REVIEW PAPER

Nanoencapsulation Techniques for Food Bioactive Components: A Review

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Abstract The protection and controlled release of bioactive compounds at the right time and the right place can be implemented by encapsulation. Nanoencapsulation remains to be the one of the most promising technologies having the feasibility to entrap bioactive compounds. Nanoencapsulation of bioactive compounds has versatile advantages for targeted site-specific delivery and efficient absorption through cells. However, researches in the application of nanotechnology in the food industry have been very limited and there are only a few review articles that explored the nanoencapsulation technology. This review focuses on the various nanoencapsulation techniques such as emulsification, coacervation, inclusion, complexation nanoprecipitation, emulsification–solvent evaporation, and supercritical fluid for food ingredients. Drying techniques such as spray drying and freeze drying for stabilization of nanoparticles are also discussed. Current state of knowledge, limitations of these techniques, and recent trends are also discussed. Finally, safety and regulatory issues in the nanoencapsulation of bioactive compounds are also highlighted.

Keywords Nanoencapsulation · Bioactive compounds · Nanoemulsions · Biopolymers · Drying techniques

Introduction

Nature had created the building blocks of life in nanoscale such as DNA, amino acids, sugars, and hormones (Weiss et al. 2006). Inspired by nature's creation, man had engineered nanomaterial for the progress and prosperity of mankind. In 1959, Richard Feynman proposed the concept of nanostructures, and in 1974, Nario Taniguchi coined the term nanotechnology for manipulation of submicron particles. The term "nano" refers to a magnitude of 10^{-9} m (Quintanilla-Carvajal et al. 2010). British Standards Institution defined nanotechnology as the design, characterization, production, and application of structures, devices, and systems by controlling the shape and size at the nanoscale (Bawa et al. 2005). Nanotechnology has emerged as one of the most promising scientific fields of research in decades. It deals with the production, processing, and application of materials with sizes less than 1,000 nm (Sanguansri and Augustin 2006). Reduction in particle size to nanoscale range increases surface-to-volume ratio, which consecutively increases their reactivity by many folds with change in mechanical, electrical, and optical properties. These properties offer many unique and novel applications in various fields (Neethirajan and Jayas 2010). For example, silicon chips have been made by nanotechnology for over 20 years. It leads to advances in electronics, computing, and communications, which revolutionized the world and changed the horizons of human life.

Nanotechnology has been touted as the next revolution in many industries, including agriculture and food industry. Nanotechnology has been revolutionizing the entire food system from production to processing, storage, and development of innovative materials, products, and applications. The application of nanotechnology to the food sector could generate innovation in the macroscale characteristics of food, such as texture, taste, other sensory attributes, coloring strength, processability, and stability during shelf-life, leading to a great number of new products. Moreover, nanotechnology can also improve the water solubility, thermal stability, and oral bioavailability of bioactive compounds (Huang et al. 2010; McClements et al. 2009; Silva et al. 2012). At present, applications of nanotechnology in food

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industries are nanocomposites in food packaging material for controlling diffusion and microbial protection, nanobiosensors for detection of contamination and quality deterioration, and nanoencapsulation or nanocarrier for controlled delivery of nutraceuticals (Chen et al. 2006a; Sanguansri and Augustin 2006; Sozer and Kokini 2009; Weiss et al. 2006). Currently, the market of nanotechnology products in the food industry approaches US$1 billion (most of this on nanoparticle coatings for packaging applications, health-promoting products, and beverages) and it has the potential to grow to more than US$20 billion in the next decade (Chau et al. 2007).

Many reviews and research papers have been published on the application of nanotechnology in foods (Weiss et al. 2006; Sekhon 2010; Neethirajan and Jayas 2010; Sozer and Kokini 2009; Graveland-Bikker and De Kruif 2006; Sanguansri and Augustin 2006; Silva et al. 2012). However, only few works were focused on nanoencapsulation of food ingredients (Quintanilla-Carvajal et al. 2010; Augustin and Hemar 2009; Mozafari et al. 2006; Fathi et al. 2012). Therefore, the main aim of this review is to discuss the various nanoencapsulation techniques and their advantages, flaws, and variations, as well as to appraise the interesting emerging technologies and trends in this field along with regulatory issues. In this way, we had analyzed the research tendency in these encapsulation techniques since year 2000 until now.

Nanoencapsulation

Encapsulation is a rapidly expanding technology with a lot of potential applications in areas including pharmaceutical and food industries. It is a process by which small particles of core materials are packed within a wall material to form capsules. Encapsulation method was employed to protect bioactive compounds (polyphenols, micronutrients, enzyme, antioxidants, and nutraceuticals) and in the finished application to protect them from adverse environment and also for the controlled release at targeted sites (Gouin 2004). Microcapsules are particles having a diameter between 3 and 800 μm (Meena et al. 2011). Nanoparticles are colloidal-sized particles with diameters ranging from 10 to 1,000 nm and are expressed both as nanocapsules and nanospheres (Konan et al. 2002). Nanocapsules are vesicular systems in which the bioactive compound is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems where the bioactive compound is uniformly dispersed (see Fig. 1) (Couvreur et al. 1995) Nanoencapsulation is defined as a technology to encapsulate substances in miniature and refers to bioactive packing at the nanoscale range (Lopez et al. 2006). The delivery of any bioactive compound to various sites within
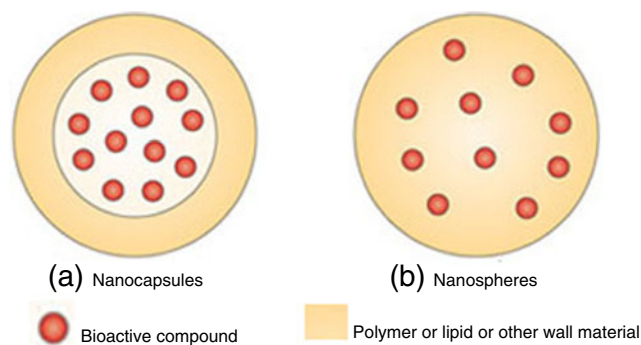

(a) Nanocapsules   (b) Nanospheres

● Bioactive compound   ▬ Polymer or lipid or other wall material

Fig. 1 Schematic structure of **a** nanocapsules and **b** nanospheres (Orive et al. 2009)

the body is directly affected by the particle size (Kawashima 2001; Hughes 2005). Thus, nanoencapsulation has the potential to enhance bioavailability, improve controlled release, and enable precision targeting of the bioactive compounds in a greater extent than microencapsulation (Mozafari et al. 2006).

Wall and Core Materials Used for Nanoencapsulation

Nutraceuticals are used in foods to impart health benefits. The effectiveness of nutraceuticals in preventing disease depends on preserving the bioavailability of bioactive ingredients until their release at targeted sites (Chen et al. 2006a). Reducing the particle size may improve the bioavailability, delivery properties, and solubility of the nutraceuticals due to more surface area per unit volume and thus their biological activity (Shegokar and Muller 2010). The bioavailabilities of these nutraceuticals are increased as a nanocarrier allows them to enter the bloodstream from the gut more easily. The nanoscale nutraceuticals were coined together as "nanoceutical" and carriers were called nanocarriers due to their size (Chen et al. 2006a). These nutraceutical compounds can be classified into lipophilic and hydrophilic types based on their solubility in water. Hydrophilic compounds are soluble in water but insoluble in lipids and organic solvents. Some of the nanoencapsulated hydrophilic nutraceuticals are ascorbic acid, polyphenols, etc. (Lakkis 2007; Teeranachaideekul et al. 2007; Dube et al. 2010; Ferreira et al. 2007). Lipophilic compounds are insoluble in water but soluble in lipids and organic solvents. Nanoencapsulated lipophilic nutraceuticals include lycopene, beta-carotene, lutein, phytosterols, and docosahexaenoic acid (DHA) (Lakkis 2007; Heyang et al. 2009; Zimet and Livney 2009; Leong et al. 2011). The solubility of the bioactive ingredients determines the release rate and release mechanism from a polymeric matrix system. Hydrophilic compounds show faster release rates and their release kinetics is determined by the appropriate combination of diffusion and erosion mechanisms. Lipophilic compounds often

resulted in incomplete release due to poor solubility and low dissolution rates by an erosion mechanism (Kuang et al. 2010; Kumar and Kumar 2001; Varma et al. 2004). However, lipophilic compounds are highly permeable through the intestine via active transport and facilitated diffusion, whereas hydrophilic compounds have low permeability and are absorbed only by active transport mechanism (Acosta 2009).

Nanocarrier food systems such as lipid or natural biodegradable polymer-based capsules are most often utilized for encapsulation (Chen et al. 2006a). Nanoliposomes, archaeosomes, and nanocochleates are three types of lipid-based nanocarrier systems with application in pharmaceutical, cosmetic, and food industries. Natural polymers such as albumin, gelatin, alginate, collagen, chitosan, and α-lactalbumin were used for the formulation of nano delivery systems (Reis et al. 2006; Graveland-Bikker and De Kruif 2006). There is tremendous growth observed in the development of food nanocarrier system in the last decade such as various products developed like whey protein which is used as nanocarrier to improve the bioavailability of nutraceuticals, nanodrops mucosal delivery system of vitamins, and nano-based mineral delivery system (Chen et al. 2006a, b).

