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Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds

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ARTICLE INFO

Article history:

Received 19 November 2010

Accepted 14 April 2011

Keywords:

Structured delivery systems

Biopolymers

Proteins

Polysaccharides

Nutraceuticals

Functional foods

Structural design

Encapsulation

Microparticles

Nanoparticles

ABSTRACT

Food-grade biopolymers, such as proteins and polysaccharides, can be used to create a diverse range of delivery systems suitable for encapsulating, protecting, and delivering lipophilic functional components, such as ω -3 rich oils, conjugated linoleic acid (CLA), oil-soluble vitamins, flavors, colors, and nutraceuticals. This article provides an overview of a number of different approaches that can be used to create structured delivery systems based on biopolymers, including molecular complexation, coacervation, thermodynamic incompatibility, moulding, and extrusion methods. These delivery systems can be produced from food-grade ingredients using simple processing operations (e.g., mixing, homogenizing, and thermal processing). The structure, production, performance, and potential applications of each type of structured delivery system are discussed.

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1. Introduction

There has been growing interest within the food industry in the development of colloid-based delivery systems to encapsulate, protect, and release various lipophilic food components (Aguilera, 2000; Augustin, Sanguansri, Margetts, & Young, 2001; McClements, Decker, & Weiss, 2007; Mezzenga, Schurtenberger, Burbidge, & Michel, 2005; Sanguansri & Augustin, 2006). These systems should be prepared from food-grade ingredients using economical and reliable processing operations. One of the most promising approaches to create food-grade colloidal particles is to use biopolymers, such as proteins and polysaccharides, as building blocks (Benichou, Aserin, & Garti, 2002; Burey, Bhandari, Howes, & Gidley, 2008; Chen, Remondetto, & Subirade, 2005; Dickinson, 2003; Malone & Appelqvist, 2003; Norton & Frith, 2001; Sundar, Kundu, & Kundu, 2010). Biopolymer colloidal particles can be assembled from proteins and polysaccharides using various bottom-up and top-down methods, including controlled biopolymer aggregation, segregation, and/or disruption (Aguilera & Stanley, 1999; Benichou et al., 2002; Tolstoguzov, 2007; van der Goot & Manski, 2007). These particles must be carefully designed

so that they exhibit the required functional attributes within the final product.

This review is divided into four sections. In the first section, we discuss various types of polysaccharides and proteins that can be used to form biopolymer particles along with some important factors to consider before selecting one or more biopolymers for particle fabrication. We then review a number of different approaches to form biopolymer particles. In general, these approaches can be classified as either “physicochemical” or “processing operation” methods. Physicochemical methods rely primarily on the utilization of physical forces (such as hydrogen bonds, electrostatic interactions, or steric exclusion effects) to control biopolymer particle formation while processing operation methods require the use of specific processing operations (such as injection, extrusion, homogenization, or evaporation). In practice, most particle formation methods often rely on a combination of both approaches. In the final section, we examine many of the fundamental properties of biopolymer particles and how these properties influence particle functionality and stability.

It should be noted that proteins and polysaccharides can also be used as building blocks to create other kinds of structures that are useful for creating functional properties in foods, such as molecular or interfacial complexes (Guzey & McClements, 2006; Livney, 2010; McClements, 2006, 2010). This work has recently been reviewed elsewhere, and so will not be covered in the current article.

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Table 1

Summary of important molecular characteristics of food-grade proteins for assembling biopolymer particles. Adopted from Jones and McClements (2010b).

Name	Source	Main structural type	pI	~ <i>T_m</i> (°C)
Bovine serum albumin	Bovine blood/milk	Globular	4.7	70–90
Caseins	Milk	Rheomorphic	~4.6	125–140
Gelatin	Animal or fish collagen	Linear	7–9.4 ^a ; 4.8–5.5 ^b	40
Ovalbumin	Egg white	Globular	4.5–4.7	74; 82 ^c
Soy glycinin	Soybean	Globular	~5	67 ^d ; 87 ^e
β-Lactoglobulin	Whey protein	Globular	4.8–5.1	75
Lactoferrin	Whey protein	Globular	~8–9	~60 and ~90

^a Type A gelatin.

^b Type B gelatin.

^c S-type ovalbumin.

^d 7S soy glycinin fraction.

^e 11S soy glycinin fraction.

2. Biopolymer particle composition

A variety of food-grade proteins and polysaccharides can be used to fabricate biopolymer particles, including whey proteins, casein, soy proteins, gelatin, zein, starch, cellulose, and various other hydrocolloids (Tables 1 and 2). As with any polymer, the type, number and distribution of monomers along the polymer chain determine the overall molecular characteristics, such as molecular weight, conformation, and electrical charge. It is essential that the molecular structure of the biopolymer be well understood as this structure ultimately determines how the biopolymer functions in complex foods. An excellent illustration of how molecular structure and composition influences biopolymer functionality is the different gelation methods for high-methoxy pectin (HMP) *versus* low-methoxy pectin (LMP). In the case of HMP, high concentrations of sugar along with a low pH environment are required for gel formation as these conditions allow for the formation of hydrogen bridges along linear sections of the polymer chain and hydrophobic interactions between methoxyl groups. On the other hand, LMP can gel upon addition of calcium ions as these cations form cross-links between free carboxyl groups along neighboring polymer chains. Calcium induced gelation is not possible for HMP as many of the free carboxyl groups present in low-ester pectin are methylated and thus will not interact with positively charged calcium ions (Willats, Knox, & Mikkelsen, 2006).

In addition to the biopolymers present within a particle, there will usually be other components present, such as water, lipids, minerals, and sugars. Some of these components may be essential for assembling the biopolymers into particles (e.g., multivalent mineral ions that promote gelation), whereas others may have other functions (e.g., lipid droplets trapped within the biopolymer matrix may act as a reservoir for lipophilic components).

The selection of particular proteins, polysaccharides and other components to form biopolymer particles depends on a number of factors: (i) the ability of the components to be assembled into particles; (ii) the functional requirement for the particles (e.g., size, charge and stability to environmental conditions); (iii) legal status, cost, ease of use, and consistency of the ingredients and processing operations. For many applications it is important to design biopolymer particles so that their compositions lead to the desired physicochemical and functional characteristics. For example, if one were creating a biopolymer particle to deliver a flavor component, then it should be designed to breakdown in the mouth and release the encapsulated flavor molecules. Conversely, if one were creating a biopolymer particle to delivery an anti-cancer component to the colon, then it should be designed to resist disruption within the mouth, stomach and small intestine, but breakdown in the colon.

2.1. Protein selection

Proteins are biological polymers comprised of amino acids that come in a variety of different general structures (Fig. 1), e.g., random coil, fibrous and globular proteins (Belitz, Grosch, & Schieberle, 2009). The molecular structure adopted by a particular protein depends on its amino acid sequence, prevailing environmental conditions, and environmental history, e.g., exposure to different temperatures, pressures, solvents, pH values, and ionic compositions (Phillips, Whitehead, & Kinsella, 1994). Proteins tend to adopt a structure that minimizes the overall free energy of the system, provided there are no kinetic constraints (energy barriers) that prevent them from reaching this low energy state.

A number of factors must be considered when selecting a suitable protein or combination of proteins to fabricate biopolymer-based delivery systems. First, it is important to establish the conditions where the protein molecules are able to associate with other protein or non-protein structure-forming molecules, e.g., environmental and solution conditions. This usually requires knowledge of specific physicochemical characteristics of the proteins involved, such as thermal denaturation temperatures (for globular proteins), helix–coil transition temperatures (for gelatin or collagen), isoelectric points (pI), sensitivities to specific monovalent or multivalent ions, or susceptibility to specific enzyme or chemical cross-linking or degradation reactions. Second, it is often important to establish the electrical characteristics of the protein molecules involved since electrostatic interactions are often utilized in structure formation, which can be conveniently described by the ζ-potential *versus* pH profile. The electrical charge on proteins goes from positive below their isoelectric point (pI), to zero at the pI, to negative above the pI. Even though the net charge on a protein is zero at its pI, the protein still has both positive and negative regions on its surface, and thus, it can be involved in attractive and/or repulsive electrostatic interactions. Proteins can vary widely in their isoelectric points depending on their biological

Table 2

Summary of important molecular characteristics^a among common food-grade polysaccharides for assembling biopolymer particles.

