

Chapter 2

Nanobiotechnology Methods to Incorporate Bioactive Compounds in Food Packaging

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Abstract Plastics have been successfully used as packaging materials, but increased environmental concerns lead to a growing interest on alternative biocompatible materials. The development of bionanocomposites for application in the food packaging sector is a major emergent fields in nano-research. Encapsulation can be done using procedures such as spray drying, spray cooling and chilling, fluidized bed coating, coacervation, liposome entrapment, co-crystallization, nano-emulsion, air suspension, freeze-drying, interfacial polymerization, molecular inclusion and sol-gel. Lipid-based nanostructures such as liposomes and solid-lipid nanoparticles are used for the delivery of bioactive molecules showing antimicrobial and antioxidant activities. Fat-soluble vitamins can be incorporated in nano-emulsions, which, added to polymeric nanoparticles, are suitable vehicles to phytochemicals like quercetin and curcumin. Nanofibers can also be used to incorporate bioactive substances in food packaging. This chapter presents methods for encapsulation of bioactive compounds into nanostructures, which can then be incorporated into packaging materials to improve food quality and safety.

Keywords Active packaging • Antimicrobial • Antioxidant • Bioactive molecules • Encapsulation • Liposomes • Nanocomposites • Nanoemulsion • Nanofibers • Nanostructures

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2.1 Introduction

A package can be defined as the material unit that functions as barrier between the contents and the exterior atmosphere. Packaging materials should provide containment, protection, convenience and communication, the four basic functions of traditional packaging, but also create appropriate conditions that are essential for obtaining a suitable shelf life and maintaining quality and safety during transportation and storage of products. The basic assets of packaging materials are suitable mechanical, optical, and thermal properties, but an adequate food package should also avoid gain or loss of moisture, prevent microbial contamination, avoid loss of nutrients and act as a barrier against permeation of water vapor, oxygen, carbon dioxide, and other volatile compounds such as flavors and taints (Rhim et al. 2013). The most innovative advances in packaging are associated with the development of biodegradable/biocompatible materials and “active” and “intelligent” packaging. Active packaging aims the shelf life extension and also the preservation or improvement of product quality, whereas the purpose of intelligent packaging is to provide indication and monitor the freshness of the product (Dainelli et al. 2008; Vanderroost et al. 2014).

Plastics are remarkable materials for packaging due to their low price, ease of processing and modeling, lightness and flexibility of thermal and mechanical properties. Food packaging represents the largest demanding request for plastics in the form of films, sheets, bottles, cups, tubs, trays, among others (Lagaron and Lopez-Rubio 2011; Rhim et al. 2013). The most frequently used polymers are petroleum-based materials like polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyethylene terephthalate (PET), and various grades of polyethylene (PE) (Marsh and Bugusu 2007). Although packaging can help to reduce organic waste by preserving foods, the substantial increase in the use of synthetic plastics raised several environmental concerns (Siracusa et al. 2008). Consequently, development and use of biodegradable and/or eco-friendly materials has received increasing attention. Unfortunately, biopolymers present relatively poor mechanical and barrier properties for packaging applications, which presently limit their industrial use. Since biopolymers are essentially hydrophilic, the improvement of moisture barrier characteristics becomes a special challenge. The functional properties of natural polymers have been improved by reinforcement of the biopolymer matrix with nanoparticles, resulting in a new class of materials called bionanocomposites (Lagaron and Lopez-Rubio 2011; Vanderroost et al. 2014).

These advances on bionanocomposites may be helpful for the development of eco-friendly active packaging. Active packaging is designed to intentionally incorporate compounds that would be released into or absorb substances from the packaged food or the food surrounding environment (Silvestre et al. 2011). An active compound can be incorporated inside the packaging material or onto its surface, in multilayer structures or in particular elements associated with the packaging such as sachets, labels or bottle caps (Dainelli et al. 2008; Vanderroost et al. 2014). Various applications of nanoparticles in the food industries are focused in (i) sensory

improvements (flavor/color enhancement, texture modification), (ii) increased absorption and targeted delivery of nutrients and bioactive compounds, (iii) stabilization of active ingredients such as nutraceuticals in food structures, (iv) packaging and product innovation to increase shelf life, (v) sensors to assess the safety of food, (vi) as an antimicrobial against food borne pathogenic bacteria (Ranjan et al. 2014).

All of the agricultural activities and the corresponding manufacturing ones generate considerable quantities of different types of by-products and waste. There is a worldwide concern regarding the use of industrial waste. Such waste is the part of raw material that is rejected during processing in the food industry. These residues are rich in nutrients as bioactive compounds; thus, they can be improved and incorporated into human diet or other industries processes (da Silva and Jorge 2014; Naziri et al. 2014).

Through encapsulation, bioactive compounds such as antimicrobials, antioxidants and enzymes can be protected against harsh processing conditions of package manufacture and may have improvement in controlled release behavior during storage (Sant'Anna et al. 2011; McClements 2014). Thus, the objective of this paper is to discuss different methods for encapsulation of bioactive compounds into nanostructures, which can be incorporated into packaging materials to improve food quality and safety.

2.2 Encapsulation of Bioactive Molecules

Figure 2.1 illustrates a survey of the last 10 years considering the keywords *encapsulation*, *bioactive* and *food* and shows an increasing trend with regard to the number of publications, of which 23 articles have already been published in the six first months of 2015.

Encapsulation is a useful tool to improve delivery of bioactive molecules (e.g. antioxidants, antimicrobials, minerals, vitamins, phytosterols, lutein, fatty acids, lycopene) and living cells, e.g. probiotics, into foods (Nedovic et al. 2011). The addition of bioactive ingredients or living cells should not cause undesirable effects on the sensory properties, color or flavor of food products. A number of encapsulation techniques can be used to produce nano or microparticulate systems, being emulsification, evaporation, spray-drying and coacervation the most extensively used (Champagne and Fustier 2007; Gómez-Mascaraque et al. 2015).

In general, encapsulation of active agents aims at: (i) stabilizing the material through the formation of enveloping wall; (ii) tuning the structure of the encapsulating material to promote the suitable leaching/leakage and to avoid the insertion/migration of unwanted components within the encapsulating device. The development of edible nano- or microencapsulation matrices has been envisaged as a plausible option to protect these biologically active compounds against adverse conditions. The classical systems developed in nano- or microencapsulation are based on reservoir or matricial particles (Fang and Bhandari 2010; Gómez-Mascaraque et al. 2015). These systems may constitute a physico-chemical barrier

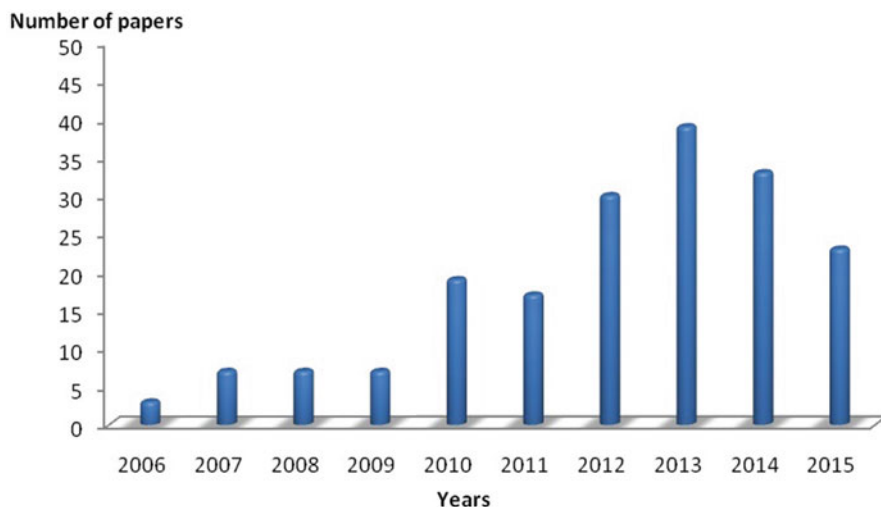


Fig. 2.1 Survey of the last 10 years considering the keywords *encapsulation*, *bioactive* and *food*. An increasing trend in publications can be observed from 2010. Values for 2015 correspond to the six initial months (Database: Scopus. Available at: www.scopus.com)

against prooxidant elements such as free radicals, oxygen or UV. Encapsulation may also represent a mean to improve biological efficiencies such as shelf life, control of delivery of active components, and could prevent the onset of side effects (Gonnet et al. 2010).

Many encapsulation procedures have been proposed but none of them can be considered as a universally applicable technique for a bioactive or food component. This is because individual food components have their own typical molecular structure. Encapsulation processes should lead to particles with a high encapsulation degree, good polydispersity index (PDI) and elevated shelf life (Gonnet et al. 2010; Fang and Bhandari 2010). Sophisticated shell materials and technologies have been developed and an extremely wide variety of functionalities can now be achieved through microencapsulation. In most cases, encapsulation refers to a technology in which the components are completely enveloped, covered and protected by a physical barrier, without any protrusion of the components. Also, encapsulation has been defined as a technology of packaging solids, liquids, or gaseous materials into small capsules that release their contents at controlled rates over prolonged periods and under specific conditions (Gouin 2004; Nedovic et al. 2011).