Nanoencapsulation Techniques

In general, the physicochemical properties such as particle size, size distribution, surface area, shape, solubility, and encapsulation efficiency, and releasing mechanisms were reported to be altered by the encapsulation technique and delivery system. Therefore, it is more essential to select the appropriate encapsulation technique based on the required size, physicochemical properties, nature of the core material, and wall material. Moreover, the techniques used for achieving nanoencapsulation are more complex than microencapsulation. It is mainly due to the difficulty in attaining a complex morphology of the capsule and core material and the demands of releasing rates of nanoencapsulates (Chi-Fai et al. 2007). Various techniques have been developed and used for microencapsulation purpose. However, emulsification, coacervation, inclusion complexation, emulsification–solvent evaporation, nanoprecipitation, and supercritical fluid technique are considered as nanoencapsulation techniques since they can produce capsules in the nanometer range (10–1,000 nm).

Nanoencapsulation techniques use either top-down or bottom-up approaches for the development of nanomaterials. A top-down approach involves the application of precise tools that allow size reduction and structure shaping for desired application of the nanomaterials being developed. In the bottom-up approach, materials are constructed by self-assembly and self-organization of molecules, which were influenced by many factors including pH, temperature,

concentration, and ionic strength (Augustin and Sanguansri 2009). Techniques such as emulsification and emulsification–solvent evaporation are used under the top-down approach. On the other hand, supercritical fluid technique, inclusion complexation, coacervation, and nanoprecipitation are used in the bottom-up approach (as shown in Fig. 2) (Sanguansri and Augustin 2006; Mishra et al. 2010). These nanoencapsualtion techniques can be used for encapsulation of various hydrophilic and lipophilic bioactive compounds. Emulsification, coacervation, and supercritical fluid technique are used for encapsulation of both hydrophilic and lipophilic compounds (McClements et al. 2009; Chong et al. 2009; Leong et al. 2009). However, inclusion complexation, emulsification–solvent evaporation, and nanoprecipitation techniques are mostly used for lipophilic compounds (Reis et al. 2006). Table 1 and the following subsections will discuss in detail the different techniques used for the nanoencapsulation process.

Emulsification Technique

Emulsion technology is generally applied for the encapsulation of bioactive compounds in aqueous solutions through the production of nanoemulsions. Nanoemulsions are colloidal dispersions comprising two immiscible liquids, of which one is being dispersed in the other, with droplet sizes ranging from 50 to 1,000 nm (Sanguansri and Augustin 2006). They offer great potential to encapsulate a high concentration of oil-soluble nutraceuticals or bioactive food supplements into a wide range of foodstuffs. Lipophilic active agents such as β-carotene, plant sterols, carotenoids, and dietary fats can be encapsulated and delivered by oil in
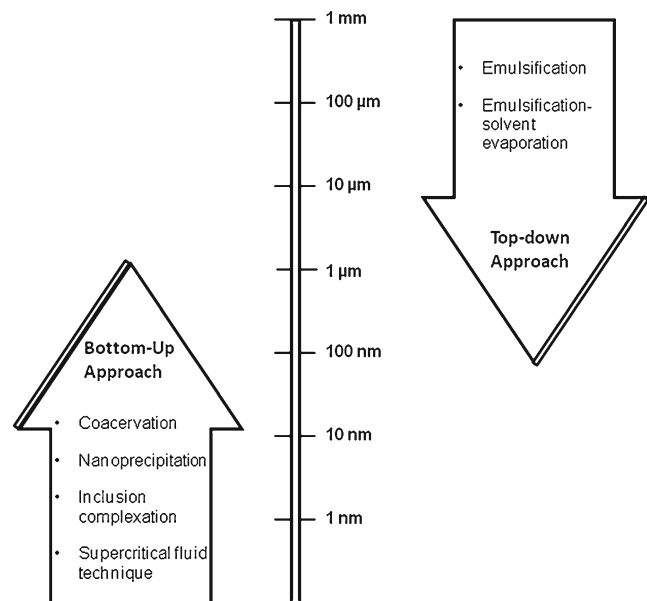

Fig. 2 Top-down and bottom-up approaches in nanoencapsulation techniques

Table 1 Nanoencapsulation techniques of various bioactive compounds

Nanoencapsulation techniques	Important raw materials used	Bioactive compounds	Particle size	Purposes	References
Emulsification	Wall materials: maltodextrin; emulsifiers: modified starch (Hi-Cap 100)	D-Limonene (L)	543–1,292 nm	Protecting the droplets from recoalescence	Jafari et al. 2007b
	Emulsifiers: Tween-40	Flax seed oil (L)	135 nm	Optimizing operating conditions to prevent the droplet from coalescence and cavitational bubble cloud formation	Kentish et al. 2008
	Emulsifiers: Tween-80, Span-80, and sodium dodecyl sulfate	Sunflower oil (L)	40 nm	Optimizing the conditions to produce nanoemulsion	Leong et al. 2009
	Emulsifiers: Tween-20	Curcumin (L)	79–618 nm	Enhancing the anti-inflammation activity	Wang et al. 2008a, b
	Wall materials: OSA starch, chitosan, and lambda-carrageenan	MCT (L)	130 nm	Improving the stability for use in food or pharmaceutical industry	Preetz et al. 2008
	Emulsifiers: Tween-20, Tween-40, Tween-60, and Tween-80	β-Carotene (L)	132–184 nm	Improving physical stability and commercial application	Yuan et al. 2008b
	Emulsifiers: Tween-20	β-Carotene (L)	121–177 nm	Improving the stability	Yuan et al. 2008a
	Other materials: marine lecithin, α-tocopherol, quercetin, chloroform, methanol, diethylic ether, hexane	Salmon oil (L)	160–207 nm	Increasing the oxidative stability	Belhaj et al. 2010
Coacervation	Wall materials: gelatin, maltodextrin and tannins; emulsifiers: Tween-60; other material: glutaraldehyde	Capsaicin (L)	100 nm	Masking the pungent odor, giving biocompatibility and biodegradation	Wang et al. 2008a, b
	Wall materials: gelatin, acacia, and hydrolysable tannins; emulsifiers: hydroxylethyl, cellulose; other material: glutaraldehyde	Capsaicin (L)	300–600 nm	Improving the efficiency and delaying the release property	Xing et al. 2004
	Wall materials: gelatin, acacia, and tannins; emulsifiers: Tween-60; other material: glutaraldehyde	Capsaicin (L)	100 nm	Masking its pungent odor and improving the stability	Jincheng et al. 2010
	Wall materials: chitosan, poly (ethyleneglycol-ran-propyleneglycol); other material: sodium tripolyphosphate	BSA (H)	200–580 nm	Controlling the release of encapsulated protein	Gan and Wang 2007
Inclusion complexation	Wall materials: beta-lactoglobulin and low methoxyl pectin	DHA (L)	100 nm	Formation of transparent solution, improve the colloidal stability, protection against degradation and useful for enrichment of acid drinks	Zimet and Livney 2009
	Wall materials: α- and β-cyclodextrin	Linoleic acid (L)	236 nm	Improving the thermal stability	Hadaruga et al. 2006
Nanoprecipitation	Wall materials: monomethoxy poly (ethylene glycol)-poly (3-caprolactone) micelles	Curcumin (L)	27 nm	Improving the solubility	Gou et al. 2011
	Wall materials: poly (lactide-co-glycolide); emulsifiers: polyethylene glycol-5000	Curcumin (L)	81 nm	Improving the bioavailability, bioactivity, encapsulation efficiency and enhancing the cellular uptake	Anand et al. 2010
	Wall materials: ethyl cellulose and methyl cellulose	Curcumin (L)	117 and 218 nm	Improving oral bioavailability and sustainability	Suwannateep et al. 2011
		β-carotene (L)	80 nm	Improving physical, chemical stability and bioavailability	Ribeiro et al. 2008

Table 1 (continued)

