol; as a mixing agent of catechin	Jufri et al. (2012), Jeirani et al. (2013), Gadkari and Balaraman (2015) And Ricaurte et al. (2016)
Basil oil	Ultrasonic emulsification	Bactericidal activity	Ghosh et al. (2013)
Rosemary oil	Phase inversion method	Larvicidal activity	Duarte et al. (2015)
Oregano oil	Ultrasonic emulsification	Control of foodborne bacteria on fresh lettuce	Bhargava et al. (2015)
Lemon oil	High pressure homogenization	Emulsion size, stability and turbidity	Rao and McClements (2012)
Tea tree oil	Spontaneous emulsification	Solubility and bioavailability	Zhang (2013)
Avocado oil	Spontaneous emulsification	Stability, viscocity and storage modulus	Mohamed Salama and Ahmad Mustafa (2013)
Sunflower oil	Phase inversion method	Shelf life and quality of *Scomberomorus guttatus* steaks	Joe et al. (2012)
Castor oil	Spontaneous emulsification	Compatibility with a soft contact lens	Katzer et al. (2014)
Soybean oil	Solvent-diffusion method/	Increasing bioavailability of doxorubicin	Jiang et al. (2013)
	Sonication	Increasing bioavailability of cisplatin	Hwang et al. (2009)
Safflower oil	Sonication	Increasing bioavailability of Saquinavir	Vyas et al. (2008)
Peanut oil	Spontaneous emulsification	Increasing bioavailability of Griseofulvin	Ofokansi et al. (2009)
Corn oil	Ultrasonic emulsification/	Food-grade albumin-stabilized nanoemulsion/	Tabibiazar et al. (2015)
	High pressure homogenization	Encapsulation of deltamethrin	Nguyen et al. (2013)

(continued)

Table 4.1 (continued)

Plant oil	Method of production	Activity assessed	References
Coconut oil	Ultrasonic emulsification	Effect of operating parameters and chemical compositions on stability	Ramisetty et al. (2015)
	Emulsion inversion point method	Nano-emulsion whitening cream	Al-Edresi and Baie (2009)
Almond oil	Sonication	Preparing superparamagnetic iron oxide-loaded Nanoemulsion	Ahmadi Lakalayeh et al. (2012)
Walnut oil	Ultrasonic emulsification	Preparing stable nanoemulsion by response surface method	Homayoonfal et al. (2014)
Orange oil	Sonication	Preparing stable nanoemulsion by response surface method	Nano-emulsification of orange peel essential oil using sonication and native gums

4.7 Minerals

Minerals exist in food material in different forms such as ions, compounds, complexes or as chelates. The solubility and other functional property of minerals change for each form. Many diseases are associated with deficiency or overconsumption of minerals. Thus, consumers preferably choose a food product with adequate amount of minerals present in it. It should be noted that changing the mineral composition of food emulsions to improve their nutritional aspects may cause undesirable changes in their physicochemical and sensory properties.

High concentrations of minerals can have an adverse affect on the aggregation stability of O/W emulsions containing electrostatically stabilized droplets due to electrostatic screening and ion binding effects. These effects can occur at relatively low mineral concentrations (less than 5 mM) when multivalent counter ions are present, for example, Ca2+ in an emulsion containing negatively charged droplets. Certain mineral ions may also promote undesirable chemical reactions that lead to product deterioration, for example, iron and copper ions can promote lipid oxidation. In these systems, it is usually necessary to add chelating agents to sequester the mineral ions and prevent them from causing chemical instability. Certain types of minerals also influence the functional properties of other food ingredients. For example, the ability of many biopolymers to thicken or gel a solution is strongly dependent on the type and concentration of mineral ions present. Careful selection and control of the mineral ions present in food emulsions is therefore important when formulating a successful product (McClements and Li 2010; Piorkowski and McClements 2013).

4.8 Weighing Agents

Weighing agents are the additives that are added to nanoemulsions to minimize the separation of oil droplets. It can be noted that the densities of vegetable and flavor oils are significantly lower than the aqueous phase as well as the aqueous sugar solutions and the nanoemulsions containing these oil droplets tends to move upward. The upward movement of oil droplets in aqueous solutions leads to ringing – oil droplet ring formation at the product surface. The best way to minimize ringing can be possible by using the hydrophobic higher density materials than the oil droplets. These higher densities materials are the weighing agents which increases the density of oil droplets which matches with aqueous phase.

Few of the commercially available weighing agents are sucrose acetate isobutyrate (SAIB), brominated vegetable oil (BVO) and dammar gum and ester gum *etc*. SAIB is a synthesized by the esterification of sucrose with acetic and isobutyric anhydrides. SAIB is a high viscosity liquid with transparency which can be mixed with the oil phase before homogenization. It has good stability to lipid oxidative degradation. Food and drug administration, United State of America (FDA, USA) restricted the SAIB level as 300 ppm per serving in the United States. BVO is made after adding when bromine in the cotton seed, corn, olive and/or soybean oil by the formation of double bond between bromine and triacylglycerol molecules of the oils with a allowed usage level of 15 ppm per serving in the United States. Although, usage of BVO is allowed in the United States but it is banned in the Asia and European Union. Ester gum is a hydrophobic polymer obtained from the natural resources. It is obtained from the esterification of glycerol and gum rosin harvested from trees – mainly from pine trees. It can be noted that, because of its supply in crystalline form and the esterification steps it has not been considered as natural weighing agent or natural food additives. According to FDA, USA the allowed usage level for ester gum is 100 ppm per serving in the United States. Dammar gum is a natural weighting agent – isolated from the plants Caesalpinaceae and Dipterocarpaceae shrubs mainly their exudates.. Dammar gum is permitted as a food additive many countries. However, recently it got approved in the USA and thus it lacks the GRAS status (Jain et al. 2016). From the above discussion it is clear that three factors which influence the selection weighing agents are – physiochemical factors, physical/synthetic source of weighing agents and the legal/labeling factors.

4.9 Emulsifiers

Emulsifiers are molecules active at surface level which are mainly used for easing the droplet breakup from top-down approach – thus helps in smaller droplet size formation – and also for prevention of aggregation of these droplets – thus helps in maintaining long term stability (Ranjan et al. 2014, 2016a, b; Dasgupta et al. 2015, 2016; Jain et al. 2016; Komaiko et al. 2016). During the fabrication of nanoemulsions, the

emulsifiers get absorbed to the O/W interface which ultimately reduces the interfacial tension causes ease in disruption of droplets. After facilitating the droplet disruption the emulsifiers must play a role of a protective coating around the oil droplets to prevent aggregation and coagulation. It can be importantly noted that, the concentration of emulsifiers should be in appropriate to cover all the O/W interfaces and rate of coating around the oil droplets by emuslfiers should be faster than the rate of coalescation (Komaiko et al. 2016). There are number of brief as well as detailed reviews have been published dealing with emulsifiers, their types, mechanism of actions, factors influencing the selection of emulsifiers, legal and health concerns (Amenta et al. 2015; Jain et al. 2016; Sodano et al. 2016). Dealing the same thing will be beyond the scope of this chapter, instead the readers are advised to refer the above mentioned articles.

4.10 Sweeteners

Nanoemulsions are get sweetened by natural (preferably) or artificial compounds which interacts with the tongue taste receptors to give the sensitivity of sweetness – primarily sugar based compounds are such compound to give perception of sweetness for mammals (Komaiko and McClements 2015; Puri et al. 2015). From the number of such compounds, sucrose has been considered of Sweetness of 100 and can be used to analyze sweetness using cautiously controlled sensory tests. The right bond orientation and dimensions of fructose is the major cause for it to be the sweetest compound. Additionally, many other natural and artificial compounds are available to have more sweetness than sucrose. Thus it can be concluded that, the amount of sugars needed decreases with increase in it's comparative sweetness. The comparative sweetness, physicochemical stability and flavor profile of the sugars are the factors which decide its use in nanoemulsions as a sweetening agent. Once compared with sugars (glucose, fructose, sucrose etc.) and sugar alcohols (sorbitol, xylitol, mannitol and erythritol) the later are considered as useful for development of reduced calorie product. The reason behind this statement is that the human body breaks down sugar alcohol with slower rate and lesser efficiency, which results in the lesser calories per gram in them. Other than sugar alcohols many of the high-intensity sweeteners are there with favorable flavor profile, low−/medium-calorie nanoemulsions e.g. natural sweetener as Stevia and artificial sweeteners as − Saccharin, Aspartame, Cyclamate, Sucrolose, Neotame and Acesulfame K (Cano-Sarabiaa and Maspocha 2015; Delaveau et al. 2015; Al-Nemrawi and Dave 2016; Baker Jr. et al. 2016).

4.11 Thickening Agents

These are the polymeric comounds which are used in nanoemulsions to increase the viscosity of aqueous phase which ultimately leads the decrease the rate of droplet creaming as well as alters the mouthfeel. These are the biocompatible polymers which will get dissolved in the aqueous phase and covers the larger volume than the

original volume of the polymer chain. The oxidative stability of the polymer and the molecular characteristics (molecular weight, interactions and confirmation) are the main characteristics which should be mainly considered for selecting the thickening agents. Many of the natural and synthetic polymers are discussed and summarized in recent reviews (Piorkowski and McClements 2013; El-Monem et al. 2014; Argenta et al. 2016; Wang et al. 2016) and few of the extraordinary books which provide detailed overview on it (McClements 2004, 2014, 2015b; Bawa et al. 2016).

4.12 Phase Diagram

Phase diagram is used to determine the existence zone of nanoemulsion. It graphically depicts the ratios of the three variables as positions in an equilateral triangle. Usually, only three components are used to form a nanoemulsion, thus these three components occupy the three different sides of the triangle. The three components are each found at apex of the triangle, where their corresponding volume fraction is 100%. Moving away from that corner reduces the volume fraction of that specific component and increases the volume fraction of one or both of the two other components. These points combine to form regions with boundaries between them, which represent the "phase behavior" of the system at constant temperature and pressure (Kumar et al. 2012).

To produce such diagrams, a large number of samples of different composition are prepared. To construct a phase diagram, oil phase is first mixed with surfactant (and co-surfactant) in different ratios. Then, this mixture is titrated with distilled water until a clear, isotropic and thermodynamically stable dispersion with low viscosity is obtained. The ternary phase diagram is constructed by plotting the different values obtained from the experiment. The titration begins by fixing two components and varying the third component. The titration procedure begins with zero loading of water and ends at a point of 100% water loading. Usually, three different forms of phases are observed while constructing a ternary phase diagram- emulsion state, liquid crystal and coarse emulsion. Emulsion state is the area of our interest and can be easily identified by its transparent appearance. Liquid crystal is a translucent gel like state where partition between oil and water phase can be still visible. Coarse emulsions are the unstable emulsions and appear as milky white. The boundary lines between the three states are drawn according to their appearance (Wang and Pal 2014).

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Chapter 5
Food Engineering for Developing Food-Grade Nanoemulsions

Abstract The conventional food-engineering equipments such as evaporators, drier, extruders have been identified to have major applications in developing food-grade nanoemulsions. These engineering aspects also helped industrialists to scale-up the laboratory scale formulation to the industrial scale via pilot-plat scale. Nanoencapsulation can be defined as a process to pack active compound using miniature making techniques such as nanoemulsification, nanocomposite, and nanoestructuration. It will maintain the product functionability during storage and also may lead to targeted delivery and controlled release of the core i.e. active compound encapsulated. Here in this review, (i) The food engineering aspects for developing food-grade nanoemulsions have been discussed; (ii) The major advantages, important changes required and other challenges have been discussed (iii) possibilities of developing food-grade nanoemulsions from lab-scale formulations have been discussed.

Key-words Food-Engineering • Nano-emulsion formulation • Lab to industry • Pilot-plant scale • Scaling up

5.1 Liquid-Based Nanoencapsulation Techniques

Nanoencapsulation is one among the potential and challenging new technologies which have the viability to deceive bioactive compounds. Liquid-based nanoencapsulation has dynamic benefits for efficient absorption after targeted site-specific delivery at cellular level. This section covers the diversed techniques for such nanoencapsulation e.g. emulsification-solvent evaporation, coacervation, nanoprecipitation, inclusion encapsulation and supercritical fluid techniques. The recent research trends, gaps, challenges and limitations are also discussed for these fields.

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5.1.1 Solvent Evaporation Emulsification Technique

It includes the aqueous phase emulsification of polymer solution followed by polymer solvent evaporation which leads to precipitation of polymer in the form of nanospheres (Ghaderi et al. 2015). The sphere size and size distribution depends on many factors like viscosity of organic/aqueous phase, stirring rate, type as well as amount of the dispersing agent and temperature. The polymers which are used frequently are as polylactic acid (PLA), poly lactic-co-glycolic acid (PLGA), cellulose acetate phthalate, ethyl cellulose, β-hydroxybutyrate, and polycaprolactone (Cavallaro et al. 2015; Fornaguera et al. 2015). High energy method – high-speed ultrasonication as well as homogenization – are often employed in order to produce small nanospheres (Rebolleda et al. 2015; Scholz and Keck 2015). Sowasod and tean have encapsulated curcumin in crosslinking of chitosan with tripolyphosphate using a multiple emulsion technique with freeze drying – a low energy approach – with a spherical shaped curcumin nanocapsules of size range 254–415 nm (Sowasod et al. 2012). In these nanocapsules, the tripolyphosphate crosslinking along with the amino group of chitosan, was confirmed by Fourier transform infrared spectroscopy (FTIR). Recently in Eudragit S 100 – synthetic polymer, researchers have encapsulated the curcumin by using solvent evaporation method and further followed by freeze-drying and the nanocapsules of spherical shape was thus obtained showed almost double the anti-cancerous activity compared with curcumin when analyzed on cell lines (Li et al. 2015b). Curcumin-loaded PLGA nanospheres with the smaller mean particle diameter (45 nm) was achieved by Mukherjee and Vishwanathan (Mukerjee and Vishwanatha 2009) and Akl et al. (2016). Notably, these nanocapsules exhibited higher drug entrapment efficiency and yield as well as sustained curcumin delivery including higher intracellular uptake and efficient anticancerous activity on human prostate cancer cell lines. Likewise, the nanospheres (synthesized by solvent emulsion-evaporation technique) then free dried end product has shown significantly improved action as compared with control (curcumin without any modification) when analyzed for in vivo anti-malarial studies.

Similarly, curcumin-loaded PLGA nanocapsules were synthesized by high pressure emulsification-solvent evaporation and subsequent freeze-drying which shows 22-fold higher oral bioavailability (Tsai et al. 2011, 2012; Klippstein et al. 2015). In the same way, Xie and team have increased the water colubility by 640-folds than naïve curcumin comparative to that of native curcumin (Xie et al. 2011). In a recent study, PLGA and PLGA–PEG blend nanocapsules (lesser than 200 nm droplet mean size) containing curcumin was produced by using single-emulsion solvent evaporation method with sonication and consequent freeze-drying (Khalil et al. 2013; Akl et al. 2016). In the comparative study pharmacokinetic activity of curcumin aqueous suspension, the PLGA and PLGA–PEG nanocapsules has shown increased bioavailability of curcumin by 16- and 55-fold, respectively.

Recently, quercetin was encapsulated in PLA by solvent evaporation and subsequent freeze-drying to obtain the encapsulates of 130 nm mean diameter, 97% of encapsulation efficiency. The in vitro observation on release kinetics under

physiological conditions has shown an initial burst release and subsequent sustained and slow release. The complete release and maximum quercetin release of 88% were at 72 and 96 h, respectively (Kumari et al. 2010a, b, 2011). Further, the same work has been continued to improve the solubility, permeability, and stability of this molecule by encapsulating quercitrin in PLA by a solvent evaporation method (Kumari et al. 2011). Likewise, an alpha-tocopherol nanodispersion have been fabricated using emulsification evaporation with the 90–120 nm as mean droplet diameters and further storage for 3 months with no significant changes. The nanocapsules have been prepared under different permutations of the processing parameters as well as the aqueous to organic phases ratios. It has been concluded that the processing cycle did not significantly influence on the droplet diameter as well as size distribution of the fabricated nanodispersion (Cheong et al. 2008; Campardelli and Reverchon 2015). For the various applications in food and pharma, many of the nanoemulsions have been fabricated e.g. nanodispersion of astaxanthin with a droplet size range of 110–165 nm (Anarjan et al. 2011); phytosterol nanodispersions with the droplet size range of about 50–282 nm (Leong et al. 2011a, b); β-carotene of size range 9–280 nm (Hélder et al. 2011). Despite these, many other nanoemulsions and nanodispersions prepared through this method which exhibited good stability. Though, the technique greatly depends on an appropriate emulsification methods e.g. high-pressure or high-speed homogenization techniques and microfluidization. It also relies on a appropriate drying technique for producing nanocapsules (Kotta et al. 2015; Sharma et al. 2015; Bai and McClements 2016; Calligaris et al. 2016; Uluata et al. 2016).

5.1.2 Coacervation

It is a capable and typical encapsulation method to achieve higher payloads of approx 99% and an efficient plausibility to achieve sustained release based on temperature, mechanical stress. (Wong 2016; Zhao and Wang 2016). This technique carries the phase separation of a single polyelectrolyte and/or mixture of polyelectrolytes from a solution further subsequent deposition of the newly formed coacervate phase around the active ingredient. Later, for increasing the coacervate strength – hydrocolloid shell can be crosslinked with a suitable enzymatic or chemical crosslinker e.g. transglutaminase or glutaraldehyde (Rathore et al. 2013; Kailasapathy 2015). Depending upon the number and types of polymer types used, coacervation can be termed as (i) simple caocervation – only one type of polymer – or (ii) complex coacervation – two or more types of polymer. Many other factors e.g. type of the biopolymer (charge, flexibility and molar mass), pH, ionic strength, biopolymers ratio and the concentration influence the interaction among the biopolymers as well as nature and strength of the complex formed (Hosseini et al. 2015; Zou et al. 2016).

Except the above mentioned interaction, other interactions as hydrophobic, opposite charge interactions or the hydrogen bonding between biopolymers can also have considerable contribution in complex formation. Complex coacervation technique has been used to encapsulate capsaicin in gelatin and acacia. The nano-

capsules (with spherical morphology and mean diameter300–600 nm) were achieved by treating the encapsulated capsaicin along with hydrolysable tannins, crosslinking with glutaraldehyde, and further freeze-drying which exhibited higher drug loading capacity, encapsulation efficiency and good dispersion (Xing et al. 2005; Nakagawa and Nagao 2012). Capsaicin has been coacervated by simple (Wang et al. 2008) as well as complex method (Jincheng et al. 2010). Consistent to this, earlier researchers have encapsulated bovine serum albumin (a model protein), in chitosan and tannic acid by an incubation or incorporation method using the polymers like polyanion tripolyphosphate (TPP) and chitosan as the coacervation crosslinking agent. The bovine serum albumin loaded chitosan–TPP nanoparticles prepared under different conditions were 200–580 nm in the size range (Li et al. 2015a; Lomova et al. 2015; Wang et al. 2016).

5.1.3 Nanoprecipitation Technique

This is the polymer precipitation from an organic solution and further aqueous medium diffusion of the organic solvent (Chidambaram and Krishnasamy 2014; Vuddanda et al. 2014). This method is anchored in the spontaneous emulsification of the dissolved polymer, drug holding organic internal phase and organic solvent in aqueous external phase. The biodegradable polymers normally used in this are as poly(alkylcyanoacrylate) (PACA), poly(ε-caprolactone) (PCL), PLA, PLGA, and Eudragit (Vuddanda et al. 2014; Bacinello et al. 2015; Cauteruccio et al. 2015; Mahalingam and Krishnamoorthy 2015). The different nanoprecipitation technique for the active compound nanoencapsulation are summarized earlier by (Ezhilarasi et al. 2013) few important of which have been described in this section. Curcumin was encapsulated (76–560 nm, diameter size range) in PLGA in the presence of poly(l-lysine) and PVA stabilizers using nanoprecipitation followed by freeze-drying techniques (Chen et al. 2014; Chow et al. 2015). This encapsulated curcumin also have showed the more efficient anticancer potential in cell proliferation and clonogenic assays, when compared with free curcumin control. Likewise, curcumin was encapsulated in PLGA with PEG-5000 as stabilizer using the nanoprecipitation method and were informed to have enhanced and efficient cellular uptake (Anandharamakrishnan 2014a; Zhang et al. 2014). Additionally, the curcumin nanocapsules exhibited increased bioavailability (in vivo) and enhanced bioactivity (in vitro) as compared with free curcumin control. Single or multi-step nanoprecipitation methods (using single or many bio/synthetic polymers) have been used to encapsulate curcumin for enhanced anticancer effect, sustained release behavior, mucoadhesive properties and cellular uptake (Gou et al. 2011; Suwannateep et al. 2011; Liu et al. 2012; Klippstein et al. 2015).

