Chapter 1

Structural Design Principles for Improved Food Performance: Nanolaminated Biopolymer Structures in Foods

David Julian McClements

Biopolymers and Colloids Research Laboratory, Department of Food Science, University of Massachusetts, Amherst, MA 01003

The bulk physicochemical, sensory and physiological attributes of most foods are determined by the characteristics, interactions and structural organization of the various ingredients they contain. Biopolymers are important functional ingredients in many foods, contributing to their overall texture, stability, appearance, flavor and nutritional quality. An improved understanding of the molecular and physicochemical basis of biopolymer functionality in foods can lead to the design of improved or novel functional attributes into foods. This chapter describes how nanolaminated layers can be formed from food biopolymers, and highlights their potential applications within the food industry. Electrostatic layer-by-layer (LbL) deposition of charged biopolymers can be used to form nano-structured interfacial layers with specific properties, *e.g.,* charge, thickness, porosity, permeability, responsiveness. These layers may be formed around macroscopic, microscopic or nanoscopic materials, and are therefore applicable to a wide range of food categories. Systematic manipulation of interfacial properties can be used to create materials with novel functional attributes, *e.g.,* improved stability to environmental stresses or controlled release characteristics. The potential of this technique is highlighted using recent studies on the formation of nanolaminated coatings on microscopic lipid droplets and macroscopic hydrogel surfaces.

Huang et al.; Micro/Nanoencapsulation of Active Food Ingredients
ACS Symposium Series; American Chemical Society: Washington, DC, 2009.

Introduction

There are a wide number of different lipophilic components within the food industry that need to be delivered in an edible form, including bioactive lipids, vitamins, flavors, antimicrobials and antioxidants *e.g.,* ω-3 fatty acids, phytosterols, lycopene, lutein, β-carotene, coenzyme A, vitamins A and D, citrus oils, essential oils [1-4]. In many cases, it is advantageous to deliver these lipophilic components in an aqueous medium because this increases their stability, palatability, desirability and bioactivity. For example, an active lipophilic component might be incorporated into a beverage or food that could easily be consumed by drinking or eating. Nevertheless, there are often a variety of technical challenges that need to be overcome before an active lipophilic component can be successfully incorporated into an aqueous-based delivery system. Lipophilic active components come in a wide variety of different molecular forms, which lead to differences in their physicochemical and physiological properties, such as chemical stability, physical state, solubility, rheology, optical properties, and bioactivity. Consequently, different delivery systems are usually needed to address specific molecular, physicochemical and physiological concerns associated with each active component. In general, an edible delivery system must have a number of attributes:

- It must be capable of efficiently encapsulating an appreciable amount of functional agent and keeping it entrapped.
- It may have to protect the functional agent from chemical degradation so that it remains in its active state.
- It may have to control the release of the functional agent, *e.g.,* the release rate or the specific environmental stimuli that triggers release.
- It may have to be compatible with the surrounding food or beverage matrix, without causing any adverse affects on product appearance, rheology, mouth feel, flavor or shelf life.
- It may have to resist the environmental stresses foods or beverages experience during their production, storage, transport and utilization *e.g.,* heating, chilling, freezing, dehydration, or shearing.
- It should be prepared completely from generally recognized as safe (GRAS) ingredients using simple cost-effective processing operations.
- It should not adversely impact the bioavailability of the encapsulated material.

A wide variety of different types of delivery system have been developed to encapsulate lipophilic functional agents, including simple solutions, association colloids, emulsions, biopolymer matrices, powders, *etc.* Each type of delivery system has its own advantages and disadvantages for encapsulation, protection

and delivery of functional agents, as well as in its cost, regulatory status, ease of use, biodegradability, biocompatibility *etc.*

This chapter will begin by introducing the basic principles of structural design for creating delivery systems with improved stability and novel functional performance. We will then focus on a particular structural design principle based on layer-by-layer (LbL) electrostatic deposition that can be used to form nanolaminated coatings around microscopic and macroscopic objects. The potential of this method for creating emulsion-based delivery systems with improved stability to environmental stresses will then be demonstrated. Finally, the potential of using the LbL technique to form laminated functional coatings on macroscopic food surfaces (such as fruits, vegetables, fish and meats) will be highlighted.