Most microcapsules are small spheres with diameters ranging from few micrometers to few millimeters. However many of these microcapsules bear little resemblance to those of simple spheres. In fact, both size and shape of formed microparticles depend on the materials and methods used to prepare them (Champagne and Fustier 2007; Gharsallaoui et al. 2007). As particle size affects texture, the addition of large particles is undesirable in most cases, explaining why encapsulation becomes crucial in the food sector. It must be stressed, however, that the food industry is severely

limited with respect to the compounds that can be used for encapsulation. Compatibility with the food components is not the only requirement that an encapsulation procedure has to meet. It also should have specific characteristics to withstand influences from the environment (de Vos et al. 2010).

The current encapsulation systems include different types of micro- or nanoparticles (capsules and spheres) produced from a wide range of wall materials (monomers and/or polymers) by a large number of different processes. These include spray drying, spray cooling and chilling, extrusion, fluidized bed coating, coacervation, liposome entrapment, centrifugal suspension separation, co-crystallization, nanoemulsion, air suspension, freeze-drying, interfacial polymerization, molecular inclusion, sol-gel, among other. However, many techniques used in the non-food sectors cannot be simply extended to food ingredients. Thus, encapsulation techniques can be performed considering the limited options of approved food-grade compounds (Gharsallaoui et al. 2007; Champagne and Fustier 2007; Fang and Bhandari 2010).

When encapsulation is aimed to prevent excessive degradation of a sensitive ingredient or to reduce flashing off volatile flavors, the cost-in-use must be lower than the non-encapsulated ingredient. However, if encapsulation provides the ingredient a special property, the cost-in-use can be slightly higher than the non-encapsulated ingredient. To protect food from microbial spoilage and prevent food borne outbreaks one possible approach is the application of encapsulated antimicrobial coatings on food contact materials or incorporation of antimicrobial compounds in food packaging to kill or inhibit the contaminating microorganisms and ensure the safety of the food during storage as well as distribution (Gouin 2004; Gharsallaoui et al. 2007).

Figure 2.2 illustrates in more detail the bioactive delivery characteristics of some of the more commonly used nanosystems, namely liposomes, nanocapsules, nanospheres and micelles or nanoemulsions. Bioactive compounds are encapsulated in different ways according to the synthesized frame: through hydrophobic and hydrophilic regions of the liposomes; the formation of a spherical wall for the nanocapsules; aggregation of the components within or on the surface of nanospheres; or stabilization liquid and solid agents or hydrophilic and hydrophobic within the micelles. Particle size is crucial in determining the particle's kinetics and biodistribution. Particle diameters of 10–100 nm accumulate more readily within cells than larger particles (Brandelli 2012; Brandelli and Taylor 2015).

In the following sections, some strategies for incorporating bioactive compounds into nanostructures are presented. Diverse nanostructures, such as nanoliposomes, polymeric nanoparticles, nanoemulsions, nanofibers and nanocomposite polymers are available for encapsulation of antimicrobial, antioxidant and other bioactive molecules.

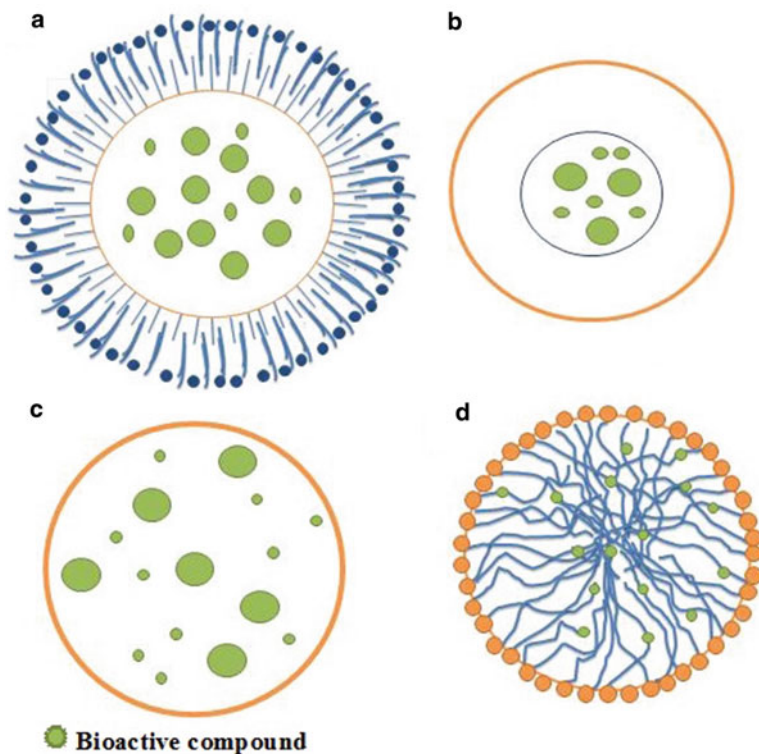


Fig. 2.2 Schematic representation of structural characteristics of some of the more commonly used nanosystems, namely: (a) liposomes; (b) nanocapsules; (c) nanospheres and (d) micelles or nanoemulsions

2.3 Lipid Nanoparticles

2.3.1 Liposomes

Liposomes are spherical structures in the colloidal system where phospholipids are dispersed in aqueous solution followed by forming the bilayer membrane of particles through self-assembling. These structures may be used for a wide variety of applications, including the entrapment and controlled release of drugs or nutraceuticals compounds and as model cells or membranes (Thompson et al. 2006; Tan et al. 2013).

The main methods for liposomes preparation have been reviewed (Patil and Jadhav 2014). The authors divided methods in eight major groups listed in Table 2.1. Several liposomal formulations are already in the market, while quite a few are still in the pipeline. Conventional techniques for liposome preparation and size reduction remain popular as these are simple to implement and do not require

Table 2.1 Methods for preparation of liposomes^a

Major methods	Specific techniques
(1) Conventional methods for preparing giant unilamellar vesicles	(A) Gentle hydration of a phospholipid film
	(B) Electro-formation
	(C) Coalescence of small vesicles
(2) Conventional methods for preparing multilamellar vesicles	(A) Hydration of a phospholipid film under hydrodynamic flow
	(B) Solvent spherule method
	(C) Hydration of proliposomes
(3) Conventional methods for preparing small and large unilamellar vesicles	(A) Reverse phase evaporation
	(B) Injection of organic solvent with dissolved phospholipids into an aqueous phase
	(C) Detergent dialysis
	(D) Reduction of size and lamellarity of multilamellar vesicles
(4) Microfluidic methods for liposome formation	(A) Micro hydrodynamic focusing
	(B) Microfluidic droplets for formation of giant vesicles
	(C) Pulsed jet flow microfluidics for giant vesicles
	(D) Thin film hydration in microtubes
(5) Supercritical fluids for liposome formation	
(6) Modified electroformation for preparation of giant vesicles	
(7) Size reduction of multilamellar vesicles and giant unilamellar vesicles	
(8) Other novel methods for preparing liposomes	(A) Freeze drying of double emulsions
	(B) Membrane contactor for preparation of liposomes
	(C) Hydration of phospholipids deposited on nanostructured materials
	(D) Liposome preparation by curvature-tuning

^aCompiled from Patil and Jadhav (2014)

sophisticated equipment. However, not all laboratory scale techniques are easy to scale-up for industrial liposome production (Patil and Jadhav 2014).

Liposomes have been used as carrier systems to deliver drugs and other bioactive substances in animals and humans. The main advantages of using liposomes as delivery systems are the potential reducing of undesirable secondary-effects and ensuring good cellular uptake, since liposomes resemble the structure of biomembranes (Chang et al. 2008). The low physical stability and high semi-permeability of membranes are challenging tasks for the incorporation of liposomes into foods. For an efficient application of liposomal carriers as food delivery systems, it is necessary to obtain deeper insights into the solubilization site, stability and impact of encapsulated compounds on bilayer membrane properties, such as fluidity,

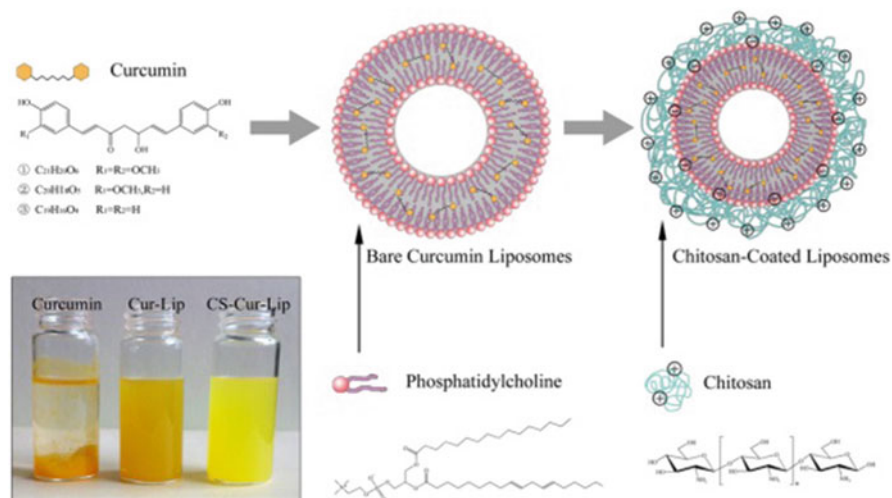


Fig. 2.3 Schematic representation of the production of liposomes incorporating curcumin and chitosan-coated liposomes. The inset photograph shows images of free curcumin, liposomes incorporating curcumin and liposomes incorporating curcumin coated with chitosan (Reprinted with permission from reference Liu et al. (2015)). *Cur-Lip* liposomes incorporating curcumin, *CS-Cur-Lip* liposomes incorporating curcumin coated with chitosan

micropolarity and molecule dynamics. The reactions occurring in phospholipid vesicles (liposomes) have received considerable attention because both stability and reactivity of the liposome are potentially regulated by keeping an intact molecular structure. However, the use of liposomal systems exhibits additional advantages amongst simple micelles and emulsions, when those systems are applied as delivery systems in food (Yoshimoto et al. 2007; Frenzel and Steffen-Heins 2015).