A schematic representation was illustrated in Fig. 5.1 to fabricate β-carotene-loaded nanodispersions (average droplet size of lesser than 80 nm with narrow distribution of size) using the solvent displacement method by encapsulation of beta-carotene into PLGA and PLA followed by freeze-drying (Ribeiro et al. 2008;

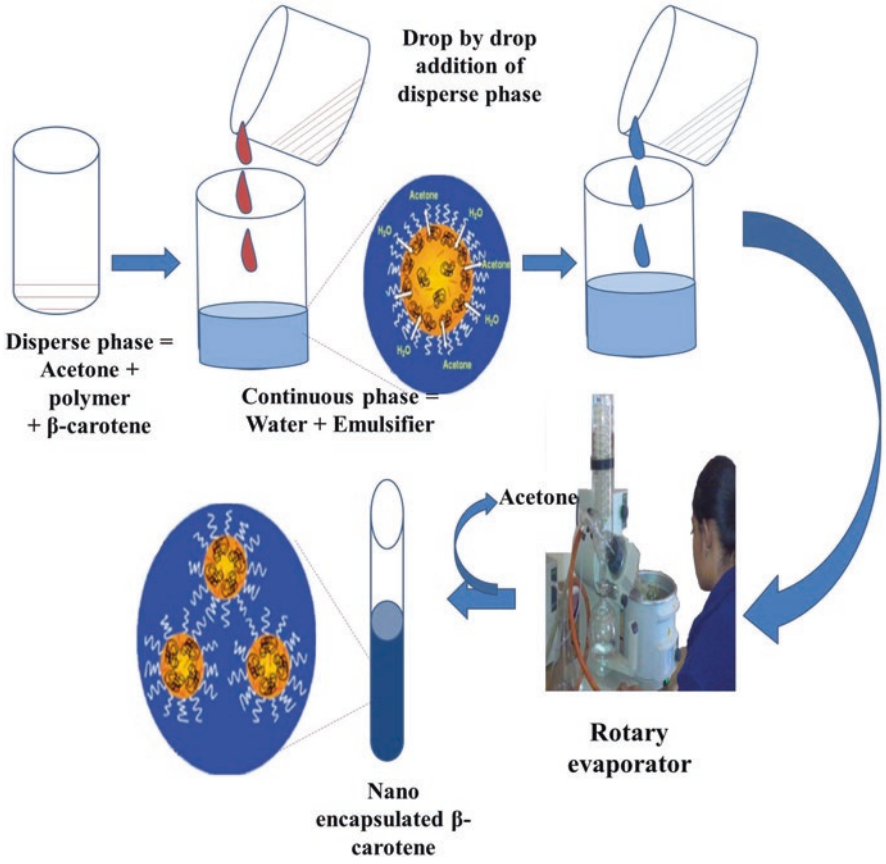

Fig. 5.1 Schematic representation for production of beta-carotene nanodispersion by the solvent displacement tecgnique. A schematic representation to fabricate β-carotene-loaded nanodispersions – with narrow size distribution – using the solvent displacement method by encapsulation of beta-carotene into PLGA and PLA followed by freeze-drying

Martín et al. 2015). Notably, the nano-encapsulated end product thus obtained is found stable against coalescence and ringing. Many compounds have been encapsulated using this method e.g. astaxanthin was encapsulated by using solvent displacement method followed by with freeze-drying (Chandra Bhatt et al. 2016); similarly, vitamin-E-loaded nanocapsules (165–172 nm) was fabricated by Khayata and co-workers with the nanoprecipitation method using the membrane contactor technique. These nanocapsules showed higher encapsulation efficiency, enhanced stability against degradation, increased cellular uptake, efficient sustained release, and more bioavailability (Khayata et al. 2012a, b; Hategekimana et al. 2015). Notably, the end results greatly depend on a suitable drying technique i.e. freeze drying technique and suitable polymer-based wall material is advised to be used e.g. PLGA and PEG. Additionally, suitable non solvent and solvent phases

should be chosen, since it might differ for each active component. Use of this technique is limited to water-soluble solvents, with sufficient diffusion rate to produce spontaneous emulsification.

5.1.4 Inclusion Complexation

The method of encapsulating a encapsulated ingredient – supramolecular linkage of a ligand – to a shell material (a cavity bearing substrate) with entropy-driven hydrophobic effect, van der Waals force or hydrogen bonding (Karoyo and Wilson 2015; Aree and Jongrungruangchok 2016). Notably, the molecular entities with molecular-level cavities are rarely available for applications in food. Earlier researchers have increased the thermal stability of alpha- and beta-cyclodextrin (α- and β-CD) by encapsulating by the inclusion complexation method. These α- and β-CD complexed nanocapsules gave the yields of about 88% and 74%, respectively (Hădărugă et al. 2006). Likewise, Lira et al. (2009) have encapsulated usnic acid (UA) in β-CD by inclusion complexation along with freeze drying. The complex of UA in β-CD (UA:β-CD) was further integrated into liposomes by hydration of a thin lipid film followed by sonication with the intention of producing a targeted drug delivery system which exhibited efficient drug encapsulation (99.5%) and 4 months stability in a suspension form. The complex Liposomes containing UA:β-CD, exhibited a more prolonged release profile of free usnic acid as compared to usnic acid-loaded liposomes (Lira et al. 2009). Considering another example of molecular inclusion, similarly Zimet and Livney have fabricated a stable nanocomplex (colloidal) of docosahexaenoic acid (DHA)-loaded protein beta-lactogloglobulin (β-Lg) with low methoxy pectin. The nano-complex of DHA-loaded β-Lg has shown an improved protection against degradation of DHA when an accelerated shelf-life stress test experiment has been performed i.e. 100 h at 40°C. Comparatively, about 80% lost was observed in the unprotected DHA (Zimet and Livney 2009; Ron et al. 2010). The inclusion complexation technique is exclusively used for the encapsulation of volatile organic molecules i.e. essential oils and vitamins in order to make and preserve odors, flavors and aromas. Recently many works have been done to encapsulate these biomolecules (Zhu et al. 2014; Hosseini et al. 2015; Perez et al. 2015; Rajendiran et al. 2015; Ha et al. 2016). Although, this technique yielded more stability of the core component along with higher encapsulation efficiency of only few molecules – like beta-lactoglobulins and beta-cyclodextrin – are apposite for encapsulation using this method.

5.1.5 Supercritical Fluid Technique

Supercritical fluids are those fluids which shows the transitional properties between liquid and gases in terms of its lower viscosity as well as density, diffusivities, higher solvating power, and higher mass transfer rates above the critical point.

Carbon dioxide, propane, water, nitrogen, etc. are the most common compounds which can be converted into supercritical state (Wang et al. 2013). To encapsulate thermally sensitive compounds supercritical fluids are used, similar to spray drying. When the supercritical fluid technique is being used in nanoencapsulation, then the polymer and the bioactive compound(s) are solubilized in a supercritical fluid and the solution is expanded through a nozzle. The process is followed by evaporation of supercritical fluid during spraying process which ultimately results the precipitation of solute particles (Duarte et al. 2015; Rodríguez-Meizoso and Plaza 2015). Taking carbon dioxide as supercritical fluid and using supercritical anti-solvent precipitation lutein has been encapsulated (size range of 163–219 nm) in hydroxypropyl methyl cellulose phthalate (HPMCP) (Jin et al. 2009). The main target of this study was to minimize its light and thermal degradation and to enhance the bioactivity of lutein. It can be noted that, the operating procedure affected product yield such as lutein loading, particle size and encapsulation efficiency. Using rapid expansion from supercritical solution and carbon dioxide as supercritical fluid the phytosterol nano-encapsulates (size below 500 nm) were synthesized (Türk and Lietzow 2004; Delgado-Zamarreño et al. 2016). In these works it has been reported that the particle size distribution was influenced by surfactant type and its concentration. The main disadvantage of using supercritical fluid technology is the initial capital investment for high-pressure equipment (Reverchon et al. 2015). Moreover, the electrospraying and electrospinning are the other techniques used for nanoencapsulation – these techniques have been discussed in further section.

5.2 Electrospraying and Electrospinning Techniques: Nanoencapsulation

In past decade, electrospinning and electrospraying have attracted a wide interest for its application in nanoemulsions in the field of food and drug industries particularly for efficient delivery system. Electrospraying and electrospinning – the electrohydrodynamic processes – have huge potential for making nanosized fibers, spheres and particles. On the other hand, more researches are required in process optimization to encapsulate various bioactive compounds in nanospheres. Additionally, the efficiency and properties of these nanoencapsulates should be analyzed to improve their applications in food and beverage industries. Electrospraying and electrospinning techniques use a consistent electrohydrodynamic force to break the liquids into fine droplets and are extremely cost-effective extremely (Ghorani and Tucker 2015; Lim 2015; Zhu et al. 2015). Additionally, these two technologies are one among the capable techniques which are attracting industries' and researchers' interestdue to their possible and unique application in efficient delivery of the bioactive compounds of food and beverages. Although both of these functionality is based on same principle but it is hereby important to point out the minor differences which are as: in electrospraying the polymer is transformed into nanosized particles

of the polymer, while in electrospinning, the polymer is transformed into continuous nanosized polymer threads. It can be noted that the electrospraying is the modified version of electrospinning (Fung 2015). Electrospinning and electrospraying techniques have been elaborated later in this section.

5.2.1 *Nanoencapsulation and Electrospraying*

Under the influence of an electric field, the low viscous polymer solution is allowed to pass through a syringe to generate a fine mist. This applied voltage generates the force which pushes the solution. As a result the solution to get ejected out of the syringe in the form of jet and further the nanoparticle formation of nanoparticles takes place. Kindly note, the amount and duration of applied voltage and the distance of the syringe are the main factors to influence the size of the nanoparticles formed. As illustrated in Fig. 5.2, the liquid forms a Taylor cone after getting charged at the nozzle end (Jaworek and Sobczyk 2008; Lim 2015; Moghaddam

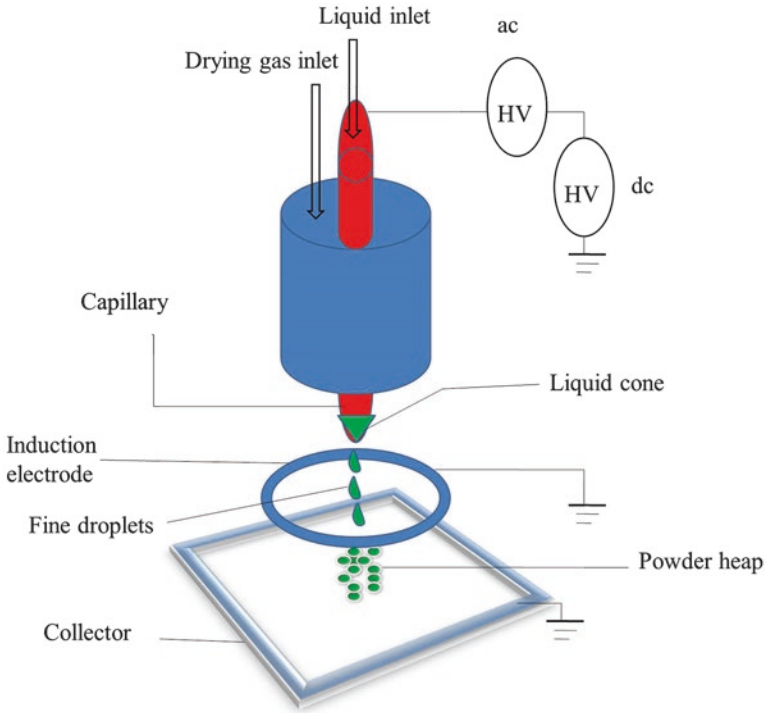

Fig. 5.2 Schematic illustration of electrospraying technique for nanoencapsulation. It can be noted that, the liquid forms a Taylor cone after getting charge at the end of the nozzle. HV indicates higher voltage, AC indicates alternating current and DC indicates direct current

et al. 2015b). Further, liquid is forced to continue acquiring more and more charge. After the threshold of the endured charges, the liquid disperses into several micro or nano-sized droplets and falls onto the opposite charge surface. Once after the movement of these droplets, the solvent preset at their surface evaporates which ultimately causes shrinkage of the droplets to further reduce the droplet size (Chen 2007; Anandharamakrishnan 2014b; Moghaddam et al. 2015a).

As mentioned earlier, the droplet size formed solely depends on the liquid flow rate and the charge acquired due to voltage variation. Monodispersed micro or nano- particles cab be engineered using this technique. Arya and team have optimized different process parameters of this method for fabricating chitosan micro−/nanospheres loaded with ampicillin. The main parameters considered were needle gauge, electrospraying voltage, electrospraying distance and concentration. It was reported that 26 g of needle gauge and 7 cm working distance fabricated nano size ranged spheres with 80% encapsulation efficiency, 128.2 mV zeta potential and with improved stability (Arya et al. 2009). Likewise, omega-3 fatty acid (docosahexaenoic acid, DHA) was encapsulated with electrospraying technique using ultrathin zein and more stability across changes in relative temperature and humidity has been claimed for the nano-capsules thus formed (Torres-Giner et al. 2010). In another study, researchers have encapsulated beta-carotene using whey protein concentrate at the nano-, submicro-, and microscale by electrospraying. They have concluded that concentration of whey proteins, additional component glycerol, and range of pH influenced the capsule morphology and size (López-Rubio et al. 2009; López-Rubio and Lagaron 2012). Likewise, curcumin containing zein solutions was electrosprayed and the spherical nanoparticles (175 and 250 nm) was obtained with improved encapsulation efficiency (85–90%), enhanced dispersibility to the active compound and its stability (Gomez-Estaca et al. 2012, 2015).

5.2.2 Nanoencapsulation and Electrospinning

Polymer fibers in the range of 100 nm diameter can be fabricated by a unique and easy approach – electrospinning. It works on the similar principle as electrospraying but in electrospinning higher voltage is used which acts as the reason to solidify the liquid and further generation of the polymer fiber takes place (Chen 2007; Anandharamakrishnan 2014b; Moghaddam et al. 2015a). The main factors which affect the electrospinning are voltage, solution flow rate, concentration, polymer molecular weight, and nozzle-to-ground distance in the electrospinning set up. The effects of process parameters and solution characteristics have been summarized in Table 5.1. Notably other than food and drug, electrospinning is also extensively used in the field of tissue engineering to engineer number of tissues such as neural, vasculature, tendon/ligament, and bone (Danie Kingsley et al. 2013; Jayaraman et al. 2015).

Electrospinning is mainly used in delivery of bioactive compounds of food and drug. The fiber is the main place where the bioactive compound encapsulation takes

Table 5.1 The dependence of fibre morphology on different parameters i.e. process parameters and solution characteristics

S. No.	Process parameters	Fiber morphology	Reference
1	At constant electric potential, flow rate, and concentration,	Fiber diameter is inversely proportional to screen distance (cm)	Chen (2007); Sill and von Recum (2008), Danie Kingsley et al. (2013), Anandharamakrishnan (2014b), Jayaraman et al. (2015) and Moghaddam et al. (2015a, b)
2	At constant flow rate, screen distance, and concentration	The fiber diameter is inversely proportional to electric potential (kV)	
3	At constant electric potential and screen distance and concentration is constant	Fiber diameter is directly proportional to flow rate (mL/h)	
4	At constant electric potential and screen distance and flow rate is constant	Fiber diameter is directly proportional to to concentration (wt%)	
5	Increase in applied voltage	Fiber diameter increase initially and then decrease	Chen (2007), Sill and von Recum (2008), Danie Kingsley et al. (2013), Anandharamakrishnan (2014b), Jayaraman et al. (2015) and Moghaddam et al. (2015a, b)
6	Increase in flow rate	Fiber diameter increase Note: Beaded morphologies occur if the flow rate is too high	
7	Increase in distance between capillary and collector	Fiber diameter increase Note: beaded morphologies occur if the distance between the capillary and collector is too short	
Solution characteristics			
1	Polymer concentration	Directly proportional to viscosity and surface tension.	Chen (2007), Sill and von Recum (2008), Danie Kingsley et al. (2013), Anandharamakrishnan (2014b), Jayaraman et al. (2015) and Moghaddam et al. (2015a, b)
2	Solvent volatility	Influences solvent evaporation between the tip and collector	
3	Solvent volatility	Influences the phase separation during the flight of the solvent	
4	Solution conductivity	Directly affects the tensile strength of the fiber produced	

(continued)

Table 5.1 (continued)

S. No.	Process parameters	Fiber morphology	Reference
5	Increase in polymer viscosity or concentration	Fiber diameter increases	Chen (2007), Sill and von Recum (2008), Danie Kingsley et al. (2013), Anandharamakrishnan (2014b), Jayaraman et al. (2015) and Moghaddam et al. (2015a, b)
6	Increase in solution conductivity	Fiber diameter decreases	
7	Solvent volatility	Fibers exhibit microtexture i.e. pores on their surfaces, which increase the surface area	

place for targeted controlled release. Two-phase electrospinning also been used for encapsulation which carries a biphasic suspension. This biphasic suspension is formed by mixing the aqueous solution of bioactive compound in an organic-polymer solution. Once after electrospuning the suspension leads to the nanoencapsulation of aqueous pools within the polymer fibers. This process has been used for encapsulating growth factors, hormones, cytochrome C and proteins in a biocompatible polymer (Dong et al. 2009). It can be noted that, the encapsulated final product may be either micro or nano-size. Few important examples for nanoencapsulated products fabricated by elecrospinning have been listed below.

Zhang and team members have naoencapsulated bovine serum albumin (BSA) into biodegradable PCL by coaxial electrospinning, this product has potential application in medical field (Zhang et al. 2006). Researchers also used the electrospinning for incorporating and fabricated encapsulation (average size as 200 nm) of two different model proteins – bovine serum albumin and epidermal growth factor fluorescently labeled with Texas Red and AlexaFluor 488, respectively – into the same coat material in two different domains. Poly(lacticco-glycolic acid) and tecophilic polyurethane and were used as coat material (Dong et al. 2009). Likewise, bifidobacteria was encapsulated in ultrathin PVA electrofibers with average droplet size of 150 nm which was found capable of forming a high oxygen barrier which results in an improved shelf life with regards of bacterial concentration (López-Rubio et al. 2009; López-Rubio and Lagaron 2012). Recently, researchers have used electrospinning and fabricated nanoencapsulated curcumin (average diameter of 250–350 nm) loaded in beta-cyclodextrin and PVA through inclusion complexation. Notably, the PVA/complex fibers showed comparatively faster release than PVA/curcumin (Sun et al. 2013). Fernandez and co-workers have encapsulated β-carotene (a light sensitive compound) in ultrafine fibers of zein prolamine, the mean diameter was found in nano-range of 1140 nm and the fabricated complex was found to possess increased oxidative and light stability (Fernandez et al. 2009). As curcumin has been encapsulated or emulsified by most of the methods and so by using electrospinning it has been encapsulated in Zein nanofibers with an average size of 310 nm by Brahatheeswaran and team (Brahatheeswaran et al. 2012). They obtained smooth surfaced curcumin nanoencapsulates with anti-oxidant activity and were

efficient in bioactive compound sustained release. In an another studies it was found that the curcumin and cellulose acetate based nanofibers (size range of 314–340 nm) retained their chemical identity for 4 months of storage period. It was found to be nontoxic for human dermal fibroblasts (Suwantong et al. 2007, 2008, 2010). Additionally, electrospinning techniques was also used to encapsulate aromatic, volatiles and fragrance compounds. Kayaci and Uyar have fabricated the nanofibers of PVA (average diameters rage as 120–230 nm) containing a vanillin-cyclodextrin inclusionn complex and the encapsulated vanillin was observed to be chemically and thermally stable (Kayaci and Uyar 2012a, b).

5.3 Nanoencapsulation and Drying Techniques

Nanosuspensions active compounds with a encapsulation or coating with wall materials (dried or liquid form) can be fabricated using nanoemulsion techniques. It can be noted that, it is desirable to convert nanouspensions into dried form because of their main disadvantage of chemical instability, irreversible aggregation, leaching of bioactive compounds from the nanocapsules. The other advantages of using drying techniques are easier handling, ease in storage and efficient dispersibility in aqueous solution. Apart from comparative higher stability than the original nanosuspensions, the dried powders have the capability to promote and control sustained release of bioactive compound. However, during processing, drying worsen additional stress on the nanocapsules. This section discuss about various drying techniques – Freeze and Spray drying – used for designing the dried form of nanoemulsions and the recent researches in each field including the pros and cons.

5.3.1 Freeze-Drying

The process in which water in a suspension or solution is crystallized at lower temperatures is called freeze drying or lyophilization. Further, it can be sublimed directly into the vapor phase (Ishwarya et al. 2015; Rey 2016). Due to the avoidance of heat damage of the bioactive compounds, the freese dried products are preferred in comparision to dehydrated products (Ishwarya et al. 2015). The freeze dried products are rapidly soluble because of its larger surface area; stable in dry form and elegant or designer because of uniformly colored cake. The four main steps in freeze drying are freezing, primary drying, secondary drying and final treatments. A detailed overviews on freeze drying processes have been discussed earlier scientific articles (Anandharamakrishnan 2014c).