Nanoencapsulation techniques	Important raw materials used	Bioactive compounds	Particle size	Purposes	References
	Wall materials: poly(D,L-lactic acid) and poly(D,L-lactic-coglycolic acid); emulsifiers: gelatin or Tween-20	Astaxanthin (L)	300–320 nm	Improving the solubility and bioavailability	Tachaprutinun et al. 2009
Emulsification–solvent evaporation	Wall materials: poly (ethylene oxide)-4-methoxycinnamoylphthaloylchitosan, poly(vinylalcohol-co-vinyl-4-methoxycinnamate), poly(vinylalcohol), and ethyl cellulose	Curcumin (L)	254–415 nm	For controlled release	Sowasod et al. 2008
	Wall materials: chitosan cross-linked with tripolyphosphate; emulsifiers: Span-80 and Tween-80; other materials: acetic acid and ethanol	Curcumin (L)	100 nm	enhance absorption and prolong the rapid clearance of curcumin	Dandekar et al. 2010
	Wall materials: hydroxyl propyl methyl cellulose and polyvinyl pyrrolidone; emulsifiers: D-α-Tocopheryl polyethylene glycol 1000 succinate, Tween-80, Tween-20, cremophor-RH 40, pluronic-F68, pluronic-F127	Quercetin	170 nm	Improving the controlled release and encapsulation efficiency	Kumari et al. 2010
	Wall materials: poly-D,L-lactide and polyvinyl alcohol	Coenzyme Q10 (L)	40–260 nm	Improving the reproducibility, stability and target drug loading yield	Kwon et al. 2002
	Wall materials: poly (-methyl methacrylate) and polyvinyl alcohol	Phytosterol (L)	50–282 nm	Optimize the operating conditions and reduce phytosterol loss	Leong et al. 2011
	Emulsifiers: Tween-20; other materials: hexane, isopropyl alcohol, ethanol, and acetone	α-Tocopherol (L)	90–120 nm	Minimizing the recoalescence, improve the physical stability and solubility	Cheong et al. 2008
	Emulsifiers: Tween-20	Astaxanthin	115–163 nm	Optimizing the processing condition and improving bioavailability	Anarjan et al. 2011
	Emulsifiers: sodium caseinate	β-carotene (L)	9–280 nm	Improving the physical stability	Silva et al. 2011
	Wall materials: poly(D,L-lactide-co-glycolide) and polyvinyl alcohol; other materials: chloroform and ethanol; emulsifiers: Tween-20	Curcumin (L)	45 nm	It gives smooth, spherical PLGA nanospheres formation, high yield, drug entrapment efficiency with a narrow size range and sustained delivery	Mukerjee and Vishwanatha 2009
Supercritical antisolvent precipitation	Wall materials: hydroxylpropyl methyl cellulose phthalate	Lutein (L)	163–219 nm	Bioactivity, promote to food industry and to avoid thermal/light degradation	Heyang et al. 2009
Spray drying	Wall materials: carbohydrate matrix and maltodextrin; other materials: acetone	Catechin (H)	80 nm	Increasing the stability, protecting from oxidation and incorporation into beverages	Ferreira et al. 2007
	Wall materials: modified n-octenyl succinate-starch; other materials: ethyl acetate	β-carotene (L)	300–600 nm (droplet size); 12 um (particle size)	Improving dispersibility, coloring strength and bioavailability	De Paz et al. 2012
	Wall materials: maltodextrin; emulsifiers: Hi-Cap, whey protein concentrate, and Tween-20	D-Limonene (L)	0.2–1.2 μm (emulsion droplet size); 21–53 μm (dried particle size)	Increasing the retention, stability during process	Jafari et al. 2007a

Table 1 (continued)

Nanoencapsulation techniques	Important raw materials used	Bioactive compounds	Particle size	Purposes	References
	Wall materials: maltodextrin; emulsifiers: modified starch (Hi-Cap)/whey protein concentrate	Fish oil (L)	0.21–5.9 μm (droplet size); 25–41 μm (particle size)	Minimizing the un-encapsulated oil at the surface and maximizing encapsulation efficiency	Jafari et al. 2008
Freeze drying	Wall materials: β- cyclodextrin, polycaprolactone; emulsifiers: pluronic F68; other materials: ethyl acetate	Fish oil (L)	183–714 nm	Preventing oxidation and masking the odor	Choi et al. 2010
	Wall materials: poly-ε-caprolactone	Fish oil (L)	200–350 nm	Increasing the oxidative stability and encapsulation efficiency	Bejrapha et al. 2010
	Wall materials: poly-ε-caprolactone and gelatin; emulsifiers: pluronic F68	Capsicum oleoresin (L)	152 nm	Improving the stability and Rehydrating to study the dispersion characteristics and gel network formation prevents the denaturation	Nakagawa et al. 2011
	Wall materials: poly-ε-caprolactone; emulsifiers: pluronic F68; other materials: trehalose, D-sucrose, D-mannitol, dextrose, D-sorbitol gelatin and κ-carrageenan	Capsicum oleoresin (L)	163–1,984 nm	Effect of excipients on the stability and particle size of nanocapsules	Bejrapha et al. 2011
	Wall materials: poly-ε-caprolactone; emulsifiers: pluronic F68	Capsicum oleoresin (L)	320–460 nm	Extending the shelf-life, minimizing environmental stress and can apply to the food products	Surassamo et al. 2010
	Wall materials: chitosan, zein; emulsifiers: Tween-20	α-Tocopherol (L)	200–800 nm	Improving stability and Protecting from environmental factor	Luo et al. 2011
	Wall materials: polyethylene glycol; emulsifier: Tween-80	Vitamin E (L)	164 nm	Increasing the stability, retention percentage and extending the shelf-life	Zhao et al. 2011
	Emulsifiers: dioctyl sodium sulfosuccinate, poloxamer 188, glyceryl monostearate	Curcuminoids (L)	450 nm	Improving the stability	Tiyaboonchai et al. 2007
	Wall materials: chitosan and sodium tripolyphosphate	Catechin (H)	163 and 165 nm	Protecting catechin from degradation	Dube et al. 2010

L lipophilic, H hydrophilic

water emulsion (o/w), while water in oil emulsion (w/o) was used to encapsulate water-soluble food active agents such as polyphenols (Zuidam and Shimoni 2010). Nanoemulsions can either be used directly in the liquid state or be dried to powder form using drying techniques such as spray drying and freeze drying after emulsification. Moreover, nanoemulsions possess high kinetic stability due to their extremely small emulsion droplet sizes (Solans et al. 2005; Sonneville-Aubrun et al. 2004). The high kinetic stability of nanoemulsions is a real benefit for encapsulation purposes and plays a critical role in the retention of surface oil content of the product (Jafari et al. 2008). Nanoemulsions, being non-equilibrium systems, cannot be formed spontaneously and consequently it needs energy input, generally from mechanical devices or from the chemical potential of the components. Therefore, nanoemulsion formation is generally achieved using high-energy emulsification methods like high shear stirring, high-speed or high-pressure homogenizers, ultrasonicator, and microfluidizer. These methods supply the available energy in the shortest time and possess the most homogeneous flow to produce the smallest droplet sizes (Walstra 1996). The use of nanoemulsion technology for delivering food components and nutraceuticals has been comprehensively reviewed by, Augustin and Hemar (2009), McClements et al. (2009), and Silva et al. (2012).

Microfluidisation uses very high pressure (up to 20,000 psi) to force the liquid through the interaction chamber, which consists of microchannels of a special configuration. The emulsion feeds through the microchannels into a collision chamber, leading to formation of fine nanoscale emulsion droplets (Fathi et al. 2012). Jafari et al. (2007a, b, 2008) used microfluidisation, ultrasonication, and silverson (a typical colloid mill with a stator composed of a metal grating in which 2-mm holes are drilled) for emulsification of d-limonene and fish oil and further spray drying to obtain particles. It was reported that microfluidisation was the best emulsification method due to maximum encapsulation efficiency and smaller emulsion droplet size. Jafari et al. (2007a) encapsulated d-limonene using maltodextrin combined with a surface-active biopolymer, i.e., modified starch (Hi-cap), whey protein concentrate (WPC), and Tween 20 as wall materials. Microfluidisation resulted in powder with highest retention (86 %) of d-limonene, smaller emulsion droplets of (700–800 nm), and narrower distribution, which also had good stability during the process. Hi-cap was found to be better than WPC due to less surface oil content and more retention of d-limonene. Moreover, Tween 20 significantly reduced the emulsion droplet size (<200 nm) but resulted in poorest encapsulation efficiency. Further, Jafari et al. (2008) encapsulated fish oil in maltodextrin combined with surface-active biopolymers (Hi-Cap 100 and whey protein concentrate) in 3:1 ratio and achieved emulsion

droplet size of 210–280 nm by microfluidisation. Jafari et al. (2007b) reported that the main problem with microfluidisation was with increased energy input, beyond moderate pressure (40–60 MPa), and cycle (one to two), leading to "over-processing" of emulsion droplets due to recoalescence and resulting in larger emulsion droplet size. However, in case of emulsification with ultrasound, increasing the energy input helps to reduce the emulsion size with minimum recoalescence of new droplets. Kentish et al. (2008) studied both a batch and continuous focused flow-through ultrasonic cell for emulsification using flax seed oil. Emulsions with a mean droplet size as low as 135 nm was achieved using a surfactant of Tween 40 using ultrasonication. They also reported that the batch cell produced better results and continuous equipment is likely to be more viable in a commercial environment.