Liu et al. (2015) presented the structure stability of chitosan-coated curcumin liposome and the *in vitro* release of curcumin as shown in Fig. 2.3. The ethanol injection method was used to prepare curcumin liposome. The chitosan solution (1%) was prepared by dissolving chitosan in distilled water containing 0.1% acetic acid and then added drop-wisely into bare curcumin liposome suspension. The *in vitro* release study showed that the cumulative release rate was faster with temperature increase and chitosan could decrease the release rate.

Bioactive and nutraceutical substances have been incorporated into liposomes for delivery in food systems. Table 2.2 presents some examples of studies in recent years that have used liposomes as the primary method of encapsulation of bioactive compounds.

According to Marsanasco et al. (2015), liposomes provide a significant protective effect to thermolabile folic acid, remaining half of this vitamin active after pasteurization. The phosphatidylcholine based liposomes revealed a low peroxidative trend, and liposomes incorporating vitamins remained stable after pasteurization. Furthermore, adding vitamins to phosphatidylcholine:stearic acid and

Table 2.2 Examples of bioactive compound encapsulated by liposomes

Bioactive	Liposome	Reference
ω -3 and ω -6 fatty acids, folic acid, vitamin E and vitamin C	Phosphatidylcholine	Marsanasco et al. (2015)
Quercetin	Soy phospholipid and whey protein isolate	Frenzel and Steffen-Heins (2015)
Bioactive peptides	Phosphatidylcholine	Mosquera et al. (2014)
Pediocin	Soybean phosphatidylcholine	de Mello et al. (2013)
Tea polyphenol extract	Milk phospholipids	Gülseren et al. (2012)
Nisin	Phospholipid	Taylor et al. (2005) and Malheiros et al. (2010a)

phosphatidylcholine:calcium stearate systems favored liposomal aggregation and membrane rigidity, respectively.

Physical stability of liposomes benefits from whey protein isolate coating, as indicated by prolonged shelf life, elimination of osmotic effects in the presence of salts or sugars, and a lower sensitivity towards low pH values during simulated *in vitro* gastric digestion. The use of whey protein isolate as liposome coating material is a new approach in food science, while chitosan has already been investigated as a coating biopolymer for liposomes and is permitted for food applications (Frenzel and Steffen-Heins 2015).

In addition to physical characterization, the influence of encapsulated tea polyphenol on the viability of adenocarcinoma cell line HT-29 was investigated and compared to that of soy phospholipid liposomes (Gülseren et al. 2012). The average size of the milk phospholipid liposomes was lower than 200 nm and all liposomes were visibly stable over the experimental time. Both liposome types were negatively charged, which has not only implications for their colloidal stability, but could potentially influence their absorption. The work clearly demonstrated that tea polyphenols can be encapsulated in liposome preparations, and milk phospholipids represent an appropriate delivery vector. Although both vesicles showed high bioefficacy and delivery of polyphenols, there were differences in their behavior. It was concluded that milk phospholipids could represent an alternative source to soy phospholipids for liposome preparation and that the differences in composition and charge may allow further fine tune the cellular uptake of the bioactive.

A peptide fraction from sea bream scales hydrolysate showing both antioxidant and antihypertensive activity was encapsulated into phosphatidylcholine liposomes. The liposomes showed a mean diameter of 90 nm and the biological activities were maintained after encapsulation (Mosquera et al. 2014). A low-molecular-weight peptide fraction (lower than 1 kDa) showing antihypertensive activity, obtained from hydrolyzed squid tunics, was encapsulated into phosphatidylcholine liposomal nanovesicles. The peptide concentration affected the encapsulation efficiency and the stability of the resulting liposomes, and the peptide amount that provided maximum stability was established as 1.75 g/L. Liposomes obtained with this peptide concentration showed an average diameter of 70.3 nm and proved to be stable

in the pH range 3–7 at 4 °C. These liposomes were incorporated into fish gelatin without detriment of rheological properties and thermal stability of the gels, indicating an interesting characteristic for application in edible films and coatings (Mosquera et al. 2015).

Nisin shows a broad antimicrobial activity against Gram-positive bacteria and is approved for food use in many countries. However, its activity may be impaired in food systems due to undesirable interaction with some food components or enzyme inactivation (Cleveland et al. 2001). Liposomal systems containing nisin have been shown to be effective against food pathogens (Malheiros et al. 2010a). Some examples of application in food systems were described and phosphatidylcholine liposomes containing nisin were able to control *Listeria monocytogenes* in milk (Malheiros et al. 2010b) and cheese (Malheiros et al. 2012). Liposomes containing nisin were embedded into hydroxypropyl cellulose films, using a film forming solution containing both free and encapsulated nisin. This film inhibited the growth of *L. monocytogenes*, representing an innovative concept of biodegradable active films that may be useful for improving food safety (Imran et al. 2012).

Pediocin is a broad-spectrum bacteriocin from lactic acid bacteria that belongs to the Class II bacteriocins, a group known as “antilisterial” or “*Listeria*-active” peptides (Papagianni and Anastasiadou 2009). This bacteriocin was incorporated into liposomes and the effect of free and nanovesicle-encapsulated pediocin was evaluated against *Listeria* species. Encapsulation of pediocin into PC liposomes was carried out by the thin-film hydration method with bath-type sonicator. The efficiency of encapsulation was 80 % and low polydispersity index values were observed, indicating an adequate size distribution of liposomes. The stability of liposome vesicles showed potential for application in foods. The antimicrobial activity of the pediocin was preserved by encapsulation. Pediocin-loaded nanovesicles may provide an important tool for controlling spoilage and pathogenic organisms in food and may improve pediocin stability and efficacy in food matrices (de Mello et al. 2013).

2.3.2 Solid Lipid Nanoparticles

Chevalier and Bolzinger (2013) presented the classical description of well-established Pickering emulsions. Pickering emulsions are emulsions of any type, either oil-in-water (o/w), water-in-oil (w/o), or even multiple, stabilized by solid particles in the place of surfactants. Solid stabilizing particles are necessarily smaller than emulsion droplets. Solid particles of nanometric size (or sub-micron, about 100 nm) allow the stabilization of droplets as small as few micrometers diameter; stabilization of larger droplets is possible as well. The solid particles adsorbed at the oil–water interface stabilize the droplets in place of the surfactant molecules. Figure 2.4 shows the structure of Pickering emulsions in comparison to the classical structure of emulsions.

Solid lipid nanoparticles are interesting systems for delivery of hydrophobic bioactive compounds that can be prepared using food grade lipids. Qian et al. (2013)

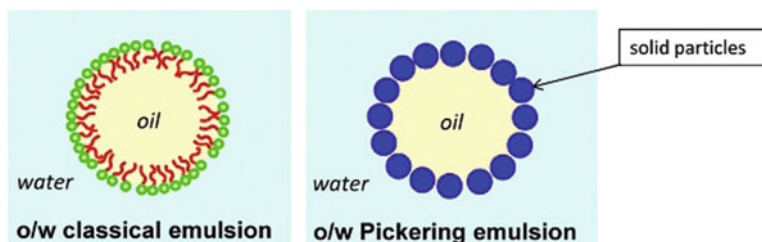


Fig. 2.4 Drawing of a classical (surfactant-based) emulsion and a Pickering emulsion (Reprinted with permission from reference Chevalier and Bolzinger (2013))

aimed to produce solid lipid nanoparticles that had a more disordered crystal structure by mixing two lipid phases with different melting characteristics together: cocoa butter and hydrogenated palm oil. They hypothesized that by blending these two types of lipids it would be able to produce physically stable solid lipid nanoparticles with improved ability to inhibit the chemical degradation of an encapsulated lipophilic component (β -carotene). Solid lipid nanoparticles and liquid lipid nanoparticles were prepared using a hot high-pressure homogenization method. Liquid lipid nanoparticles were produced by directly cooling the hot nanoemulsions since this temperature is above the crystallization temperature of the mixed lipid systems. Solid lipid nanoparticles were produced by cooling the hot nanoemulsions since this is below the crystallization temperature of the mixed lipid phase, and then heating the sample back since this is below the melting temperature of the mixed lipid phase. Liquid lipid nanoparticles had better stability to droplet aggregation and β -carotene degradation than solid lipid nanoparticles after storage for 8 days: the droplet diameter increased around 35 % for solid lipid nanoparticles and around 1 % for liquid lipid nanoparticles. The high stability of this system can be attributed to the ability of the non-ionic surfactant used to generate a strong steric repulsion between the liquid lipid nanoparticles and also to the ability of the fat crystals within the lipid nanoparticles to promote partial coalescence and expulsion of carotenoids to the particle exterior.