Shaikh and his research team have nano-encapsulated curcumin (mean diameter – 264 nm; encapsulation efficieny – 77%; loading – 15% and stability – 3 months) by using emulsion–diffusion–evaporation technique along with freeze-drying. It has been found by in vivo pharmacokinetics studies that these nano-entrapped curcumin showed nine fold increased oral bioavailability as compared to curcumin adminis-

tered with piperine as absorption enhancer (Shaikh et al. 2009). Likewise, in another study curcumin-loaded O-carboxymethyl chitosan nanoparticles have been fabricated which indicated slow, controlled and sustained release of curcumin from the nanocapsules. Thus fabricated curcumin also showed enzyme-triggered degradation and release in the presence of lysozyme and toxic behavior against cancer cell lines (Anitha et al. 2011a, b, 2012). Similarly, curcumin has been encapsulated with biodegradable thermoresponsive chitosan-g-poly(N – vinylcaprolactam) by a simple ionic crosslinking method using tripolyphosphate along with freeze-drying and the same was found to show specific toxicity to cancer cell lines (Sanoj Rejinold et al. 2011a, b). Other than curcumin several other bioactive compounds have been nanoencapsulated by using freeze drying combined with polymers and other methodologies to achieve several objectives. Few of such examples are as – Fish oil (Bejrapha et al. 2010; Choi et al. 2010), capsicum oleoresin (Quintanar-Guerrero et al. 1998; Surassmo et al. 2010, 2011; Bejrapha et al. 2011; Hebbalalu et al. 2013) miglyol 829 oil (Abdelwahed et al. 2006; Frank et al. 2015; Brown 2016; Zhang et al. 2016), (+)-catechin and (−)-epigallocatechin gallate (EGCG) (Dube et al. 2010; Gadkari and Balaraman 2015), tocopherol (Luo et al. 2012; Hosseini et al. 2013).

Although, freeze drying provides the improved characteristics of nanoemulsions e.g. stability, efficient delivery, sustained and controlled release, more encapsulation efficiency etc. but all these depend on the selection of high-energy emulsification technique and other encapsulation techniques to break down the droplets into nanosized form. It can importantly be noted that, use of cryoprotectants (sucrose, trehalose, or mannito etc) is necessary to conserve the particle size and to avoid aggregation during freeze-drying and additionally, the various temperature for freeze drying has been reported which has been summarized by (Ezhilarasi et al. 2013; Anandharamakrishnan 2014a, b).

5.3.2 Spray Drying

Spray drying in used widely in food and drug industries for producing dry powders in continuous mode. The feed – solutions/suspensions – in fluid form will be transformed into dried particulate form by spraying the feed into hot drying medium. It takes lesser time and is mocre economical as well as producing fine particles (Nandiyanto and Okuyama 2011) and because of these properties it is widely used in industries. Wide range of food ingrediemts e.g. flavors, vitamins, minerals, colors, fats, and oils will get encapsulated using spray drying to extend the storage shelf life and protect it from surrounding environment(Murugesan and Orsat 2012). It has a best potential to be considered as a best nanoencapsulated method. Encapsulation is obtained by dissolving, dispersing or emulsifying the core substance in an aqueous carrier material solution, followed by atomization and spraying of the mixture into a hot chamber (Trinh et al. 2015). It can be noted that, the wall material has to be soluble in water and some of the generally available wall materials are gum acacia, modified starch, polysaccharides (alginate, carboxymethylcellulose, guar gum), maltodextrins, and proteins (whey proteins, soy proteins, and sodium caseinate).

Recently many researchers have used spray drying for encapsulation oil through nanoemulsion production. Modified starch (Hi-Cap) and whey protein was used as a wall materials by Jafari and co-workers (Jafari et al. 2007, 2008) and by few others (Paramita et al. 2012; Fisk et al. 2013; Drosou et al. 2016; Santana et al. 2016). Though their studies identified that the nano-ranged emulsion droplets will get converted into micro-sized droplet after or when applying spray drying technique. In another researche catechin has been encapsulated in a carbohydrate matrix with a size range of 80 nm with spherical and smooth droplets and was found stable after spray drying techniques i.e. higher negative zeta potential (Ferreira et al. 2007; Majeed et al. 2015). This nano-encapsulated catechins was found to be stable after altering pH and the carbohydrate layer helps to prevent catechins from oxidizing and is also increasing its bioavailability. A combination of processes have been used i.e. emulsification evaporation ans spray drying techniques to encapsulate β-carotene in formulated nanosuspensions using modified n-octenyl succinate starch. It was observed to be of higher encapsulation efficiency (65–90%) and antioxidant activity, with particle size range of 300–600 nm. It this research also the increase in size (in the range of 10 micro meter) was observed after using spray drying technique (de Paz et al. 2014). In a work conducted by Liang and co-workers, though the nanoencapsulated size of β-carotene (using Hi-Cap) has been converted in micro-range but the porwder showed good dissolution in water and reconstituted emulsion had similar particle size range to the fresh nanoemulsions which ultimately suggests that the spray drying is not affecting the original size of the fresh nanoemulsion (Liang et al. 2013). Though spray drying is the single step process and is more economical and less time consuming than the freeze drying process – as it is not altering the droplet size range of fresh nanoemsulsion. It can be noted that conventional spray drying technology should not be followed instead prior nanoemulsion should be performed before going for spray drying.

From the literature it can be concluded that the change in particle size and degradation of the encapsulated compound depends on many factors such as (i) the concentration of wall materials, (ii) the organic solvent/water flow ratio (iii) the temperature used (iv) time duration (v) emulsion density and viscosity, (vi) film property of the matrix (vii) moisture sorption property (viii) glass transition temperature of the powder (ix) film oxygen permeability and (x) humidity etc. Overall, it can be concluded that in spray drying the particle size can be controlled by optimizing these parameters.

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Chapter 6
Research Updates on Different Vitamins Based Nanoemulsions and Characterization of Nanoemulsions

Abstract Developing and marketing vitamin rich food products are major challenge because of efficient nutritional delivery system, longer stability, and shelf life. Nanoemulsified and nanoencapsulated vitamin based nanoemulsions have been developed to overcome these issues. Recently, vitamin based nanoemulsions have been developed and also been marketed. Here in this review, (i) several vitamin enriched nanoemulsions have been discussed in which different oils have been used to meet specific need (ii) the opportunities of such products in markets and research have been discussed; (iii) the conventional nanoemulsion characterization techniques have been discussed and also (iv) non-conventional and newly developed characterization techniques have been discussed to show their future and impact on nanoemulsion characterization.

Keywords Vitamin enriched nanoemulsions • Future • Characterization • Conventional characterization techniques • Recent characterization techniques • Advantages

6.1 Introduction

Nanotechnology has been explored widely all around the globe for enhancing the bioavailability of poorly soluble vitamins like vitamin A,D,E,K by encapsulating vitamins using various types of encapsulating compounds. In this section, major focus has been laid on the use of oils to protect vitamins and to delay their release in the body system. Till now, many studies have been conducted involving the fabrication of nanoemulsion using different combinations of vitamin and oils. An overview of such studies has been provided in the tabular chart below (Table 6.1) with some detailed explanation thereafter.

Researchers have performed emulsification studies to compare and contrast the results obtained by using different emulsifiers. Protein (Whey protein isolate) and polysaccharides (gum Arabic) have been used as emulsifiers to formulate vitamin E acetate emulsions. They observed that protein emulsifiers are more effective in producing smaller size droplets in the range of 110 nm as compared to polysaccharide

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Table 6.1 Tabular representation of types of oils and vitamins used for nanoemulsion preparation

Encapsulated compound	Encapsulating material	Emulsifier	Preparation method	Droplet size	Brief summary	References
Vitamin E	Orange oil	Natural surfactant: (a) Whey protein isolate (WPI) (b) Gum Arabic (GA)	High pressure homogenization	(a) 0.11 μm (b) 0.38 μm	WPI was successful in producing small droplets at low emulsifier concentration and GA was more stable to environmental stress	Ozturk et al. (2015a)
Vitamin D	Corn oil, Fish oil, Orange oil and Mineral oil	Natural surfactant: Quillaja saponin	High pressure homogenization	140–190 nm	Long chain fatty acids serve as better delivery system for nutrients	Ozturk et al. (2015b)
Vitamin E	Short chain triglycerides (SCT): Glycerol tributyrate Medium chain triglyceride (MCT): Caprylic triglyceride Long chain triglyceride (LCT): Olive oil	Non-ionic synthetic surfactant: Tween 80	Emulsion phase inversion	83–110 nm	Synthesized nanoemulsions were found stable to heat, ph and salt but significant degradation was seen when exposed to heat shock and long storage conditions	Hategekimana et al. (2015)
Vitamin E	Canola oil	Synthetic and Natural surfactant mixture: Tween 80:soya lecithin (3:1)	High pressure homogenization	132–160 nm	Response Surface Methodology was used to optimise nanoemulsion synthesis process. The effect of emulsifying conditions on droplet size and stability of nanoemulsions was studied	Mehmood (2015)

Vitamin D	Medium chain triglyceride (MCT)	Non-ionic synthetic surfactant: Tween	Spontaneous emulsification	<200 nm	Effect of emulsifying conditions on the stability of synthesized vitamin D nanoemulsion was analyzed. In this study, it has been concluded that the thermal stability of nanoemulsions can be increased by adding a co-surfactant	Guttoff et al. (2015)
Vitamin E	Orange oil	Natural surfactants: Quillaja saponin and lecithin	High pressure homogenization	≈ 120–130 nm	The effect of emulsifying conditions (surfactant concentration, oil concentration) and environmental conditions (temperature, ph or ionic strength) on the formation and stabilization of nanoemulsion has been demonstrated	Ozturk et al. (2014)
Vitamin E	Canola oil	PEG-40 hydrogenated castor oil and Sorbitan monoleate	Mechanical stirring	–	The release rate of nanoemulsion system was measured and compared via dialysis sac, reverse dialysis sac and USP apparatus 4 fitted with dialysis adapter	Morais and Burgess (2014b)

(continued)

Table 6.1 (continued)

Encapsulated compound	Encapsulating material	Emulsifier	Preparation method	Droplet size	Brief summary	References
vitamin E	Medium chain triglyceride (MCT)	Non-ionic synthetic surfactant: Tween 80	Spontaneous emulsification	≈ 50–100 nm	With increasing concentration of glycerol, the amount of surfactant required to fabricate vitamin E nanoemulsion could be reduced.	Saberi et al. (2013)
vitamin E	Medium chain triglyceride (MCT)	Non-ionic synthetic surfactant: Tween 80 and Brij 35	Membrane emulsification	106 nm	Membrane emulsification technique has been successfully chosen as an alternative for the formation of vitamin E nanoemulsion	Laouini et al. (2012)
Lipophilic compounds	MCT, flavour oil, LCT	Tween 80	Spontaneous emulsification	–	To understand the influence of emulsifying conditions on the formation and stability of nanoemulsion.	Komaiko and McClements (2014)
Vitamin E and Paclitaxel drug	Chloroform	Soy lecithin and sodium deoxycholate	High pressure homogenization	≈89–217 nm	In this study, drug encapsulation has been demonstrated by incorporating the paclitaxel drug into vitamin E nanoemulsion to improve its efficiency against breast cancer	Pawar et al. (2014)

Vitamin E	Canola oil	PEG-40 hydrogenated castor oil and Sorbitan monoleate	Low energy method: Wash out method	87–103 nm	In this study, a practical RP-HPLC method was developed for vitamin E acetate estimation in lipid based nanoemulsions	Morais and Burgess (2014a)
Vitamin A	Polyoxyethylene hydrogenated castor oil	Non-ionic surfactant and liquid paraffin	–	–	The stability of vitamin A in different emulsion systems (simple and complex) was studied and compared	Yoshida et al. (1999)
Vitamin E	Mustard oil	Non-ionic surfactant: Tween 80	Wash-out method	86.45 ± 3.61 nm	Researchers formulated vitamin E nanoemulsion and performed its activity analysis	Nandita et al. (2015)
Vitamin E	MCT and orange oil	Tween series, whey protein isolate, sucrose monopalmitate, casein and quillaja saponin	Emulison phase inversion (EPI) and microfluidization	< 200 nm	This research was done to demonstrate the effect of surfactant to oil ratio (SOR) on the formation of nanooemulsion and specifically on its particle size. A detailed comparison of low and high energy methods has also been done	Mayer et al. (2013)

emulsifiers (380 nm). This difference has been explained by using different emulsifiers. On the other hand, Gum Arabic has been found to produce more stable emulsions, especially at elevated temperatures as compared to whey protein isolate. This can be attributed to the fact that protein surfactants get denatured in protein based emulsions (Ozturk et al. 2015a). This study shows that how different emulsifier can cause change in stability of the formed emulsions.

A study performed by Ozturk et al. 2015b, shows that long chain triglycerides are far more effective at increasing vitamin D bioavailability and pose higher stability to lipid digestion. This can be attributed to the fact that long chain free fatty acids protectively surround lipid droplets and thus, prevent them from lipase action. Stable Nanoemulsions in the range of 140–190 nm were synthesized using natural surfactants by High pressure homogenization technique. A comparative study of different oil-based nanoemulsions has been provided based on vitamin D release in simulated gastro-intestinal conditions. The effect of carrier oil type on lipid digestion and vitamin D release has been demonstrated in this study (Ozturk et al. 2015b).

A study has been conducted by Mehmood 2015 where canola oil was used to encapsulate vitamin E acetate using High energy approach to understand the changes in nanoemulsion droplet size and its stability with the change in the emulsifying conditions which includes homogenization pressure, oil, surfactant and vitamin E concentration. Moreover, it suggests the application of response surface methodology technique to optimize the nanoemulsion formulation process (Mehmood 2015).

Recently, researchers have formulated vitamin E nanoemulsion using edible mustard oil via low energy approach. A wide number of analytical tests had been performed such as stability test, anti-oxidant analysis, HPLC for measuring encapsulating efficiency of vitamin E, anti-microbial test to synthesize an effective emulsion system. The prepared nanoemulsion was found to be stable for more than 15 days with significantly higher anti-oxidant property of 62.55%. Its high antibacterial property makes it suitable for enhancing the shelf life of health drinks. High encapsulating efficiency (99.65%) proves that the synthesized nanoemulsion is highly effective in encapsulating and protecting vitamin E from unfavourable conditions (Nandita et al. 2015). This study shows the advantages of using nanoemulsion system as a health supplement in beverage industries.

6.2 Trends in Detection, Identification, Visualization and Characterization of Nanoemulsions

6.2.1 Conventional Characterization Techniques for Nanoemulsion

6.2.1.1 Separation Techniques

In the theme of chemical engineering separation technology is generally referred as a method to achieve any mass transfer phenomenon that converts a mixture of substances into two or more distinct product mixtures – which is generally referred to

as fractions. Rarely, separation techniques may fully divide the mixture into its pure constituents. In colloidal food science separation technology is mainly used to identification and quantification of the active compounds the different compounds present. Many researchers have established the use of the separation techniques in nano-colloidal food science i.e. food grade nanoemulsions or nanoencapsulated products. The details on which have been discussed below.

Size Exclusion Chromatography

It is a chromatographic method in which molecules in solution are separated by their size, and in some cases molecular weight. It is usually applied to larger molecules or macromolecular complexes such as proteins and industrial polymers. It can be noted that size is the main characteristics of nanoemulsions, so it is the most suitable type of liquid chromatography for the separations of nanoemulsions from food matrix (Saifullah et al. 2016). Size exclusion chromatography has been used in nanoemulsion based drug delivery analysis.

The uptake of nanoemulsions by phagocytic cells is critically dependent on their respective size. Thus, development of a nanoemulsion should focus on droplets with a well defined size, i.e. with a narrow size distribution. Otherwise, contaminations with droplets of large size that are taken up more efficiently and/or carry a higher perfluorocarbon load may affect both the specificity and quantity of the perfluorocarbon label. As can be derived by Grapentin and co-workers, perfluorocarbon -nanoemulsion generated by use of phospholipids usually show a broad size distribution – a problem which cannot be overcome by the conventional manufacturing process. However, the formation of defined perfluorocarbon -nanoemulsion is feasible by combining centrifugation with size exclusion chromatography. Size exclusion chromatography shows that they were able to successfully separate well defined fractions of a perfluorodecaline emulsion *via* a Toyopearl HW-75S column (Grapentin et al. 2014). Similarly, this technique has been used for characterization of nanoemulsion-based drug delivery by ketene based polyester synthesized using electron rich carbon/silica composite surface (Swarnalatha et al. 2008). The use of size exclusion chromatography technique in nanoemulsion characterization is rare now and if used then in drug delivery system (Bae and Chung 2014; Vezocnik et al. 2015).

Ion Exchange Chromatography

It is a chromatography process that separates ions and polar molecules based on their affinity to the ion exchanger. It works on wide variety of charged molecule – including large proteins, small nucleotides, and amino acids. Simultaneously, as charge size is also one among the main characteristics of nanoemulsions, so it is also the most suitable type of liquid chromatography for the separations of nanoemulsions from food matrix (Saifullah et al. 2016). In past one decade rarely few researchers have used ion exchange chromatography for special forms of fabricated nanoemulsions e.g. analyzing encapsulation efficiency of plasmid nanoemulsion

(Zhang et al. 2007); purifying the fusion proteins in fabricated nanoemulsions (Middelberg and Zeng 2013). Ion exchange chromatography has been used as purifier in nanoemulsion which includes sucrose fatty acid ester (Bromley 2011).

6.2.1.2 Physical Characterization Techniques

UV-Vis Spectrophotometer

As UV-Visible spectrophotometer analysis is based on the optical density and the amount of light transmitted or refracted, it is mainly used to identify the compound and quantify their concentration. It can be noted that, for quantification and identification the result should be confirmed with the control and also it can not give the structural confirmation. The structural confirmation can not be concluded by UV-Visible spectroscopy. Earlier researchers have used this method for various estimation e.g. detection of oily content in final nanoeulsion (Costa et al. 2013); to estimate the curcumin encapsulated (Rachmawati et al. 2014); to measure the Percentage transmittance of nanoemulsions (Jaiswal et al. 2015). Though it is conventional technique, but it is widely used for initial analysis for identifying and quantifying.

Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC)

FTIR and DSC are the commonly used instrumental techniques for analyzing the structural identity of the compounds or mixture of compounds. In colloidal research – mainly in nanoemulsion – these techniques are used to identify the structural identity of active compound present in food composition. FTIR and DSC are normally used for assessing surface morphology and active compound encapsulation, analyzing the integrity of these active compounds, and to ensure the compatibility among the ingredients (Pathan and Mallikarjuna Setty 2011, 2012; Ahmad et al. 2014). Additionally, these tests may be used for analysing the impact and penetration of nanoemulsions with bioactive compounds in cells and their fate after some period of incubation (Shakeel et al. 2008; Sari et al. 2015; Tabibiazar et al. 2015). Overall, FTIR and DSC are mainly used in characterisation of the active compounds present in nanoemulsions and also used in analyzing their fate at cellular and sub-cellular level.

X-Ray Diffraction (XRD)

XRD is critically used to confirm the crystallinity of any compound. Researchers have analyzed the crystallinity of nano-fibre formation from nanoemulsion by using electrospinning technology (Gordon et al. 2015; Sugumar et al. 2015) and few have designed specific nanoparticles with the help of nanoemulsions and have analyzed

the crystal structure for thus fabricated nanoparticle (Shams and Ahi 2013; Filipović et al. 2015; Soltani et al. 2016). Further extending the applications of XRD in core-nanoemulsion characterization, few researchers have used it on derived forms of nanoemulsions by using several food engineering steps (Ahmad et al. 2014; Zhou et al. 2014). Additionally, the protocol has been established to analyze the presence of ions in nanoemulsions (Mahendran and Philip 2013). Other X-ray based technology also is being used to identify lipid based nanoparticles or nanoemulsion based solid nanomaterials – small-angle X-ray Scattering (Jenning et al. 2000; Alaimo et al. 2015; Truong et al. 2015; Uzun et al. 2016).

6.2.1.3 Imaging Techniques

Atomic Force Microscopy (AFM)

As many of the advanced microscopic techniques have been evolved but conventionally AFM is easier to use for characterizing as nanoemulsions are in colloidal film on slides and the AFM images will give clear idea about the shape and size of the nanoemulsions droplet. Additionally it can be noted that, AFM results must be confirmed by using other characterization techniques e.g. dynamic light scattering and other imaging techniques which will be discussed later in this section.