Structural Design Principles

In this section, a brief outline of the major building blocks available to create food grade delivery systems, as well as the major molecular interactions and structural design principles that can be used to assemble them into functional systems is given.

Building Blocks

The major building blocks that can be used to assemble food-grade delivery systems are outlined below:

- *Lipids.* Lipids are predominantly non-polar substances that are highly hydrophobic. In the food industry, the main sources of lipids are triacylglycerols, which may come from animal, fish, or plant origins. Lipids can be used to solubilize non-polar lipophilic components in foods, and are commonly used in delivery systems based on emulsions or microemulsions.
- *Surfactants.* Surfactants are surface-active molecules that consist of a hydrophilic head group and a lipophilic tail group. The functional performance of a specific surfactant depends on the molecular characteristics of its head and tail groups. Food-grade surfactants come in a variety of different molecular structures. Their head groups may vary in physical dimensions and electrical charge (positive, negative, zwitterionic or non-ionic), while their tail groups may vary in number (typically one or two), length (typically 10 to 20 carbons per chain) and degree of saturation (saturated or unsaturated). Surfactants are typically used to form emulsions,

association colloids or biopolymer complexes that are suitable for use as delivery systems.

- *Biopolymers.* The two most common classes of biopolymer used as structure forming materials in the food industry are proteins and polysaccharides. Ultimately, the functional performance of food biopolymers (*e.g.,* solubility, self-association, binding and surface activity) is determined by their unique molecular characteristics (*e.g.,* molecular weight, conformation, flexibility, polarity, hydrophobicity and interactions). These molecular characteristics are determined by the type, number and sequence of the monomers that make up the polymer chain. The monomers vary according to their polarity, charge, physical dimensions, molecular interactions and chemical reactivity. Biopolymers may adopt a variety of conformations in food systems, which can be conveniently divided into three broad categories: globular, rod-like or random coil. Globular biopolymers have fairly rigid compact structures, rod-like biopolymers have fairly rigid extended structures (usually helical), and random-coil biopolymers have highly dynamic and flexible structures. In practice, many biopolymers do not have exclusively one type of conformation, but have some regions that are random coil, some that are rod-like and some that are globular. Biopolymers can also be classified according to the degree of branching of the chain. Most proteins have linear chains, whereas polysaccharides can have either linear or branched chains. In solution, biopolymers may be present as individual molecules or they may be present as supra-molecular structures where they are associated with one or more molecules of the same or different kind. Finally, it should be mentioned that biopolymers may undergo transitions from one conformation to another, or from one aggregation state to another, if their environment is altered, *e.g.,* pH, ionic strength, solvent composition or temperature. The conformation and interactions of biopolymers play a major role in determining their ability to form structured delivery systems.

Some of the most important food-grade components that are available as building blocks to form structured delivery systems are listed in Table 1. The choice of a particular food-grade component depends on the type of structure that needs to be formed, as well as its legal status, cost, usage levels, ingredient compatibility, stability and ease of utilization.

Molecular Interactions

Knowledge of the origin and nature of the various molecular forces that act between food components is also important for understanding how to assemble delivery systems with specific structures from food grade ingredients:

Table 1. Major food-grade structural components that can be used to construct delivery systems for nutraceuticals.

Name	Important Characteristics	Examples
Lipids	Chemical stability Melting profile Polarity	*Animal fats*: beef, pork, chicken *Fish oils*: cod liver, menhedan, salmon, tuna *Plant oils*: palm, coconut, sunflower, safflower, corn, flax seed, soybean *Flavor oils*: lemon, orange
Surfactants	Solubility (HLB) Charge Molecular geometry Surface load	*Non-ionic*: Tween, Span *Anionic*: SLS, DATEM, CITREM *Cationic*: Lauric Arginate Zwitterionic: lecithin
Biopolymers	Molar Mass Conformation Charge Hydrophobicity Flexibility	*Globular Proteins*: whey, soy, egg *Flexible Proteins*: casein, gelatin *Non-ionic Polysaccharides*: Starch, Dextran, Agar, Galactomannans, Cellulose *Anionic Polysaccharides*: Alginate, Pectin, Xanthan, Carrageenan, Gellan, Gum Arabic *Cationic Polysaccharides*: Chitosan