Helgason et al. (2009) examined the effect of surfactant concentration on stability and crystal structure of suspensions of solid lipid nanoparticles composed of a model triacylglycerol (tripalmitin) stabilized by a non-ionic surfactant (Tween 20). Emulsion droplets after homogenization had a mean particle diameter of 134 nm and a polydispersity index of 0.08. Solid lipid nanoparticle dispersions rapidly gelled due to aggregation of particles driven by hydrophobic attraction between insufficiently covered lipid crystal surfaces. The Tween 20 concentration in the aqueous phase decreased after tripalmitin crystallization suggesting additional surfactant adsorption onto solid surfaces. At higher Tween 20 concentrations, solid lipid nanoparticles have increasingly complex crystal structures. The results suggested that surfactant coverage at the interface might influence crystal structure and stability of solid lipid nanoparticles via surface-mediated crystal growth.

2.4 Micro and Nanoemulsions

Emulsions can be in the form of oil drops in water or water drops in oil, but in both cases the drops need to be stabilized to prevent them from re-coalescing. Emulsion droplet stabilization is often achieved by the addition of amphiphilic molecules such as surfactants or emulsifiers, which act by decreasing the interfacial tension between the phases, increasing the steric hindrances and/or the electrostatic repulsion between the droplets (Rayner et al. 2014). One of the main advantages of nanoemulsions is that considerably less surfactant is required to their formation when compared with conventional emulsions. Food-grade nanoemulsions can be prepared by high-energy methods, such as high-pressure homogenization or sonication, or low-energy methods, such as phase inversion temperature, spontaneous emulsification, or emulsion phase inversion. Such nanoemulsions have good stability against droplet aggregation because the range of attractive forces acting between the droplets decreases with decreasing particle size, whilst the steric repulsion is less dependent on particle size (Gulotta et al. 2014; Ranjan et al. 2014). Table 2.3 presents examples of recent studies that have used micro or nanoemulsion as the primary method for encapsulation of bioactive compounds.

Zeeb et al. (2015) studied the stability of protein-based nanoemulsions fabricated using a high-pressure emulsification approach and the influence of small chain alcohols on their formation. The addition of alcohol, regardless of type, had a major impact on the final droplet size. Presence of 5–10% alcohol led to the formation of smaller oil droplets compared to the equivalent alcohol-free emulsions - a phenomena that might be attributed to changes in the interfacial tension. The mean particle diameter decreased with increasing alcohol concentrations from 0 to 10%, but extensive droplet aggregation occurred at higher levels. This phenomenon was attributed to enhanced protein–protein interactions between the adsorbed emulsifier

Table 2.3 Examples of bioactive compound encapsulated by micro or nanoemulsion

Bioactive	Micro/nanoemulsion	Reference
None	(1) Caseinate; (2) Fish gelatin; and (3) Whey protein isolate. With or without alcohol	Zeeb et al. (2015)
Vitamin D3 and ω -3 fatty acid	Flaxseed oil stabilized with calcium caseinate with or without lecithin	Mehmood (2015)
Essential oils	Sodium alginate	Salvia-Trujillo et al. (2015)
Quercitin	Blend of lipids: glyceryl monostearate, glycerol monolaurate and caprylic capric triglyceride	Ni et al. (2015)
Quercitin	Ethyl acetate-water	Gonçalves et al. (2015)
Curcumin	Whey protein concentrate	Sari et al. (2015)
Vitamin E acetate	Edible mustard oil and Tween 80	Dasgupta et al. (2016)
β -carotene	Anhydrous milk fat	Zhang et al. (2013)

molecules in the presence of alcohol leading to droplet flocculation. This study demonstrated that the addition of alcohol might be a useful tool for producing protein-stabilized nanoemulsions suitable as delivery systems for lipophilic bioactives, and the addition of short-chain alcohols to such emulsions prior to homogenization can reduce the droplet size, thereby facilitating nanoemulsion formation.

Nanoemulsions were developed for delivery of vitamin D3 and ω -3 fatty acid in cheddar cheese. Recovery level of vitamin D3 in cheese was 91 and 84% in the presence or absence of lecithin, respectively. The fortification of foods with encapsulated vitamins and other bioactive molecules may have a positive impact on the composition, yield and chemical stability of the resultant product (Mehmood 2015). Vitamin E acetate nanoemulsion was produced using mustard oil and Tween 80 and flocculation was not observed in 15 days. This nanoemulsion showed improved antioxidant and antimicrobial bioactivities and could be potentially applied to increase the shelf life of fruit juices (Dasgupta et al. 2016).

The formation of transparent dispersions of nanostructured lipid carriers using the phase-inversion temperature method was studied by Zhang et al. (2013). To form a coarse emulsion, anhydrous milk fat was heated for complete melting, followed by mixing with warm aqueous phase under agitation. β -carotene was dissolved in warm anhydrous milk fat before emulsification. The degradation of β -carotene encapsulated in nanostructured lipid carriers was significantly reduced when compared to its encapsulation in the soybean-oil-based nanoemulsion, which is associated to the lower mobility in the amorphous lipid core. β -Carotene degraded much faster in melted anhydrous milk fat (79.0%) than in soybean oil (28.9%) at 65 °C after 16 days, suggesting soybean oil having better antioxidant properties. At 21 °C, β -carotene did not show obvious degradation in both lipids during storage. The degradation tests in bulk lipids support the conclusion that enhanced stability of β -carotene in nanostructured lipid carriers is due to the amorphous lipid structures of anhydrous milk fat core.

Microfluidized nanoemulsions incorporating essential oils were characterized in terms of droplet size, size distribution, viscosity and color, besides in vitro antimicrobial activity against *Escherichia coli* in comparison with conventional emulsions. Further processing of blends by microfluidization after a previous high shear homogenization rendered nanoemulsions with a reduced droplet size. Moreover, strong differences between emulsion droplet size, size distribution and viscosity of coarse emulsions were observed, depending on the essential oil used in the formulation. The ζ -potential of nanoemulsions indicated a strong electrostatic repulsion of the dispersed oil droplets in the aqueous phase. Emulsions and nanoemulsions containing lemongrass, clove, thyme or palmarosa essential oils showed the strongest antimicrobial activity (Salvia-Trujillo et al. 2015). Nanoemulsions of clove bud and oregano essential oils were incorporated into water-soluble methylcellulose films and their antimicrobial activity was tested in sliced bread. Antimicrobial films containing emulsified essential oils caused a reduction in the counts of yeasts and molds in sliced bread during 15 days (Otoni et al. 2014).

Quercetin is a polyphenolic compound that has been associated with multiple biological effects, including antioxidant, radical scavenging and antimicrobial

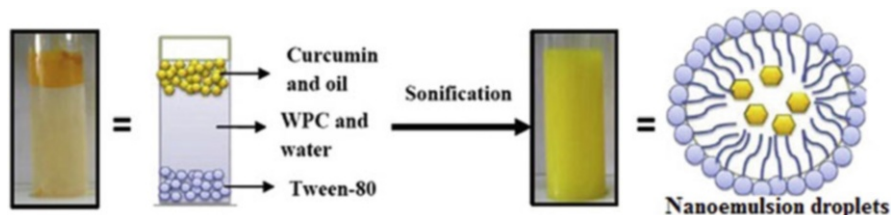


Fig. 2.5 Methodology for preparation of curcumin encapsulated nanoemulsion. A suspension of curcumin dispersed in carrier oil was mixed with an aqueous solution of whey protein concentrate, and sonicated in presence of the surfactant Tween 80, resulting in homogeneous nanoemulsion (Reprinted with permission from reference Sari et al. (2015)). WPC whey protein concentrate

activities. An *in vitro* digestion model consisting of mouth, gastric and small intestinal phases was used to determine the potential biological fate of the quercetin-nanostructured lipid carrier. The *in vitro* digestion of free or encapsulated quercetin was performed under stirring and maintained at 37 °C throughout the digestion process. The model indicated that bioaccessibility of quercetin under simulated small intestine conditions was improved by encapsulation. The formulated quercetin-nanostructured lipid carrier showed high encapsulation efficiency (93.5 %) and the stability analysis indicated that the nanocarriers remained stable during 60 days of storage. The possible reason for higher encapsulation efficiency of quercetin in nanostructured lipid carriers could be the hydrophobic character of this polyphenol, leading to higher drug partitioning into the lipid matrix of nanoparticle and lesser into the external aqueous phase (Ni et al. 2015).