Name	Source	Main structure type	Major monomer	Gelation Mechanism
Alginate	Algal	Linear	β-D-Mannuronic Acid	Calcium cross-linking
Beet pectin	Sugar beet pulp	Branched coil with protein	Glucuronate (backbone)	Sugar/heat (HM); calcium (LM)
Carrageenan	Algal	Linear/helical	Sulfated galactan	Cooled set
Chitosan	Crustaceans, invertebrates	Linear	2-Amino-2-deoxy-β-D-glucose	No common application
Gum arabic	Acacia sap	Branched coil domains on protein scaffold	Galactose	Conc. dependent
Inulin	Plants or bacteria	Linear with occasional branches	β-D-Fructose	Conc. dependent
Methyl cellulose	Wood pulp	Linear	Methylated glucose	Heat-set (rev.)
Pectin	Plant cell walls	Highly branched coil	Glucuronate (backbone)	Sugar/heat (HM); calcium (LM)
Xanthan gum	<i>Xanthomonas campestris</i> exudate	Linear/helical (high MW)	β-D-glucose (backbone)	None; thickens with concentration

^a Polysaccharide ingredients available commercially generally possess appreciably different molecular and functional properties; the listed information describes general characteristics for industrial usage.

Fig. 1. Simplistic schematic representation of different structures that protein and polysaccharide molecules may have in solution.

origin, and the processing procedures used to extract them (Table 1). Third, it is usually important to have knowledge of the nature of the biopolymer particles that can be formed after protein association, such as their morphology (fibrous, globular), physical properties (density, refractive index), size, charge, and stability (e.g., to pH, ionic strength, temperature, and enzymes). These factors will determine how the particles may impact the optical, rheological, stability, and functional characteristics of the products into which they are incorporated (LaClair & Etzel, 2010).

2.2. Polysaccharide selection

Like proteins, the molecular structure of polysaccharides depends on their monomer sequence, the prevailing environmental conditions, and their history. Polysaccharides are often classified as either homo-polysaccharides (consisting of only one type of monosaccharide) or hetero-polysaccharides (consisting of different types of monosaccharide). Polysaccharides differ from one another chemically in terms of the type, number, sequence, and bonding of the monosaccharides within the polymer chain. These chemical differences lead to differences in molecular properties, such as molecular weight, degree of branching, structure, flexibility, electrical charge, and interactions. In turn, these molecular differences lead to differences in functional properties, such as solubility, thickening, gelation, water holding capacity, surface activity, emulsification, and digestibility (Belitz et al., 2009; Glicksman, 1982).

There are a number of factors that must be considered when selecting a suitable polysaccharide or combination of polysaccharides to fabricate a biopolymer-based delivery system. It is important to establish the environmental and solution conditions where the polysaccharide molecules can associate with other polysaccharide or non-polysaccharide structure-forming molecules. This usually requires knowledge of the physicochemical properties of the polysaccharides involved, such as helix–coil transition temperatures (for carrageenan, alginate, pectin), electrical properties (pK_a values), sensitivity to specific monovalent or multivalent ions, or susceptibility to enzyme or chemical reactions (BeMiller & Whistler, 1996). For some applications, it is important to establish the electrical characteristics of the polysaccharide molecules used (ζ -potential versus pH), since electrostatic interactions may be used to assemble specific biopolymer structures. The electrical charge on polysaccharides depends on the nature of the ionic groups along the chain background, as well as solution conditions. Some polysaccharides are neutral (starch, cellulose), some are anionic (alginate, carrageenan, xanthan, gum arabic) and some are cationic (chitosan). The magnitude of the electrical charge on ionic polysaccharides depends on the pH relative to the pK_a value of the charge groups. Anionic polysaccharides tend to be neutral at pH values sufficiently below their pK_a value but negative above, whereas cationic polysaccharides tend to be neutral at pH values sufficiently above their pK_a value but positive below. The most common charged groups on polysaccharides are sulfate groups (e.g., carrageenan), carboxyl groups (e.g., pectin, alginate, xanthan, carboxymethylcellulose) and amino groups (e.g.,

chitosan): $-\text{SO}_4\text{H} \leftrightarrow -\text{SO}_4^-$ ($pK_a \approx 2$); $-\text{CO}_2\text{H} \leftrightarrow -\text{CO}_2^-$ ($pK_a \approx 3.5$); $-\text{NH}_3^+ \leftrightarrow -\text{NH}_2$ ($pK_a \approx 6.5$). The electrical charge on polysaccharides may also be altered by interactions with other ionic species in their environment. These interactions typically involve monovalent or multivalent ions such as sodium or calcium that bind to oppositely charged groups on the biopolymer chain, altering overall charge characteristics. Third, it is usually important to establish the nature of the biopolymer particles that can be formed by the polysaccharides used, such as their morphology, density, refractive index, size, charge, and stability to pH, salt, temperature and enzymes. In addition, knowledge of the type of environmental and solution conditions present within a particular food is often important for selecting the most appropriate polysaccharide building blocks. For example, pectin can start to depolymerize when exposed to neutral or alkaline conditions as a result of a base-catalyzed β -elimination reaction that breaks down its sugar chain. Over time, this breakdown can result in a decline in viscosity and loss of texture (Sila et al., 2009).

3. Biopolymer particle formation methods: physicochemical approaches

Biopolymer particles can be prepared using a number of approaches that primarily rely on the manipulation of the physicochemical properties of the system as opposed to specific processing operations. This section provides a brief overview of some of the most important physicochemical approaches for fabricating biopolymer particles.

3.1. Formation of molecular complexes

Many types of biopolymer molecules are capable of binding lipophilic molecules and forming molecular complexes. The lipophilic molecules may be bound to individual biopolymer molecules, or they may be incorporated within clusters formed by a single type or mixed types of biopolymers (Fig. 2).

3.1.1. Individual biopolymer molecules

Lipophilic molecules may bind to individual biopolymer molecules at one or more active sites, by either specific or non-specific interactions with different molecular origins. Globular proteins, such as β -lactoglobulin and BSA, have been shown to bind surfactants and other bioactive lipophilic molecules such as resveratrol, docosahexaenoic acid (DHA), and conjugated linoleic acid (CLA) to hydrophobic pockets on their surfaces (Kelley & McClements, 2003; Liang, Tajmir-Riahi, & Subirade, 2008; Zimet & Livney, 2009). Flexible proteins, such as caseinate, have been shown to bind certain kinds of lipophilic molecules and form molecular complexes that remain dispersed in aqueous solutions (Semo, Kesselman, Danino, & Livney, 2007). Advances in the formation of complexes between milk proteins and lipophilic bioactives have recently been reviewed (Livney, 2010).

The driving force for protein binding is usually either hydrophobic or electrostatic in origin. The non-polar regions of surfactants and lipids may bind to non-polar regions on the protein surface through hydrophobic attraction. The head groups of ionic surfactants may bind to oppositely charged groups on the protein surface through electrostatic attraction. Surfactants may bind to proteins either as individual monomers or as micelle-like clusters depending on the nature of the interaction and the surfactant concentration. Once a surfactant has bound to a protein it may either stabilize or destabilize its structure. Depending on the type and concentration of surfactant as well as environmental conditions, this interaction may either promote or prevent protein aggregation.

Fig. 2. Illustration of various types of molecular complexes that may be formed between lipophilic molecules and biopolymers.

Individual polysaccharide molecules that have ionic or non-polar side groups may also bind lipids and surfactants. Surfactants may bind through electrostatic or hydrophobic interactions to ionic or non-polar side groups. For example, anionic and cationic surfactants have been shown to bind to cationic, anionic and neutral polysaccharides through electrostatic and hydrophobic interactions (Bao, Li, Gan, & Zhang, 2008; Merta & Stenius, 1999). Starch components (amylose) and starch derivatives (maltodextrins and cyclodextrins) are able to form helices that have a hydrophobic interior, which are capable of binding non-polar molecules including fatty acids and ionic surfactants with appropriate molecular dimensions through hydrophobic interactions (Wangsakan, Chinachoti, & McClements, 2001, 2004; Wangsakan, McClements, Chinachoti, & Dickinson, 2004; Zabar, Lesmes, Katz, Shimoni, & Bianco-Peled, 2010). Surface active lipids may bind to polysaccharides either as monomers or as micelle-like clusters depending on their concentration and the molecular origin of the interaction.