High-pressure homogenization is a technique in which a mixture is pushed with high pressure (100 to 2,000 bar) and high shear stress, which resulted in the disruption of particles down to the nanometer range. Homogenization may be performed either at elevated temperature (hot homogenization) or below room temperature (cold homogenization) (Mueller et al. 2000). Using high-pressure homogenization technique, Yuan et al. (2008a) formulated β-carotene nanoemulsions (o/w) and investigated the influence of emulsifying conditions on the properties and stability of the nanoemulsions. Yuan et al. (2008a) optimized the conditions for preparing β-carotene nanoemulsions (o/w) by response surface methodology. The optimum conditions were homogenization pressure (129 MPa), temperature (47 °C), β-carotene concentration (0.82 %), and emulsifier concentration (8.2 %) for producing emulsion droplet size in the range of 120–177 nm. Apart from this, the physical stability of the nanoemulsions decreased with the elevated temperature but increased with pressure (up to 100 MPa) and homogenization cycle (up to three cycles). Moreover, during storage at 25 °C, β-carotene degraded after 4 weeks with greater loss. Further, Yuan et al. (2008b) reported that Tween 20 has produced the smallest droplet sizes (Fig. 3) and narrowest size distribution compared to other emulsifiers (Tween 40, Tween 60, and Tween80). The droplet sizes decreased with increase in homogenization pressure, cycle, and temperature (≤50 °C). Recently, Belhaj et al. (2010) prepared nanoemulsions of salmon oil (Salmo salar) using high-pressure homogenization (1,700 bars) technique and reported the droplet size to be 160–207 nm. Wang et al. (2008b) compared the high-speed homogenization and high-pressure homogenization techniques by producing curcumin nanoemulsion. They reported that curcumin nanoemulsion produced by the high-pressure homogenization technique had a lower mean droplet size (80 nm) and higher anti-inflammation activity than the high-speed homogenization technique (619 nm).
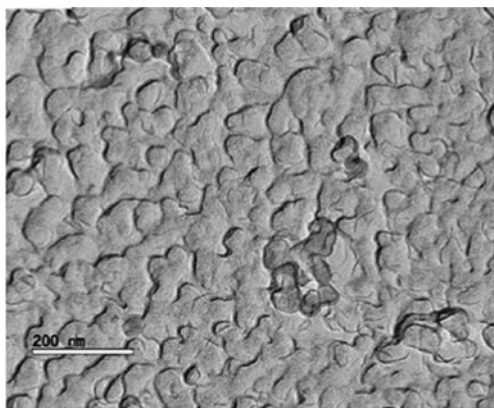
Fig. 3 Transmission electron micrographs of β-carotene nanoemulsions prepared with Tween 20 at a final concentration of 10 % (*w/w*), 100 MPa, and 50 °C (sample analyzed immediately after preparation) (Yuan et al. 2008b)

Garti and Benichou (2001, 2004) stated that the multiple emulsions can also be used as delivery systems with novel encapsulation and delivery properties. The most common examples of this are oil-in-water-in-oil (O/W/O) and water-in-oil-in-water (W/O/W) emulsions. Functional food components could be encapsulated within the inner water phase, the oil phase, or the outer water phase, thereby making it possible to develop a single delivery system that contains multiple functional components. Alternatively, it could be used to protect and release an aqueous-phase component trapped within the inner water droplets to a specific site such as the mouth, stomach, or small intestine (Weiss et al. 2006). Dupeyron et al. (2009) encapsulated bovine serum albumin into an enteric copolymer (methacrylic acid/ethyl acrylate blended with PEG) by modified double emulsion (W/O/W) technique and vacuum drying. They produced 90 % nanoparticles with 77–78 % encapsulation efficiency, indicating successful control of the process parameters.

Microfluidisation was an efficient emulsification technique, yielding less surface oil on encapsulates, but when energy input increased beyond moderate pressure it leads to over-processing due to recoalescence. Comparably, ultrasonication was also a better emulsification technique and increasing energy input resulted in the reduction of emulsion droplet size with minimum recoalescence. High-pressure homogenization yielded smaller droplet sizes with the temperature increased up to 50 °C than high-speed homogenization. All these emulsification methods were capable in reducing the droplet size to a larger extent. Hence, the emulsification technique proved to be one of the effective nanoencapsulation techniques but depends on a good drying technique to produce these encapsulates in powder form. Moreover, the nanoparticle size, distribution, emulsion size stability, and other parameters have been altered by various emulsion preparation techniques and its operating conditions like speed, shear, pressure, temperature, type of emulsifiers, and emulsifier concentration.

Coacervation

The coacervation technique involves the phase separation of a single or a mixture of polyelectrolyte from a solution and the subsequent deposition of the newly formed coacervate phase around the active ingredient. Further, a hydrocolloid shell can be cross-linked using an appropriate chemical or enzymatic cross-linker such as glutaraldehyde or transglutaminase, mainly to increase the robustness of the coacervate (Zuidam and Shimoni 2010). Based on the number of polymer type used, the process can be termed as simple coacervation (only one type of polymer) and complex coacervation (two or more types of polymer). Many factors including the biopolymer type (molar mass, flexibility, and charge), pH, ionic strength, concentration, and the ratio of the biopolymers affect the power of the interaction between the biopolymers and the nature of the complex formed (Tolstoguzov 2003; De Kruif et al. 2004; Turgeon et al. 2007). Apart from the electrostatic interactions between biopolymers of opposite charges, hydrophobic interactions and hydrogen bonding can also contribute significantly to the complex formation. Gouin (2004) stated that coacervation is a distinctive and promising encapsulation technology because of the very high payloads achievable (up to 99 %) and the possibilities of controlled release based on mechanical stress, temperature, or sustained release.

Wang et al. (2008a) encapsulated capsaicin using the simple coacervation process in gelatin by cross-linking with glutaraldehyde and drying in vacuum oven. The obtained nanocapsules were of size 100 nm. Moreover, the melting point and the thermal pyrolysis temperature of the nanocapsulates were improved due to encapsulation of the cross-linked gelatin over the surface of capsaicin. Xing et al. (2004) encapsulated capsaicin in gelatin and acacia using complex coacervation technique. The nanocapsules were obtained by treating encapsulated capsaicin with hydrolysable tannins and cross-linking with glutaraldehyde along with freeze drying technique. The mean diameters of nanocapsules were found to be 300–600 nm with a spherical morphology. A total of 81 % encapsulation efficiency with good dispersion property was observed in this study. Moreover, the addition of hydrolysable tannins in the system had an important influence on the morphology and particle size distribution of the nanocapsules due to the synergistic actions of hydrogen bonding and hydrophobic effects. Recently, Jincheng et al. (2010) also encapsulated capsaicin by a similar complex coacervation method as shown in Fig. 4 and drying in vacuum oven. The authors optimized the operating parameters and reported that higher shearing force (15,000 rpm agitation rate), lower gelatin
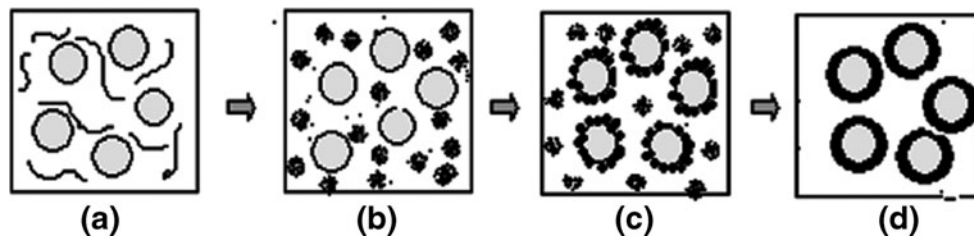
Fig. 4 Formation process of the nanoencapsulated capsaicin agents: **a** dispersion of capsaicin in gelatin solution, **b** coacervation of gelatin with acacia in the solution, **c** coacervation of insoluble complex on the surface of the capsaicin, **d** shell formation by the addition of glutaraldehyde solution (Jincheng et al. 2010)

viscosity (15–20 cP s), suitable cross-linking time (40–80 min), utilization of tannins, and other experimental conditions had influenced the synthesis of nanoencapsulates. The obtained nanocapsulates had a mean diameter of about 100 nm with a spherical morphology, increased melting point (75 to 85 °C), and improved degradation properties. Their results were consistent with earlier studies. Gan and Wang (2007) encapsulated a model protein bovine serum albumin (BSA) in chitosan by incorporation or incubation method using polyanion tripolyphosphate (TPP) as the coacervation cross-link agent. The BSA-loaded chitosan–TPP nanoparticles prepared under varying conditions were found to be in the size range of 200–580 nm. Detailed sequential time frame transmission electronic microscope (TEM) imaging of the morphological change of BSA-loaded particles showed a swelling and particle degradation process. Their study demonstrated that the poly ionic coacervation process can be conditioned to exert control over protein encapsulation efficiency and subsequent release profile.

The nanocapsules produced using the coacervation technique were in the range of 100–600 nm, but it depends on a suitable drying technique such as vacuum drying and freeze drying. Gelatin, gum of acacia, and chitosan have been used as wall materials in this technique. Moreover, the treatment of nanocapsules with tannins influenced their morphology (good dispersion and shape) and particle distribution. Cross-linking with glutaraldehyde for a particular time period had increased the melting point and thermal stability of the nano-encapsulates. The major problem recognized in this technique lies in commercializing the coacervated food ingredient due to the usage of glutaraldehyde for cross-linking, which must be carefully used according to the country's legislation. Nevertheless, at present, so many suitable enzymes are being developed for cross-linking (Gouin 2004).