Water-soluble formulations of quercetin through pressurized ethyl acetate-water emulsion technique have been also developed. The dissolution is achieved by mixing a quercetin suspension in pressurized ethyl acetate at ambient temperature, with a stream of preheated and pressurized ethyl acetate, using a T-mixer. Formulations of quercetin carried out with lecithin yielded the best results with encapsulation efficiencies around 76 % and a micellar particle size in the range of nanometers. The aqueous suspensions of emulsified quercetin presented concentrations of this flavonoid up to 630 ppm, being 315-fold higher soluble when compared with pure quercetin in water (Gonçalves et al. 2015).

The preparation, physicochemical characterization and *in vitro* digestion kinetics of curcumin nanoemulsion was studied by Sari et al. (2015). The coarse emulsion was prepared using magnetic stirring at room temperature for different time intervals, and fine emulsion was prepared by ultrasound processing the coarse emulsion as shown in Fig. 2.5.

Curcumin, being highly unstable and hydrophobic, is difficult to incorporate in aqueous food systems. For this reason, curcumin was encapsulated inside carrier oil in an emulsion form. An encapsulation efficiency of 90 % was achieved, and the more stable formulation maintained the initial properties for 27 days storage at 25 °C. *In vitro* release kinetics of curcumin from nanoemulsion by simulated gastrointestinal studies showed that the curcumin nanoemulsion was relatively resistant

to pepsin digestion, but pancreatin causes release of curcumin from nanoemulsion. The digestion of encapsulated bioactives in the gastrointestinal tract is a complex process and its impact on the release of bioactive component plays a major role in the uptake, distribution as well as bioavailability of the component (Sari et al. 2015).

Similarly, it can be assumed that addition of nanoemulsified bioactives like quercetin and curcumin to films and coatings should improve stability and controlled release, representing a beneficial to the food packaged in these systems.

2.5 Polymeric Nanoparticles

Molecules composed of regions, typically one hydrophobic and another hydrophilic monomer, with opposite affinities for an aqueous solvent can form polymeric nanoparticles. Protein-based nano-encapsulated materials are particularly interesting because they are relatively easy to prepare and can form complexes with polysaccharides, lipids, or other biopolymers. They are called nanocapsules when used as vehicles for delivery of essential nutrients or pharmaceuticals. There are six classical methods for the preparation of nanocapsules: nanoprecipitation, emulsion-diffusion, double emulsification, emulsion-coacervation, polymer-coating and layer-by-layer. Polymeric nanoparticles are named nanocapsules when they contain a polymeric wall composed of non-ionic surfactants, macromolecules, phospholipids and an oil core. These are prepared mostly by two techniques: the interfacial polymerization and interfacial nano-deposition. A wide variety of nutrients can also be incorporated (Kothamasu et al. 2012; Ranjan et al. 2014; Dasgupta et al. 2015).

Although the use of polymeric nanoparticles for delivery of bioactive molecules has been described (Zhang et al. 2010), the focus of this section is mainly on the nanoparticles developed from biopolymers. Diverse examples of nanoparticles based on protein and/or polysaccharides with potential food applications are discussed below.

Table 2.4 illustrates some studies in recent years that have used polymeric nanoparticles as the primary method of encapsulation of bioactive compounds.

Nanoparticles of whey protein isolate and beet pectin were prepared for anthocyanin encapsulation. Biopolymers were assembled using a combination of two physicochemical phenomena: thermal treatment of protein-polysaccharide mixtures to induce thermal denaturation and aggregation of the globular protein molecules as nanoparticles and pH adjustment to promote coating of protein nanoparticles by oppositely charged polysaccharide molecules. The biopolymer nanoparticles were loaded with an anthocyanin-rich extract. This process led to the formation of relatively small anionic biopolymer nanoparticles (200 nm) at pH 4. A higher loading efficiency was observed when anthocyanin was added before heating the whey protein isolate-beet protein solution, which was attributed to increased protein-polyphenol interactions. This study has shown that biopolymer particles can be produced by thermal processing and electrostatic complexation of whey protein-pectin mixtures. However, the biopolymer particles were only physically stable over

Table 2.4 Examples of bioactive compound encapsulated by polymeric material.

Bioactive	Polymeric material	Reference
Anthocyanin	Whey protein isolate and beet pectin	Arroyo-Maya and McClements (2015)
–	Zein and alginate	Hu and McClements (2015)
Chitosan/fucoidan	Polystyrene	Pinheiro et al. (2015)
Trimethylsilila (TMCS)	Silica aerogel	Oh et al. (2015)
Curcumin	k-carrageenan	Xu et al. (2014)
Quercetin	Chitosan/lecithin	Souza et al. (2014)
Linoleic acid	Chitosan/ β -lactoglobulin	Ha et al. (2013)
Curcumin	Casein	Pan et al. (2013)
–	Gelatin and shellac	Patel et al. (2013)
Lipase	Silica shell	Macario et al. (2013)
Quercetin	Polyvinyl alcohol and Eudragit®	Wu et al. (2008)

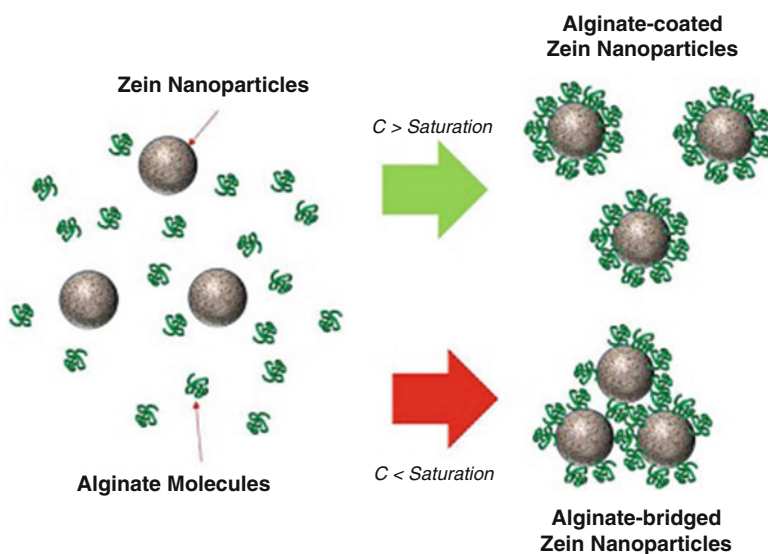


Fig. 2.6 Proposed mechanism of sodium alginate stabilization of zein nanoparticles. The nanoparticles had a core diameter of about 80 nm and a shell thickness of about 40 nm. The amount of alginate required to saturate the surfaces of zein nanoparticles was 2.0 mg/m². Alginate-bridged zein nanoparticles are formed with lower alginate concentration (Reprinted with permission from reference Hu and McClements (2015))

a limited range of pH conditions (pH \approx 3.8 to 4.2), which was attributed to changes in electrostatic, hydrophobic, bridging, and depletion interactions at lower and higher pH values (Arroyo-Maya and McClements 2015).

Protein-polysaccharide nanoparticles from zein and alginate were prepared using a combined antisolvent precipitation/electrostatic deposition method (Fig. 2.6). In addition, the influence of pH, ionic strength, and heating on the stability of these

nanoparticles was investigated to assess their potential for commercial applications. Zein nanoparticles were highly susceptible to aggregation when they were prepared in the absence of non-ionic surfactant (Tween 80), which can be attributed to hydrophobic attraction between non-polar patches on zein surfaces. Their stability was greatly improved in the presence of Tween 80 since the non-polar tails of the surfactant molecules adsorb to these non-polar patches, while the polar head groups generate a steric repulsion. The yield of the zein-alginate particles was 95 %, which mean that the majority of the zein added to the water phase formed nanoparticles, also exhibiting superior storage stabilities during room temperature and refrigerator storage. These core/shell biopolymer nanoparticles have potential to be used as nano-delivery systems for bioactive molecules in food and pharmaceutical formulations (Hu and McClements 2015).

Fucoidan is mainly composed of fucose and uronic acids, whereas chitosan is mainly composed by D-glucosamine and is known by its antimicrobial activity. Layer-by-layer deposition of chitosan/fucoidan on a sacrificial template allows the production of biodegradable hollow nanocapsules with potential antioxidant and antimicrobial activity and with great potential to act as a controlled delivery system for bioactive compounds. The resulting nanocapsules also showed a high antioxidant activity at a concentration of 1 mg/mL fucoidan. Thus, chitosan/fucoidan nanocapsules are a promising delivery system for water soluble bioactive compounds, showing a great potential for application in food and pharmaceutical industries (Pinheiro et al. 2015).