Recently, many researchers have avoided the other imaging techniques but followed AFM imaging followed confirmation using dynamic light scattering (Makidon et al. 2008; Ghosh et al. 2013; Ma et al. 2016; Nandita et al. 2016), while others have further confirmed the AFM results with other imaging techniques (Salvia-Trujillo et al. 2013; Neeru et al. 2014; Singh et al. 2015b; Song et al. 2016).

6.2.2 Recent Trends in Nanoemulsion Characterization

Though several conventional techniques exist but for better efficiency, précised results, lesser time taking and more user friendly techniques have been developed or the protocol with respect to nanoemulsion characterization have been developed according to the need. These are described in this section.

6.2.2.1 Separation Techniques

The main challenge for food grade nanoemulsion is the in situ characterization of them; nevertheless, in most cases it is not possible to detect them in the food matrixes. Therefore, separation techniques are necessary to isolate the nanoemulsion from food prior to their characterization. This section describes the most important separation techniques for isolation of nanoemulsions.

High Performance Liquid Chromatography (HPLC)

Though HPLC method was highly explored in different field of biological research – mainly in identifying and quantifying the active compound. It has also been highly explored to identify the presence of some unidentified compounds in extracts e.g. leaf, fruit or stem samples including environmental samples such as soil, water and other samples. It mainly works on the principle of separation based technique.

The efficiency and precise results of HPLC is now preferred by researcher in nanoemulsions to identify and quantify the encapsulated compounds. Recently, Dasgupta and co-workers have used HPLC to quantify non-encapsulated vitamin E and have calculated encapsulation efficiency for food grade mustard oil nanoemulsion. it was first of its kind article in the field of food grade nanoemulsions (Nandita et al. 2016). During the similar time period many other researchers have also used HPLC for characterization different nanoemulsion and other than encapsulation efficiency it has also been used in analyzing the stability of particular compound in nanoemulsion (Li et al. 2015; Panatieri et al. 2016; Youssof et al. 2016). Overall, like other chromatographic techniques, HPLC was also used to identify and quantify the active compounds and was found more precise, easier and efficient for nanoemulsions characterization.

Field Flow Fractionation (FFF)

FFF is a separation technique where a field is applied to a fluid suspension or solution pumped through a long and narrow channel, perpendicular to the direction of flow, to cause separation of the particles present in the fluid, depending on their differing "mobilities" under the force exerted by the field. Different forms of FFF are there to perform different functions e.g. asymmetric FFF which can be adopted based on the need. Asymmetric flow field-flow fractionation might be capable of separating nanoemulsion droplets and liposomes by their size, in combination with static light scattering a deeper insight could be gained. Vezocnik and team have established the process for FFF coupled to a multi-angle light-scattering detector and have analyzed various samples of lipid droplets and lipid vesicles e.g. nanoemulsions (Vezocnik et al. 2015) and Grapentin and co-workers have analyzed perfluorocarbon using asymmentric FFF (Grapentin et al. 2015). In nanoemulsion research the Ostwald ripening can be analyzed by sedimentation FFF (Kabalnov and Shchukin 1992). Many in vitro and in vivo test has been performed for different nanoemulsions using FFF or integrated FFF, which also have been summarized and reviewed by many researchers (Esposito et al. 2015; Martins et al. 2015; Pan and Zhong 2016).

Gas Chromatography (GC)

GC is a separation technology which can be used for analyzing the compounds that can be vaporized without decomposition. Many researchers have tried trapping the aroma or other volatiles in nanoencapsulated form and the level of this nano-entrapment could be analyzed by GC. Recently, researcher's interest has increased to analyze volatiles by GC which are nano-entrapped volatiles in nanoemulsions or the cellular level penetration can be efficiently analyzed (Hoscheid et al. 2015; Lucca et al. 2015; Maher et al. 2015).

6.2.2.2 Physical Characterization Techniques

This physical perspective of the nanoemulsion characterization techniques has been described in this section – e.g., size, size distribution, zeta potential, crystallinity, viscocity, stability etc. of the nanoemulsions.

Dynamic Light Scattering (DLS)

DLS is commonly used for determining the size distribution profile of small particles in suspension or polymers in solution. In this technique the temporal fluctuations are usually analyzed by means of the intensity or photon auto-correlation function – so DLS is also termed as photon correlation spectroscopy or quasi-elastic light scattering. Recently, DLS analysis becomes the basic but compulsory analysis to measure the hydrodynamic size of nanoemulsion droplets. This is so because of the one step process of DLS size measurement and precise estimation of hydrodynamic size range of nanoemulsion droplets. Many food grade nanoemulsions have been fabricated recently and commonly almost all of them have used this analysis in their research (Dasgupta et al. 2014; Guttoff et al. 2015; Komaiko and McClements 2015; Ma et al. 2016; Nandita et al. 2016). It has been reviewed and summarized in many recent scientific articles (Azeem et al. 2009; Rajpoot et al. 2011; Shakeel et al. 2012; Ranjan et al. 2014, 2016a, b; Dasgupta et al. 2015, 2016; Jain et al. 2016). Now a days, the DLS is getting integrated with the zeta potential analyzer in which changing the normal DLS cuvette to the double electrode integrated cuvette, which will analyze the zeta potential of the nanoemulsion (Jain et al. 2016; Nandita et al. 2016).

Disc Centrifuge

Disk centrifuge is an excellent tool for particle size analysis of virtually any material between 5 nm to 75 μm. It can be used as wide range of particle sizing applications which also includes nanoemulsions. In this technique the sizing is performed based on differential sedimentation method i.e. settling of different sized material at different rate. The gravitational sedimentation rate of droplets follows Stoke's law

i.e. sedimentation velocity increases as the square of the droplet diameter. Which ultimately indicates that the minor difference in droplet size has much impact in gravitational sedimentation velocity and it makes the droplets to get detectable. Though it is not in fashion and rarely used technique in nanoemulsion based researches but it has potential to hit the research trend (Fielding et al. 2012; Fissan et al. 2014; Jain et al. 2016). The reason behind its rare use is that using this is more time taking than DLS and is tedious.

Viscometer

Viscometer is an instrument used to measure the viscosity of a fluid. Nanoemulsions are mainly characterized by using rotational viscometer. Rotational viscometers use the idea that the torque required to turn an object in a fluid is a function of the viscosity of that fluid. They measure the torque required to rotate a disk or bob in a fluid at a known speed. It is very important to know the viscosity of the food grade nanoemulsions if ingested and passing through gstro-intestinal tract or applied transdermally. Positively charged nanoemulsion based steroidal drug for transdermal application has been analyzed using rotary viscometer (Da Costa et al. 2014). Similarly, many researchers have used rotary viscometer for analyzing food and drug grade nanoemulsions (Tsai et al. 2014). Recently, few reviews have highlighted the recent characterization technologies for nanoemulsions (Jaiswal et al. 2015; Jain et al. 2016).

Nuclear Magnetic Resonance (NMR) and Magnetic Resonance Imaging (MRI)

As the nanoemulsions are the stable emulsion and it is very difficult to analyze the structural identity of the compounds in its hydrodynamic forms. NMR and MRI are well-established technologies based on an intrinsic quantum property of atomic nuclei called “spin.” While non-invasive and capable of providing information in a wide range of applications, NMR/MRI techniques have long been plagued by a lack of sensitivity (Berkeley Labs 2016).

Researchers have shown that tiny bubbles of nanoemulsions carrying hyperpolarized xenon gas hold big promise for NMR and its sister technology, MRI, as these xenon carriers can be used to detect the presence and spatial distribution (i.e. voids) of specific molecules with far greater sensitivity than conventional NMR/MRI. Applications include molecular imaging of complex solid or liquid chemical and environmental samples, as well as biological samples, including the detection and characterization of lung cancer tumours at an earlier stage of development than current detection methodologies (Schroder 2013; Stevens et al. 2013). Additionally in the same time-period another researchers have successfully developed fluorine based 19F–MRI technique for precise and efficient characterization for nanoemulsions in vitro and in vivo (Janjic et al. 2008, 2009; Janjic and Ahrens 2009; Patel et al. 2013). Development of these techniques is a major breakthrough of nanoemul-

sion characterization techniques or colloidal food science characterization techniques. It will take time to adopt these throughout the world because of several scientific, economical and political reasons (Takegami et al. 2015).

6.2.2.3 Stability and pH Analysis of Nanoemulsion

Stability of the nanoemulsion can be analyzed only visually and the main forms of instability of nanoemulsions are gravitational separation, flocculation, coalescence and phase separation. It can be noted that one has to be very precise while analyzing stability through naked eyes or the magnifying glasses, particularly in the case of coalescence – because stable nanoemulsion and coalescence has very minute difference of instability. It can be noted that, analyzing the stability for nanoemulsion is entirely different than analyzing stability for nanoencapsulation because in the case of former the separation of oil and water phase has to be analyzed while in later case the encapsulation has to be analyzed. Similar to stability analysis, pH analysis for final nanoemulsion product has to be tested, particularly for food and drug grade nanoemulsions, because they are going to interact with the biological metrices which ultimately depends upon the pH. Though, simple pH meter or pH pens are to be used for analyzing the pH of the nanoemulsions but it is always advised to properly caliberate the pH meter and dip in the nanoemulsion for the longer time till the readings are not getting stable (Jain et al. 2016).

6.2.2.4 Imaging Techniques

Microscopy can be used as a direct imaging technique for nanoemulsions; nevertheless the type of microscopy used depends on the kind of matrix to be analyzed. This technique enables information regarding the size, shape, and aggregation state of the nanoemulsions. There are several instruments imbedded with basic microscopy to provide more precise imaging techniques. Some of the imaging methods (non-imbedded as well as imbedded) that are used for the characterization of nanoemulsions systems are presented below.

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

TEM is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through it. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device. While in the case of SEM the scanned electrons are responsible for image formation. TEM & SEM are efficiently used for nanoparticles from early decade of twenty-first century, but recently after several instrumental advancements now researchers are performing

TEM and SEM images for nanoemulsions too. (Klang et al. 2012; Jaiswal et al. 2015). It the current decade several protocol has been established for SEM and TEM analysis of nanoemulsions and many food grade and drug grade nanoemulsions have been characterized by SEM and TEM (Da Costa et al. 2014; Singh et al. 2015a, b; Lee et al. 2016). Further, it can be noted that SEM/TEM need the perfection – instruments as well as operator side – for analyzing translucent nanoemulsion droplets by using SEM/TEM. It can be noted that other microscopy techniques e.g. multi-photon confocal microscopy, confocal laser scanning microscopy, two-photon excitation microscopy has also been used in nanoemulsion based researches but not for the characterization of nanoemulsions but to analyze the effect of nanoemulsions on different cells or tissues as these microscopes are working on the excitation wavelengths which ultimately come from the several standard dyes used (Choi et al. 2014; Attia et al. 2015; Liuzzi et al. 2016).

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Chapter 7
Food-Grade Nanoemulsions: Review on the Possible Market

Abstract Nanoemulsions are thermodynamically stable, low viscosity, transparent, and isotropic dispersions consisting of water and oil stabilized by an interfacial film of surfactant molecules, in conjunction with a cosurfactant. Nanoemulsions (so-called due to their small particle size; 5–100 nm) have found application in a wide variety of systems, such as pharmaceutical, food, agriculture, environment and oil recovery. Understanding the market of the basic research will lead to development of products with real-life use. The same have been explained in brief with the aim to focus on the future market of food-grade nanoemulsions to attract researchers, scientists and industrialists.

Keywords Nano-emulsified foods • Benefits • Future • Beverages • Dairy • Confectionary

7.1 Introduction

Food-grade nanoemulsions constitute one of the most promising systems for improving the solubility, bioavailability, stability, and functionality of many bioactive compounds (Fig. 7.1). These improved properties of nanoemulsions can be exploited for many novel and technological innovations that find various industrial applications.

7.2 Beverages

The industrially produced emulsified beverages are typically divided into two major groups: flavor emulsions and cloud emulsions. Flavor emulsions contain lipophilic compounds that are primarily present to provide taste and aroma to a beverage product (such as lemon, lime, or orange oils). On the other hand, cloud emulsions are used to provide specific optical properties to certain beverage products, i.e., to increase their turbidity ("cloudiness"). Cloud emulsions are typically prepared

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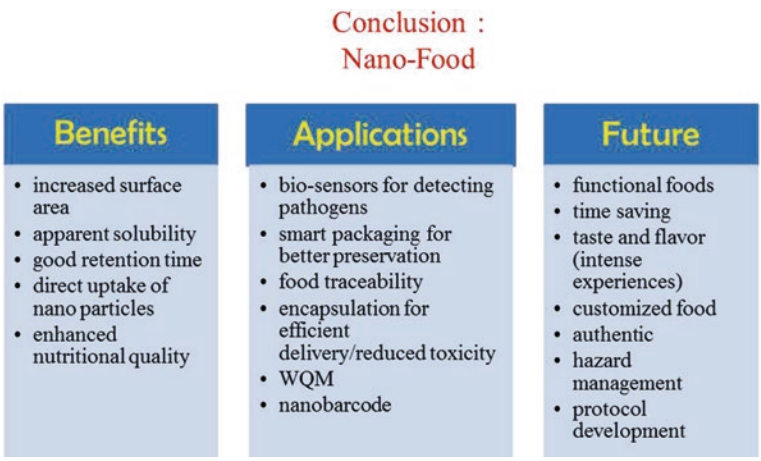

Fig. 7.1 The beneficial properties – increased surface area, apparent solubility, good retention time, direct uptake of nanomaterials, and enhanced nutritional quality – open the ample scope for the nanotechnology with different applications and have best future to cover the market (*Courtesy:* Dasgupta et al. 2016)

using an oil phase that is highly water-insoluble and that is not prone to chemical degradation, such as flavorless vegetable oils. In addition, the size of the droplets within cloud emulsions is designed so that they have dimensions where strong light scattering occurs, but are not too large to undergo gravitational separation. Cloud emulsions are often added to beverages that only contain a relatively low percentage of juice and provide a desirable cloudy appearance that hides sedimentation and ringing. Ron *et al*. have used beta-lactoglobulin and pectin nanocomplex for the encapsulation of vitamin D2, suitable for enrichment of clear beverages and non-fat foods (Ron et al. 2010).

Recently, there is a push to fortify many beverage products with ingredients that are perceived as giving added health benefits, such as vitamins, minerals, antioxidants, peptides, proteins, dietary fibers, and u-3 fatty acids. These nutraceutical additives vary greatly in their molecular, physicochemical, and biological properties, such as physical state, solubility, charge, polarity, interactions, and flavor profiles. For example, carotenoids and polyunsaturated lipids may oxidize during storage and produce undesirable flavors or changes in color. Multivalent mineral ions (such as calcium) may precipitate from solution, promote precipitation of other components (such as proteins), or cause undesirable mouthfeel. Dietary fibers may increase the viscosity of a product, promote depletion flocculation, or give a slimy mouthfeel. Consequently, each beverage product must be carefully formulated to take into account the specific types of nutraceutical components it contains.

7.3 Dairy Industry

Common examples of emulsions in dairy industry are margarines, fatty spreads and homogenized milk. In homogenized milk, the fat globules are reduced in size and dispersed uniformly. The dairy industry utilizes three basic microsized and nanosized structures (casein micelles, fat globules, whey proteins) to build all sorts of foams (ice cream and whipped cream), emulsions (butter), complex liquids (milk), plastic solids (cheese), and gel networks (yogurt). In a study, ice cream was enriched with alginate nanoparticles including Fe and Zn salts (Sharifi et al. 2013). Unilever has produced ice cream by lowering fat content from 16% to 1% without compromising on taste through the application of nanoemulsions (Hélder et al. 2011). Caesin micelles and whey proteins are used as food grade emulsifying agent for entrapment, protection and delivery of sensitive hydrophobic nutraceuticals within other food products. Anarjana and Tan have developed astaxanthin enriched milk in which they have incorporated astaxanthin nanoemulsions in skimmed milk and observed significant cellular uptake and stability of the nanodispersions (Anarjan and Tan 2013). Fortified yogurt with the help of nanoemulsion is also developed by supplementing with Omega-3 Polyunsaturated Fatty Acids (McCowen et al. 2010) or rice bran oil.

Sometimes biopolymers, such as gums or gelatin, are added to whipping cream to improve its whipping properties by increasing the viscosity of the aqueous phase, thereby slowing down the movement of air bubbles. The addition of sugars to whipping cream can be either detrimental or advantageous to foam quality (Hartel and Hartel 2001; Ron et al. 2010). If the sugars are added prior to whipping, the foam volume and stability may be adversely affected because the sugars stabilize the globular structure of the whey proteins, thereby retarding protein surface denaturation and aggregation and reducing the tendency for a viscoelastic protein membrane to form around the air bubbles. On the other hand, if the sugars are added after whipping the foam stability is often improved because they increase the viscosity of the aqueous phase and therefore retard the movement of the air bubbles. Small molecule surfactants are often added to the ice cream mix prior to aging because they displace proteins from fat globule surfaces, which facilitate partial coalescence of the fat globules during the subsequent freezing/agitation process.

7.4 Confectionary

Chocolate is a suspension of non-fat particles (sugar, cocoa solids and milk solids) in a continuous fat phase (cocoa butter). Emulsifiers affect various properties such as bloom, the stability against fillings, and oxidization. However, the main advantage of emulsifier application in chocolate is the improvement of flow parameters, thus allowing to minimize cacao butter addition and to reduce production costs. However application of ‘nano’ emulsion in confectionaries is very limited. Recently,

few patents have been filed or granted for NEs with future applications in confectionary industries (Hung 2011a, b). Interestingly, except the two of the above mentioned two patents, none of the granted patents have been found in Google Patent search engine. In the meanwhile it can be noted that though reports are not found on the applications of nanoemulsions in confectionary industries but as per our expectations nanoemulsions have potential future to be applied in the below discussed confectionary fields too.

The confectionary system that most commonly relies on emulsifiers is toffee. A typical toffee has a continuous phase of high solids sugar syrup with milk proteins present. The disperse phase may be all milk fat, a mixture of vegetable fat and milk fat, or purely vegetable fat. The interface between the two phases is likely to be formed of some of the milk protein and any added emulsifier. Another type of emulsion of interest in confectionary is that of foams and these can be regarded as a dispersion of air within a liquid. Some confectionary products such as whipped montelimars are a fat-in-sugar syrup emulsion with air whipped in.

The usual use of emulsifiers in confectionary is to keep oils or fats dispersed. A subsidiary effect is also to alter the texture since the texture of a product is affected by the size of any dispersed fat globules and a related effect to this is the handling of the product during manufacture since the product will need to flow and probably be shaped and cut. Adding the wrong emulsifier or an excess of emulsifier can cause handling problems. In this section, emulsifier means a material that is on the list of permitted emulsifiers and stabilizers – there are some materials that are extremely effective emulsifiers but which, in fact, do not qualify as emulsifiers in food law.

Caramels and Toffees: In these products, the emulsifiers are used to assist in dispersing the fat. Toffees or caramels can be made without adding any emulsifier but only at the expense of using a larger quantity of skim milk solids in the recipe. Chewy Confectionery: These products are usually made by dispersing some fat in a mixture of sugar and glucose, the resulting product being flavoured with a fruit or possibly mint flavor. Some of these products contain egg albumen or other proteins. This type of confectionary normally contains an emulsifier to assist with fat dispersion and to give the desired texture. In chewing gum, emulsifiers can act as a plasticizer. This is not too surprising as chewing gum is effectively a chewable polymer product. Tabletted products: In these systems, emulsifiers can be used to disperse oily ingredients such as flavours. Another use is to make it easier for the granules to flow during processing.

7.5 Some Important Marketed Nano-emulsified Nutraceuticals

Nanoemulsion production for encapsulation and delivery of functional compounds is one of the major fields of nanotechnology applied to food industry. Applications of this technology is described in examples are given below.

Unilever has made ice cream healthier without compromising on taste through the application of nanoemulsions. The objective is to produce ice cream with lower fat content, achieving a fat reduction from the actual 16–1% (Silva et al. 2012; Ranjan et al. 2014). Other applications of nanoemulsions into the food industry include antimicrobial nanoemulsions for decontamination of food equipment, packaging or food which have been provided in our review (Ranjan et al. 2014; Dasgupta et al. 2015).

Aquanova has developed a nanotechnology-based carrier system using 30 nm micelles to encapsulate active ingredients such as Vitamins C and E and fatty acids which can be used as preservatives and aids (Aquanova undated). Aquanova markets its micelles as "NovaSol" and claims that the nanoscale carrier system increases the potency and bioavailability of active ingredients. Aquanova in collaboration with Zyme are offering omega 3 in 30–40 nm size range nano-capsules which is 4000 times smaller to the existing product in market (Halliday 2011; Silva et al. 2012; Ranjan et al. 2014; Dasgupta et al. 2015). NovaSol portfolio is divided into two categories: healthy functional compounds (coenzyme Q10, DL-a-tocopherol acetate, vitamins A, D, D3, E, and K and omega three fatty acids) and natural colourants (β-carotene, apocarotenal, chlorophyll, curcumin, lutein and sweet pepper extract) (Silva et al. 2012). Novasol has been used as an optimum carrier system of hydrophobic substances for a higher and faster intestinal and dermal resorption and penetration of active ingredients. Aquanova claims enhanced stability (both in terms of pH and temperature) of encapsulated functional compounds and standardised additive concentrations (Silva et al. 2012; Ranjan et al. 2014).