Inclusion Complexation

Inclusion complexation generally refers to the encapsulation of a supra-molecular association of a ligand (encapsulated ingredient) into a cavity-bearing substrate (shell material) through hydrogen bonding, van der Waals force, or an entropy-driven hydrophobic effect. In the food industry,

molecular entities having suitable molecular-level cavities are rarely available. Hadaruga et al. (2006) encapsulated linoleic acid in α- and β-cyclodextrin (α- and β-CD) by inclusion complexation technique to improve its thermal stability. The nanocapsules of α- and β-CD complexes had a yield of about 88 and 74 %, respectively. Similarly, Lira et al. (2009) encapsulated usnic acid (UA) in β-CD and formed a complex (UA/β-CD) by inclusion complexation along with freeze drying. The complex (UA/β-CD) was further incorporated into liposomes in order to produce a targeted drug delivery system for exploiting the antimycobacterial activity of UA. Liposomes containing UA/βCD were prepared using hydration of a thin lipid film method with subsequent sonication. Formulations of liposomes containing UA/βCD exhibited a drug encapsulation efficiency of 99.5 % and remained stable for 4 months in suspension form. Interestingly, the encapsulation of UA/βCD into the liposomes resulted in a modulation of in vitro release kinetics of UA. Liposomes containing UA/β-CD indeed presented a more prolonged release profile of free usnic acid compared to usnic acid-loaded liposomes (Lira et al. 2009). Another example of molecular inclusion was the milk protein β lactogloglobulin (β-Lg). Zimet and Livney (2009) produced a colloidally stable nanocomplex of DHA-loaded β-Lg along with low methoxyl pectin. The entrapment by β-Lg and the formation of nanocomplexes with pectin provided good protection against degradation of DHA during an accelerated shelf-life stress test and only about 5–10 % lost during 100 h at 40 °C.

The inclusion complexation technique is mainly used in the encapsulation of volatile organic molecules (essential oils and vitamins); it is useful to mask odors and flavors and preserve aromas. This technique yielded higher encapsulation efficiency with higher stability of the core component. However, only few particular molecular compounds like β-cyclodextrin and β-lactogloglobulin are suitable for encapsulation through this method.

Nanoprecipitation Technique

The nanoprecipitation method is also called solvent displacement. It is based on the spontaneous emulsification of

the organic internal phase containing the dissolved polymer, drug, and organic solvent into the aqueous external phase. Nanoprecipitation technique involves the precipitation of polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium (Galindo-Rodriguez et al. 2004). The solvent displacement forms both nanocapsules and nanospheres. Biodegradable polymers are commonly used, especially polycaprolactone (PCL), poly (lactide) (PLA), and poly (lactide-co-glicolide) (PLGA), eudragit, poly (alkylcyanoacrylate) (PACA) (Reis et al. 2006).

Recently, Anand et al. (2010) encapsulated curcumin in PLGA with stabilizer PEG-5000 using nanoprecipitation technique followed by freeze drying technique. The nanocapsules had mean particle diameter of about 81 nm (Fig. 5) and reported to have enhanced cellular uptake, and increased bioactivity in vitro and in vivo study. Similarly, solvent displacement method in combination with freeze drying technique was used by Suwannateep et al. (2011) for encapsulation of curcumin in a mono polymeric carrier made from ethyl cellulose and a dipolymeric carrier (ECMC). The obtained curcumin loaded ethyl cellulose (C-EC) and curcumin loaded ECMC (C-ECMC) nanocapsules with mean diameter of 281 nm and 117 nm, respectively. Similarly, Gou et al (2011) also encapsulated curcumin using single-step nanoprecipitation method along with freeze drying technique. Encapsulation efficiency of 99 % with mean particle size of 27 nm was obtained. It also exhibited strong anticancer effect than free curcumin on in vivo study.

Tachaprutinun et al. (2009) encapsulated astaxanthin by solvent displacement along with freeze drying technique. It yielded reasonably good encapsulation efficiency (98 %) at a loading of 40 %. Moreover, the freeze-dried astaxanthin-encapsulated nanospheres showed good dispersibility in
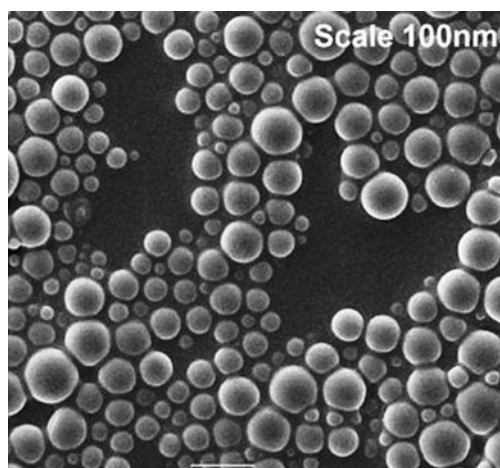

Fig. 5 SEM picture of nanoencapsulated curcumin (Anand et al. 2010)

water, yielded stable aqueous suspensions of nanoparticles of about 300–320 nm. Using solvent displacement method (schematic flow diagram is shown in Fig. 6), Ribeiro et al. (2008) produced β-carotene loaded nanodispersions by encapsulating β-carotene into PLA and PLGA along with freeze drying technique. Gelatin and Tween 20 were used as stabilizing hydrocolloids in the continuous phase. The diameter of the droplets was reported to be below 80 nm and the dispersions were more stable against ostwald ripening and coalescence. Moreover, on redispersion of lyophilized powder in water there was no significant change in droplet size.

Nanoprecipitation seems to be an efficient technique for producing nanocapsules of around 100 nm and below. Moreover, the nanoencapsulates were exhibiting good stability against degradation, higher encapsulation efficiency, sustained release increased cellular uptake and bioavailability during in vivo studies. However, it depends on a good drying technique (freeze drying) and only polymer based wall material can be used (PEG and PLGA). Appropriate solvent and non solvent phase needs to be selected, which may vary for each bioactive component and moreover polymer and solvent needs to be of food grade. The usefulness of this simple technique is limited to water-miscible solvents, in which the diffusion rate is enough to produce spontaneous emulsification. This is an efficient method to nanoencapsulate lipophilic drugs because of the miscibility of the solvent with the aqueous phase.

Emulsification–Solvent Evaporation Technique

Emulsification–solvent evaporation technique is a modified version of solvent evaporation method. It involves emulsification of the polymer solution into an aqueous phase and evaporation of polymer solvent inducing polymer precipitation as nanospheres (Reis et al. 2006). The drug is finely dispersed in the polymer matrix network. The size of the capsules can be controlled by adjusting the stir rate, type and amount of dispersing agent, viscosity of organic and aqueous phases, and temperature (Tice and Gilley 1985). Frequently used polymers are PLA, PLGA, ethyl cellulose, cellulose acetate phthalate, PCL, and poly (h-hydroxybutyrate). In order to produce a small particle size, often high-speed homogenization or ultrasonication may be employed (Zambaux et al. 1998).

Sowasod et al. (2008) encapsulated curcumin in chitosan by cross-linking with tripolyphosphate using multiple emulsion/solvent technique along with freeze drying technique. The obtained nanocapsules were spherical in shape and the particle sizes were ranging from 254 to 415 nm. The yield of nanoencapsulated curcumin ranged from 19 to 96 % and FTIR analysis confirmed the cross-linking between tripolyphosphate and the amine group of chitosan in nanocapsules.