3.1.2. Biopolymer molecular clusters: single biopolymer type

Clusters of biopolymer molecules are also capable of encapsulating certain types of lipophilic molecules. These clusters may be formed from a single type of biopolymer or from mixtures of different types of biopolymers. It was recently shown that casein micelles, which are clusters of casein molecules ($d = 100\text{--}300\text{ nm}$), are capable of encapsulating and protecting non-polar molecules, such as hydrophobic nutraceuticals, vitamins and drugs (Portnaya et al., 2006; Semo et al., 2007; Shapira, Assaraf, & Livney, 2010). These delivery systems are formed by dissolving the desired lipophilic bioactive in an organic solvent such as ethanol and then adding this solution drop wise into an aqueous solution of sodium caseinate. The casein micelles are then re-assembled by adding calcium, phosphate and citrate to the caseinate solution around neutral pH to promote cluster formation. The encapsulation of

vitamin D in caseinate micelles improved its stability to photochemical degradation, when compare to non-encapsulated vitamin D (Semo et al., 2007).

3.1.3. Biopolymer molecular clusters: mixed biopolymer type

Proteins and ionic polysaccharides form molecular clusters at pH values where there is a slight electrostatic attraction between them. Recently, it has been shown that bioactive lipids (ω -3 fatty acids) can be encapsulated inside molecular complexes of a globular protein (β -lactoglobulin) and an anionic polysaccharide (pectin) (Zimet & Livney, 2009). Encapsulating these ω -3 fatty acids in biopolymer clusters was shown to improve their oxidative stability. Similar complexes of β -lactoglobulin and pectin have also been used to encapsulate and protect vitamin D₂. The stability of vitamin D₂ incorporated into these mixed biopolymer complexes was superior to both unprotected vitamin D₂ and single complexes of vitamin D₂ and β -lactoglobulin (Ron, Zimet, Bargaram, & Livney, 2010).

3.2. Formation of biopolymer nanoparticles or microparticles

Many types of biopolymers are capable of forming biopolymer nanoparticles or microparticles that can be used to incorporate lipophilic compounds (Fig. 3). These particles can be formed from individual types of biopolymers or from mixed biopolymer systems depending on the fabrication mechanism.

3.2.1. Biopolymer particles: single biopolymer type

Biopolymer particles can often be formed from aqueous solutions containing a single biopolymer by promoting self-association (Fig. 3a). To encourage self-association, solution conditions are altered such that biopolymer–biopolymer interactions are favored over biopolymer–solvent interactions. In this section, we review a number of approaches to fabricate this type of particle.

Fig. 3. Examples of some different kinds of internal structures that may be formed within biopolymer particles.

Biopolymer particles can be formed when globular protein solutions are heated above their thermal denaturation temperature (T_m) under conditions where there is a relatively weak attraction between the protein molecules (Jones, Decker, & McClements, 2010; Jones & McClements, 2010b). The size and charge of these biopolymer particles can be controlled by altering the initial biopolymer concentration, holding temperature, holding time, pH and ionic strength. Recently, this approach was used to encapsulate functional food components such as EGCG ((-)-Epigallocatechin-3-gallate), a relatively unstable tea polyphenolic that has beneficial bioactivity (Shpigelman, Israeli, & Livney, 2010).

Biopolymer particles can also be formed from thermally denatured globular proteins using a cold-set particle formation technique (Chen & Subirade, 2009; Nicolai & Durand, 2007; Wu, Xie, & Morbidelli, 2005). In this technique, a globular protein solution is heated above T_m under conditions where there is a strong repulsion between the protein molecules so that the molecules unfold but do not extensively aggregate. Typical solution conditions that promote repulsion include a pH far from the pI of a protein and a low ionic strength. The solution conditions are then altered to promote protein self-association. Attractive forces between protein molecules are enhanced by adjusting the pH towards pI or by adding monovalent or divalent counter-ions such as NaCl and CaCl₂ respectively (Bryant & McClements, 1998). The lipophilic component to be encapsulated can be mixed with the heat-denatured protein solution prior to promoting particle formation.

Biopolymer particles can also be formed by changing the *quality* of the solvent surrounding the biopolymer molecules in solution. In most cases, solvent quality is altered by adding alcohol to an aqueous biopolymer solution. The biopolymer molecules present in solution will tend to spontaneously self-associate once some critical alcohol content has been exceeded, leading to the formation of biopolymer aggregates. Protein nanoparticles consisting of BSA (Rahimnejad, Jahanshahi, & Najafpour, 2006), gelatin (Mohanty & Bohidar, 2003) and caseinate (Gupta & Bohidar, 2005; Tsioulpas, Lewis, & Grandison, 2007) have been formed by the addition of ethanol to aqueous protein solutions. Biopolymer particles have also been formed from chitosan by the addition of methanol (Peniche, Arguelles-Monal, Peniche, & Acosta, 2003).

Conversely, non-polar biopolymers can be induced to form biopolymer particles by dissolving them in an organic solvent, and then adding sufficient quantities of a polar solvent such as water to promote self-association. This approach has been used to form biopolymer particles with an approximate diameter of 500 nm from the wheat protein gliadin to encapsulate retinoic acid (Duclairoir et al., 1999). Gliadin nanoparticles have also been successfully used to encapsulate vitamin E (Renard et al., 2002). Recently, this technique has been used to encapsulate lysozyme, an antimicrobial protein, into particles derived from corn zein (Zhong, Jin, Davidson, & Zivanovic, 2009). Gelatin can also be desolvated by certain salt solutions (Izmailova et al., 2001). Casein is another candidate for this technique as it is known to aggregate in the presence of ethanol and cationic solutions.

Desolvation is generally performed by introducing large quantities of aqueous solution and other polar co-solvents (Duclairoir, Nakache, Marchais, & Orecchioni, 1998) or salts (Lazko, Popineau, & Legrand, 2004; Mauguet et al., 2002) to an ethanol-dispersion. The size of the particles formed during this process can be adjusted using salts and surfactants (Duclairoir et al., 1999). In theory, other alcohol-soluble biopolymer materials, such as celluloses, could be used with this methodology. A similar approach was recently used to form biopolymer particles by promoting the precipitation of cellulose using acetone and water (Hornig & Heinze, 2008).

Biopolymer particles can also be formed by adding a cross-linking agent to an aqueous solution with a biopolymer concentration below that required to form a macroscopic gel. Examples of such cross-linking agents include chemicals (such as glutaraldehyde or formaldehyde), enzymes (such as transglutaminase or laccase), and mineral ions (such as potassium, calcium, or tripolyphosphate). Alternatively, cross-linking can be promoted by altering environmental conditions, such as temperature or pressure. The cross-links formed may be either chemical or physical in nature, which often plays an important role in determining the reversibility of the system. Chemical cross-linking involves the formation of covalent bonds between polymers, while physical cross-linking involves the formation of non-covalent bonds, such as hydrogen bonding, hydrophobic association, and ion bridging (Williams, 2007). Both proteins and polysaccharides are capable of gelation although the gelation mechanism and properties of these two types of gels are often quite different (Renard, van de Velde, & Visschers, 2006).

3.2.1.1. Protein gelation. Globular proteins are commonly used to form biopolymer particles through controlled cross-linking. These particles are usually formed by heating globular proteins above their thermal denaturation temperature, which promotes protein self-association through hydrophobic attraction and disulfide formation. The nature of the particles formed can be controlled by manipulating the intermolecular interactions, through controlling pH, ionic strength, and heating conditions. Biopolymer particles can also be formed by controlled aggregation of more flexible random coil proteins, such as casein and gelatin. Casein can be gelled by adjusting the pH to close to the proteins isoelectric point, by adding multivalent counter-ions, or by adding rennet (Cooper, Corredig, & Alexander, 2010). Gelatin can be gelled by cooling a biopolymer solution below the helix–coil transition temperature to promote the formation of helices and hydrogen bond cross-links (Izmailova et al., 2004). Enzymes such as transglutaminase can also be used to form covalent cross-links between amino acids on various protein substrates (DeJong & Koppelman, 2002), which has recently been used to form biopolymer delivery systems for lipophilic components (Huppertz & de Kruif, 2008; Song, Zhang, Shi, & Li, 2010).