NutraLease, a technology start-up company by a scientific team is working to improve the bioavailability of functional compounds. Some functional compounds like lutein, lycopene, β-carotene, vitamins A, D3 and E, Q10, phytosterols, and lastly isoflavones are available contained in beverages. Their technology is derived from self-assembled (implying low energy approach) nanoemulsions which then achieves a better encapsulation rate as well as an improved bioavailability in the human body (Halliday 2011; Silva et al. 2012; Ranjan et al. 2014; Dasgupta et al. 2015). NutraLease nanoemulsions can protect flavour compounds from manufacturing conditions and this continues all through the beverages' shelf life. It is claimed that nanoemulsions can capture the flavour and protect it from temperature, oxidation, enzymatic reactions and hydrolysis and are thermodynamically stable at a wide range of pH values (Silva et al. 2012). The product brand name is nano-self-assembled structured liquids (NSSL) under the category of genetic food additive which contains nano-micelles for encapsulation of nutraceuticals. NSSL is used for improved bioavailability means nutraceuticals are released into membrane between the digestive system and the blood.

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Chapter 8
Nanotechnology in Food Packaging

Abstract Packaging of the food products are the major and unavoidable stage of food supply chain as the better packaging leads to longer durability, shelf life and maintenance of mechanical, physical, chemical and physio-chemical properties of food products. Here in this review, (i) a details of nano-packaging have been discussed; (ii) different forms of nano-packaging systems have been discussed to meet specific requirements; (iii) additionally, the recent trends, future and opportunities of nano-food packaging have been discussed.

Keywords Nano-packaging • Sensors • Antimicrobials • Shelf life • Durability

8.1 Introduction

Nanotechnology in food packaging materials may increase the shelf life of the food by using high barrier packaging that improves the thermal and mechanical properties. Nanosensors can also be incorporated into the packaging systems to indicate the spoilage of the food, alert the consumers that the food is unfit for consumption, repair the damages in packaging, and release preservatives to extend the shelf life of the food. Encapsulation techniques can be used to incorporate nanosupplements for nutritional enhancement and targeted drug delivery systems. The advantages provided by nutritional enhancement overpower the disadvantages of the technology (Chellaram et al. 2014). Food industries are always on the search for cheaper methods for food production and preservation. This takes us into the realm of nanotechnology. Recent trends in food packaging rely on nano-reinforcement, nanocomposite active packaging and nanocomposite smart packaging. Different reinforcement methods using nanoclays, cellulose are used to enhance the tensile strength of the food packaging.

Food packets can also be integrated with different agents like antimicrobial agents, oxygen scavenging agents and enzyme immobilization systems to form nanocomposite active packaging. Nanocomposite smart packaging involves sensors, e.g., gas detectors, time–temperature integrator (TTI) and other nanosensors (Ranjan et al. 2014).

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8.2 Nanoreinforcements in Food Packaging Materials

Food packaging, now, relies mainly on the use of polymers instead of conventional materials like glass, metals, ceramics, paper etc.). However, polymers face some limitations like they are not as resistant to deformations as compared to ceramics and metals. Another limitation of polymeric packaging is its permeability to gases and vapors (Arora and Padua 2010). Nanoreiforcement fills the gaps between the polymer molecules to increase the strength and viability of the packaging material. Polymer nanocomposites provide the advantage of better filling than the conventional composites (Luduena et al. 2007). Composite properties are significantly enhanced by the use of nano fillers. They enhance the, thermal stability, mechanical strength and barrier properties of the composites. Properties of nano-fillers that play a vital role in modifying the properties of the composite are; their size and shape; their affinity towards matrix material and filler loading. Nanocomposites with nanoclay filled polymer matrix have gained quite an attention in the materials industry for their improved performance since exfoliated clays could exhibit superior barrier properties, higher strength and modulus compared to unfilled polymer matrix (Goettler et al. 2007). Filler size has now become quite important with respect to the polymer size. There has been a surge in the interest in nanotechnology since they can exhibit unique characteristics that are difficult to achieve with their conventional micro-scale equivalents (Kriegel et al. 2008). Nanoclay material finds a lot of application in the packaging industry owing to their low cost, easy processing good performance and easy availability. Carbon-based packaging materials like graphene nanosheets and carbon nanotubes are also being developed (Arora and Padua 2010). Nanofiller incorporation and distribution in the polymer matrix increases the matrix/filler interfacial area, which improves its barrier properties, mechanical, thermal properties (glass transition temperature- Tg) and restricts the mechanical mobility of the matrix. Fillers with higher aspect ratios provide better reinforcing effects due to their higher specific surface area (Dalmas et al. 2007). The aspect ratio is as the ratio of the largest to the smallest dimension of filler An interphase region with decreased mobility surrounds each nanofiller and this plays an important role in improving the composite performance (Qiao and Brinson 2009). When there's constant filler content, the number of filler particles increase with the reduction in the particle size, which brings them closer to one another, causing the overlapping of the interface layers from adjacent nanoparticles. This alters the bulk properties to a great extent (Jordan et al. 2005).

8.2.1 Nanoclays Reinforcement

A novel class of clay filled polymers, called PCNs has been developed over the past two decades. They performance of polymers used in food packaging can be improved by the use of PCNs. In the conventional tactoid structure of

microcomposites, the clay tactoids and polymers remain immiscible (Luduena et al. 2007; Alexandre et al. 2009). However, the interaction between polymers and layered silicates may produce two types of nanoscale composites, namely: intercalated nanocomposites in which the polymer layers penetrate into the interlayer region of the clay, to produce an ordered multilayer structure with alternating polymer/inorganic layers (Weiss et al. 2006), and exfoliated nanocomposites, in which there is extensive penetration of the polymers into the delaminated and randomly dispersed layers of clay (Luduena et al. 2007).

8.2.2 Cellulose Nanoreinforcements

Cellulose nanoreinforcement is a profitable material to prepare lightweight, low cost, and high-strength nanocomposites (Podsiadlo et al. 2005). Microfibrils are cellulose chains synthesized in living organisms (mainly plants). They are bundles of elongated molecules (2–20 nm in diameter and micrometric in length) stabilized by hydrogen bonds (Mattoso et al. 2009). Each microfibril, has crystalline and amorphous regions. The crystalline parts consist of nanocrystals or nanowhiskers whose aspect ratios are related to the origin of cellulose and its processing conditions. They can be isolated by acid hydrolysis (Azizi Samir et al. 2005).

Cellulose reinforcement has a positive effect on tensile properties like modulus of polymers, but the decrease the elongation. According to Helbert et al. (1996), the effect of cellulose nano-reinforcements on modulus due to the presence if a fibrillar network within the polymer matrix, hydrogen bonding between the cellulose nanoreinforcement, stiffness and geometry of the fillers (Helbert et al. 1996). The presence of nano-reinforcements increases the resistance of the diffusivity path for the permeants and hence, lowers the polymer permeability (Sanchez-Garcia et al. 2008). Studies suggest that the barrier properties of polymers are improved by the addition of cellulose nano-reinforcement. If the filler has less permeability, is well dispersed and has a high aspect ratio, the barrier properties are enhanced more (Lagaron et al. 2004).

8.2.3 Other Nano-Reinforcements

Carbon nanotubes have a high value of elastic modulus and high aspect ratio. They may be in the form of single walled nanotubes that are one atom thick or they multiwalled nanotubes that consist of a number of concentric tubes (Zhou et al. 2004). Carbon nanotubes have been found to increase the tensile strength/modulus of several polymers such as polyethylene naphtalate (Kim et al. 2008), polypropylene (Prashantha et al. 2009), polyamide (Zeng et al. 2006) and polyvinyl alcohol (Chen and Evans 2005). Silica nanoparticles ($nSiO_2$) improve the tensile strength and oxygen barrier property of polypropylene (Vladimirov et al. 2006). It also improves the

tensile strength of starch (Xiong et al. 2008), and decreases the water absorption by starch (Tang and Liu 2008; Xiong et al. 2008) and (Vladimirov et al. 2006). Nanocomposites of polyvinyl alcohol with $nSiO_2$ were prepared by Jia et al.(2007) using radical copolymerisation of vinyl silica nanoparticles and vinyl acetate and they were found to exhibit better thermal and mechanical properties than pure polyvinyl alcohol because of the strong covalent bonding between SiO_2 and the polymer matrix (Jia et al. 2007).

8.3 Nanocomposite Active Food Packaging

An active food packaging provides a twofold benefit, it acts as a barrier system for food spoilage microorganisms and at the same time, it interacts with the food in a beneficial way, for example, eliminating the factors that cause food spoilage (such as water vapor and oxygen), releasing beneficial compounds like antioxidants or antimicrobials etc. Such interactions bring about stability in the food and food packaging, food stability. Some of the main examples of food packaging systems are given in the below sections (Ranjan et al. 2014). Some other nanotechnology packaging based products have been given in Table 8.1.

8.3.1 Antimicrobial Systems

Nanoparticles have also been patented as antimicrobial agents. Several particles have been used for antimicrobial activity such as nanoparticulated silver, gold, zinc, copper, cerium (Ismail et al. 2008), aluminum, iron, cadmium, palladium, rhodium or chromium (Park and Kim 2003). These nanometals inactivate the proteins in the microorganisms by releasing metal ions. The inactivation of the protein causes the death of the microorganism by disrupting cellular function, membrane synthesis and function, hindering the replication of DNA (Ismail et al. 2008). Antimicrobial systems control the growth of pathogenic and spoilage causing organisms on food surfaces. Nanocomposites naturally have different properties when compared to their macroscale counterparts, because of which they are greatly being put into use. This is because of the higher surface to volume ratio of nanoscale materials when compared to microscale products. The high surface area to volume ratio allows the attachment of more number of microbial cells and molecules (Luo and Stutzenberger 2008). Nanomaterials acts as antimicrobial agents by inhibiting the growth, killing the cells (Huang et al. 2005; Kumar and Münstedt 2005) or by their antibiotic activity (Gu et al. 2003; Cioffi et al. 2005).

Silver has a multifold action as a food packaging material; it is toxic towards many microorganisms (Liau et al. 1997), possesses high temperature stability and has low volatility (Kumar and Münstedt 2005). A larger surface area provides a greater interaction with microbial cells (Kvitek et al. 2008), an example

Table 8.1 Details on the Nano-food processing products or process which has been implemented in market

Product	Company	Details	Applications	Reference
FresherLonger™ Miracle food storage	Sharper Image® USA	Food Storage containers — infused with naturally antibacterial silver nanoparticles (25 nm)	it's easy to keep foods like fruits, vegetables, herbs, breads, cheeses, soups, fresher three or even four times longer.	The Project on Emerging Nanotechnology (2013)
FresherLonger™ Plastic storage bags	Sharper Image® USA	Re-sealable zip-top food storage bags are infused silver nano-particles	To reduce the growth of bacteria, mold and fungus and thereby increases their shelf life.	
Nano silver baby mug cup	Baby Dream® Co., Ltd. Korea	Silver nanoparticles infused with containers.	To reduce the growth of bacteria, mold and fungus by 99.9% and thereby maintain freshness.	
Samsung® Refrigerator	Samsung® Korea	Used silver nanoparticles	Interior coating of refrigerators for effective sterilization deodorization and anti-bacterial effects.	
Nano silver NS-315 water bottle	A-DO Global Korea	Used silver nanoparticles	Antibacterial, Antibiotic effect by Nano Silver.	
			No smell, harmless to food by silver	
Beer bottle plastics	Voridian USA	Imperm (a plastic) incorporated with clay nano-particles	Provide barrier to CO_2 which trying to escape out and O_2 which trying to enter inside bottle	
Daewoo® Refrigerator	Daewoo Korea	Nano Silver Poly technology – Nano silver particle imbedded in plastic resin	Restrain the growth and increase of a wide variety of bacteria and to suppress odours	
EcoSphere	Ecosynthetix USA	Starch nanoparticles	Use for adhesive- requires less water and less time and no use of other chemicals like other adhesive	
Food container	A-DO Global Korea	Used silver nanoparticles	Antimicrobial activity	
Hite Brewery beers	Honeywell USA	Nano-nylon materials	Oxygen-scavenging activity	

(continued)

Table 8.1 (continued)

Product	Company	Details	Applications	Reference
N-Coat	Constantia Multifilm	A thin layer of nano-sized clay particles added to polyester	Gas barrier properties developed primarily for dry foods	
	USA			
Nano flagon – Moon drunker	Top Nano Technology Co., Ltd.	Used nanoclay particles	Drastically increasing aroma, Less peculiar smell and spicy taste, Decreasing content of alcohol, less toxicity	
	Taiwan			
Nano plastic wrap	SongSing Nano Technology Co., Ltd.	Nano-ZnO mixed with plastic	Anti-UV, Reflecting IR,Sterilizing and anti-mold, Having better temperature, tolerance, Fire-proof, Bearing grinding	
	Taiwan			
Nano-silver storage box	Quan Zhou Hu Zheng Nano Technology Co., Ltd.®	Nano-silver incorporated into preservation box	Preservation can be extended for fruits, dry goods, vegetables, fruit juice and other foodstuffs	

of which is the better antimicrobial activity of silver nanoparticles in comparison to bulk silver (Yu et al. 2007). Silver is often used for nanocomposite formation due to its antimicrobial activity which follows the given mechanism: (a) cell surface adhesion, increasing the membrane permeability by degrading the lipopolysaccharides and forming 'pits' in the membrane (Sondi and Salopek-Sondi 2004), (b) penetration into the bacterial cell, DNA damage (Li et al. 2009b) and (c) dissolution of silver nanoparticles and the subsequent release of Ag^+ ions (Morones et al. 2005). The released Ag^+ ions bind to several electron donor groups in biological systems, containing oxygen, nitrogen or sulfur (Kumar and Münstedt 2005; Morones et al. 2005). Use of silver nanoparticles also extends the shelf life of fruits and vegetables by decomposing ethylene which causes premature ripening (Li et al. 2009a).

Nanosilver has been widely used for development of contact materials for preservation of foods for longer time by preventing growth or killing the microorganism.For example, BlueMoonGoods LLC has developed fresh box super airtight containers using silver nanoparticles that reduce the bacteria up to 99% and prevent the spoilage of food. This increases the shelf life of the food product. Nanoparticles of silver have anti-bacterial, anti-microbial and anti-fungal properties, which when incorporated into the food packaging or container, provide the consumers fresher and better quality of food which increased shelf life and also decreases food wastage by preserving the food for longer duration (The Project on Emerging Nanotechnologies 2013). Costa et al. (2011) demonstrated that the addition of Ag-MMT nanoparticles to propylene boxes preserves the fresh food salad for a long duration (Costa et al. 2011). It was also tested by the same research group that shelf life of carrot can be increased by more than 2 months when stored under 4 ± 1 °C (Costa et al. 2012).

A Nanostructed calcium silicate (NCS)-Ag complex was prepared by the adsorption of Ag^+ ions from a solution using nanostructured calcium silicate (Johnston 2010). This complex exhibited significant anti-microbial activity even at low levels of silver (10 mg/kg) and was suitable to incorporate into the food packaging. Titanium dioxide (TiO_2) exhibits photocatalysis and is widely used to disinfect surface coatings (Fujishima et al. 2000). Photolysis of TiO_2 causes the peroxidation of the phospholipid cell membranes (Maness et al. 1999) which inactivates the food pathogens (Robertson et al. 2005). A TiO_2 powder-coated packaging film capable of reducing E. coli contamination on food surfaces (Chawengkijwanich and Hayata 2008). Gelover et al. (2006) TiO_2-coated films, after exposure to sunlight, can inactivate faecal coliforms in water, This was demonstrated by (Gelover et al. 2006). Metal doping improves the visible light absorbance of TiO_2 (Anpo and Takeuchi 2001), and increases its photocatalytic activity under UV radiation (Choi et al. 1994). Photocatalytic bacterial inactivation of TiO_2 is enhanced by doping it with silver (Reddy et al. 2007). Cheng et al. (2006), reported significant antibacterial activity from a polyvinyl chloride nanocomposite incorporated with TiO_2/Ag^+ nanoparticles(Cheng et al. 2006). Chitosan nanoparticles also exhibit anti-bacterial properties due to the electrostatic interactions that occur between the positively

charged chitosan molecules and the negatively charged cell membrane. Such interactions make the cell membrane more permeable, causing leakage and rupturing of the intracellular material Qi et al. (2004) also demonstrated that both chitosan and its engineered nanoparticles are ineffective at pH lower than 6, because of the protonation of the amino groups. Chitosan can be added with sorbic and benzoic acids and their nano-sized solubilisates are used to prepare antimicrobial coatings for meat (Cruz-Romero et al. 2013).

Rabea et al.(2003), proposed two another anti-microbial mechanisms: chitosan mediated chelation of trace metals which causes inhibition of enzyme activities of the microorganism; and (in fungal cells) penetrating the cell membrane and cell wall followed by DNA binding and inhibition of RNA synthesis (Rabea et al. 2003). Carbon nanotubes also possess antimicrobial properties. The thin and long nanotubes puncture the cell membrane and cause leakage of the intracellular material and permanent damage to the cell. However, some studies suggest that carbon nanotubes may have cytotoxic effect on human cells, especially on contact with skin (Monteiro-Riviere et al. 2005) or lungs (Warheit et al. 2004). It is important to know the health effects of carbon nanotubes since they can migrate into the food from the packaging material.

8.3.2 O_2 Scavengers

Oxygen (O_2) participates in several forms of food deterioration. Low levels of O_2 can be maintained by incorporating O_2 scavenging systems into food packaging. Low levels of O_2 prevent direct oxidation reactions that result in browning reactions and rancidity, food deterioration by indirect action of O_2 occurs by the action of aerobic microorganisms Xiao-e et al. (2004) developed oxygen scavenger films by adding TiO_2 nanoparticles to different polymers (Xiao-e et al. 2004). Many oxygen sensitive food products can be preserved by the use of nanocomposite materials as packaging films. A major limitation of using TiO_2 is that it requires UVA light for its photocatalytic action (Mills et al. 2006). Commonly used polymer polythene is known for moisture and barrier applications. But it shows poor oxygen barrier properties and is unsuitable to package oxygen sensitive food products. To overcome this limitation, iron containing kaolinite has been incorporated into the high density polythene films to create an oxygen barrier (Busolo et al. 2010).

A nanocomposite named Imperm is made by Voridian collaboration with Nanocor, is used in plastic beer bottles and makes bottles as hard as glass but a more stronger and lighter. Further the nanoparticles is designed in a way that it minimizes the loss of carbon dioxide from the beer and restricts the entry of oxygen into the bottle, therefore, retains the freshness of the beer and extends its shelf life (The Project on Emerging Nanotechnology 2013). Another product developed by Honeywell (USA)"Aegis® OX. They developed Multilayer polyethylene tere-

phthalate (PET) bottles that make use of nanocomposites (nylon resin) to enhance the barrier properties and extended shelf life (The Project on Emerging Nanotechnologies 2013). Another hybrid plastic Durethan KU2-2601 (Bayer AG) is developed by incorporatingNanocor's clay to produce a film that is stronger, more hear resistant and lighter than conventional packaging materials. The film prevents food spoilage by preventing the entry of oxygen and other gases and retaining the moisture inside the bottle (Han Wei et al. 2011; Gruère 2012).

8.3.3 Enzyme Immobilization Systems

Enzymes have a various applications in food industry. Specificity of enzyme catalysts assure improvements in many applications, like sugar and corn syrups, dairy, baking products, alcohol drinks, meat tenderness or cheese ripening, but due to their short lifetime limit their usefulness in many applications (Perez-Esteve et al. 2013). However, often the enzymes cannot be directly added to the food items because of their sensitivity to the manufacturing conditions. Immobilization improves the enzyme stability to temperature, pH, denaturing compounds and proteases. They also facilitate its repeated use and controlled release (Lopez-Rubio et al. 2006). By incorporating enzymes like cholesterol reductase and lactase to packaging material, the value of the food product is improved and it also makes up for enzyme deficiencies in customers (Fernández et al. 2008). Due to the higher surface area to volume ration, nanoscale enzyme immobilization systems perform better than their conventional counterparts (Fernández et al. 2008).