- *Electrostatic interactions*. Electrostatic interactions are important for food components that have an electrical charge under the utilization conditions, *e.g.*, proteins, ionic polysaccharides, ionic surfactants, phospholipids, mineral ions, acids and bases. Electrostatic interactions may be either attractive or repulsive depending on whether the charge groups involved have opposite or similar signs. The sign and magnitude of the charge on food components usually depends on solution pH, since they have weak acid or base groups. The strength and range of electrostatic interactions decreases with increasing ionic strength due to electrostatic screening effects. The most common means of manipulating the electrostatic interactions between food components are therefore to alter the pH and/or ionic strength of the aqueous solution. Alternatively, electrostatic bridging interactions may be used to assemble food components.
- *Hydrophobic interactions*. Hydrophobic interactions are important for food components that have appreciable amounts of non-polar groups, and they manifest themselves as a tendency for the non-polar groups to associate with each other in water. Hydrophobic interactions may be manipulated somewhat by altering the temperature or changing the polarity of an aqueous solution (*e.g.*, by adding alcohol).
- *Hydrogen bonding*. Hydrogen bonding is important for food components that have polar groups that are capable of forming relatively strong hydrogen bonds with other polar groups on the same or on different molecules. Hydrogen bonds tend to decrease in strength as the temperature is increased, and they often form between helical or sheet-like structures on the same or different biopolymers.
- *Steric exclusion*. Steric exclusion effects are important for food components that occupy relatively large volumes within a system, because they exclude other components from occupying the same volume, thereby altering the configurational and/or conformational entropy of the system.

The relative importance of these interactions in a particular system depends on the types of food components involved (*e.g.*, molecular weight, charge density *vs.* pH profile, flexibility, hydrophobicity), the solution composition (*e.g.*, pH, ionic strength and dielectric constant) and the environmental conditions, (*e.g.*, temperature, shearing). By modulating these parameters it is possible to control the interactions between the food components and therefore assemble novel structures that can be used as delivery systems.

Structural Design Principles

In this section, some of the major structural design principles that can be used to assemble novel structures from food components are highlighted.

- *Phase separation.* When two different materials are mixed together they may be completely miscible and form a single phase, or they may separate into a number of different phases, depending on the relative strength of the interactions between the different types of molecules present (compared to the thermal energy). There are a number of examples of phase separation involving food components that can be used to create novel structures. The most common example is the phase separation of oil and water due to the fact that oil-water interactions are strongly unfavorable (compared to the average of water-water and oil-oil interactions), which is the basis for the formation of emulsion systems. Another example is the phase separation of mixed biopolymer solutions as a result of relatively strong thermodynamically unfavorable interactions between the different types of biopolymers (*e.g.,* electrostatic repulsion and/or steric exclusion). As a result of this type of phase separation the mixed biopolymer system separates into two different phases: one phase is enriched with one type of biopolymer and depleted with the other type, while the opposite situation occurs in the other phase.
- *Spontaneous Self-assembly.* Under appropriate environmental conditions, certain types of food components spontaneously assemble into well-defined structures since this minimizes the free energy of the system, *e.g.,* micelles, vesicles, fibers, tubes, liquid crystals. The driving force for self-assembly is system dependent, but often involves hydrophobic attraction, electrostatic interactions and/or hydrogen bond formation. Association colloids, such as micelles, vesicles and microemulsions, are some of the most common types of self-assembled structures in food materials. The primary driving force for the spontaneous formation of these structures is the hydrophobic effect, which causes the system to adopt a molecular organization that minimizes the unfavorable contact area between the non-polar tails of the surfactant molecules and water.
- *Directed self-assembly.* Directed self-assembled systems do not form spontaneously if all the components are simply mixed together. Instead, the preparation conditions (*e.g.,* order of mixing, temperature-, pH-or ionic strength-time profiles) must be carefully *controlled* to direct the different components so that they are assembled into a particular metastable structure. The driving force for directed-assembly of food structures is also system-dependent, but again hydrophobic, electrostatic and hydrogen bonding interactions are common. A widely used directed self-assembly method is layer-by-layer (LbL) electrostatic deposition of polyelectrolytes and other charged substances onto oppositely charged surfaces due to electrostatic attraction (which will be covered in this chapter). Another example of this method is the formation of hydrogels from biopolymers. For example, when a solution of gelatin is cooled below a certain temperature a coil-to-helix transition occurs, which is followed by extensive hydrogen bond formation