3.2.1.2. Polysaccharide gelation. With the exception of starch-based polysaccharides and cellulose derivatives, most gelling polysaccharides are composed of more than one type of sugar unit and are thus hetero-polysaccharides. The combination of multiple sugar units along with various side chains for some polysaccharides means that gelation methods can vary widely among polysaccharides. Some of the most common gelation methods include cold-setting gels and heat-setting gels (Morris, 2007). Ionotropic gelation is also another important gelling mechanism for polysaccharides (Burey et al., 2008). Some types of modified cellulose will self-associate upon heating, which has been attributed to an increase in the strength of the hydrophobic attraction between them. Other polysaccharides, such as alginate and carrageenan, self-associate when they are cooled below their thermal transition temperature due to helix formation and association through hydrogen bonding. The enzyme laccase has been shown to form gels from sugar beet pectin by forming covalent cross-links between phenolic groups (Minussi, Pastore, & Durán, 2002).

3.2.2. Biopolymer particles: mixed biopolymer type

When two different biopolymers are mixed together they may either form a one-phase or a two-phase system depending on the nature of the biopolymers involved, the solution composition and the prevailing environmental conditions (Fig. 4). In a one-phase

Fig. 4. Possible interactions for a two component biopolymer system, e.g., a protein–polysaccharide mixture.

system, the two biopolymers can exist either as individual molecules or as soluble complexes that are evenly distributed throughout the entire system. In a two-phase system, the solution separates into two distinct phases that have different biopolymer compositions. Phase separation can occur through two different physicochemical mechanisms: associative and segregative separation (Fig. 4).

In *associative separation*, there is a relatively strong *attraction* between the two different kinds of biopolymers which causes them to associate with each other. The most common example of this type of interaction for food biopolymers is the electrostatic attraction between molecules with opposite electrical charges. The resulting two-phase system consists of a phase that is rich in both biopolymers and a phase that is depleted in both biopolymers. The biopolymer-rich phase may either form a *coacervate* or a *precipitate*, depending on the strength of the attraction and the nature of the polymers involved (Fig. 4). Recently it has been shown that biopolymer particles can be formed by heating mixtures of proteins and polysaccharides together under conditions where they form molecular complexes (Jones, Decker, & McClements, 2009; Jones, Decker, et al., 2010; Jones, Lesmes, Dubin, & McClements, 2010; Jones & McClements, 2008, 2010a).

In *segregative separation*, there is a relatively strong *repulsion* between the two different kinds of biopolymers, i.e., there is a relatively high positive (unfavorable) free energy of mixing. The molecular origin of this effect is usually the steric exclusion effect. This type of phase separation often occurs when one or both of the biopolymers are uncharged, or when both biopolymers have similar charges. At sufficiently low biopolymer concentrations, the two biopolymers are intimately mixed and form a one-phase solution. Once the biopolymer concentration exceeds a certain level, phase separation occurs and a two-phase solution is formed with each phase being rich in one type of biopolymer and depleted in the other type of biopolymer (Fig. 4).

A variety of microstructures can be created in phase-separated biopolymer systems by varying the preparation conditions or by shearing the system. Examples of these systems include “water-in-water” emulsions (Fig. 5) or “oil-in-water-in-water” emulsions (Fig. 6). Once a particular microstructure has been formed, it is often possible to trap the system in a kinetically stable state, and thus create novel food microstructures and rheological properties (Norton & Frith, 2001). For example, kinetic trapping can be achieved by changing solution or environmental conditions so that one or both of the phases thickens or gels. If this process is carried out in the presence of shear, it is possible to produce a wide variety of

Fig. 5. Schematic representation of production of a water-in-water (W/W) emulsion from a two-phase system consisting of two aqueous biopolymer phases.

Fig. 6. Schematic representation of production of an oil-in-water-in-water (O/W/W) emulsion from a two-phase system consisting of two aqueous phases.

different microstructures such as spheres, tear-drops or fibers. Alternatively, it may be possible to adsorb another biopolymer around the water droplets that form the dispersed phase in a W/W emulsion, thereby stabilizing them.

More sophisticated biopolymer particle structures can be formed by carrying out these steps sequentially or by combining different methods. For example, a biopolymer particle may be formed first, and then a shell of another material could be deposited around it to form a core–shell structure (Fig. 3b).

4. Biopolymer particle formation methods: process operation methods

In this section, we focus on methods of biopolymer particle formation that primarily rely on specific processing operations to form and stabilize biopolymer particles.

4.1. Molding techniques

Molds formed by soft lithography techniques can be used to create particles with well-defined sizes and morphologies (Dang et al., 2009; McGuigan, Bruzewicz, Glavan, Butte, & Whitesides, 2008). In this technique, a biopolymer solution is poured into a mold with a specific size and shape (Fig. 7). Solution or environmental conditions are then adjusted to promote biopolymer gelation. Thus, one can think of this method as a micro- or nano-scale version of the formation of gelatin gels that is routinely carried out in home kitchens. In the past, the production of micro- or nano-sized cavities was limited by the available technology. Recently, developments in soft lithography techniques have enabled the formation of polymer molds from finely tooled metal

plates (Xia & Whitesides, 1998). The biopolymer solution can be trapped between a mold and a flat plate until it hardens into a gel. A number of different molding technologies have been developed, including replica molding, micro-contact printing, micro-transfer molding, capillary micro-molding, and solvent-assisted micro-molding (Xia & Whitesides, 1998).

Polydimethylsiloxane (PDMS) molds are commonly used for traditional soft lithography techniques. Unfortunately, PDMS molds have some negative features such as substrate adhesion. Moreover, simple PDMS molds can only be used to create embossed films as opposed to discrete particles. In the case of substrate adhesion, polymeric spacers such as monolayers of hexa-(ethylene glycol) or bovine serum albumin have been used to minimize this problem (Tang, Golden, & Tien, 2003). In the PRINT (Particle Replication In Nonwetting Templates) method, nonwetting mold materials, such as photocurable perfluoropolyether (PFPE), are employed to minimize the adhesion of hydrophilic substrates to the mold. Using PRINT, particles between 200 and 500 nm have been created (Rolland et al., 2005). Recently, an adaptation of the PRINT method was used to fabricate monodisperse protein particles from albumin and insulin ranging in size from 200 nm to 5 μ m. In this approach, an aqueous protein solution was deposited onto a PFPE mold, the filled mold was lyophilized, and the resulting protein particles were harvested (Kelly & DeSimone, 2008). In theory, a variety of gelation methods could be combined with PRINT methods to create food-grade particulates. The possibility of forming biopolymer particles with different morphologies and compositions using molding methods that are suitable for food applications has been investigated (Malone & Appelqvist, 2003).

To the authors knowledge, this technique is not currently used in the food industry because of the high cost and difficulty in scale-up. However, some closely-related systems have been investigated. For instance, alginate particles have been made by placing a calcium-releasing plate on one side of the mold (TaleiFranzese, Ni, Ling, & Khademhosseini, 2006). Diffusion of the calcium ions out of the plate caused gelation of the alginate within the mold. Photosensitive biopolymers, such as hyaluronic acid, have been similarly created using UV-radiation for cross-linking and particulate formation (Yeh et al., 2006).

4.2. Spray drying

Spray drying has been used for many years to encapsulate various kinds of food ingredients (Fellows, 2000). A liquid solution or suspension is passed through a small nozzle, which leads to the formation of a mist of fine drops. The outlet of the nozzle is located in controlled high temperature environment so that the volatile liquid phase (usually water) within the drops quickly evaporates. Spray driers typically operate at temperatures between 150 and 300 °C depending on the nature of the material being prepared. The actual temperature experienced by the material within the drops is

Fig. 7. Schematic diagram of molding method for forming filled biopolymer particles. An O/W emulsion containing a biopolymer in the aqueous phase is poured into a mold. The gelation of the biopolymer molecules is then promoted by changing environmental conditions or adding a gelling agent. The particles can then be removed from the mold.

considerably less than this because of the latent heat associated with liquid evaporation. In addition, the high surface-to-volume ratio of the drops allows for rapid drying, which also minimizes thermal damage. Spray drying is capable of continuous operation and produces a dry powdered product. The diameters of the particles within the spray-dried powder are usually in the 10–100 μm range, so that each particle usually contains many different lipid droplets or biopolymer molecules. Spray drying can be used to convert a suspension of biopolymer particles into a powder that can be reconstituted prior to use.