Encapsulation efficiency of quercetin into chitosan/lecithin nanoparticles presented values higher than 95 % for all tested concentrations. Moreover, encapsulated quercetin showed improved antioxidant properties. Nanoparticles presented a spherical morphology and stability studies showed that nanoparticles are stable to temperatures ranging between 5 and 70 °C and a pH variation from 3.3 to 5.0. The results suggest that quercetin-loaded chitosan/lecithin nanoparticles can be used in the manufacture of functional foods (Souza et al. 2014). Other authors also describe the development of nanoparticulate systems for encapsulation of quercetin, which has been used as a model molecule for encapsulation studies. Ha et al. (2013) produced linoleic acid-modified chitosan/ β -lactoglobulin nanoparticles by the modified ionic gelation method. Spherical shape nanoparticles in the range of 170 and 350 nm were successfully formed, and encapsulation efficiency of quercetin was enhanced with increased amount of linoleic acid and decreased temperature. A novel quercetin nanoparticle system was prepared by Wu et al. (2008) with a simple nanoprecipitation technology with Eudragit® and polyvinyl alcohol as carriers. The utilization of a weight ratio quercetin: Eudragit:polyvinyl alcohol at 1:10:10 resulted an encapsulation efficiency were over 99 %. In addition, the antioxidant activity of the encapsulated quercetin was more effective than pure quercetin on 2,2-diphenyl-1-picrylhydrazyl scavenging, superoxide anion scavenging, and lipid peroxidation assays. This study also established that the release mechanisms of quercetin were attributed to the reduction of drug particle size, formation of high-energy amorphous state, and intermolecular hydrogen bonding.

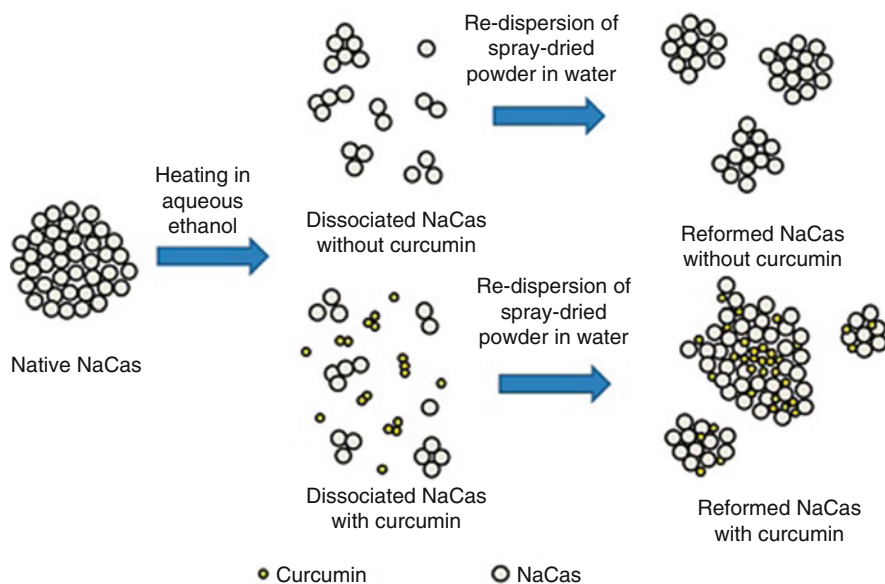


Fig. 2.7 Schematic mechanism of the structural changes of sodium caseinate subjected to heating in aqueous ethanol and spray-drying for encapsulation of curcumin. The increased nanoparticle dimension, together with fluorescence and FTIR spectroscopy results, suggested that curcumin was entrapped in the nanoparticle core through hydrophobic interactions (Reprinted with permission from reference Pan et al. (2013). NaCas, sodium caseinate)

The characteristics of curcumin encapsulation and protection in a k-carrageenan and lysozyme system have been studied. The results indicated that the spherical shaped k-carrageenan/lysozyme complex with 1 μm diameter was spontaneously formed by one spot incubation. The encapsulation efficiency of curcumin by the k-carrageenan/lysozyme complex (2:1 ratio) were 71%. The results demonstrated that the protein/polysaccharide complex has great potential to deliver sensitive amphiphilic bioactive compounds, protecting them against unfriendly environment (Xu et al. 2014).

Encapsulation of curcumin was also studied by spray-drying dispersions with casein according to the diagram in Fig. 2.7. The spray-dried powder contained 16.7% (dry basis) curcumin and the encapsulation efficiency was about 83%. The encapsulation caused the loss of crystallinity of curcumin because it was entrapped in the nanoparticle core through hydrophobic interactions. Free curcumin has a potentially high antioxidant activity because its phenolic -OH groups play a major role in the oxidation reactions. However, pristine curcumin has a low antioxidant activity when dissolved in 95% ethanol followed by dilution in water, because the low solubility limits the amount of dissolved curcumin molecules to react with free radicals in the aqueous phase. The curcumin encapsulated in casein nanoparticles had higher biological activity, as assessed by antioxidant and cell proliferation assays, than pristine curcumin, likely due to the improved dispersibility (Pan et al.

2013). This simple approach may be applied to encapsulate various lipophilic bioactive compounds.

Patel et al. (2013) successfully demonstrated the generation of novel microcapsules from two natural products gelatin and shellac, both of which are edible and approved by the Food and Drug Administration. Shellac, a natural biodegradable resin from insect origin (*Kerria lacca*), is a complex mixture of polar and non-polar components consisting of polyhydroxy polycarboxylic esters, lactones and anhydrides with the main acid components being aleuritic and terpenic acids. The all-natural microcapsules were generated using a simple extrusion method wherein the gelatin-shellac was dropped in acidic medium resulting in an instantaneous generation of spherical microcapsules that retained their shape on drying. The formation of the microcapsules was basically due to the strong interactions between two oppositely charged polymers and the instant precipitation of acid-resistant shellac.

Macario et al. (2013) reported the synthesis of organic–inorganic nanoparticles with spherical morphology where lipase was encapsulated as active compound. These nanospheres are composed of a purely organic internal liposomal phase in which the bioactive molecule (lipase) is encapsulated in a microaqueous environment. The liposomal phase is covered with porous inorganic silica shell. The synthesis of hybrid nanospheres occurs in two stages: preparation of liposome nanospheres encapsulating the enzyme and formation of an inorganic porous silica shell around the organic nanospheres. Maintaining the ratio between liposome and silica equal to 1, the highest enzyme immobilization efficiency (93–95 %) was achieved. Silica can stabilize the internal liposomal phase and, consequently, isolate and protect the bioactive molecules. The tests showed excellent catalytic performance, near to those of free lipase. This suggests that the enzyme has been immobilized in its active form and the silica shell protects the organic phase, because its activity is preserved after the second reaction cycle. This fact may be associated to the higher density of the external microporous silica shell, which could avoid the decomposition of the enzyme after successive catalytic runs.

Gram-negative *Salmonella typhimurium* LT2 and *Salmonella typhimurium* 14082 s and Gram-positive *Listeria innocua* NADC 2841 were utilized by Oh et al. (2015) in order to study the interactions of the developed food-contact surfaces with bacteria through dip inoculation. Silica (SiO₂) aerogel was synthesized by the sol–gel polymerization of tetraethylorthosilicate via hydrolysis and condensation reaction using ammonium hydroxide as catalyst. The reaction was allowed to take place for 24 h and the silica aerogel formed was dried using supercritical carbon dioxide. This resulted in hydrophilic silica aerogel, which was submerged in 6 % trimethylsilyl chloride solution to functionalize silica surfaces with trimethylsilyl chloride. Figure 2.8 illustrates the change of hydrophilic to hydrophobic behavior of silica aerogel. Compared with the negative control, the positive control and silica aerogel led to decreased counts of *salmonellae* by 1.2 ± 0.1 and 3.1 ± 0.1 log units, corresponding to 93.2 and 99.9 % reduction, respectively. The log reductions in the number of *L. innocua* were 1.3 ± 0.0 and 3.0 ± 0.0 for the positive control and silica aerogel, which represents a reduction of 94.8 % and 99.9 %, respectively. Overall, bacterial anti-adhesion property as well as other distinctive properties such as

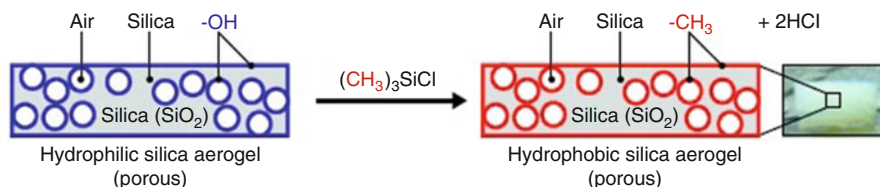


Fig. 2.8 Schematic of representation of the modification of hydrophilic to hydrophobic character of silica (SiO₂) aerogel via methylation reaction using trimethylsilyl chloride (Adapted with permission from reference Oh et al. (2015))

superior thermal insulation and ultra-lightweight make hydrophobically-modified silica aerogel an attractive candidate as a novel food-contact surface.