between helices on different gelatin molecules. These hydrogen bonded regions act as physical cross-links between the gelatin molecules that may eventually lead to gelation. The gelatin molecules therefore self-assemble under the prevailing environmental conditions, but the precise details of the structures formed (*i.e.,* the number, position and length of the cross-links) depends on the specific preparation conditions used (*e.g.,* time-temperature profile, shearing).

- *Directed assembly.* In principle, it is also possible to form structured delivery systems by physically bringing molecules together in well-defined ways, *e.g.,* by micro-manipulation methods. Nevertheless, most of these technologies are unlikely to find widespread use in the food industry, at least in the foreseeable future, due to a variety of economic and practical constraints, such as the fact that expensive equipment is needed to fabricate and characterize the structures formed, and the throughout of fabricated structures is likely to be extremely low.

Structured Delivery Systems

Structural design principles can be used to create a variety of different delivery systems that can be utilized to encapsulate lipophilic components. Some structured delivery systems that can be created using food-grade ingredients and common unit operations that are based on emulsion technology are highlighted in Figure 1.

- *Conventional Emulsions.* Conventional oil-in-water (O/W) emulsions consist of emulsifier-coated lipid droplets dispersed in an aqueous continuous phase. They are formed by homogenizing an oil and water phase together in the presence of a hydrophilic emulsifier.
- *Multiple Emulsions.* Multiple water-in-oil-in-water (W/O/W) emulsions consist of small water droplets contained within larger oil droplets that are dispersed in an aqueous continuous phase. They are normally produced using a two-step procedure. First, a W/O emulsion is produced by homogenizing water, oil and an oil-soluble emulsifier. Second, a W/O/W emulsion is then produced by homogenizing the W/O emulsion with an aqueous solution containing a water-soluble emulsifier.
- *Multilayer Emulsions.* Multilayer oil-in-water (M-O/W) emulsions consist of small oil droplets dispersed in an aqueous medium, with each oil droplet being surrounded by a nano-laminated interfacial layer, which usually consists of emulsifier and biopolymer molecules. They are normally formed using a multiple-step procedure. First, an oil-in-water emulsion is prepared by homogenizing an oil and aqueous phase together in the presence of an ionized water-soluble emulsifier. Second, an oppositely charged polyelectrolyte is added to the system so that it adsorbs to the droplet