Spray drying is often used to dry foods and ingredients that are sensitive to heat such as proteins, flavor oils, and lipid droplets (Abdul-Fattah, Kalonia, & Pikal, 2007; Ameri & Yuh-Fun, 2006; Desai & Hyun Jin, 2005). Excellent reviews exist on spray drying oils for encapsulation (Jafari, Assadpoor, He, & Bhandari, 2008; Vega & Roos, 2006). In terms of product functionality, it is important that these powders are easy to reconstitute. Caking, a process associated with the transformation of a free flowing powder to an agglomerated material, must be minimized during storage. During the process of wetting, submersion, dispersion, and dissolution, particles are subjected to increasing levels of water. The speed and conditions of these steps are important for proper reconstitution (Vega & Roos, 2006). For many spray-dried capsules, the ultimate purpose is to have the encapsulated material released at a controlled rate when the powder is dispersed in a product or comes into contact with saliva during oral ingestion.

When fabricating spray-dried biopolymer particles containing lipid droplets, parameters such as the amount of dispersed oil, the quantity of biopolymer, the composition of the wall material, and the heating conditions can be manipulated to form different types of particles. For instance, studies have shown that higher oil contents will increase the total amount of encapsulated oil but will also decrease encapsulating efficiency (Adamiec & Kalemba, 2006). Research conducted on the encapsulation of flavor oils found that oil type had only a modest impact on entrapped oil content. Oil type did, however, have an effect on the surface oil content. The type of biopolymer used as an encapsulant also influenced particle size and surface oil content (Baranauskienė, Venskutonis, Dewettinck, & Verh, 2006). Encapsulant type can also impact the storage stability of spray-dried encapsulated oil. In a recent study where conventional encapsulants such as gum arabic were compared with protein-based encapsulants, flavor retention was highest for samples encapsulated by gum arabic however samples encapsulated by protein showed lower rates of oxidative deterioration (Charve & Reineccius, 2009).

Spray drying has been used to prepare zein microparticles that encapsulate various antimicrobial components such as thymol and lysozyme (Jin, Davidson, Zivanovic, & Zhong, 2009; Zhong & Jin, 2009). In this case, ethanol rather than water was used as the carrier fluid since zein is a non-polar protein. Another less common drying process that can be used for oil encapsulation is freeze drying. In an interesting application of the positively charged biopolymer chitosan, a coating of chitosan in combination with a negatively charged surfactant (citrem) was used to encapsulate a freeze dried emulsion of the flavor oil carvone. The flavor retention of these coated freeze dried emulsions was found to be quite high. There was also little change in the particle size of these emulsions following reconstitution (Kaasgaard & Keller, 2010).

4.3. Solvent desorption

The formation of biopolymer particles using solvent desorption techniques rely on changes in the solubility of biopolymers under different solvent conditions (Horn & Rieger, 2001). In this process, both the biopolymer and the active component to be encapsulated

are solubilized in a particular solvent. The solvent conditions are then adjusted so that solvent desorption is favored, and biopolymer particle formation is induced. Examples of these changes to solvent conditions include adding incompatible co-solvents, adding aggregating reagents, or evaporating the solvent. A fraction of the active components dispersed within the initial solution become entrapped within the biopolymer particles during solvent desorption (Galindo-Rodriguez, Allemann, Fessi, & Doelker, 2004).

A number of encapsulation methods described in the literature can be classified as techniques involving solvent desorption. These methods include nano-precipitation (Barichello, Morishita, Takayama, & Nagai, 1999; Duclairoir et al., 1999; Jimenez, Pelletier, Bobin, Martini, & Fessi, 2004; Lee, Park, & Choi, 1999; Leo, Brina, Forni, & Vandelli, 2004), interfacial deposition/solvent displacement (Mosqueira et al., 2001; Mosqueira, Legrand, Pinto-Alphandary, Puisieux, & Barratt, 2000; Murakami, Kobayashi, Takeuchi, & Kawashima, 1999), salting-out (Galindo-Rodriguez et al., 2004), simple coacervation (Mauguet et al., 2002; Mohanty & Bohidar, 2003), and emulsification–diffusion (Cirpanli, Unlu, Calis, & Atila Hincal, 2005; Galindo-Rodriguez et al., 2004; Lai & Tsiang, 2005). Solvent desorption can also be used to deposit polymers onto the surfaces of particles, such as lipid droplets (Cosgrove & Heath, 1987). The primary distinction between these different techniques is the method used to induce solvent desorption and biopolymer particle formation. Examples of these methods include adjusting polymer polarity, use of various salts, use of various solvents, speed of solvent addition, and removal of solvent (e.g. evaporation). Nevertheless, all of these methods promote desorption of the solvent from the biopolymer to induce aggregation and entrapment of the compound of interest. The entrapment efficiency depends on the biopolymer used and the method of solvent desorption.

4.4. Injection methods

Injection or extrusion methods involve the injection of a biopolymer solution into another solution that promotes gelation (Fig. 8). Depending on the method of gelation, this second solution may contain a gelling agent such as ions or enzymes. In the case of thermal gelation, this second solution could be heated or chilled to promote heat-set or cold-set gelation. This method has often been used to form alginate microbeads as alginate will form a physical gel in the presence of calcium ions (Li, Dobraszczyk, & Wilde, 2004). To create these microbeads, individual droplets of an alginate solution are injected into a calcium solution bath where gelation occurs and the beads harden (Amici, Tetradis-Meris, de Torres, & Jousse, 2008; Liu, Ding, Liu, Chen, & Zhao, 2006; Shin et al., 2007). The microbeads formed have shown great promise as microencapsulation devices for pharmaceutical drugs (Jiao et al., 2002; Shanmugasundaram, Sundaraseelan, Uma, Selvaraj, & Babu, 2005; Tonnesen & Karlsen, 2002) and probiotics (Sheu & Marshall, 1993). Since this method is both economical and relatively easy to control, it is quite feasible to produce alginate microbeads on an industrial scale (Shin et al., 2007). Besides alginate, microbeads could be formed from other biopolymers such as injecting a pectin solution into a calcium solution (ionic gelation), injecting a chitosan solution into a tripolyphosphate solution (ionic gelation), injecting a whey protein solution into a hot liquid (heat-set gelation), or injecting a gelatin solution into a cold liquid (cold-set gelation).

4.5. Microfluidic methods

Microfluidics is finding widespread application for the production of polymer particles with well-defined characteristics. In some respects, this method is a special case of the injection methods

Fig. 8. Schematic representation of injection method used to produce filled hydrogel beads. An O/W emulsion containing a biopolymer in the aqueous phase is injected into an aqueous solution containing a gelling agent or different environmental conditions. Droplets of the O/W emulsion are formed at the end of the inner cylinder, which are gelled by the gelling agent.

described previously (Fig. 9). A recent trend in microfluidics is the fabrication of uniform droplets using precise microchannels often created by modern lithographic techniques (Oh, Drumright, Siegwart, & Matyjaszewski, 2008; Seo, 2005; Zhang, Tumarkin, Sullan, Walker, & Kumacheva, 2007). In this technique, dispersed phase flow creates pressure at the tapered ends of the channels, forcing the solution into specific droplet shapes. Droplets can be expelled into a bulk carrier solution or alongside carrier solution from a parallel microchannel. The use of co-eluting parallel streams allows for a purely mechanical atomization effect, resulting in droplets on the order of tens of microns (Gañán-Calvo & Barrero, 1999). By selecting the appropriate stabilization method, the morphology and shape of the droplet can be retained (Oh, Kim, Baek, Seong, & Lee, 2006; Oh et al., 2008; Zhang et al., 2007). Often, microspheres are on the micron-scale (Nisisako, Torii, & Higuchi, 2004). The great advantage of microfluidics is the production of highly reproducible droplet sizes (Utada et al., 2005) and the capability of producing multiple phase layers (Okushima, Nisisako, Torii, & Higuchi, 2004; Utada et al., 2005). Nevertheless, they may not be appropriate for widespread utilization within the food industry due to scale-up problems.