2.6 Nanofibers

Recently, electrospinning has received great attention in functional food and active food packaging systems. This simple technique allows the production of fibrous polymeric unwoven membranes formed by polymer fibers with nanometric diameters. Electrospinning process takes place at ambient conditions, and electrospun fibers are more suitable to encapsulate thermally labile substances than fibers prepared by conventional melt spinning or films produced by extrusion process or other encapsulation methods (Wei 2012). Figure 2.9 illustrates the electrospinning process and drug loading on nanofiber surface. Bioactive compounds can be loaded on the surface of nanofibers by simple physical adsorption or adsorption to surface functionalized nanofibers (Brandelli 2012; Mascheroni et al. 2013). Table 2.5 presents some recent studies that have used nanofibers as the primary method of encapsulation of bioactive compounds.

Aceituno-Medina et al. (2013) evaluated the feasibility of producing electrospun fibers from different amaranth protein isolate:pullulan blends and the influence of Tween 80 (non-ionic surfactant) on the morphology and molecular organization of the electro-deposited material. Addition of Tween 80 significantly improved the fiber morphology for the blends with greater protein content, resulting in defect-free smooth electrospun fibers for the 70:30 and 80:20 amaranth protein isolate:pullulan compositions. Presence of pullulan in the blends resulted in increased viscosity and lower conductivity of the solutions, related to a better chain entanglement and decrease in the polyelectrolyte protein character, respectively, both factors needed for fiber formation. The solutions were stable, which could be attributed to the formation of protein-polysaccharide soluble complexes through the formation of hydrogen bonds (C=O···HO, OH···OH, HO···HN), hydrophobic interactions, and/or ionic bonds.

Aceituno-Medina et al. (2015a) investigated the potential of amaranth-based structures for encapsulation and protection of two potent antioxidant compounds,

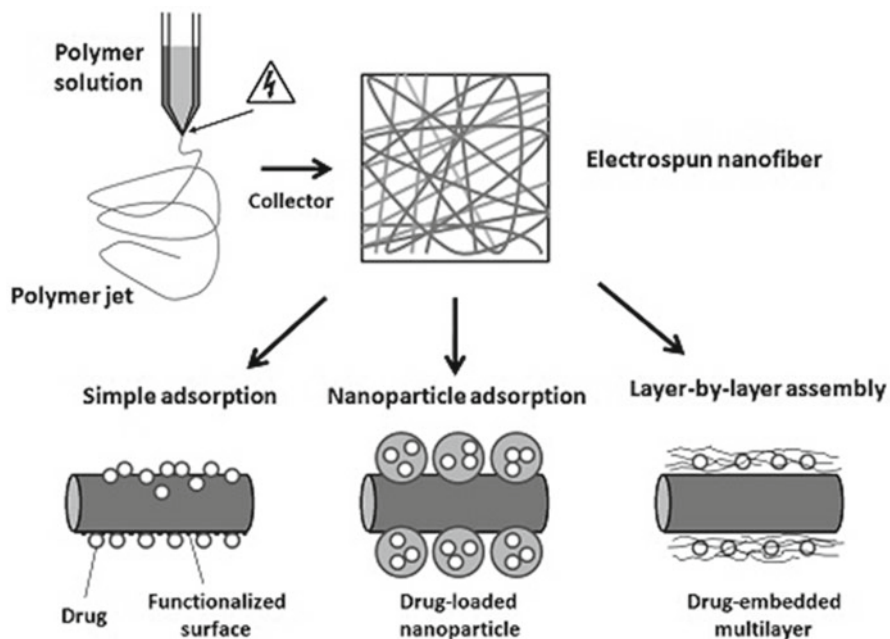


Fig. 2.9 Schematic representation of electrospinning and methods of drug loading on the surface of electrospun nanofibers. The bioactive molecules can be loaded to the nanofibers by simple physical adsorption, by assembly of nanoparticles on the nanofiber surface, multilayer assembly, or through chemical immobilization (Reprinted with permission from reference Brandelli (2012))

Table 2.5 Examples of bioactive compound encapsulated with nanofibers

Bioactive	Nanofiber	Reference
Folic acid	Amaranth protein isolate and pullulan	Aceituno-Medina et al. (2015a)
Quercetin and ferulic acid	Amaranth protein isolate and pullulan	Aceituno-Medina et al. (2015b)
Plantaricin 423	Polyethylene oxide and poly(lactic acid)	Heunis et al. (2011)
Nisin	Polyethylene oxide and poly(lactic acid)	Heunis et al. (2013)

quercetin and ferulic acid, to be incorporated into functional foods. In order to develop the electrospun fibers for encapsulation of the bioactives, 50:50 and 80:20 amaranth protein isolate:pullulan blends with the surfactant Tween 80 were prepared using 95% formic acid as solvent. Smooth ultrathin electrospun fibers were obtained in which the antioxidants were homogeneously distributed. Thermal analysis showed that the degradation temperature within the nanofibers is still far from the usual temperatures used for food processing. Smooth, defect-free structures were obtained, which contained the antioxidants evenly distributed along the fibers.

Efficiency obtained for quercetin was higher, but it should be considered that the concentration of bioactive in this case was half the concentration of ferulic acid used. Therefore, the developed electrospun structures show promissory results in terms of bioactive protection for application in functional foods.

Same authors also investigated the potential of amaranth-based structures for encapsulation and photoprotection of folic acid for food fortification (Aceituno-Medina et al. 2015b). In order to develop the electrospun fibers for encapsulation of folic acid, a blend of 80:20 amaranth protein isolate:pullulan with the surfactant Tween 80 was prepared. Addition of 100 mg of folic acid per g of biopolymer to the polymeric solution used for electrospinning resulted in increased apparent viscosity and, thus, in thicker electrospun fibers. Folic acid was encapsulated through electrospinning in amaranth protein isolate:pullulan ultrathin fibers with encapsulation efficiency higher than 95%. Even though no specific chemical interactions were shown to be established between the vitamin and the matrix materials. Therefore, the API:pullulan structures have demonstrated a great potential for encapsulation and protection of photosensitive bioactives for food-related applications.

Nanofibers have been used as a delivery platform for bacteriocins as well. A gelatin nanofiber incorporating nisin produced by electrostatic spinning inhibited *Lactobacillus plantarum*, *S. aureus* and *L. monocytogenes*. Although the nanofiber mats were easily broken and dissolved in water, cross-linking with glutaraldehyde strengthen the mat. This antimicrobial material was stored for up to 5 months at 25 °C, maintaining the antibacterial activity after this time (Dheraprasart et al. 2009). Another antimicrobial nanofiber was generated by electrospinning nisin (Nisaplin) into polyethylene oxide and poly(lactic acid) (50:50) blend nanofibers. Active nisin diffused from the nanofiber wound dressings for at least 4 days *in vitro*, as shown by consecutive transfers onto plates seeded with strains of methicillin-resistant *S. aureus* Heunis et al. (2013). Same authors successfully used this same polymer blend to incorporate other antimicrobial peptides, namely plantaricin 423 and bacteriocin ST4SA into nanofiber mats Heunis et al. (2011).

Nanofibers can be very interesting nanostructures for developing functional high-performance packaging materials. The ability to produce non-woven mats composed of nanofibers with diameters below 100 nm from biopolymer solutions can be used to produce food packaging materials with superior mechanical properties and very large specific surface area (Kriegel et al. 2008). These nanofibers based on biopolymers can be loaded with large amounts of bioactive compounds, such as antimicrobials, antioxidants, enzymes, and reinforcing materials resulting in eco-friendly active packaging. For instance, starch-chitosan hybrid films are entirely green since they are produced from renewable and fully degradable biopolymers. Addition of chitosan in packaging can enable to increase the food shelf life due to its antimicrobial activity, and incorporation of cellulose nanofiber into the film matrix caused an increase in tensile strength of starch-chitosan films. In addition, antimicrobial analysis showed that the addition of cellulose nanofiber could increase the effect towards Gram positive bacteria (Salehudin et al. 2014).

2.7 Gels and Films

An edible coating or film has been defined as a thin, continuous layer of edible material formed or placed on or between foods or food components. Furthermore, acting as protective barriers, edible films can be used as carriers of bioactive compounds, thus enhancing the functional properties of the food product by promoting health benefits. Edible films and coatings generally exhibit lower moisture barriers due to their hydrophilicity. To improve moisture barrier characteristics, hydrophobic compounds such as surfactants could be added to hydrophilic materials. Edible films and coatings made from carbohydrates or chitosan have been shown to improve physicochemical and microbiological quality of fresh-cut fruits and vegetables (Cé et al. 2012; Ramos et al. 2012). Table 2.6 illustrates some recent studies dealing with films and coatings as the primary method of encapsulation of bioactive compounds.