Different approaches are required for enzyme adsorption into nanoclays incorporated to polymers (Rhim and Ng 2007), because of the high affinity of nanoparticles for proteins and also because they are good enzyme carriers (Gopinath and Sugunan 2007). Conductive polymers are often used for the immobilization of biomolecules (Ahuja et al. 2007), as reported by Sharma et al. (2004). He also immobilized glucose oxidize onto films of poly (aniline-co-fluoroaniline) (Sharma et al. 2004). Lactate dehydrogenase and glutamate dehydrogenase have shown remarkable enzyme activity on being immobilized on SiO_2 nanoparticles (Qhobosheane et al. 2001).

Very few patents product are available in the market at the moment (Table 8.2). According to literature silica and nanoporous silica material have been used in enzyme immobilization. Porous silica has various unique properties like high stability and biocompatibility, very large surface/volume ratio therefore large load capacity, tailor-made pores, the capability to avoid mechanisms of inactivation, and have a well-known and easy functionalization chemistry that can lead to a control of both size and pore polarity. Carbon nanotube (electrochemical biosensing applications) (Gu and Cole 2011), ZnO (Lu et al. 2005) and gold nanoparticles have been used as nanoplatforms for enzyme immobilization (Perez-Esteve et al. 2013).

Table 8.2 List of important patents filed or granted recently, which has potential to hit the food market

Enzyme	Immobilization nanoplatform	Aim	Patent No.	References
Trypsin, aprotinin, obalbuminmucin or lipase	Nanomegnetic chitosan	High protein load and high catalytic activity	CN1904043A Patent application	Xiaoning (2007)
Protease	Nano carbon-tube	High stability and efficient transport of protease.	CN102373192A Patent application	Liu Yang et al. (2012)
Cellulase,Amylase, glucoamylase, xylanase, glucoseisomerase	Zeolites and silica gel	Increase activity and stability for the use in industrial processes.	WO2004081208	Saville and Khavkine (2007)
Thermolysin	Silica gel	Produce low molecular weight protamine (LMWP).	WO2007050100	David and Yang (2007)
Hydrolases, perhydrolases, haloperoxidases	Silica-based nanomaterial or carbon nanotubes	Produce oxygen metabolites at concentrations sufficient to kill or inactivate a variety of different infectious agents.	WO2010123534	Dinu et al. (2010)
Lipase, protease, peroxidase, cellulose, laccase and oxidative dehydrogenase	Nano chitosan fibre composite	High surface area, good bioavailability, stability,	CN1948474A Patent application	Huang Xiaojun (2007)

The application of gold nanoparticle (Au nanoparticles) as protein immobilization is widely used in food industry. Free cystein is required for immobilization of protein on Au nanoparticles surface (Cedervall et al. 2007). α-Amylase activity is retain by immobilization of enzyme on Au nanoparticles(Rangnekar et al. 2007), and the enzymes on the Au nanoparticles can prevent particle agglomeration, thereby increase stability of enzyme. We can alter this approach for use in food industry for detects starch digestion by enzyme when α-amylase catalysed starch-gold nanoparticle. The enzyme was subsequently immobilized on the Au nanoparticle surface via thiol linkages after digestion of the starch (Deka et al. 2008). This approach has been used to notice starch digestion by the α-amylase (Agyei et al. 2015). Luo et al. demonstrated that glucose oxidase immobilization on gold nanoparticles enhance the stability of the biosensor in the time, since gold

nanoparticles were able to strongly adsorb the enzyme and thus inhibit the leakage of enzyme (Table 8.3) (Scognamiglio 2013).

Graphene and its derivatives is known for advantage over other nanomaterial is uniform nanosheets of single carbon atoms, which provides a enormous and uniform area for the biomolecule (enzymes) attachment (Shan et al. 2009; Zhang et al. 2010) and used as suitable carrier for enzyme immobilization (Agyei et al. 2015). Nanosheets of graphene derivatives used as immobilization of α and β-galactosidase enzyme using cysteamine as the spacer arm and glutaraldehyde as the cross-linker (Singh et al. 2014). Separation of these nano-biocatalysts from reaction can be done by low-speed centrifugation or sedimentation methods with good recovery. Immobilization of α-galactosidase using graphene showed enhanced thermal stability from 50 to 80 °C. Optimum temperature for soluble and immobilized Cicer α-galactosidase is 50 and 80 °C respectively. The soluble enzyme retained more than 90% activity when kept at 50°Cfor 15 min but lost 90% activity within 2 min when kept at 70 °C, whereas immobilized Cicerα-galactosidase retained almost 90% activity at 80 °C for 60 min. And also retained 70% residual activity after 12 repeated uses. The immobilized enzyme has been used for the hydrolysis of the raffinose family of oligosaccharides from soybean derived food products, reducing the gas problems in alimentary canal caused by consumption of soybean products(Singh et al. 2014). Similarly immobilization of β-galactosidase on nanosheets of grapheme was used for the hydrolysis of whey lactose, a by-product of the cheese industry. Immobilization of β-galactosidase had shown 92% activity after 10 successive reuse (Kishore et al. 2012).

8.4 Nanocomposite Smart Packaging Systems

The use of nanotechnology to established a system (nanosensor system) which can detect minor changes in environment such as temperature change, changes in humidity or oxygen exposure during storage, chemical changes due degradation of food components or due to contamination by microorganism (Bouwmeester et al. 2009). A ‘smart’ food packaging system has the ability to that ‘perceive’ some property of the packaged food and register and communicate information about the quality of the food with respect to its edibility, safety and digestion. Nanosensors incorporated into food packaging systems can be used to detect spoilage-related changes, contaminations by pathogens and chemical contaminations such as veterinary drugs,pesticides, phytotoxins and marine toxins, contaminations from processing etc. and eliminate the dependence on inaccurate expiry dates, and provide accurate status on the freshness of the food (Liao et al. 2005).

Nanosensor incorporated with nanofibrils of perylene-based fluorophores can confirm spoilage by detecting gaseous amines in fish and meat. Nanocomposites of zinc oxide and titanium oxide can also work as sensor system and detect volatile organic compounds. This makes nanocomposite sensors very useful to the consumers by providing accurate data on the quality of the food and also for the producers

Table 8.3 Important details on enzyme immobilization using nanotechnology, which shows recent research trends in up-stream process in food engineering

Enzyme	Nanoparticle	Objective	Applications	References
α-Amylase	Gold nanoparticles	prevent particle agglomeration, thereby increase stability of enzyme	Starch digestion	Deka et al. (2008)
Trypsin	Nanodiamond prepared by detonation (dND)	To enhance thermal and chemical stabilities	Proteolysis	Wei et al. (2010)
α-Amylase	TiO_2nanoprticles	Improved thermal stability	Starch hydrolysis	Ahmad et al. (2013)
β-galactosidase	On nanosheets of grapheme	Increased stability	Hydrolysis of whey lactose	Kishore et al. (2012)
Alcohol dehydrogenase from Thermoanaerobiumbrockii (TbADH)	Gold and silver	Increased stability	Alcohol synthesis	Petkova et al. (2012)
α-Chymotrypsin	Polystyrene nanoparticles	Affect the mobility of the enzyme	Proteolysis (cleaves peptide amide bonds)	Jia et al. (2003)
Cholesterol oxidase	Fe_3O_4 nanoparticles	Enhance stability and retention of activity	Analysis of total cholesterol in Serum	Kouassi et al. (2005)
Mucorjavanicus lipase	silica nanoparticles	Increase hydrolytic activities, resistant to temperature inactivation	Catalyses the hydrolysis of triacylglycerol to glycerol and fatty acids	Kim et al. (2006)

Candida rugosa Lipase	γ Fe2O3 magnetic nanoparticles	Easily separation of enzyme from medium, protect enzyme from denaturation	Used in synthesis of enantioenriched monomers and macromers and for polymerization reactions	Ulman and Gross (2003)
α and β-galactosidase	Graphenenanosheets	Enhanced thermal stability	Hydrolysis of the raffinose family of oligosaccharides from soybean derived food products and whey lactose respectively.	Singh et al. (2014) and Kishore et al. (2012)
Acid phosphatase	nanoporous silica	Enhanced activity and thermal stability	Hydrolyses organic phosphates at an acid pH	Yen Wei et al. (2001)
β-galactosidase	Carbon nanotubes	Improved catalytic activity	*O*-glucosyl compounds releasing glucose and an aglycon	Gómez et al. (2005)

in terms of rapid distribution and validate the quality of the food product (Chellaram et al. 2014). Nanosensors using single particles, such as carbon nanotubes, or arrays of nanoparticles, such as quantum dots, have been used for detecting target analytes. For instance carbon nanotubes are used to detect target analytes, such as pesticides, toxins, or contaminants (Boussaad et al. 2006). Antibodies conjugated to nanomaterials, like quantum dotscan be used to detect food spoilage bacteria in the food (Mihindukulasuriya and Lim 2014). Another type of nanosensor involves an array of nanosensors which detect the gases released when the food begins to spoil due to microbial contamination, detection leads to a colour change on the sensor strip, indicating the consumer of the spoilage of the food. One such device, popularly known as the 'electric tongue' has been developed by Kraft Foods to beware the consumer of unhealthy spoilt food (Momin et al. 2013). Another nanosensor has been developed by AgroMicron, named NanoBioluminescence Detection Spray which binds to luminous protein, and the surface of bacteria such as Salmonella and E.coli (Dur´an and Marcato 2013).

8.4.1 Time Temperature Integrators and Moisture Indicators

For determining the kinetics of physical, chemical and microbial spoilage in food products, the most concerning environment factor is temperature. Time–temperature indicators or integrators (TTIs) are designed to monitor, translate and record whether the food is fit for consumption after checking the temperature history of the food. It checks if the food has exceeded the threshold temperature, and also the minimum time it has spent above the threshold temperature. TTT play a very important role in the detection of food quality when the food has been stored in conditions that are not optimal for it. For example, if a food product is supposed to be stored at freezing temperature, a TTI can check and tell if it has been exposed to high temperature and also the duration of exposure The TTIs are categorized into three basic types,, partial temperature history indicators, abuse indicators, and full temperature history indicators. Critical temperature indicators and abuse indicators tell whether the threshold temperature has been reached Partial temperature history indicators find the time-temperature history only when the temperature exceeds a critical predetermined value. Full temperature history indicators provide a continuous information of temperature changes with time (Singh). This is indicated by a color development, due to the migration of a dye through a porous material when there's a change in the temperature or a color change that occurs as a result of a physical or chemical reaction. An example of such an indicator is the Timestrip, which uses gold nanoparticles to detect temperature changes. When the temperature is above the freezing temperature, the color is red and if or when the temperature falls below the freezing temperature, the particles get agglomerated and the red color is lost (Robinson and Morrison 2010).

Other factor which is responsible for the degradation of food is Moisture. Moisture enters through package and accelerates the food degradation. Thus, sen-

sors are used to check the humidity levels in the food package so that the food is not spoilt by moisture. Iridescent technology has been used to develop humidity indicators (Zhou 2013). A thick iridescent nanocrystalline cellulose film was formed by the self-assembly of rigid rod crystallites, leading to chiral nematic texture iridescent films are structures to interact with the electromagnetic field. Color of the dry film is blue-green and it changes to red-orange on exposure to high humidity or water in less than 2 s (Mihindukulasuriya and Lim 2014).

8.4.2 Freshness Indicators

The real time information of products which includes actual product safety and quality during storage and distribution can be provided by Freshness indicators to producer, retailer and/or consumers. Food products can be tested to check whether a food is spoiled or not by detecting the metabolites released by the spoilage microorganisms like volatile sulphides and amine and these metabolites or compounds presents in food products when a food products is spoiled. A technique involved deposition of a transition metal (silver and/or cupper) coating of 1 to 10 nm thick on paper packaging structures or a plastic film which can check the quality of meat, is invented by Smolander et al. (2004). The distinctive dark colour appears when this thin coating reacts with sulphide volatiles components present in spoiled food products (Smolander et al. 2004). Peptide receptor based a portable "bioelectronic nose" is created to check the freshness of food by detecting trimethylamine which present only when the raw seafood is spoiled (Jh et al. 2016).

8.4.3 Detection of Gases

Gas produced during the metabolism of food spoilage microorganisms can be detected by several types of gas sensors that translate the chemical interactions between the gas molecules and surface particles into response signals. For the detection of gases, nanosensors mainly use metal oxides or conducting polymer nanoomposites, which are able to identify or quantify microorganisms based on their respective gas emissions. Conductive polymers usually have conducting particles embedded into an insulated polymer matrix (Arshak et al. 2007). Conducting polymers have unique electronic, magnetic, electrical, and optical properties, which are due to their conjugated π electrons (Ahuja et al. 2007). There is a change in the resistance of the sensing particles on coming in contact with the gas molecules which produces a pattern that is typical of the gas under investigation (Arshak et al. 2007). Polyaromatic and polyene conducting polymers like polyacetylene, polypyrrole and polyaniline, have been studied extensively (Ahuja et al. 2007). The ability of electrochemically polymerized conducting polymers to alternate between the conducting oxidized (doped) state and insulating reduced (undoped) state finds its

application in many sensing systems (Rajesh et al. 2004). Arshak et al. (2007) developed carbon black and polyaniline containing nanosensors that have the ability to detect and identify three food borne pathogens by producing a specific response pattern for each microorganism (Arshak et al. 2007).

8.4.4 O_2 Sensors

Nowadays, both producers and consumers require complete absence of O_2 in oxygen free food packaging. Hence, there exists a huge demand for nontoxic oxygen free packaging systems and irreversible O_2 sensors with packaging done under nitrogen or vacuum. One such example of an O_2 sensor is an UV-activated colorimetric O_2 indicator developed by Lee et al. (2005). It consists of TiO_2 nanoparticles which use UV-A light to photosensitize the reduction of methylene blue (MB) by triethanolamine in a polymer encapsulation matrix. The sensor bleaches when irradiated by UV-A light and its blue color is restored on exposure to oxygen (Lee et al. 2005). The rate of color change is proportional to the level of O_2 exposure (Gutie'rrez-Tauste et al. 2007). Mills and Hazafy (2009) developed a colorimetric O_2 indicator using nanocrystalline SnO_2 as a photosensitizer. It comprised of a redox dye (MB), sacrificial electron donor (glycerol) and an encapsulating polymer (hydroxyethyl cellulose). When exposed to UV-B light, the indicator undergoes photobleaching and the MB is photoreduced by SnO_2 nanoparticles. The indicator film remains colorless when not exposed to oxygen and on exposure it turned blue depending on the level of O_2 (Mills and Hazafy 2009). Mihindukulasuriya and Lim (2013) developed an UV activated oxygen indicator membrane by electrospinning method. The active compounds – TiO_2 nanoparticles, glycerol, methylene blue, were encapsulated in polyethylene oxide fibers while they were electrospun. Polyethyelenefibers are submicron in diameter which increased the sensitivity of the membrane as compared to the cast membrane carrier (Mihindukulasuriya and Lim 2013).

8.4.5 Detection of Microorganism

Another role of nanosenors is the detection of pathogen and toxin in food products when incorporated with food packaging (Bhattacharya et al. 2007). Fluorescent nanoparticles method is the one of the method to detect pathogens and toxin in food (Burris and Stewart 2012). The foodborne pathogenic bacteria species (such as *S.* Typhimurium, *S. flexneri*, and *E. Coli*O157:H7) can be detected by quantum dot coupled with immunomagnetic (Fe_2O_3 magnetic nanoparticles) separation in milk and apple juice (Zhao et al. 2008). Another technique which is used in detecting of *Escherichia coli* in food samples based on the light scattering by cell mitochondria, measurement of light scattering is confers the presence of *E.coli*. The silicon chip contains the protein of a known and well characterised bacterium. This protein can

bind with any other E.coli that has contaminated the food sample and upon binding, a nanosized light is scattered which is detected and analysed by a digitalizing system (Horner et al. 2006). Fu et al. (2008) developed a technique for detection of *Salmonella* is present in food products. Silicon/gold nanorod is incorporated with anti- *Salmonella* antibodies and fluorescent dye, which become visible when comes to attach with bacteria (Fu et al. 2008).

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Chapter 9
Nano-Food Toxicity and Regulations

Abstract Food nanotechnology has seen exponential growth in last decade due to its unique physicochemical properties; however, the risk associated with this emerging technology has withdrawn ample attention in the past decade. Developing novel food-product for better efficiency is important but analyzing the short term as well as long term toxic effects is more important and that is why rules, regulations and other controlling measures. Nanotoxicity is majorly contributed to the small size and large surface area of nanomaterials, which allow easy dispersion and invasion of anatomical barriers in human body. Unique physio-chemical properties of nanomaterials make the investigation of their toxic consequences intricate and challenging. Nano-toxicity has various effects on human health and diseases as they can easily enter into the humans *via* different routes, mainly respiratory, dermal and gastrointestinalroutes.

This review focuses on the nanomaterial-cell interactions leading to toxicological responses. Different mechanisms involved in nanoparticle-mediated toxicity with the main focus on oxidative stress, genotoxic and carcinogenic potential has also been discussed. This review provides a better understanding of the current scenario of the nanotoxicology, disease progression due to nanomaterials, and their use in the food industry and medical therapeutics. Briefly, the required rules, regulations and the need of policy makers has been discussed critically. Here in this review, (i) the toxicity of nano-materials used in foods are discussed in brief; (ii) Safety regulations drafted in context of nano-foods have been discussed; (iii) globally many countries e.g. India do not have any strict regulations to control nano-food products, in this regard, the regulations and recommendations which may fit with nano-foods have been pointed out here. This review will be brief note for the policymakers when the nano-toxicology, nano-regulations will come in lime-light.

Keywords Nano-toxicology • Side-effects • Food-grade nanomaterials • Regulations • Recommendations • Safety

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9.1 Introduction

Modern technologies bring their own set of disadvantages along with their benefits. Nanoparticles possess unique properties (Danie Kingsley et al. 2013) due to their small size and large surface area. However, the same property imparts toxicity to nanoparticles as they can easily diffuse and disperse through biological barriers. Due to their small size, nanoparticles can easily enter the food chain via processed foods, nutrients, environmental pollutants, pesticides etc., increasing the toxicity in food products and ecosystem (Rico et al. 2011), Therefore, there needs to be a thorough investigation on the life cycle of nanoparticles, their uptake by plants, entry in the food chain, bio-distribution etc. before nanoparticles are employed in the agricultural or the food sector. Many factors have to be considered before using nanoparticles to gauge the impact of their exposure on human health (Jasmine et al. 2010). There needs to be a better understanding of nanoparticle based products for their technological development and acceptance in the commercial world and the society. A dynamic, participatory, and responsive nanotechnology policy and coordinated risk management strategy needs to be developed if the Indian agriculture and food system intends to benefit from the economic benefits of nanotechnology (Kalpana et al. 2010; Kalpana Sastry et al. 2013). The small size of nanoparticles imparts some unique properties to them but it also makes them biologically unpredictable and can lead to unexpected outcomes on contact with biological structures. Smaller size also imparts a dissimilar bio-kinetic behaviour and makes them capable to reach extra distal sections of the body (Oberdörster et al. 2005).

Environmental contamination is another risk of the use of nanoparticles. There are many concerns about the undesirable and harmful effects of engineered nanomaterials on the environmental and human health. The importance of nanomaterial risk assessment and management is being recognized by scientists and government regulatory authorities all over the world. Figure 9.1 depicts the interlinked different factors for determining environmental and health risks due to exposure of engineered nanomaterials.