Fig. 6 Schematic representation of the production of β-carotene nanodispersion by solvent displacement method (Ribeiro et al. 2008)
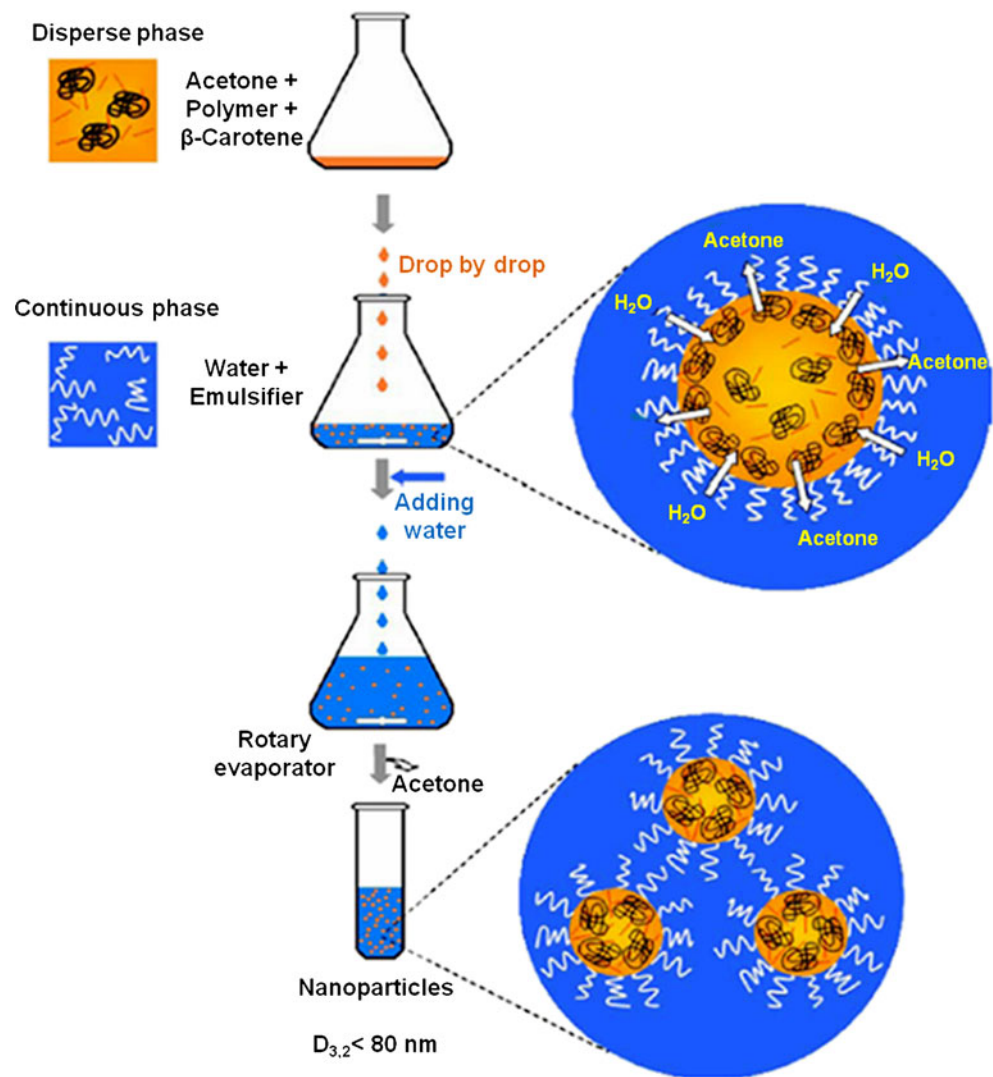
Likewise, curcumin was encapsulated in Eudragit S 100 (polymer) using solvent evaporation method followed by freeze drying technique (Dandekar et al. 2009). The obtained nanocapsules were spherical, with encapsulation efficiency of about 72 %. Furthermore, the nanocapsules reported almost double the inhibition of cancerous cells as compared to using curcumin alone. Similarly, using the emulsification–solvent evaporation technique, Mukerjee and Vishwanatha (2009) prepared curcumin-loaded PLGA nanospheres. The nanospheres were smooth, spherical, and exhibiting high yield and drug entrapment efficiency, with a mean particle diameter of 45 nm. Further, they reported higher intracellular uptake and efficient action in prostate cancer cell lines. Recently, Dandekar et al. (2010) also encapsulated curcumin in a nanocarrier by solvent emulsion–evaporation technique. These nanoparticles were observed to be around 100 nm in size, with a fairly narrow distribution and encapsulation efficiency of 72 %. This

optimized system was further subjected to freeze drying technique. The freeze-dried product on reconstitution exhibited a size and distribution similar to that before freeze drying. In their in vivo anti-malarial studies, a significant superior action of nanoparticles over curcumin control was revealed.

Kwon et al. (2002) prepared coenzyme Q10-loaded nanoparticles using emulsification–solvent evaporation technique (as shown in Fig. 7) with microfluidization. The nanoparticles were in the range of 40 to 260 nm. Despite a very high target drug loading yield of around 39 %, the actual loading efficiency reached above 95 %, and the mean diameter of the nanoparticles was highly influenced by the kind of surfactants used and the recycling number of the microfluidization process. In a recent study, Kumari et al. (2010) encapsulated quercetin in PLA by solvent evaporation method along with freeze drying technique. The mean diameter of the nanoparticles obtained was 130 nm.
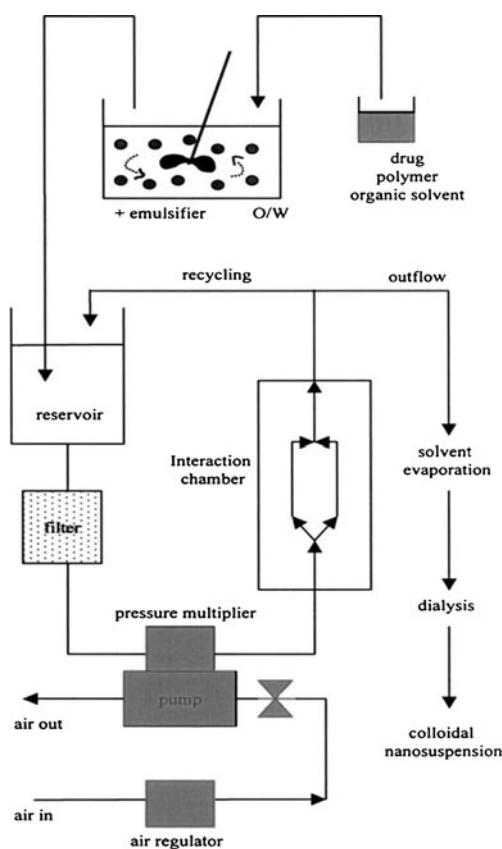
Fig. 7 Preparation procedure of nanoparticles by emulsification–solvent evaporation method (Kwon et al. 2002)

Moreover, encapsulation efficiency was found to be 97 % and maximum release of quercetin was 88 % at 96 h.

Nanodispersions, nanoemulsions, and nanosuspensions are also prepared through emulsification–evaporation technique. Cheong et al. (2008) prepared nanodispersion of α-tocopherol using emulsification–evaporation technique under various combinations of the processing parameters and the ratio of aqueous and organic phases. Droplet diameters were in the range of 90–120 nm and there were no significant changes in mean diameters during the storage period of 3 months. Likewise, using emulsification evaporation technique, Anarjan et al. (2011) prepared a nanodispersion of astaxanthin. The obtained nanodispersions were in the range of 110–165 nm. They optimized the processing conditions and reported that the most desirable nanodispersion was obtained by using a high-pressure homogenizer at 30 MPa with three passes followed by evaporation at 25 °C. Recently, Leong et al. (2011) compared conventional homogenization with high-pressure homogenization in the preparation of water-soluble phytosterol nanodispersions using emulsification–evaporation technique. Phytosterol loss after high-pressure homogenization ranged from 3 to 28 %, and losses increased with increasing homogenization pressure. Similarly, Silva et al. (2011) produced nanoemulsions of β-

carotene using high-energy emulsification–evaporation technique. The obtained β-carotene nanoemulsions presented a volume surface diameter ranging from 9 to 280 nm. The process parameters such as time and shear rate of homogenization significantly affected the particle size distribution and storage stability of nanoemulsions.

Emulsification solvent evaporation technique also remains to be an efficient technique for producing nanocapsules of sizes below 100 nm. Most of the nanocapsules were exhibiting a spherical shape, having a high drug loading content and encapsulation efficiency of about 75–96 % with sustained release and increased absorption. Apart from this, the nanodispersions and nanoemulsions prepared through this method were showing good stability. However, it depends on a suitable emulsification technique such as microfluidisation, high-speed and high-pressure homogenization techniques, and the other operating conditions alter the particle size to a large extent. It also relies on a suitable drying technique for producing nanocapsules. The limitations are imposed by the scale-up of the high energy requirements in homogenization. Most probably, it involves the application of a polymer-based wall material; only lipophilic core material can be encapsulated and the solvent to be utilized should be of food grade.

Supercritical Fluid Technique

A supercritical fluid can either be a liquid or gas and used above its thermodynamic critical point of temperature and pressure (Jung and Perrut 2001). Supercritical fluids exhibit properties intermediate between those of liquids and gases such as low viscosity, low density, high solvating power, high diffusivities, and high mass transfer rates above the critical point. A number of compounds can be brought to a supercritical state, such as carbon dioxide, water, propane, nitrogen, etc. (Gouin 2004). Some of the methods under supercritical fluid technology such as rapid expansion from supercritical solution, gas antisolvent, supercritical antisolvent precipitation, aerosol solvent extraction, and precipitation with a compressed fluid antisolvent have been utilized in recent years (Kikic et al. 1997). Supercritical fluids are used for the encapsulation of thermally sensitive compounds in a process similar to spray drying. In this technique, the bioactive compound and the polymer were solubilized in a supercritical fluid and the solution is expanded through a nozzle. Then, the supercritical fluid was evaporated in the spraying process, and solute particles eventually precipitate (Reis et al. 2006). This technique has been widely used because of its low critical temperature and minimum use of organic solvent.

Using supercritical antisolvent precipitation, Heyang et al. (2009) encapsulated lutein in hydroxyl propyl methyl cellulose phthalate (HPMCP) to maintain its bioactivity

and to avoid thermal/light degradation. Various operating parameters affected the yield, such as, lutein loading, encapsulation efficiency, particle size, and distribution of the nanocapsule. They reported that the mean diameter of a lutein-loaded HPMCP nanocapsule was in the range from 163 to 219 nm. The highest lutein loading of 16 % and encapsulation efficiency of 88 % were obtained under the operating conditions of 11 MPa pressure at 40 °C temperature with 5:1 ratio of HPMCP and lutein. Using rapid expansion from supercritical solution technique, Turk and Lietzow (2004) synthesized phytosterol nanoparticles (below 500 nm) with long-term stability. Moreover, the surfactant type and concentration were reported to influence the particle size distribution. However, this process requires a high initial capital investment for high-pressure equipment (Gouin 2004).