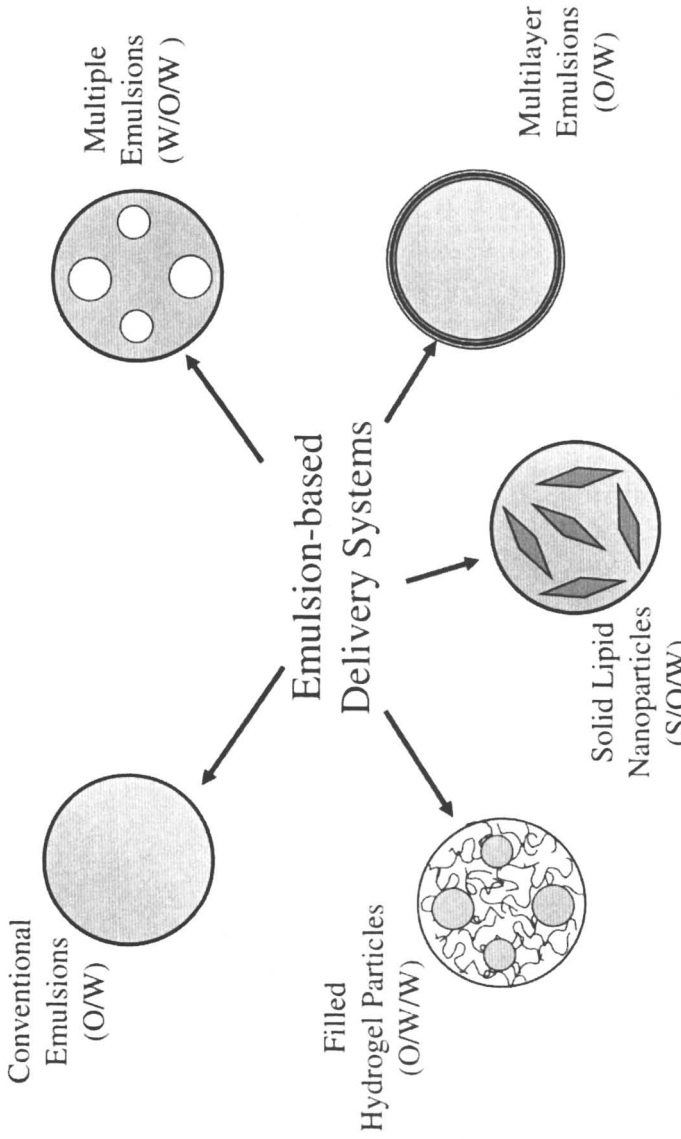

Figure 1. Some emulsion-based structured delivery systems that could potentially be used to encapsulate and delivery lipophilic functional agents.

surfaces and forms a two-layer coating around the droplets. This procedure can be repeated to form oil droplets coated by nano-laminated interfaces containing three or more layers by successively adding polyelectrolytes with opposite charges.

- *Solid Lipid Nanoparticles.* Solid lipid nanoparticles (SLN) are similar to conventional emulsions consisting of emulsifier-coated lipid droplets dispersed in an aqueous continuous phase. However, the lipid phase is either fully or partially solidified, and the morphology and packing of the crystals within the lipid phase may be controlled. SLN are formed by homogenizing an oil and water phase together in the presence of a hydrophilic emulsifier at a temperature above the melting point of the lipid phase. The emulsion is then cooled (usually in a controlled manner) so that some or all of the lipids within the droplets crystallize.
- *Filled Hydrogel Particles.* Filled hydrogel particle emulsions consist of oil droplets contained within hydrogel particles that are dispersed within an aqueous continuous phase. They can therefore be considered to be a type of oil-in-water-in-water (O/W1/W2) emulsion. There are a number of different ways to form this kind of system based on aggregative or segregative phase separation of biopolymers in solution.

The functional performance of a particular delivery system can be controlled by varying the properties of the structured particles (Figure 1): composition (*e.g.,* ratio of oil, water and biopolymer, oil type, biopolymer type); dimensions (*e.g.,* particle radii, film thicknesses), physical state (*e.g.,* solid or liquid); permeability; polarity *etc.*

Multilayer Emulsion-based Delivery Systems: Lipid Droplets Coated by Nanolaminated Coatings

In this section, the focus will be on the development of multilayer emulsions that could be used as delivery systems for lipophilic functional components. Conventionally, oil-in-water (O/W) emulsions are created by homogenizing oil and aqueous phases together in the presence of an emulsifier [5, 6]. The emulsifier adsorbs to the surfaces of the droplets formed during homogenization, where it reduces the interfacial tension and facilitates further droplet disruption. In addition, the adsorbed emulsifier forms a protective coating around the droplets that prevents them from aggregating. Many different kinds of emulsifiers are available for utilization in food products, with the most important being small molecule surfactants, phospholipids, proteins, and polysaccharides. Each type of emulsifier varies in its effectiveness at producing small droplets during homogenization, and its ability to prevent droplet aggregation under different environmental stresses, such as pH, ionic strength,

heating, freezing and drying. Food emulsifiers also differ in their cost, reliability, ease of utilization, ingredient compatibility, label friendliness and legal status. For these reasons, there is no single emulsifier that is ideal for use in every type of food product. Instead, the selection of a particular emulsifier (or combination of emulsifiers) for a specific food product depends on the type and concentration of other ingredients that it contains, the homogenization conditions used to produce it, and the environmental stresses that it experiences during its manufacture, storage and utilization.