There also needs to be proper understanding of the in-flow and out-flow of nanoparticles into the cells to understand and manage their toxicity. This would also help to improve the bio-medical applications of nanoparticles and formation of regulatory guidelines to reduce the risks associated with their toxicity. The European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has looked into the existing information/data and problems to be considered in conducting risk assessment on nanomaterials (Ranjan et al. 2014; Dasgupta et al. 2015). The European Commission's Scientific Committee on Consumer Products (SCCP) released an article titled "Opinion on Safety of Nanomaterials in Cosmetic Products". They raised concern on ineffectiveness of existing methodologies for the evaluation and management of risks associated with nanoparticle, huge data gaps, and insufficient information about nanoparticles on skin amalgamation in both abnormal (diseased) and normal skins. Different

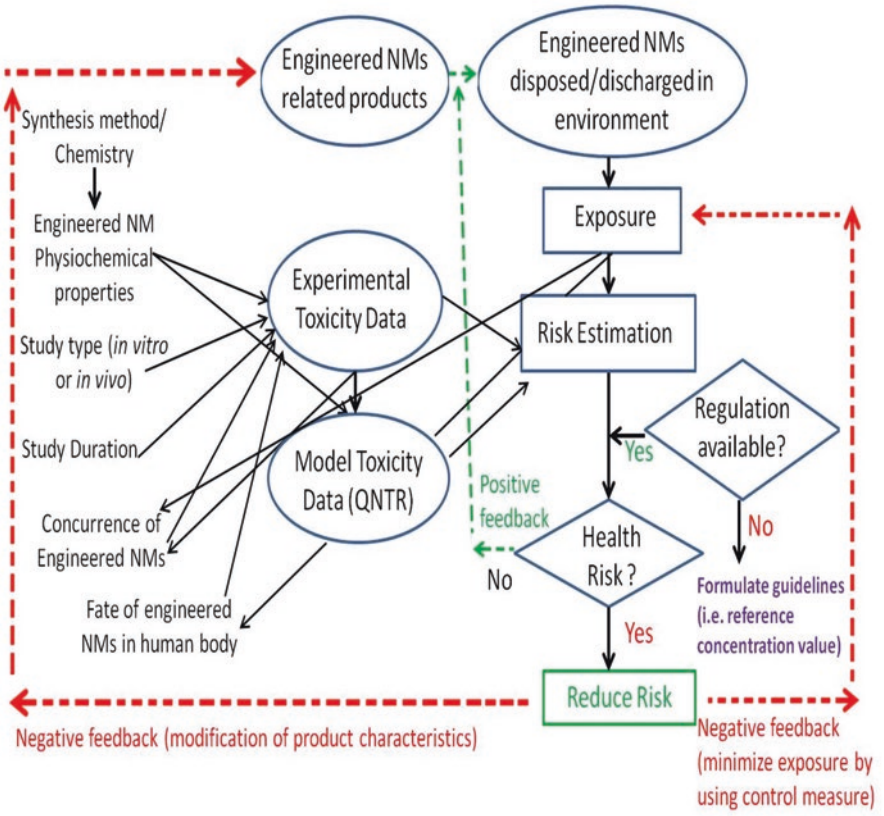

Fig. 9.1 Interlink of different factors for determining environmental and health risks due to nanomaterials exposure – a schematic representation (Adopted with permission: Kumar et al. 2014)

scientific groups propose different methods for the safe handling of nanomaterials (Dhawan et al. 2009, 2011; XpertArena 2015).

9.2 Exposure to Nanomaterials

People are exposed to both anthropogenic and natural nanomaterials on an almost daily basis. The manufacturing units of nanomaterials also pose a threat to the environment and human health. Spillage and effluent discharge from research facilities and industries further add up to the contamination. Inhalation, ingestion or dermal exposures are some of the direct routes of nanaomaterial exposure by way of cosmetics, personal care products and food items. Wash off from these products, then, contaminates the environment (Mihranyan et al. 2012; Ashutosh and Alok 2013a, b).

One of the most common and direct route of exposure is inhalation (Bakand et al. 2012). Workers engaged in nanomaterial manufacturing units face severe health hazards due to the inhalation of the powder during nanomaterial synthesis, processing and packaging. Absence of regulatory guidelines and monitoring leads to the release of these nanoparticles into the environment and possess serious health hazards be entering the body through the respiratory system (Kim et al. 2009; Jasmine et al. 2010). Any airborne particle can induce toxicity mainly in three regions of the respiratory system – nasopharyngeal, trachea-bronchial, and alveolar regions. Particles also face several clearance mechanisms, especially, in epithelial and alveolar macrophages. Fine and coarse particles can be phagocytised by the alveolar macrophages but singlet nanoparticle s escape this clearance and find their way into the interstitial sites and the regional lymph nodes. They are dispersed to other organs like liver and spleen via blood (Dhawan et al. 2011). These nanoparticles are either retained in the body and translocated to other organs or are eliminated out of the body.

Another direct route of exposure is via dermal exposure. Possible routes for nanoparticle entry can be trans-appendageal, inter-cellular and trans-cellular system (Wu et al. 2013; Yan and Chen 2013). Lipid soluble nanopatricles move through the lipid membranes of skin cells in the intercellular routes, and in the transcellular route the substance penetrates into the cells. The hair follicles and sweat glands may provide the trans-appendageal route for nanomaterial entry (Crosera et al. 2009; Albanese and Chan 2011; Love et al. 2012).

Direct ingestion of NMs can occur through food packaging, drug delivery, food, and cosmetics. Removal of nanomaterials from the effluent of manufacturing units is very difficult and so they often find their way into the food chain and eventually the human body. These ingested nanoparticle s are translocated to different organs by getting absorbed into the blood through the intestinal lumen (Pietroiusti et al. 2013). The translocation of Tio_2 nanoparticle s have been reported to various organ in body through the gastrointestinal (GI) track*via* blood (Böckmann et al. 2000). To what extend that nanoparticles can be taken depends upon their size and shape. It has been reported that triangular shaped nanoparticles are more toxic than spherical nanoparticle (Huang et al. 2007; Chan et al. 2008; Dasgupta et al. 2016). In a study, 6.6% of the administered 50 nm particles, 5.8% of the 100 nm particles, 0.8% of 1 μm particles, and 0% for 3 μm particles of polystyrene particles was translocated from the Pyer's patches into the mesenteric lymph and then to systemic organs (Jani et al. 1990). This study concludes that the particle size of nano-range can easily move within our body by overcome all the barriers whereas particle of large size (micro-meter) range cannot overcome all the barriers.

9.3 Risk Assessment

In recent year it has been observed that the uses of nanomaterialsin more than 800 of products which are not limited to just one category but covered various categories such as food products, cosmetics (sunscreens), ski waxes, antimicrobial agents,

electronic devices, cigarettes filter, stain resistance fabrics and cleaning products. Nanoparticles differ from their bulk material in various parameters such as solubility, melting point, electrical conductivity, or changes in the crystalline structure of the materials. However, nanoparticles may cause the several cytotoxic and genotoxic effects due to their unique properties such aslarge surface area to mass ratio (Elder et al. 2009; Savolainen et al. 2010).

There are many risk associated with nanoparticles uses which have been realized by the regulatory agencies. The Royal Academy of Engineering and The Royal Society established in UK in June-2003 regulate by looking into the various aspects of nanomaterial such as benefits, safety and health related issues arising from usage of nanomaterials. A report published in 2004 by The Royal Society entitled "Nanoscience and Nanotechnologies: Opportunities and Uncertainties"specifying the nanoparticles or nanotubes must be treated as recent materials under the existing "notification of new substances" (NONS) rules as well as in the "registration, evaluation, authorization and restriction of chemicals" (REACH)' to set off further testing (Jones and Grainger 2009; Tervonen et al. 2009; Sharma et al. 2012b; Hirose 2013).

Another organization "United States Environmental Protection Agency" (USEPA) is also regulates the uses of nanomaterials present in food products or other products and the risk associated with these products. It also regulates the formulation of nanomaterials. Inside its document—EPA 100/B-07/001 (Nanotechnology White Paper) published in 2007, it has stated "as the use of nanomaterials in society increases, it is reasonable to assume that their presence in environmental media will increase proportionately, with consequences for human and environmental exposure". Other committees such as Committees on the Toxicity, Carcinogenicity and Mutagenicity of Chemicals in Food, Consumer Products and the Environment, also work in the identification of risk associated with nanomaterials and mentioned in their 'Joint Statement on Nanomaterials Toxicology'.

The European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has also reviewed the existing information/data and issues to be considered in conducting risk assessment on nanomaterials (Sharma et al. 2012b). European Commission's Scientific Committee on Consumer Products (SCCP) issued a document titled "Opinion on Safety of Nanomaterials in Cosmetic Products" and raised a concern about large data gaps, inappropriateness of existing characterization techniques for nanoparticle risk assessment and inadequate information regarding nanoparticles absorption and uptake in both normal and diseased skins. Guidance documents on harmless management of nanomaterials are also being outlined by researchers. Non-governmental organizations like "Friends of the Earth" and "Xpert Arena" have warned against nanotechnology in cosmetic and sunscreen products, since they may result in possible uptake of particles by human skin: if nanoparticles penetrate the skin, they can join the bloodstream and circulate around the body with uptake by cells, tissues and organs leading to cause several diseases (Heinemann and Schäfer 2009; Dhawan et al. 2011; Shivendu and Nandita 2013; XpertArena 2015).

9.4 Toxic Effects of Nanomaterials

9.4.1 Genotoxic Potential

Nanomaterials, due to their very small size are absorbed through the gastrointestinal track and come in contact thereby interact with various cells, protein and even DNA. They internalized into cells and interaction with cellular organelles and macromolecules (DNA, RNA and protein) take place. This interaction can cause the mutation in the cells or can affect the defense mechanism by disturbing the biochemical pathways. The causes of genotoxicity induce by nanomaterials are either direct interaction of nanomaterials with genetic material (DNA and RNA) or due to indirect damage by reactive oxygen species (ROS) (Kisin et al. 2007; Barnes et al. 2008). The generation of ROS by nanomaterials has been seen in both*in vitro* and *in vivo* conditions (Jones and Grainger 2009; Karlsson et al. 2009; Xie et al. 2010; Heng et al. 2011; Khan et al. 2012). It has been studied that nanomaterials interact with cytoplasmic/nuclear proteins, oxidative stress, binding with mitotic spindle or its components thereby disturb of cell cycle. Nanomaterials can also interrupt the antioxidant defense mechanism which may also leads to genotoxicity (Dhawan and Sharma 2010; Shukla et al. 2013b; Ashutosh et al. 2015; Kansara et al. 2015).

By using computational approach it has been observed that carbon nanotubes bind with the sister chromatid strand during DNA replication and gets integrated into the DNA duplex and can hinder the DNA replication process. Similarly, other nanomaterials are also show strong interaction with genetic material (DNA and RNA) (An et al. 2010; Jin et al. 2012). It has been observed by in-silico approaches that C60 fullerene affects the DNA mismatch repair mechanism when PMS2, RFC3, and PCNA proteins interact with the C60 fullerene. C60 fullerene can also hinder the enzymatic activity of human DNA topoisomerase II alpha by interacting with its ATP binding domain (Benyamini et al. 2006; Baweja et al. 2011). Nanomaterials bind to the active site of protein which leads to the change in their structural/conformation. When nanomaterials bind to the enzyme on its active sites, it competitive inhibits the enzymes so that now ligands cannot bind to the enzyme active site. Jugan et al. (2012) have shown DNA repair activity in A549 cells was weakened by TiO_2 nanoparticles. The impairment in the DNA repair protein activity by nanomaterials, leads to the ROS generation (Jugan et al. 2012; Kansara et al. 2014).

Various other interactions of nanomaterials with many proteins involve in regulation of biological functionalities of different system such as DNA replication, transcription, centriole, mitotic spindle apparatus, and other associated proteins. The interaction studies with proteins/enzyme are based on computational and *in vitro* studies. Low ROS concentration will activate the signaling pathways whereas its higher concentration can cause many damages e.g. mitochondria, cell membrane, and other macromolecules damage, lipid peroxidation *etc.* Mitochondrion is the main cause to generation of the ROSinduced oxidative

stress and oxygen free radicals. Under stress condition mitochondria secrete various pro-apoptotic factors due to depolarization of the mitochondrial inner membrane and increased permeability of its outer membrane (Cadenas and Davies 2000; Kumar et al. 2011a; Shukla et al. 2013a). The direct attack of ROS on the nucleotide bases in DNA strand modifies the base. The modified base such as 8-oxo- 7,8-dihydroguanine (8-oxoG) can cause the cancer and mutation in the cells. The presence of 8-oxoG can reflects the DNA damagedue to oxidative stress after nanomaterials exposure which has been analyzed by FPG-modified comet assay (Kim et al. 2011; Asare et al. 2012; Magdolenova et al. 2014). ROS will enhance the level of 8-oxoguanine DNA glycosylase (OGG1) which ultimately affects base excision repair mechanism of 8-oxoG.C60 fullerene treated showed increased expression of mRNA of OGG1but no increment in its repair mechanism has been seen. The genotoxicity induce by nanomaterials can be inhibited by pre-treatment with the free radical scavenger *N*-acetyll- cysteine (NAC) (Guo et al. 2011; Sharma et al. 2012a). From all the above studies we can easily understand the nanomaterials induced ROS and its effects on cellular perturbation along with DNA damage and apoptosis.

9.4.2 Carcinogenic Potential

Several *in vitro* and *in vivo* studies have proved that nanomaterials exposure to our body can cause DNA damage and mutation which lead to cancer. These studies show the carcinogenic nature of some nanomaterials. Many research and review papers explored the correlations between metallic compounds, metal oxide and organic molecules with oxidative stress, and cancer (Barchowsky and O'Hara 2003; Pulido and Parrish 2003; Valko et al. 2005; Lee et al. 2012). The excessive generation of ROS by oxidation of biomolecules increases the defense mechanism of cells. ROS can also cause the tissue degradation which ultimately causes carcinogenesis, aging, and other diseases (Luo et al. 2011). On other hand, it also affects immune system which results into increased microbial load – resulting in cell and tissue damage. Among all the free radicals species studied so far, mostly 8-OHdG showed to cause for genetic changes because of its relative premutagenic potential and ease of measurement. 8-OHdG elevation has been seen in many tumors which intensely associate such damage in the etiology of cancer. In conclusion we can say that enhancement in the level of 8-OHdG in cells because of carcinogenic potential of nanomaterials.

The induction of inflammatory responses by oxidative stress induce by nanomaterials can lead to cancer. The main factor which makes the nanomaterials high reactive is the electron presence on their boundary. Nanomaterials are responsible for the trigger cytokine release when they interact with protein or enzyme, therefor mediate inflammatory reactions and thereby initiate series of toxic reactions (Borm and Kreyling 2004; Bergamaschi et al. 2006). For instance, C60 fullerene causes similar reaction in body by causing photo-induced DNA damage by interacting with

endogenous reducing agent NADH (Wang et al. 2009; Yamakoshi et al. 2014). On other hand carbon containing nanomaterials like carbon nano tubes responsible for cardiovascular disorders due to platelet aggregation, aortic DNA damage and enhanced vascular thrombosis through inflammatory.

9.5 Toxicity Evaluation Method: Methods and Techniques Used

Genotoxicity of nanomaterials can be tested by various *in silico*, *in vitro*, *in vivo* and microbial models. The Ames test is an extensively used test for the early screening of genotoxicity (Maenosono et al. 2009; Sotto et al. 2009; Kumar et al. 2011b). It uses bacterial strains that have been mutated at the histidine locus to give rise to histidine auxotrophs and detects mutagenesis on the basis of their reversion to autotrophs (Ames et al. 1975; Mortelmans and Zeiger 2000). Histidine auxotrophs die when they are grown on a medium that does not contain histidine. However, on the reversion of mutation, the nanomaterials enable the bacterial strains to synthesize histidine. The bacterial strains grow in a minimal histidine medium. Deep rough (rfa) mutation increases the permeability of the bacterial cell wall by eliminating the polysaccharide chains of the LPS membrane. This mutation is performed to make the cell wall more permeable for the entry of nanomaterials and makes the Ames test more suitable to detect nanomaterial genotxicity.

DNA damage by nanomaterials can be tested by various mammalian cell culture assays like hypoxanthine phosphoribosyltransferase (HPRT) assay, phosphotidylinositol glycan, comet assay, thymidine kinase (TK) assay, micronucleus assay, chromosomal aberration test, class A (Pig- a) assay (He et al. 2008; Shinohara et al. 2009; Chen et al. 2014). Thus, *In vivo* studies have helped to establish the genotoxicity of the nanomaterials.

HPRT forward mutation assay is performed on V79 Chinese hamster cells (Finette et al. 2002). Their X chromosome carries a single functional copy of the HPRT gene, which is responsible for the phosphoribosylation of guanine and hypoxanthine. Cells are grown in a media containing 6-thioguanine. It is analogous to guanine but is toxic in nature. During DNA replication by HPRT enzyme, 6-thioguanine gets incorporated in the DNA duplex and leads to cell death. However, if the HPRT gene has been mutated by exposure to nanomaterials, the salvage pathway fails to occur and 6-thioguanine is not incorporated into the DNA duplex. Thus, the deleterious mutations induced by the nanomaterials is represented by the number of visible colonies on the medium, Different nanomaterials have given negative results (Chen et al. 2014).

The micronucleus assay is performed to identify the genotoxicity and carcinogenic potential of the nanomaterials. It involves scoring and comparison of the micronucleus and is easier and faster in comparison to the chromosomal aberration test. Micronucleus is formed during the anaphase of the cell cycle from frag-

ments of lagging chromosomes. This assay reduces the chances of getting false positives as the cells become binucleated due to the inhibition of cell division by cytochalasin B, a cytokinesis blocking agent However, a deposition of nanomaterials occurs if their concentration is high and this makes counting of micronucleus difficult (Li et al. 2012; Shukla et al. 2013a; Dobrzyńska et al. 2014; Magdolenova et al. 2014).

Comet assay is used for the detection of double stranded or single stranded breaks in DNA, oxidative DNA damage, DNA-protein or DNA-DNA crosslink, alakali-labile sites quantification and identifying the basic sites. Damaged nucleotide bases can also be detected by incubation of nucleoids with lesion specific endonucleases such as formamidopyrimidine DNA glycosylase (EPG) and endonuclease III which can recognize oxidized purines and pyrimidines, respectively (Karlsson et al. 2009; Stone et al. 2009; Shukla et al. 2011). A monolayer of cells is obtained by suspending single cells in low melting point agarose and spreading it onto a microscopic slide with normal melting agarose. To prevent loss of cells, a thin layer of agarose is again added to sandwich the cells. Nucleoids are obtained by alkaline lysis of the cells. Nucleoids undergo electrophoresis followed by neutralization which causes renaturation of some DNA. Fluorescent dye like ethidium bromide is then used to stain the DNA. The chromosomal DNA migrates towards the anode, the migration being more in the cells with higher DNA damage. The shape left by the migrating DNA resembles a comet when it is viewed under the fluorescent microscope. Commercial software can also be used for the quantitative and qualitative assessment. Additional DNA damage is induced by the occurrence of nanomaterials in the comet head (Karlsson 2010). It has also been found that oxidative DNA damage detection is hampered by the inhibition of FPG enzyme activity caused by the ions and nanomaterials that are released due to dissolution and get bound to the –SH groups at the active site of the enzyme (Kain et al. 2012). Another tool to detect double stranded breaks is to analyze γ-H2AX, which is a part of the nuclosome core histone H2A family. A double stranded break in the DNA results in its phosphorylation. Phosphorylation occurs by the action of ataxia telangiectasia, ataxia telangiectasia mutated (ATM) or Rad-3 related protein or DNA dependent protein kinase. The histone complexes are altered into monomers, which act as signals for the DNA repair proteins to recruit and repair the double stranded break site. The altered expression of phosphorylated γ-H2AX can be assessed by several techniques like western blot, flow cytometry and immune (Ismail et al. 2007; Lewis et al. 2010).

9.6 Effects on Human Health

Various routes such as inhalation, ingestion or by dermal penetration through which nanoparticle can enter into body (Boussaad et al. 2006; Bouwmeester et al. 2009; Baltic et al. 2013). Two major organs are known large distribution

volume for nanomaterial after ingestion and passing from intestines to circulation (Silvestre et al. 2011; Baltic et al. 2013). Elder et al.8 showed that the inhaled nanoparticle magnesium oxide reached up to brain olfactory region through the axons of olfactory nerve in the nose and may reach to other parts of the brain (Elder et al. 2009). Likewise undesirable delivery of other nanomaterials to our body organ may produce unwanted results. Therefore, targeted delivery of these nanomaterials is an area of concern. Tissue specific delivery of these nanomaterials can be obtained by preparing nanoemulsion containing nanomaterial and polymer of desired properties. For instance a colon specific delivery of drugs or nanomaterials can be done by pH sensitive polymers such as methacrylate/methacryl acid Eudragitw L and S dissolve in aqueous media at pH 7 and 6, respectively, which may be equivalent to a drug release to the distal ileum and prevent early release of drugs/nanomaterials (Lamprecht et al. 2004). So by overcoming the delivery related problems of nano food and drug satisfactory results can be achieved.