Fig. 9. Schematic diagram of microfluidic method for forming filled biopolymer particles using a co-axial device. An O/W emulsion containing a biopolymer in the aqueous phase is passed through an inner cylinder, while another aqueous phase containing a gelling agent is passed through an outer cylinder. Droplets of the O/W emulsion are formed at the end of the inner cylinder, which are gelled by the gelling agent.

Fig. 10. (a) Emulsion-templating method for forming biopolymer particles. A W/O emulsion is formed by homogenizing an oil phase (oil + lipophilic surfactant) and an aqueous phase (water + biopolymer). The water phase is then gelled by changing environmental conditions (such as temperature) or adding a gelling agent. (b) Emulsion-templating method for forming filled biopolymer particles. An O/W/O emulsion is formed by homogenizing an O/W emulsion with a water phase (water + oil-soluble surfactant). The internal water phase is then gelled by changing environmental conditions (such as temperature) or adding a gelling agent.

4.6. Emulsion-templating methods

The emulsion-templating method relies on the utilization of W/O emulsions as templates to produce biopolymer particles with specific dimensions (Fig. 10a). An aqueous biopolymer solution is homogenized with an oil phase containing an oil-soluble emulsifier to form a water-in-oil emulsion (W/O). Water droplet size can be controlled by varying either homogenization conditions (pressure and number of passes) or solution composition (oil-to-water ratio, emulsifier–water ratio). The inner water phase is then gelled using a mechanism appropriate for the particular biopolymer used, e.g., temperature change, addition of cross-linking agent, or pH/ionic strength change. Finally, the biopolymer particles can be obtained by centrifuging/filtering the W/O emulsion, collecting the particles, and then washing them with an organic solvent to remove any residual oil. The resulting biopolymer particles can then be dispersed in an aqueous solution or dried.

This approach has been used to form biopolymer particles based on alginate (Reis, Neufeld, Vilela, Ribeiro, & Veiga, 2006). An aqueous solution containing alginate and insoluble calcium salt were dispersed into a continuous oil phase containing oil and surfactant under constant agitation to form a W/O emulsion. An organic acid was then mixed with the oil phase, which caused the insoluble calcium salt to slowly dissolve and release calcium ions into the aqueous phase, leading to the formation of gelled calcium alginate particles. Following gelation, these particles were separated from the oil phase by filtration/centrifugation and then washed using an organic solvent to remove any residual oil (Reis et al., 2006). This method has been successfully used to form

alginate/whey protein microspheres for the encapsulation of riboflavin (Chen & Subirade, 2006). It has also been used to encapsulate probiotic bacteria inside milk protein particles formed by enzyme (rennet) (Heidebach, Först, & Kulozik, 2009). In this study, a solution of probiotic bacteria and milk protein was incubated at a low temperature (5 °C), and then this mixture was added to vegetable oil to form a W/O emulsion. The temperature of the oil was subsequently increased to 18–20 °C, a temperature where casein micelles treated with rennet will gel, to form spherical microcapsules.

A similar approach can be used to form filled biopolymer particles, i.e., biopolymer particles containing lipid droplets (Fig. 10b). In this case an O/W emulsion is formed, and then this emulsion is homogenized with an oil phase containing an oil-soluble emulsifier to form an oil-in-water-in-oil emulsion (O/W/O). The water phase can then be gelled, and the filled biopolymer particles removed by centrifugation and washing as described previously.

4.7. Shearing methods

Since the size and morphology of biopolymer particles can have a significant impact on their functionality in foods, it is important to understand how these characteristics are affected by shear. Shear forces can be used to break up dispersed droplets into smaller sizes, or to cause spherical particles to become increasingly elongated and aggregated. Following the application of shear, the structure of the newly formed particles is fixed using an appropriate gelation mechanism (e.g., change in temperature or addition of gelling agent).

In the case of biopolymer particles created by aggregative phase separation, research has shown that smaller coacervate particles are formed at higher stirring speeds (Jegat & Taverdet, 2000). There is a limit, however, to this effect as excessively high shear rates can result in biopolymer particle aggregation. By controlling shear forces and particle cross-linking kinetics, it is possible to generate biopolymer particles with defined size and morphology (Dobetti & Pantaleo, 2002).

Biopolymer particles can also be fabricated from segregative phase-separated biopolymer mixtures. These systems can be made to form water-in-water (W/W) emulsions by shearing, and then the inner water phase can be gelled to create biopolymer particles (Stokes, Wolf, & Frith, 2001). When subjected to low levels of shear, the dispersed phase of these aqueous emulsions tends to form spherical droplets. As shearing rates are increased, these droplets begin to elongate into first ellipsoidal structures and then fibers or threads at higher shear rates (Wolf, Scirocco, Frith, & Norton, 2000). The extent of particle deformation is related to the viscosity of the solution, the shear rate, the particle diameter, and the interfacial tension (Van Puyvelde, Antonov, & Moldenaers, 2002). Research has shown that suspensions of non-spherical biopolymer particles have significantly different rheological properties when compared to suspensions of spherical particles. The ability of non-spherical particles to modify the rheological properties of a product without altering its composition could be exploited to develop food and pharmaceutical products with novel textures and flow properties. A number of experimental techniques have been developed to create these anisotropic particles in the laboratory (Erni, Cramer, Marti, Windhab, & Fischer, 2009).

5. Biopolymer particle properties

The functional performance of a biopolymer particle within a food product depends on its structure, electrical charge, and physicochemical properties. It is therefore useful to highlight the

most important characteristics of biopolymer particles and how these characteristics influence the macroscopic properties of foods.

5.1. Particle structure

5.1.1. External structure: particle dimension and shape

The dimensions and morphology of biopolymer particles can often be manipulated by controlling the ingredients and assembly conditions used to fabricate them. Depending on how these particles are fabricated, the dimensions of these particles can range from a few nanometers to several micrometers. The dimensions of a population of particles within a colloidal suspension are usually expressed as either a particle size distribution (PSD) or as a mean particle diameter (d) and a polydispersity index (σ). Sometimes it is important to know the fraction of particles that fall above or below some critical size, i.e., the cumulative distribution (McClements, 2005).

Biopolymer particles with different shapes can often be formed by controlling the ingredients and assembly conditions used to fabricate them, e.g., spheres, spheroids, rods, or clusters. For example, non-spherical biopolymer particles can be produced by extrusion or molding methods, or by application of shear forces during particle formation (Norton & Frith, 2001; Norton, Frith, & Ablett, 2006). The appearance, rheology, mouthfeel, and release characteristics of colloidal dispersions containing non-spherical particles are often quite different from those containing a similar amount of spherical ones. Consequently, modulations of particle shape can be used to create novel or improved textures in foods.

5.1.2. Internal structure

Biopolymer particles can be designed to have different internal structures by controlling the ingredients and assembly conditions used in their fabrication (Fig. 3):

- **Homogeneous:** The interior of a biopolymer particle may be comprised of one or more types of biopolymer that are intimately mixed with each other so that they appear to be homogeneous on the length scale considered e.g., particle diameter.
- **Heterogeneous – Bicontinuous:** The interior of a biopolymer particle may consist of two or more types of biopolymer that phase separate into regions with different compositions. For example, one phase may be a protein-rich phase while another phase may be a polysaccharide-rich or solvent-rich phase.
- **Heterogeneous – Dispersion:** The interior of a biopolymer particle may consist of two or more discrete phases, with one or more of the phases being dispersed within the other. The composition, dimensions, shape, connectivity, interactions, and spatial organization of the various phases within the particle interior may vary considerably depending on particle type. For example, the dispersed phase may be lipid droplets, solid particles, or air bubbles dispersed within a biopolymer matrix. In this case, the size, concentration and location of the dispersed particles may be important.
- **Heterogeneous – Core-shell:** A biopolymer particle may consist of two or more discrete phases, with at least one of the phases forming a shell around the other phase. This shell may vary in its composition, thickness and structure, e.g., it may be single or multiple layered.

The internal structure of biopolymer particles may have a large impact on functionality. Functional properties that may be affected by changes to internal structure include encapsulation efficiency, loading capacity, permeability, integrity, environmental responsiveness, and digestibility. In particular, particle porosity may have

a major influence on the accessibility of a bioactive trapped within its matrix. In general, a highly porous structure should allow for relatively easy access and release of an encapsulated bioactive while a dense structure would limit access and release.