Using conventional food emulsifiers and homogenization techniques there are only a limited range of functional attributes that can be achieved in emulsion-based delivery systems. This has motivated a number of researchers to examine alternative means of improving emulsion stability and performance. One strategy has been to create oil-in-water emulsions containing lipid droplets surrounded by multi-component nano-laminated interfacial coatings consisting of emulsifiers and/or biopolymers [7-23]. In this "layer-by-layer" (*LbL*) electrostatic deposition approach, an ionic emulsifier that rapidly adsorbs to the surface of lipid droplets during homogenization is used to produce a *primary* emulsion containing small droplets, then an oppositely charged biopolymer is added to the system that adsorbs to the droplet surfaces and produces *secondary* emulsions containing droplets coated with an emulsifier-biopolymer interfacial layer (Figure 2). This latter procedure can be repeated to form lipid droplets covered by coatings consisting of three or more layers, *e.g.,* emulsifier – biopolymer 1 – biopolymer 2. Emulsions containing lipid droplets surrounded by multi-layered interfacial coatings have been found to have better stability to environmental stresses than conventional oil-in-water emulsions under certain circumstances (18-22). They can also be used to protect lipophilic functional components within lipid droplets from chemical degradation [16, 17], or to develop controlled or triggered release systems [8, 21].

The *LbL*-electrostatic deposition method therefore offers a promising way to improve the stability and performance of emulsion-based delivery systems. Nevertheless, the choice of an appropriate combination of emulsifier and biopolymers is essential to the success of this approach, as well as determination of the optimum preparation conditions (*e.g.,* droplet concentration, biopolymer concentration, pH, ionic strength, order of addition, stirring speed, washing, floc disruption, and temperature) [18, 20, 22]. The purpose of this section is to provide an overview of recent research that has been carried out in our laboratory on the development, characterization and application of O/W emulsions containing lipid droplets surrounded by nanolamiated coatings of emulsifier and biopolymer. In particular, we will focus on the use of the *LbL* electrostatic deposition technique to create emulsions with improved resistance to environmental stresses, such as pH, ionic strength, thermal processing, freezing, dehydration, and lipid oxidation. These multilayer emulsions may be useful for the encapsulation and delivery of lipophilic functional ingredients.