Drying Techniques for Producing Nanoparticles

The major problems of nanocapsules are irreversible aggregation and chemical instability caused by the hydrolysis of polymeric substances, resulting in the leakage of the encapsulated active ingredients (Chacon et al. 1999). Therefore, it is desirable to convert nanocapsule suspensions into the dried form since their stability can be maintained by drying (Nakagawa et al. 2011). Nanoencapsulation techniques are used to produce nano suspensions with coating or encapsulated with wall materials in liquid or dried form. However, normally, nanoencapsulation methods are used combined with drying techniques for converting encapsulated suspensions to a dried stable form. Freeze drying and spray drying techniques are commonly employed in drying nanosuspensions (Choi et al. 2004; Abdelwahed et al. 2006a). Through various researches, it was clear that the operating conditions of spray drying and freeze drying are found to be significantly important to stabilize nanocapsules (Nakagawa et al. 2011). Besides having higher stability compared to original nanoparticle suspension, the dried powders have the ability to control and sustain bioactive compound release (Guterres 2009). However, drying provokes additional stress on the nanocapsules during processing. Hence, it is necessary to investigate the relationship between the process parameters for drying and nanocapsule stability for achieving better encapsulation of bioactive ingredients (Nakagawa et al. 2011). The following subsections will discuss in detail the different drying techniques for producing nanoparticles.

Spray Drying

Spray drying involves the process of transformation of feed (solution) into a dried particulate form by spraying the feed into a hot drying medium. It yields fine particles with less processing time and also a more economical unit operation (Masters 1991). Due to its incessant production of dry powders with low moisture content, it is widely used for industrial process (Anandharamakrishnan et al. 2007; Kuriakose and Anandharamakrishnan 2010). Moreover, it is the well-established technique in food industry and widely used for encapsulation for past several decades. Spray drying is also used to encapsulate a wide range of food ingredients such as flavors, vitamins, minerals, colors, fats, and oils in order to protect them from their surrounding environment and extend shelf-life stability during storage (Pillai et al. 2012), and thus it can be considered as a good microencapsulation technique. However, in the case of nanoencapsulation, it is just capable of converting a suspension of colloidal nanoparticles into a nanostructured powder form. Spray drying technique is reported to be considered as a suitable process for consolidating nanoparticles into macroscopic compacts and submicron spherical powders with nanometer-scale properties (Okuyama and Lenggoro 2003).

Jafari et al. (2007a, 2008) reported that modified starch (Hi-Cap) was better than whey protein concentrate due to less surface oil in the encapsulated powders. In both studies, only the emulsion droplets were found to be in nano size (200–800 nm), but they were converted to micron size (above 20 μm) during spray drying. Ferreira et al. (2007) encapsulated catechin in a carbohydrate matrix using homogenization followed by spray drying at an inlet temperature of 150–190 °C. They produced spherical-shaped particles (diameter in the range of 80 nm) with a smooth surface. Moreover, encapsulation of catechins prevented them from oxidation and improved its bioavailability. Recently, De Paz et al. (2012) formulated nanosuspensions by encapsulating β-carotene using modified n-octenyl succinate starch through emulsification evaporation along with spray drying technique. The nanosuspensions were produced with various experimental operating conditions with higher encapsulation efficiency (65–90 %) and antioxidant activity, with particle size in the range of 300–600 nm. However, particles collected after spray drying were around 12 μm.

Spray drying is an efficient drying technique to stabilize the nanocapsules. In contrary to freeze drying, it is more economical and fast and is a single-step drying method. It yields uniformly spherical shaped particles, which offer complete protection of the core material being encapsulated. On the other hand, it yields particles of micron size on drying the nanoemulsions and nanosuspensions. However, the core material encapsulated inside the matrix of micron-sized particles was in nano-size range (nanosupensions and nanoemulsions) and which Jafari et al. (2007a, 2008) has considered as nanoparticle encapsulation. Moreover, nanoencapsulation of spray drying depends on other nanoencapsulation techniques (like emulsion) prior to spray drying. Therefore, conventional spray drying technique itself may

not be considered as a nanoencapsulation technique. However, in spray drying, it is possible to control the particle size and morphology by varying process parameters and formulations (Anandharamakrishnan et al. 2008). Hence, spray drying needs suitable modifications for drying of nanoemulsion and suspensions to retain their nanometer size.

Freeze Drying

Freeze drying, also known as lyophilization, is a process used for the dehydration of almost all heat-sensitive materials and aromas (Anandharamakrishnan et al. 2010). Freeze drying is a multi-stage operation stabilizing materials throughout the four main stagess: freezing, sublimation (primary drying), desorption stage (secondary drying), and, finally, storage. Freeze drying results in superior-quality products, which are easily reconstituted, having a longer shelf-life. However, energy intensiveness, long processing time (more than 20 h), and open porous structure were the main drawbacks of freeze drying (Singh and Heldman 2009). Nevertheless, freeze drying is normally used for the separation of nanoparticles (i.e., removal of water from the substances) produced by other nanoencapsulation techniques. During freeze drying, pores are formed due to the ice sublimation process. Hence, this process is not purely encapsulation as active food ingredients are exposed to the atmosphere due to pores on the particle surface. Therefore, it is difficult to use any release mechanism like diffusion or erosion technique. Currently, freeze drying technique is a widely used technique to remove water from nanocapsules without changing their structure and shape. However, spray–freeze drying technique may be an effective alternative to conventional freeze drying technique in terms of reducing the pore size and drying time (Anandharamakrishnan et al. 2010).

Choi et al. (2010) encapsulated fish oil using β-cyclodextrin (β-CD) by self-aggregation method and using PCL (Food and Drug Administration (FDA)-approved edible drug delivery material) via emulsion diffusion method along with freeze drying technique. The mean particle size of β-CD fish oil was reported to be 250–700 nm and PCL/fish oil particles were less than 200 nm. It was found that PCL/fish oil (99 %) has higher fish oil loading and encapsulation efficiency and lower fish oil leakage than β-CD (84–87 %). Recently, Bejrapha et al. (2010) compared the effects of vacuum freeze drying (vacuum pressurized freezing and drying) and conventional freeze drying (atmospheric pressurized freezing and drying) processes on the stability of fish oil-loaded nanocapsules encapsulated in a PCL. Their study indicated that the particle size of fish oil nanocapsules was reported to be below 360 nm and found to be aggregated. The encapsulation efficiency of conventional freeze drying was greater than vacuum freeze drying, except at the freezing temperature of −30 °C. In addition, the

authors revealed that the vacuum freezing process may affect the fragility of the PCL membrane due to its lower encapsulation efficiency and aggregation of particles. It was also found that conventional freeze drying process was more effective than vacuum freeze drying in improving the oxidative stability of fish oil-loaded nanocapsules.

Dube et al. (2010) encapsulated (+) catechin and (−) epigallocatechin gallate (EGCG) in chitosan–tripolyphosphate by ionic gelation method and sonication along with freeze drying technique. The effectiveness of nanoencapsulation was compared with the addition of reducing agents such as ascorbic acid, dithiothreitol, and (tris 2-carboxyethyl) phosphine (TCEP) for their potential to protect catechin and EGCG from degradation. The nanocapsules had a mean particle size of less than 200 nm. Nanoencapsulation of catechin and EGCG had provided good protection than reducing agents TCEP and ascorbic acid. Surassamo et al. (2010) encapsulated capsicum oleoresin in PCL through emulsion diffusion method (it involves the emulsion formation between water-miscible solvent containing drug and polymer aqueous phase; addition of water to the system causes the solvent to diffuse to the external phase, resulting in the formation of nanospheres (Quintanar-Guerrero et al. 1998)) followed by freeze drying. The process parameters were optimized by varying the concentration of the surfactant, pluronic F68 (PF68). The obtained nanoemulsions were in the range of 320–460 nm. The size of nanocapsule particles decreased on increasing the emulsifier concentration. On increasing the surfactant concentration, the particle size was decreased. Recently, Nakagawa et al. (2011) encapsulated capsicum oleoresin in PCL, stabilized with gelatin through emulsion–diffusion method followed by freeze drying technique, and studied its dispersibility. The mean diameter of the nanocapsules was below 200 nm. It was also found that the prepared freeze-dried capsules had different dispersion characteristics at different positions in the dried bulk sample. This heterogeneity was dependent on the cooling program used during the processing. They suggested that the gel network formation of nanocapsule gelatin would be advantageous for producing excellent nanocapsule dispersion characteristics after drying. Likewise, Bejrapha et al. (2011) also produced capsicum oleoresin-loaded nanocapsules with PCL by the modified emulsion–diffusion method combined with freeze drying. Various freezing temperatures such as −40, −20, and −15 °C were applied to study the effects of cooling temperature on the properties of the capsicum oleoresin-loaded nanocapsules. The effects of excipients such as gelatin and κ-carrageenan on the stability of capsicum-loaded nanocapsules during freeze–thawing and freeze drying procedures were also studied. Through their results, it was clear that a relatively high freezing temperature (−15 °C) had an effect on the maintenance of nanocapsule size after freeze–thawing and freeze drying. Abdelwahed et al. (2006b) studied the freeze drying of PCL nanocapsules encapsulating miglyol 829 oil,