5.2. Particle electrical characteristics

The electrical characteristics of biopolymer particles are determined by the electrical characteristics of the various components used to fabricate them, as well as by the pH, ionic composition and dielectric constant of the surrounding medium. Biopolymer particles may have electrical charges that range from highly positive to highly negative depending on their composition and environmental conditions (see Section 2).

Biopolymer particle charge is a crucial parameter for predicting the stability of colloidal delivery systems. The electrical charge on individual particles strongly influences whether or not particles aggregate or remain separated. If the charge is sufficiently large, then there will be a high electrostatic repulsion between them that may prevent aggregation. Particle charge also influences how they interact with other charged species in the surrounding medium. If a biopolymer particle has an opposite charge to another ionic ingredient within a food, then it may form an electrostatic complex that may precipitate and sediment.

Electrical charge also determines how biopolymer particles interact with the different surfaces of the human digestive system. A cationic biopolymer particle may bind to the anionic surface of the tongue thereby causing perceived astringency. Conversely, a cationic biopolymer particle could be designed to bind to a specific location within the gastrointestinal tract (“mucoadhesion”) to delay its transit through the body and release its bioactive at a particular site. Finally, the electrical characteristics of the molecules within a biopolymer particle may determine its internal structure, and might lead to either swelling or shrinking in response to environmental changes such as pH or ionic strength.

The electrical characteristics of biopolymer particles can be controlled by selecting one or more ingredients with different charge *versus* pH profiles.

5.3. Particle physicochemical properties

Physicochemical properties of biopolymer particles include parameters such as density, refractive index, rheology, polarity, and porosity. These basic properties determine how other molecular species interact with the particle, influencing such parameters as equilibrium partition coefficients (K_{OW}), diffusion coefficients (D), and permeability characteristics (P). Furthermore, macroscopic properties such as appearance, texture and physical stability are also determined by the physicochemical properties of particles. Since these properties have a significant impact on the overall utility of a given biopolymer particle system in foods, it is essential that these properties are carefully characterized, measured, and controlled. Some of the effects of biopolymer particles on the macroscopic physicochemical properties of food materials are reviewed below.

5.3.1. Particle integrity and environmental sensitivity

The “integrity” of a biopolymer particle is its ability to maintain its composition and structure under a given set of solution or environmental conditions, such as pH, ionic strength, and temperature. Typically, biopolymer particles should maintain their integrity under one set of conditions, but breakdown under another set of conditions. Loss of biopolymer integrity can occur by several different physicochemical mechanisms including simple diffusion, fragmentation, swelling, or erosion (Fig. 11). The mechanism

Fig. 11. Some common mechanisms for loss of particle integrity: diffusion; swelling; erosion; and, fragmentation.

involved depends on the type of biopolymer components present, the nature of the molecular interactions holding them together, and the environmental conditions.

5.3.2. Optical properties

Appearance is one of the most important attributes for a food product. Since biopolymer particles often interact with light waves, incorporating these particles into foods may impact their optical properties. The overall appearance of a food can be separated into two main attributes: (i) opacity, which is primarily determined by light scattering; and, (ii) color, which is primarily determined by selective absorption of light waves. The overall impact of biopolymer particles on the optical properties of a food depend on their concentration, size and refractive index (McClements, 2002). The impact of biopolymer particle radius on the turbidity of colloidal suspensions containing biopolymer particles is shown in Fig. 12. For small particles with a radius below approximately 25 nm, the turbidity of the suspension is predicted to be quite low. As particle size increases, turbidity increases to a maximum value at an approximate radius of 700 nm and then starts to decline at higher particle sizes. The turbidity or opacity will also increase as the refractive index contrast between the particle and its surrounding medium increases. This situation would occur if the total biopolymer concentration within a biopolymer particle increased, *i.e.*, the biopolymers become more closely packed.

The impact of biopolymer particles on optical properties has important consequences for their use in different types of foods. For instance, some products such as clear beverages should be transparent while other products such as yogurt and creamy dressings should be opaque. By controlling the size and refractive index of biopolymer particles, these particles can be incorporated into foods without affecting optical properties. Thus, small biopolymer particles ($r < 25$ nm) must be used for transparent foods and beverages as these particles do not scatter light strongly while larger biopolymer particles ($r \approx 200$ – 1000 nm) should be used for opaque products as these particles scatter light strongly.

Fig. 12. Theoretical prediction (Mie theory) of the influence of particle radius on the specific turbidity of biopolymer colloidal suspensions. The refractive index of the biopolymer particles was 1.5 and the surrounding liquid was 1.33. A wavelength of 500 nm was assumed.

5.3.3. Rheological properties

The rheology or texture of foods may also be positively or negatively affected by the incorporation of biopolymer particles. In general, the rheology of a colloidal suspension depends on particle concentration, shape, and interactions. The impact of biopolymer particles on the viscosity of fluid foods can be described by the following equation to a first approximation:

$$\frac{\eta}{\eta_0} = \left(1 - \frac{\phi_{\text{eff}}}{\phi_c}\right)^{-2} \quad (1)$$

Here, η_0 is the shear viscosity of the liquid surrounding the particles, ϕ_{eff} ($=R\phi$) is the *effective* volume fraction of the biopolymer particles, ϕ is the actual volume fraction of the biopolymer molecules that make up the particles, R is the effective volume ratio (the total effective volume occupied by the biopolymer particle divided by the total volume occupied by the actual biopolymer chains) and ϕ_c is the critical packing parameter (≈ 0.6) where spherical particles become close packed. The effective volume of a biopolymer particle may be much greater than the actual volume of the biopolymer molecules for a number of reasons: (i) *solvation* – biopolymer particles may trap solvent molecules such as water; (ii) *flocculation* – flocculated particles trap solvent molecules between them; (iii) *non-sphericity* – non-spherical particles have a greater effective volume than an equivalent mass of spherical particles.

This equation can be used to predict how biopolymer concentration and effective volume ratio affect the viscosity of a suspension of biopolymer particles (Fig. 13). In general, the viscosity of the system increases with increasing biopolymer concentration until the biopolymer particles become tightly packed together, i.e., the critical packing parameter (ϕ_c) is reached (McClements, 2005). Above ϕ_c , the system becomes solid-like and may have a yield stress and elastic modulus. The effectiveness of biopolymer particles at increasing the viscosity of the system increases as they entrap more solvent (higher R) within their structure (Fig. 13). This explains why biopolymers with highly open structures (such as many gums) are excellent thickening agents. The rheology of colloidal dispersions is also highly dependent on colloidal interactions. If particles are

Fig. 13. Theoretical prediction of the influence of biopolymer concentration and packing on the apparent viscosity of biopolymer colloidal suspensions. Here R is the effective volume ratio, i.e. the particle volume (biopolymer + water) divided by the biopolymer volume. It is assumed the biopolymer particles are spherical.

attracted to each other, then a suspension tends to be much more viscous or even gel-like.

Biopolymer particles may be designed to provide desirable rheological attributes to a product such as thickness or creaminess, or they may be designed to have negligible impact on the textural attributes of a product. In summary, the main characteristics of biopolymer particles that can be designed to control their impact on food texture are: composition, shape and interactions. The ability of biopolymer particles to increase solution viscosity will increase as they become more solvated, more asymmetrical, or more aggregated.

5.3.4. Stability

For any delivery system, it is essential that the system remain stable throughout the entire life cycle of the product. Furthermore, the biopolymer particles should not adversely impact the normal shelf-life of the product itself. Biopolymer particles may become unstable in a food product through a variety of mechanisms, including gravitational separation (creaming or sedimentation), aggregation (flocculation or coalescence), volumetric changes (swelling or shrinking), and dissociation (erosion or disintegration). To ensure stability, it is imperative that the major physicochemical mechanism that promotes particle instability be identified so that it can be successfully inhibited or prevented. The dominant instability mechanism depends on biopolymer particle characteristics (such as composition, size, charge, and structure), as well as environmental conditions (such as temperature, pH and ionic strength).