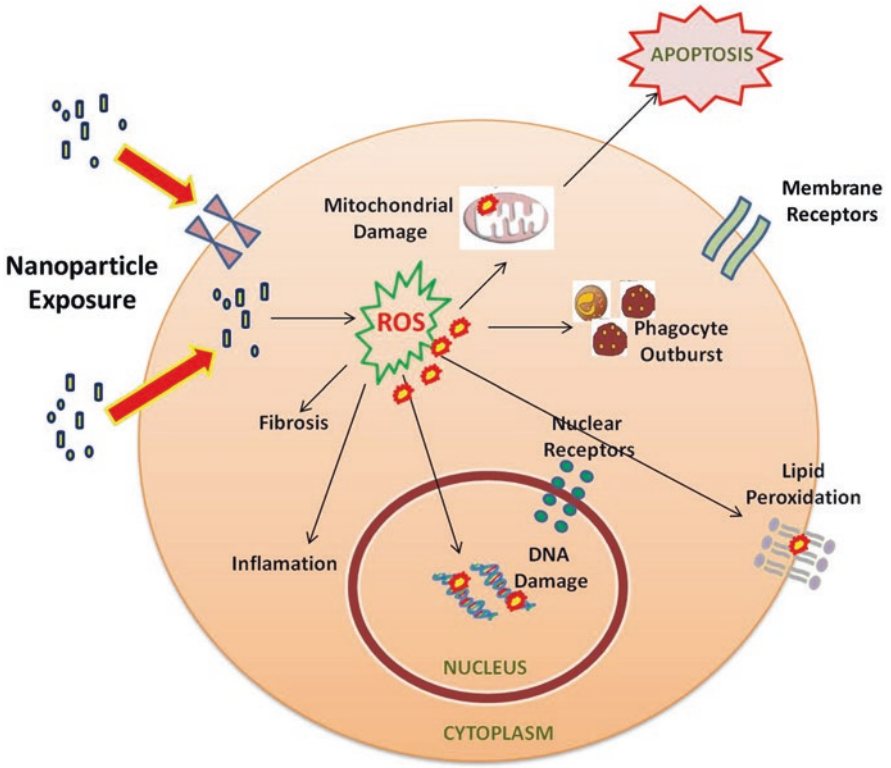

Fig. 9.2 Nanoparticle mediated cellular responses: nanoparticles mediated ROSgeneration is capable of inducing oxidative DNA damage, strand breaks, protein denaturation, and lipid peroxidation. ROS alsoresults in mitochondrial membrane damage that leads too cellular death by Apoptosis (Adopted with permission: Jain et al. 2016)

The small size and large surface area of nanoparticles is the main factor which contributes to the toxicity of nanomaterials. The small size allows them to easily pass through the biological barriers and membranes. Toxicity of nanomaterials is difficult to investigate because of their distinctive physio-chemical properties. For this, it is necessary to have a thorough knowledge of the mechanisms by which nanomaterials exhibit their toxic effects. The entry of nanomaterials into the human body can be through various routes like dermal, gastrointestinal and respiratory routes. This is one limitation of nanomaterials to be used for diagnostic or therapeutic tools. Various mechanisms have been proposed with respect to nanoparticle toxicity, most of them focus mainly on genotoxicity, carcinogenic potential and oxidative stress of the nanoparticles.

Cardiac and respiratory system have been shown toxicity with nanomaterial. Hence, it is necessary to gain more knowledge and understanding on nanotoxicity, diseases and defects caused by nanomaterials, and the application of nanomaterials in the medical therapeutics and the food industry. Nanoparticles cause cell death by various means of action such as it induces oxidative stress, apoptosis, cell wall disruption and mitochondrial damage etc. Nanoparticles may have the genotoxic and carcinogenic properties which should be evaluated prior to launch as incorporation with food products – Fig. 9.2 (Jain et al. 2016).

9.7 Nano-Foods: Safety Regulations to Be Followed

There are two sides of the same coin. This also applies to the field of Nanotechnology. In spite of the technological advantages that nanotechnology promises to provide, it also has its own set of disadvantages mainly in the form of toxic effects and the incidence of diseases on exposure to nanoparticles. The toxic effects of nanoparticles are a well-studied and an established fact. Various cardiac and respiratory diseases have been associated with the nanoparticles present in the form of aerosols (Ferin et al. 1992; Dockery et al. 1993; Schwartz 1994; Danie Kingsley et al. 2013; Nandita et al. 2016). Distinctive properties of nanoparticles like shape, size, area, reactivity and surface charge impart toxicity to nanoparticles. This multifaceted nature of nanoparticle toxicity makes its study, investigation and control very complex. Particles in the nano size have higher toxicity than their larger counterparts. Also, the toxicity and pathological response to inhaled particles depends more on the particle surface area than the particle mass (Fadeel and Garcia-Bennett 2010).

Anthropogenic or engineered nanoparticles are used in many fields like food industry, pharmaceutical industry, biomedical applications, electronic devices, material science etc. In the biomedical field, nanotechnology has given rise to a new field of nano-theranostics which incorporates both diagnostics and therapeutic application. The use of nanoproducts in pharmaceutical and medicine industry is estimated to increase over 17% each year and at a rate much higher than that of the food industry (Jones and Grainger 2009). However, it has also arisen

the need to understand the unanticipated and unexpected side effects of nanomaterials on human and environmental health and the occurrence of diseases due to nanoparticle exposure (Maynard et al. 2011). The toxic effect or response of nanomaterials differs in different organs. Hence, a proper understanding of the interactions and response of biological systems at the physiological, molecular and cellular levels is necessary to assess the toxicological effects of nanomaterials on different organs. Toxic effects can result from both direct exposures by the use of nanomaterials and indirectly by exposure to by-products from their usage. Nanoparticle exposure causes oxidative stress and inflammation at the cellular, organ and tissue level. It also leads to higher incidence of cardiovascular and respiratory diseases by affecting the function of autonomic nervous system. Nanoparticles enter the blood and reach to different organs via blood circulation. To conclude, the toxic effects of nanoparticles affect almost all the organs of the body and may result in altered blood pressure and heart rate, respiratory problems, myocardial infarctions, thrombosis, higher incidence of cardiac strokes reducing the life expectancy of the person (Künzli and Tager 2005).

Concerning the above mentioned toxicity problems it has become very necessary to have a strict rules and regulations to control the market. Country and territory-wise the perspectives and regulations varies. Though still there is a lack of defined regulations for nano-products but the earlier rules and their extended definitions may be applicable for Nano-products. Given three tables summarizes the direct or indirect laws in context of Europe and Indian context (Tables 9.1, 9.2 and 9.3). These will be applicable to control the Nano-products in food and allied market. In Table 9.2 list of regulation for various food feature modifier such as food additives, food flavorings, food enzymes, food supplements and using of biocides in food. It is given in the regulation that whether a specific compound should be added in food or not, whether it is safe to consume or not etc. The approval and authorization of nano-form compounds in food is done prior to launch any product in market. All these assessment regarding to use of nano-form compounds is necessary to control the toxicological thread of these compounds.

Table 9.1 List for few of the European Union regulations providing specific provisions for nanomaterials – directly or indirectly

S.No.	Details
Communications	
1.	European Commission 2004, "Towards a European Strategy for Nanotechnology" COM (2004)338
2.	European Commission 2005, "Nanosciences and nanotechnologies: An action plan for Europe 2005e2009"(COM (2005)243).
3.	European Commission 2007, "Nanosciences and nanotechnologies: An action plan for Europe 2005e2009. First Implementation Report 2005e2007",COM (2007)505 final
4.	European Commission 2008, "Regulatory aspects of nanomaterials" COM (2008) 366 def.
5.	European Commission 2009, "Nanosciences and Nanotechnologies: An action plan for Europe 2005e2009. Second Implementation Report 2007e2009", COM (2009)607 final
6.	European Commission 2012, "Second Regulatory Review on Nanomaterials" COM (2012) 572 final
Opinions	
7.	Opinion of the European Economic and Social Committee on the Communication from the Commission: Towards a European strategy for nanotechnology, (2005) OJ C 157/22
8.	Opinion of the European Economic and Social Committee 2009, "Nanomaterials" INT/456
Recommendations	
9.	European Commission 2008, "Code of conduct for responsible nanosciences and nanotechnologies research", COM (2008)424 final.
10.	European Commission 2011, "Definition of nanomaterial"
Resolutions	
11.	European Parliament 2006, "Nanosciences and nanotechnologies: an action plan for Europe 2005e2009" (2006/2004(INI))
12.	European Parliament 2009, "Regulatory aspects of nanomaterials"(2008/2208(INI))
Guidelines and report	
13.	EFSA 2011, "Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain", EFSA Scientific Committee, EFSA Journal 9 (5), 2140
14.	EFSA 2013. Annual report of the EFSA Scientific Network of Risk Assessment of Nanotechnologies in Food and Feed for 2013
15.	EFSA 2015 Annual report of the EFSA Scientific Network of Risk Assessment of Nanotechnologies in Food and Feed1 for 2014 (Joe et al. 2012)

Adopted from: (Jain et al. 2016; Sodano et al. 2016)

Table 9.2 Few main documents for European Union soft law for nano regulation

S. No.	Name of regulations/ directives	Specific feature	Details (in brief)	References
1	Regulation (EC) No 1333/2008	For food additives	States that a food additive already authorized but obtained using nanotechnology requires a re-evaluation before marketing	Decision (2004), Kuempel and Castranova (2011), Marrani (2013), Amenta et al. (2015) and Jain et al. (2016)
2	Regulation (EC) No 1332/2008	On food enzyme	States that a food enzyme already included in the Community list but prepared by different methods or using starting materials significantly different (It is specified that "Significantly different" could mean a change in particle size) from those included in the risk assessment of the Authority, should be submitted for re-evaluation	
3	Regulation (EC) No 1334/2008	On food Flavourings	Flavourings must undergo a common (EU-wide) assessment and authorization prior marketing and lays down a Union list of flavourings and source materials approved for use in and on foods and their conditions of use.	
4	Directive 2002/46/EC Food supplements	Food supplements	Stated that the food supplements (minerals or vitamin) can be used which are listed by EC. The use of nanoforms of minerals and vitamin requires a safety evaluation prior marketing which will be done under Novel Food Regulation, due to the differences in production, potential differences in nutritional value and bioavailability when compared to macro-scale counterparts	

(continued)

Table 9.2 (continued)

S. No.	Name of regulations/ directives	Specific feature	Details (in brief)	References
5	Regulation (EC) No 450/2009[a]	active and intelligent materials and articles intended to come into contact with food	Although nanomaterials are not directly mentioned, there is a reference to "substances deliberately engineered to particle size which exhibit functional physical and chemical properties that significantly differ from those at a larger scale"; therefore, a case-by-case analysis has to be followed for active and intelligent materials and articles containing nanomaterials	
6	Regulation (EU) No 10/2011[a]	On "plastic materials and articles intended to come into contact with food"	States that the substances in nanoform should be used only if listed in the Annex I of the regulation	
7	(EU) No 528/2012	Biocides	As of today, nanomaterials based Biocidal products are not eligible for a simplified authorisation procedure. For subsequent nanomaterials based product authorisation and approving nanomaterials as active substances, the test methods applied to the nanomaterials shall beaccompanied and standardized by an explanation addressing their exact appropriateness considering the specific characteristics ofeach nanomaterials	

Adopted from: (Jain et al. 2016; Sodano et al. 2016)

[a] It can be noted that, both regulations 450/2009 and 10/2011 state the functional barrier concept which means it is not directly applicable to nano-materials

Table 9.3 Overview of current regulatory framework affecting the development and marketing of nano materials and nanotechnology based application in India

S. No.	Legal acts and their scopes	Section number in IPC and other details	Gaps and challenges
1.	Patents Act 1970	**Patentability criteria**	**Patentability criteria**
		Section 2 (1)(j)	Patentability criteria may pose serious impediments to NT inventors applying for patents in India. Problem magnified by absence of separate classification for NT patents
		3(d)	A nano material may be a combination of many particles or technologies or a nano particle of an existing material and patents for nanostructures without substantial difference in character and industrial application could very well not pass the standard of efficacy demanded by section 3 (d)
		Section 10	Explanation to section 3(d) does not accommodate the new technological development of nanotechnology as it specifically debars patenting of 'particle size' unless it may differ significantly in properties with regard to efficacy
		Section 107 (A),	**Lack of Capabilities**
			Indian IPR regime and its implementation agencies-particularly the Indian Patent Office ill- equipped to handle nanotechnology and all that it entails in terms of prior art searches and patent examination.
			Shortage of manpower at the IPO
			No special training to patent examiners to handle NT and and lack of special examiners for handling NT patent applications
			Ineffective indexing and management of prior art databases
			Prior art databases.
			Lack of guidelines for examing nanopatent applications.

(continued)

Table 9.3 (continued)

S. No.	Legal acts and their scopes	Section number in IPC and other details	Gaps and challenges
			Lack of capacity among patent attorneys to draft NT specific claims
2.	Public Funded Intellectual Property Bill 2008	Provides the necessary boost to the commercialisation of inventions made through government-funded research by passing on the IP rights on the same to the institution responsible for that invention.	Contains very few provisions to safeguard public access, in the form of 'march-in' rights or compulsory licensing
3.	Drugs and Cosmetics Act 1940	Section 3(b).	Consideration that nano particles as either new or modified ingredients
		Section 10(A)	Second Schedule – Class 1 Drugs –Patented and Proprietary Medicine – Standard prescribed (Labeling) The formula of list of ingredients displayed in the prescribed manner on the label of the container and such other standards as may be prescribed. (No Obligation to disclose the use of nanoparticles as ingredients)
		Section 16	
		Section 18	
		Section 22	
	Regulates the import, manufacture, distribution and sale of drugs and cosmetics	Section 26	
4.	Medical Devices Regulation Bill, 2006	Section 12	Drugs and cosmetics Act includes medical devices within the definition of drugs, therefore there will be problems in the phase of transitioning the regulation from drug controller to the new medical device regulatory authority

(continued)

Table 9.3 (continued)

S. No.	Legal acts and their scopes	Section number in IPC and other details	Gaps and challenges
	To consolidate laws related to medical devices and to establish the Medical Device Regulatory Authority of India for establishing and maintaining a national system of controls relating to quality, safety, efficacy and availability of medical devices that are used in India	Section 13	Ministry of health and family welfare may be better equipped to regulate medical devices, in view of its experience and overall ministerial mandate
		Section 53	In principle the focus on risk management is commendable but a precautionary approach is not reflected adequately in the functioning of the Bill
		Section 67	
		Section 66	
5.	National Pharmacovigilance Protocol	Para. 6.1:	It is a voluntary instrument
	Postmarketing tool in ensuring the safety of pharmaceutical and related health products	Para. 6.2:	There is no framework under which the reports of adverse drug reactions can be addressed in a comprehensive manner
		Para. 6.4:	Regulatory assessment is missing of past studies of drug reactions
			Tailored monitoring andreporting of Nano based health applications
			Submission of adverse drug reports by companies – clear conflict of interest
6.	Food Safety and Standards Act, 2005	Section 3 (k)	Definition of Food Additives can accommodate nano particles used in packaging
		Sec 3 (r)	FSA is empowered to carry out food safety audits for ensuring compliance

(continued)

Table 9.3 (continued)

S. No.	Legal acts and their scopes	Section number in IPC and other details	Gaps and challenges
		Sec 3 (u)	FSA is also empowered to undertake foresight activities in the case of emerging risks The entire FSA administrative framework is required to undertake public consultations and make regular information disclosure and communication to public bodies like panachayats, consumers and interested parties. – appreciates the differentiation between groups within the public
		Sec 3 (y)	Food Commissioner is also empowered to prohibit on the basis of public health
		Sec 3 (zm)	Food standards to be determined on the basis of a risk analysis – however exceptions made for developments with uncertain scientific justifications – provisional measures
		Sec 3 (zz)	There is a provision establishing the supremacy of this act over the provisions of any other domestic statute
		Section 16	
		Section 30	
		Section 59.	
7.	Insecticides Act 1968	Section 2 (k)	Reporting requirement should be mandatory across states and not left for states to notify
	To regulate the import, manufacture, sale, transport, distribution and use of insecticides with a view to prevent risk to human beings or animals	Section 5 (1)	Reporting requirement should not be restricted to poisoning but any adverse reaction/effect. Reporting is an important post-marketing tool and in case of merging technologies, when risks may emerge at a later date, such reporting would be helpful

(continued)

Table 9.3 (continued)

S. No.	Legal acts and their scopes	Section number in IPC and other details	Gaps and challenges
		Form I Rule 6	In the absence of supporting data, the government can prohibit sale import or manufacture of an insecticide in the interest of public safety only for a maximum of thirty days
		Section 9. 3B	
		Section 17	
		Section 26	
		Section 27	
8.	Factories Act 1948	Sec 2 (cb)	Designer, manufacturer, importer or supplier of any article to ensure that the article is safe and without risks to the health of the workers. There is a clear conflict of interest here. Enjoining the beneficiaries of the trade with this responsibility would make it a difficult proposition
	Law regulating labour in factories	Section 7A	Hazardous process definition is wide enough to include nanoparticles, but it is restricted to industries in the schedule. Thus the schedule would have to be expanded
		Section 7B	Right of workers is an important empowering tool but pointless in the absence of awareness and knowledge and awareness. Training to workers about imminent risks must be provided to enable the workers to exercise their right of warning
		Section 9	
		Section 41C	
		Section 41E	
		Section 41H	

(continued)

Table 9.3 (continued)

S. No.	Legal acts and their scopes	Section number in IPC and other details	Gaps and challenges
9.	Hazardous Material (management, handling and Trans boundary Movement) Rules 2007	Section 2 (l)	Guidelines for handling of waste have to take into account the characteristics of nano-waste
		Sec 4(1)	SPCB approval required for generation, processing, treatment, package, storage, selling of hazardous waste; But are actions of SPCB and other institutions in coordination with respect to reporting of adverse impacts, latest technologies etc. for safe management of hazardous waste
	Handling of hazardous waste rules	Sec 5(1)	
10.	DCA Drugs (Control) Act 1950	Section 26	
11.	Manufacture, Storage & Import of Hazardous Chemicals Rules, 1989 U/Environmental Protection Act, 1986	Lays down certain responsibilities of the authorities responsible for manufacture, storage and import of hazardous chemicals	Positive list of hazardous chemicals laid down
			Hazardous chemicals in terms of dosage or content laid down. Need for the government to recognize risks of nano particles and metals that change properties at nano scale
	Rules for manufacture, storage and import of hazardous chemicals		
12.	The Chemical Accidents (Emergency Planning, Preparedness and Response) Rules, 1996	Rule 2 (a), (f)	Functions of the central crisis group are more with respect to post accident monitoring and evaluation
		Rule 3	There is some overlap of functions between centre and state groups for reviewing district off-site emergency plans

(continued)

Table 9.3 (continued)

S. No.	Legal acts and their scopes	Section number in IPC and other details	Gaps and challenges
		Rule 7	Local crisis groups are entrusted with the responsibility to train personnel involved in chemical accident management. However, there is no effort towards building the capacity of local actors and stakeholders in this regard
13.	Air (Prevention and Control of Pollution) Act	Sec 22	These standards are with respect to specified areas only
	Prevention, control and abatement of air pollution	Sec 17 (1) (e)	Recognition of nano particles as potential suspended particulate matters
14.	Environment Protection Act 1986	Section 2 (a, b, and e)	
	For the protection and improvement of environment	Section 4	
		Section 5(a)	
		Section 6	
15.	Water (Prevention and Control of Pollution) Act	Section 2 (e and k)	Co-ordination amongst state and central boards
	Prevention and control of water pollution and the maintaining or restoring of wholesomeness of water	Section 16	Awareness and recognition about risks associated with nano-particles with the standard setting agencies
		Section 17 (a, g, k, m)	
		Section 21	
		Section 24	
		Section 25	
		Section 32	
16.	G.S.R.384(E), [11/4/1994] – National Ambient Air Quality Standards	National Ambient Air Quality Standard: The levels of air quality necessary with an adequate margin of safety, to protect the public health, vegetation and property	

(continued)

Table 9.3 (continued)

S. No.	Legal acts and their scopes	Section number in IPC and other details	Gaps and challenges
		As per the notification, SPM concentration limit in ambient air for industrial area is 360 μg/m^3 (annual avg) and 70 μg/m^3 in sensitive areas and for RPM concentration it is 150 μg/m^3 in industrial area and 75 μg/m^3 in sensitive areas	
17.	Environmental Impact Assessement Notification-2007		Requirements for EIA are based on land area under operation. Any operation below 50 hectare does not require a mandatory EIA
	For imposing certain restrictions and prohibitions on new projects or activities, or on the expansion or modernization of existing projects or activities based on their potential environmental impacts		
18.	The Chemical Accidents (Emergency Planning, Preparedness and Response) Rules, 1996	Rule 2 (f), Rule 2 (a)	Functions of the central crisis group are more with respect to post accident monitoring and evaluation
		Rule 3	There is some overlap of functions between centre and state groups for reviewing district off-site emergency plans
	Emergency planning, preparedness and response for chemical accidents	Rule 7	Local crisis groups are entrusted with the responsibility to train personnel involved in chemical accident management. However, there is no effort towards building the capacity of local actors and stakeholders in this regard
19.	Factories Act	Section 12 (1)	
20.	The Hazardous Wastes (Managem ent and Handling) Rules, 1989	Rule 4(1)	

(continued)

Table 9.3 (continued)

S. No.	Legal acts and their scopes	Section number in IPC and other details	Gaps and challenges
21.	The BioMedical Waste (Managem ent and Handling) Rules, 1998	Rule 3(5)	Biotechnology waste recognized as a separate category of waste. Same should be done for nanotechnology waste or bio nano waste
		Rule 4	
		Rule 5(1, and 2)	
22.	The Municipal Solid Wastes (Managem ent and Handling) Rules, 2000	Rule 3	
		Rule 4	
		Rule 5	
		Rule 6	

Adopted for public awareness from (T E R I Report No. 2006ST21: D6 2009)

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