Arcan and Yemenicioglu (2014) aimed at developing a scientific basis for edible packaging materials having controlled release properties for multiple active compounds capable of showing antimicrobial and antioxidant effects not only in packed food (active packaging) but also in the human physiological system after consumption of the enriched packed food (bioactive packaging). The films were produced by slowly mixing zein and glycerol with a magnetic stirrer. The mixture was then heated under continuous mixing until it started to boil. The blend zein films has lower initial release rates for the model active compounds, lysozyme and (+)-catechin, than the zein control films, respectively. The change of fatty acid chain length affected both catechin and lysozyme release rates while the change of fatty acid double bond number affected only the catechin release rate. The film morphologies suggested that the blend films owe their controlled release properties mainly to the microspheres formed within their matrix and encapsulation of active compounds.

Wichchukit et al. (2013) evaluated the physical/mechanical properties of a release system made from whey protein/alginate gel beads in a viscous medium that mimics a food beverage. Four polymer solutions were made by mixing Na-alginate with denatured whey protein isolate. Riboflavin was then added at a concentration of 0.25 g/50 g to those gel solutions under minimum light exposure to prevent degradation. Viscoelastic characteristics were not observed in pure alginate solution; it behaved as a Newtonian solution with no measurable elastic behavior. In contrast, the combination of whey protein and alginate resulted in shear thinning behavior of solutions. Solutions with high apparent viscosity resulted in larger beads. Mechanical

Table 2.6 Examples of bioactive compound encapsulated with gels and films.

Bioactive	Components	Reference
Cinnamaldehyde	Chitosan	Rieger et al. (2015)
Lysozyme, catechins, and a group of flavonoids	Zein and glycerol	Arcan and Yemenicioglu (2014)
Riboflavin	Whey protein isolate and alginate gel	Wichchukit et al. (2013)

relaxation tests of the four types of gel beads quantified the viscoelastic property. Pure whey protein beads tended to be more solid and relaxed the least, while pure alginate beads lack sufficient structural integrity.

Rieger et al. (2015) confirmed that cinnamaldehyde has a strong antimicrobial effect against *E. coli*, *S. aureus*, and *P. aeruginosa*. Increased encapsulation of cinnamaldehyde was enabled using a model surfactant, sorbitan monooleate (SpanVR80). Chitosan/ cinnamaldehyde films were prepared by adding the various amounts of cinnamaldehyde to the chitosan solution. Qualitatively, films exhibited well-defined structural color, which quantitatively ranged from 145 to 345 nm thick. These results suggest that nanostructured chitosan- cinnamaldehyde coatings hold potential to delay bacterial colonization on a range of surfaces, from indwelling medical device to food processing surfaces. Films fabricated using SpanVR80 demonstrated an increased release that paralleled the increased cinnamaldehyde incorporation. NMR indicated that a majority of the cinnamaldehyde was physically incorporated into the hydrophilic chitosan films. Spin-coating can be used to incorporate and deliver high-loadings of a model essential oil, cinnamaldehyde, from ultrathin chitosan films. These natural bioactive films hold potential for use as bioactive coatings in food packaging.

2.8 Nanocomposite Packaging

2.8.1 Active Nanocomposite Packaging

Active packaging is intentionally designed to incorporate components that release, absorb or modify substances into or from the packaged food or the environment surrounding the food. The major developments on active nanocomposite packaging are described on antimicrobial films, oxygen scavenging systems and enzyme immobilization systems (Silvestre et al. 2011; Ranjan et al. 2014).

Several antimicrobial substances, including natural products like bacteriocins, essential oils, isothiocyanates and sorbic acid, antimicrobial enzymes, metallic nanoparticles, and some modified clay minerals have been used to develop antimicrobial nanocomposite packaging materials (Azeredo 2013; Rhim et al. 2013). Diverse food packaging systems incorporated with silver nanoparticles have been described. The incorporation of silver nanoparticles in biopolymer films such as cellulose, starch, chitosan and alginate, exhibit consistent antimicrobial activity against both Gram-positive and Gram-negative bacteria (Unalan et al. 2014). The bacteriocin nisin has been also tested in several antimicrobial packaging systems. This bacteriocin was adsorbed by coating method on poly(lactic acid) containing cellulose nanocrystals and applied on sliced cooked ham to control *Listeria monocytogenes* (Salmieri et al. 2014). Recent studies showed efficient inhibition of *L. monocytogenes* by incorporation of nisin into poly(butylene adipate-co-terephthalate) films (Zehetmeyer et al. 2016), and starch nanocomposites containing halloysite nanoclay as reinforcement (Meira et al. 2016).

Protection against oxidative damage can be achieved by developing food packaging materials containing oxygen scavengers, which results into lowering the oxygen level (Rhim et al. 2013). Active packaging based on nanocomposite films were developed for selective control of oxygen diffusion. Different polymers containing nanostructured antioxidants or oxygen scavengers can be used as active packaging for sliced meat, poultry, beverages, cooked pasta, ready-to-eat snacks, and fish (Neethirajan and Jayas 2011).

The immobilization and incorporation of enzymes in packaging materials is an alternative to its direct use in food matrix since the enzymatic activity can be inactivated by some processing conditions and/or interaction with other compounds. Nanoscale immobilization can increase the available surface area and enhance performance by improving stability to pH and temperature and resistance to proteases or other denaturing compounds, besides allowing repeated use or controlled release into food (Rhim et al. 2013; Ranjan et al. 2014).

2.8.2 Smart Nanocomposite Packaging

Intelligent packaging systems are intended to include innovative functions such as detection, recording, tracing and communication. The condition of packaged food is monitored during storage and transportation through indicators and/or sensors in the form of a package label or a print on packaging films. In addition, such devices can inform the supplier or consumer that foodstuffs are still fresh, or has spoiled, kept at the appropriate temperature along the supply chain, or whether the packaging has been opened (Silvestre et al. 2011; Ranjan et al. 2014).

Nanoparticles have been used as reactive units in composite packaging materials to inform about the condition of the package. These nanosensors are designed to detect selected chemical compounds, such as pesticides, allergens and toxins, and are able to respond to microbial contamination, spoilage products, and environmental changes on temperature, humidity, and oxygen level. In this way, nanosensors inform the consumer, providing real-time status of food freshness and eliminating the need for inaccurate expiration dates. Nanosensors can be a valuable tool for the manufacturers, protecting against food mishandling after dispatch, ensuring that the product reaches the consumer in a suitable condition (Silvestre et al. 2011).

2.8.3 Nanoreinforcements

Significant improvements in mechanical and barrier properties, dimensional stability, and solvent resistance can be achieved by including some nanofillers into biopolymer matrixes intended for packaging purposes. Nanoreinforcement materials can include clay and silicate nanoplatelets, silica nanoparticles, carbon nanotubes, cellulose nanofibers or nanowhiskers, and chitin or chitosan nanoparticles

(Sorrentino et al. 2007). Currently, only the inorganic clays have attracted some attention by the packaging industry, due to their availability, low cost, significant improvements, and easy to process.

Mineral clays are considered environmentally friendly, naturally abundant and inexpensive, and regarded as safe food additives according to Food and Drug Administration and European Food Safety Authority (Ibarguren et al. 2014). Montmorillonite, a member of the smectite group, is one of the most widely used natural clays, particularly exploited for the fabrication of polymer-clay nanocomposites (Azeredo 2013). The improved barrier properties of polymer-clay nanocomposites appear to be due to an increased tortuous path for a diffusive molecule, forcing them to travel a longer way to diffuse through the film. Montmorillonite layers were efficiently dispersed and distributed within soy-protein-based films, generating a significant strengthening of the nanolayer, leading to resistance to breakage, and decrease in extension, moisture content, solubility, and permeability to water vapor (Echeverría et al. 2014). Lee et al. (2014) showed that two types of nanoclays incorporated to sesame seed meal protein nanocomposite film enhanced their physical properties (tensile strength, elongation and water vapor permeability), indicating that the films could be applied in food packaging.

Nanocellulose structures are described as a multi-performance material with potential for application in cellulose-based food packaging, showing promising properties like firmness, biocompatibility and suitable water vapor permeability. Cellulose nanocrystal obtained from the paper-mulberry bast pulp was tested as a reinforcing agent for the preparation of agar based bio-nanocomposites. Properties of agar films like mechanical and water vapor barrier properties were significantly improved by blending with cellulose nanocrystals (Reddy and Rhim 2014). The tensile strength and tensile modulus of agar films increased by 25% and 40%, respectively, in the composite film with 5% nanocellulose, whereas the water vapor permeability decreased by 25% in nanocomposite with 3% nanocellulose.

2.9 Conclusion

In the near future, many nanostructured bioactive compounds could be developed with efficacy. Advances in the strategies for incorporation of bioactive molecules into nanostructures and development of nanocomposite materials for food packaging constitute a field of great promise in the food sector. Development of integrated nanomaterial-bioactive systems for food processing, packaging, proper delivery of nutraceuticals and quality control is a topic of utmost interest to the worldwide community. Therefore, production of food grade nanostructures conjugated with bioactive compounds or plant extracts and their incorporation in food packaging for enhanced properties using nanotechnology are some of the future directions in the field. The use of agroindustrial byproducts as a source of natural polymers for development of such nanostructures and packaging materials constitute an interesting economical and eco-friendly alternative.

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