The creaming rate of non-interacting rigid spherical particles in a dilute Newtonian liquid is given by Equation (2).

$$U = -\frac{2gr^2(\rho_2 - \rho_1)}{9\eta_1} \quad (2)$$

Here, U is the creaming velocity (positive U for creaming; negative U for sedimentation), g is the acceleration due to gravity, r is the radius of the particle, ρ is the density, η is the shear viscosity, and the subscripts 1 and 2 refer to the continuous phase and particles, respectively. More sophisticated mathematical models are available that take into account polydispersity, non-spherical particles, particle fluidity, particle–particle interactions and non-Newtonian fluids (McClements, 2005). The overall density of a biopolymer particle will depend on the densities (ρ) and

concentrations (ϕ) of the various components within the particle. To a first approximation, the overall density of a biopolymer particle containing biopolymer, water, and oil in different ratios is given by:

$$\rho_{\text{Particle}} = \phi_B \rho_B + \phi_L \rho_L + (1 - \phi_B - \phi_L) \rho_W \quad (3)$$

Here the subscripts B, W and O refer to the biopolymer, water and oil phases, respectively. In many applications, biopolymer particles may contain other components (such as minerals or solids) and so the above equation must be extended.

In the absence of lipid droplets, the sedimentation rate ($-U$) increases as the size of biopolymer particles increase (Fig. 14a). The impact of filled particle composition (biopolymer, lipid and water content) on the sedimentation stability of an aqueous biopolymer particle suspension is shown in Fig. 14b. The rate and direction of gravitational separation depends on the composition of the biopolymer particles. At low lipid droplet concentrations and high biopolymer concentrations, the particles tend to sediment ($-U$). On the other hand, at high lipid droplet concentrations and low biopolymer concentrations the particles tend to cream ($+U$). Interestingly, there are particular combinations of lipid, biopolymer and water that provide density matching between the filled

biopolymer particles and the surrounding aqueous phase, e.g., 50% lipid, 10% biopolymer, and 40% water (Fig. 14b). The use of density matched particles for low viscosity products may be imperative to preventing particle sedimentation during the shelf-life. Particle sedimentation would be less of a concern for highly viscous products such as desserts or sauces.

Mathematical models may also be useful in predicting the impact of other types of instabilities on the shelf-life of biopolymer particles. For example, the tendency for particle aggregation to occur can be predicted by calculating the relative strength of the various attractive and repulsive colloidal interactions operating between them. These interactions include van der Waals, steric, electrostatic and hydrophobic forces (McClements, 2005). When attractive forces dominate, particles have a tendency to aggregate, but when repulsive forces dominate, particles resist aggregation. The tendency for swelling, shrinking, erosion or dissociation to occur is highly system specific and will depend on the type of bonds holding the biopolymer molecules together in the particles.

5.3.5. Release characteristics

Biopolymer particles may be designed to encapsulate, protect and release a specific functional food component, such as a flavor, antimicrobial, antioxidant, or bioactive nutrient. Thus, these particles may need to be designed to release an active component at a particular site in the body. Developing a model for such processes requires an understanding of the physicochemical mechanisms leading to release. Four main mechanisms, which primarily differ in the role the carrier particle plays in controlling release, have been described (Fig. 11):

Diffusion: The active component simply diffuses into the surrounding medium through the biopolymer particle matrix, which remains intact. In this case, the mass transport rate will depend on the solubility of the substance in the particle matrix and its diffusion coefficient through the matrix. For biopolymer networks, the diffusion rate may depend on the mesh size of the biopolymer network compared to the size of the diffusing active component, as well as any specific electrostatic or hydrophobic attractions between the biopolymer network and the active component.

Erosion: The active component is released into the media due to erosion processes taking place at either the outer layer or throughout the entire volume of the biopolymer matrix. Matrix erosion may be due to physical, chemical or enzymatic degradation processes, such as dissociation of physical bonds or the hydrolysis of covalent bonds.

Fragmentation: The active component is released into the media due to the physical disruption of the carrier, which is either fragmented or fractured, by applying shear or compression forces. The bioactive will still diffuse out of the particles, but the rate of release will be quick due to the increased surface area and decreased diffusion path.

Swelling/shrinking: Core release may be induced by the uptake of solvent by the biopolymer particles, which causes the particles to swell. For example, an active component could be encapsulated within a solid biopolymer particle or within a hydrogel biopolymer particle with a pore size small enough to prevent it from leaching out. Once the particle absorbs solvent, it will swell and the active component can then diffuse out. The active component could be loaded into the biopolymer particles by initially swelling them in its presence, and then changing the solution conditions to induce shrinkage.

Mathematical theories have been developed to model different types of release mechanisms involving particulate systems

Fig. 14. (a) Theoretical prediction of the influence of biopolymer particle composition and size on the stability to gravitational separation. All biopolymer particles were considered to have 10% biopolymer, and 0–80% lipid. Assumed densities: biopolymer = 1500 kg m^{-3} ; water = 1000 kg m^{-3} ; oil = 900 kg m^{-3} . (b) Theoretical prediction of the influence of biopolymer particle composition (lipid and biopolymer concentration) on the stability to gravitational separation. Assumed densities: biopolymer = 1500 kg m^{-3} ; water = 1000 kg m^{-3} ; oil = 900 kg m^{-3} .

Fig. 15. Theoretical prediction of the release rate of lipophilic molecules from spherical biopolymer particles of different radius. It was assumed that the biopolymer particles contained 20% oil, that the oil–water partition coefficient was 1000, and that the effective diffusion coefficient of lipophilic molecules through the biopolymer particles was $1.7 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$. M_t/M_0 is the fraction of material that has been released after time t .

(Pothakamury & BarbosaCanovas, 1995; Siepmann & Siepmann, 2008). To select an appropriate model of release, one must first know the origin of the release mechanism as diffusion, erosion, swelling, or fragmentation. Some of the physical and chemical parameters required for these models include the initial particle size distribution, the concentrations of the active component within the particle and surrounding medium (equilibrium partition coefficients), and the transport rate of the active component in the system (translational diffusion coefficients). While such empirical data is often available for drug delivery systems, the data for food delivery systems are only now starting to accumulate (Serenio, Hill, Taylor, Mitchell, & Davies, 2009). The adoption of these models will help food scientists to rationalize the design, fabrication and utilization of biopolymer particle systems with specific release characteristics.

To illustrate the usefulness of mathematical models to predict bioactive release, consider the release of a lipophilic active component encapsulated within spherical filled biopolymer particles suspended in water as depicted in Fig. 15. In this example, the release mechanism is a diffusion process. In this graph, M_t represents the amount of lipophilic bioactive released at time (t) while M_0 represents the amount of bioactive present in the initial system. The mathematical equation used to make these predictions is a modified version of the Crank diffusion equation that takes into account the presence of lipid droplets within the biopolymer particles (Lian, Malone, Homan, & Norton, 2004). According to this model, bioactive release rates increase with decreasing biopolymer particle size, as would be expected since the diffusion path length will decrease as the size of the particle decreases. Based on these predictions, particles could be designed with different release properties depending on their final application.

6. Conclusions

The development of biopolymer systems for the encapsulation of lipophilic bioactives is an important area of research for the food industry. Many beneficial food bioactives such as ω -3 fatty acids, carotenoids, fat-soluble vitamins, and phytosterols are lipophilic. The hydrophobic nature of these compounds makes their

incorporation into aqueous foods and beverages challenging. In addition to incorporation problems, many of these lipophilic compounds are also chemically unstable and tend to degrade during storage when incorporated into foods. The use of biopolymers such as proteins and polysaccharides to encapsulate and protect these bioactives is thus highly desirable. At present, our understanding of how to create biopolymer particles with specific functional attributes is still rather limited, and a better understanding of structure–function relationships is needed. Identification of the most appropriate ingredients and conditions required to create these particles requires knowledge of the molecular and functional characteristics of the biopolymers used, as well as of the physicochemical mechanisms underlying particle formation. From a practical point of view, the application of biopolymer delivery systems on an industrial scale is limited to those systems that can be manufactured economically and that are robust enough for commercial applications.

Future research on biopolymer delivery systems should focus on developing new methods for fabrication and refining or adapting current methods for their application to foods. Since many of the methods used to create delivery systems have been developed by other industries such as pharmaceuticals and polymers, it is imperative that these methods be adapted so that only food-grade ingredients and economical processes are used. As for the development of new techniques, advances in this area will likely come from a more fundamental understanding of biopolymer structure and interactions. In summary, biopolymer-based structured delivery systems have tremendous potential to improve the quality of foods and beverages. The challenge to food scientists is to develop the methods and techniques that can be used to fabricate these systems.

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