which was prepared by the emulsion–diffusion method and stabilized by polyvinyl alcohol (PVA). Different parameters have been tested throughout the freeze–thawing study, including PVA and PCL concentration, cooling rate, cryoprotectant concentrations (sucrose and polyvinyl pyrolidonne), nature of encapsulated oil, and nanocapsule purification. The effect of annealing on the nanocapsule stability and the sublimation rate has also been explored. They concluded that PCL nanocapsule could be freeze-dried without a cryoprotectant if the concentration of PVA stabilizer is sufficient (5 %). The type of cryoprotectants had practically negligible effects on the size and the rehydration of freeze-dried nanocapsules and the annealing process accelerated the sublimation with the conservation of nanocapsule size. Tiyaboonchai et al. (2007) loaded curcuminoids into solid lipid nanoparticles using microemulsion technique and freeze drying. At optimized process conditions, lyophilized curcuminoids-loaded nanoparticles showed spherical particles with a mean particle size of 450 nm and incorporation efficacy up to 70 %. It was found that a variation in the amount of ingredients such as lipid and emulsifier had profound effects on the curcuminoid loading capacity and size distribution. In vitro release studies reported a prolonged release of the curcuminoids (up to 12 h) and could maintain the physical and chemical stabilities during the storage period of 6 month. Zhang et al. (2009) encapsulated trehalose in a thermally responsive pluronic nanocapsule using freeze drying technique. The nanocapsule is capable of physically withholding trehalose with negligible release in hours for cellular uptake at 37 °C and its cytotoxicity is low.

Luo et al. (2011) encapsulated tocopherol in zein and zein/chitosan complex using freeze drying technique. The particle size of the complex varied from 200 to 800 nm. The encapsulation efficiency ranged from 77 to 87 %. The kinetic release profile of the tocopherol showed burst effect followed by slow release. Compared with zein, zein/chitosan complex provided better protection of tocopherol release against gastrointestinal conditions due to chitosan coatings. Zhao et al. (2011) produced conventional liposomes and polyethylene glycol (PEG)-coated vitamin E lyophilized proliposomes (PLP) by thin-film ultrasonic dispersion and lyophilization. The mean diameter and encapsulation efficiency for PEG-coated lyophilized proliposomes were 164 nm and 84 %, respectively. Vitamin E contained within PLP exhibited better stability compared to the conventional liposomes and the retention percentage of PLP was 90 % at 4 °C after 15 days of storage.

Freeze drying seems to be an efficient drying technique for stabilizing nanocapsules. It was capable of retaining the particle size in a nanometric range even after drying (below 400 nm and exceptionally few near to 800 nm), improving the stability of core compound against degradation, and exhibiting better encapsulation efficiency of about 70 %. Moreover, it seems to be an excellent drying technique for heat-sensitive food materials and bioactive components.

However, final freeze-dried nanoparticle characteristics depend on a suitable high-energy emulsification technique and other encapsulation techniques to break down the droplets into nano form. Moreover, it requires cryoprotectants such as sucrose, trehalose, and mannitol to conserve the particle size and also to avoid aggregation during freeze drying. The various freezing temperatures were also reported to affect the nanocapsule size. In most of the studies, polymers such as PCL and chitosan were used as a wall material.

Characterization of Nanoparticles

A complete and accurate characterization of nanoparticles is an essential part of understanding both the possible benefits as well as the potential toxicity of nanoparticles in biological systems (Royal Society 2004). Characterization of nanoparticles such as state of aggregation, dispersion, sorption, size, structure, and shape can be studied by using imaging techniques like TEM, scanning electron microscopy (SEM), high-resolution transmission electron microscopy, and atomic force microscopy (Mavrocordatos et al. 2004). SEM is capable of producing high-resolution images of a sample surface (Goldstein et al. 2003). Chromatography and related techniques such as size exclusion chromatography (SEC), capillary electrophoresis, and hydrodynamic chromatography (HDC) are used for the separation of nanoparticles. SEC allows the separation of particles in different solutions based on the charge and size distribution of the components. HDC separates particles based on their hydrodynamic radius (Tiede et al. 2008). Field flow fractionation (FFF) is a highly promising technique for the size separation of nanoparticles (Hassellov et al. 2007). FFF is able to fractionate particles in the range from 1 nm to 1 mm in Brownian mode. Centrifugation and filtration techniques such as ultracentrifugation, ultrafiltration, and nanofiltration are well-established tools for the preparative size fractionation of samples. Some spectroscopic and related techniques useful for nanoparticle characterization are static light scattering, dynamic light-scattering (DLS), neutron scattering, small-angle neutron scattering, nuclear magnetic resonance, and X-ray diffraction (XRD). DLS is particularly useful for sizing nanoparticles and determining their state of aggregation in suspensions (Tiede et al. 2008). XRD is used to determine the identity of crystalline solids based on their atomic structure (Luykx et al. 2008).

Problems and Safety Issues on Nanoencapsulation

The advancement of nanoencapsulation process paves the way for protection, controlled delivery, and enhanced bioavailabilty of bioactive substances. Apart from its

advancement, it has raised a number of safety, environmental, ethical, and regulatory issues, which stem from the lack of knowledge about the impacts of nano-sized materials on human health and the environment (EFSA 2009). The important issue in regard to nanoencapsulation was the biotransformation of nanoparticles after oral administration, which was less explored. Even the excretion of nanoparticles was less known (FAO 2009). In general, the impact of nanoparticles on the body (i.e., nanotoxicity) is influenced by factors such as particle size, mass, chemical composition, surface properties, and the aggregation of individual nano particles (Nel et al. 2006; Oberdorster et al. 2005). The unforeseen complexity of nano food materials in natural systems and the uncertainty in regard to hazard and exposure assessment can be answered by building on experiences with chemical risk assessment and toxicological assessment (Donofrio 2006). A European Food Safety Authority report suggested that current risk assessment methodologies for micro/macroscale chemicals need to be modified to deal with the risks associated with nanotechnologies (EFSA 2009). The existing toxicological and eco-toxicological methods for assessing nanoparticles are not sufficient. It was reported in a study from the USA that the toxicities of nanoparticles and large particles were similar when the dose was expressed in surface area (Monteiller et al. 2007). The Scientific Committee on Emerging and Newly Identified Health Risks reported that several dose metrics such as surface area and particle number may need to be explored in addition to mass (SCENIHR 2009).

There is ambiguity for the use of nanoparticles as approved food ingredients or additives in food or food contact materials. Authorities responsible for regulation have to make clear statements about their use and they should be regarded as novel products requiring evaluation and approval (Morris 2007). In 2006, FDA has formed an internal FDA nanotechnology task force for determining regulatory approaches for the nanomaterials (FDA 2006). Moreover, the current state of knowledge about the unique properties of engineered nanomaterials does not give exact inclusion or exclusion criteria for nano-specific risk evaluation. Therefore, it may be useful in the risk assessment to consider a breadth of potential properties that may include unique biological or physical behavior along with their toxicological evaluation (FAO 2009). The dispute on the benefits and risks of applying nanotechnology in food industry will last long. Moreover, until now, there has been no conclusive data about the undesirable effect of nanotechnology in food system on health. Therefore, it is wise to have a regulatory control as a proactive approach for protecting the public from potential adverse effects of nanotechnology until proven otherwise. Furthermore, an updated scientific evidence of nanotoxicity needs to be in close link with the newly innovative nano products for the development of updated regulations for nano foods to minimize the possible impacts of nanotechnology on health (Chau et al. 2007).

Conclusion

Nanotechnology has shown greater potential in improving the effectiveness of delivery of bioactive compounds to improve human health. Currently, various techniques of nanoencapsulation are gradually emerging with their own merits and demerits. Techniques such as emulsification, coacervation, inclusion complexation, nanoprecipitation solvent evaporation, and supercritical fluid technique are enduring techniques for nanoencapsulation of food ingredients. Moreover, solvent evaporation and nanoprecipitation remains to be unique techniques for encapsulation of lipophilic bioactive compounds. However, all the encapsulation techniques ultimately depend on suitable drying techniques to produce nanoencapsulates in powder form. At present, spray drying and freeze drying are widely used drying techniques involved in the nanoencapsulation process. However, freeze drying is more expensive and needs more processing time and spray drying needs some modifications for retaining the nanoparticle size. Hence, a special design of drying equipments is needed to produce nanoencapsulates in powder form. Furthermore, each encapsulation technique has some unique operating factors, which affect the final outcome of nanoencapsulates, and those factors need to be investigated and optimized. Most nanoencapsulates have shown excellent bioavailability and few encapsulates have reported good inhibitory effect against certain targeted diseases. However, currently, the potential risks of nanomaterials to human health are unknown and need to be explored and studied. Moreover, the regulatory issues on nanofoods are still being developed, and it is expected that national bodies will increase initiatives to control, administrate, and promote the proper development of nano-sized food-related products. It can be foreseen that nano-approach in the area of delivery of bioactive food components with substantiated health benefits will meet the challenges in reducing the risks of target diseases in a population.

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