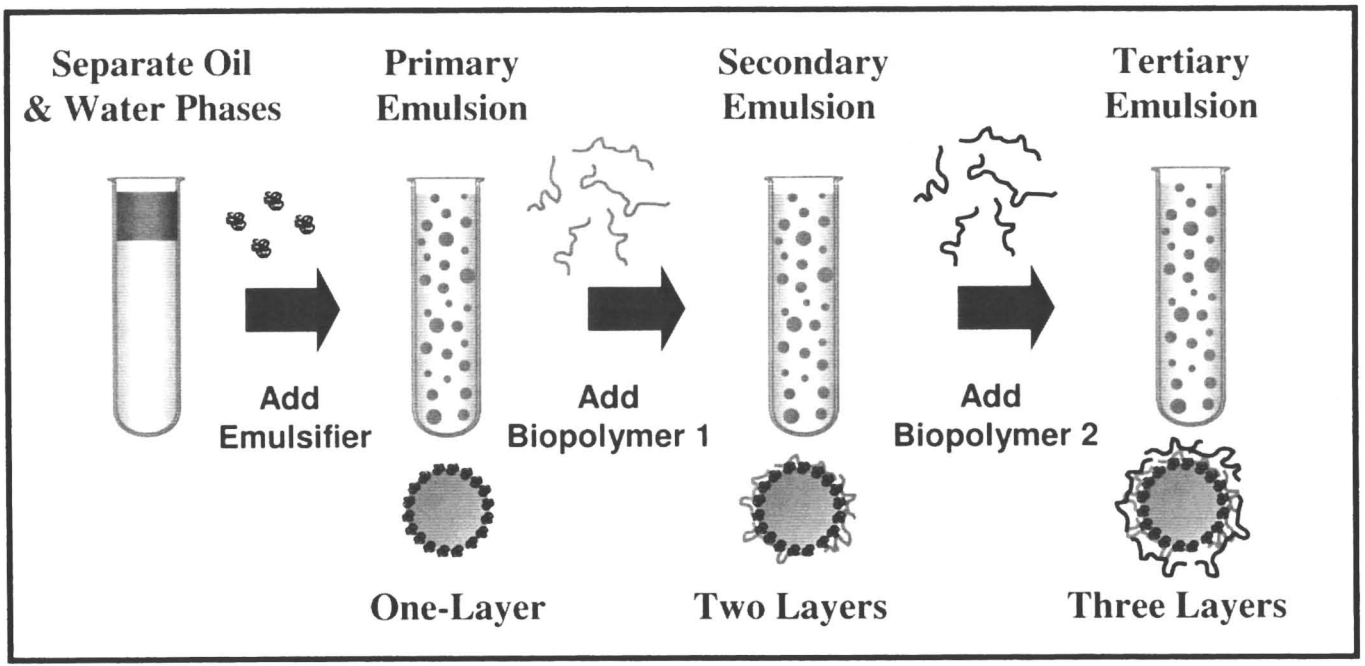

Figure 2. Schematic representation of coating lipid droplets in oil-in-water emulsions with laminated coatings using the layer-by-layer electrostatic deposition method.

Huang et al.; Micro/Nanoencapsulation of Active Food Ingredients
ACS Symposium Series; American Chemical Society: Washington, DC, 2009.

Preparation of multilayered emulsions

Oil-in-water emulsions containing oil droplets surrounded by multi-layered interfacial coatings can be prepared using a multiple-step process [12, 18]. For example, the following procedure could be used to create emulsion droplets coated by three layers, *e.g.,* emulsifier-biopolymer 1-biopolymer 2 (Figure 2). First, a *primary* emulsion containing electrically charged droplets surrounded by a layer of emulsifier is prepared by homogenizing oil, aqueous phase and a water-soluble ionic emulsifier together. Second, a *secondary* emulsion containing charged droplets stabilized by emulsifier-biopolymer 1 layers is formed by incorporating biopolymer 1 into the primary emulsion. Biopolymer 1 normally has to have an opposite electrical charge than the droplets in the primary emulsion (although this is not always necessary if there are significantly big patches of opposite charge on the droplet surface). If necessary mechanical agitation is applied to the secondary emulsion to disrupt any flocs formed because of bridging of droplets by biopolymer molecules. In addition, the secondary emulsion may be washed (*e.g.,* by filtration or centrifugation) to remove any free biopolymer remaining in the continuous phase. Third, *tertiary* emulsions containing droplets stabilized by emulsifier-biopolymer 1-biopolymer 2 interfacial layers are formed by incorporating biopolymer 2 into the secondary emulsion. Biopolymer 2 normally has to have an opposite electrical charge than the droplets in the secondary emulsion (but see above). If necessary mechanical agitation is applied to the tertiary emulsion to disrupt any flocs formed, and the emulsion may be washed to remove any non-adsorbed biopolymer. This procedure can be continued to add more layers to the interfacial coating. The adsorption of the biopolymers to the droplet surfaces can be conveniently monitored using ζ-potential measurements (Figures 3 and 4), whereas the stability of the emulsions to flocculation can be monitored by light scattering, microscopy or creaming stability measurements (Figures 3 and 4) [12-14, 20]. Since the major driving force for adsorption of biopolymers to the droplet surfaces is electrostatic in origin, it is important to control the pH and ionic strength of the mixing solution.

Unless stated otherwise, the results reported below are for oil-in-water emulsions containing lipid droplets coated by interfacial layers comprising of β-lactoglobulin (primary) and β-lactoglobulin-pectin (secondary). These emulsions were formed by mixing a β-lactoglobulin-stabilized emulsion with a pectin solution at pH 7 where the protein and polysaccharide were both negatively charged so that no adsorption occurred. Then, the pH of the solution was adjusted so that the protein-coated droplets became positively charged (or had positive patches), which promoted pectin adsorption [11, 19, 24]. The pectin concentration was controlled to avoid both bridging and depletion flocculation [11, 18, 19].

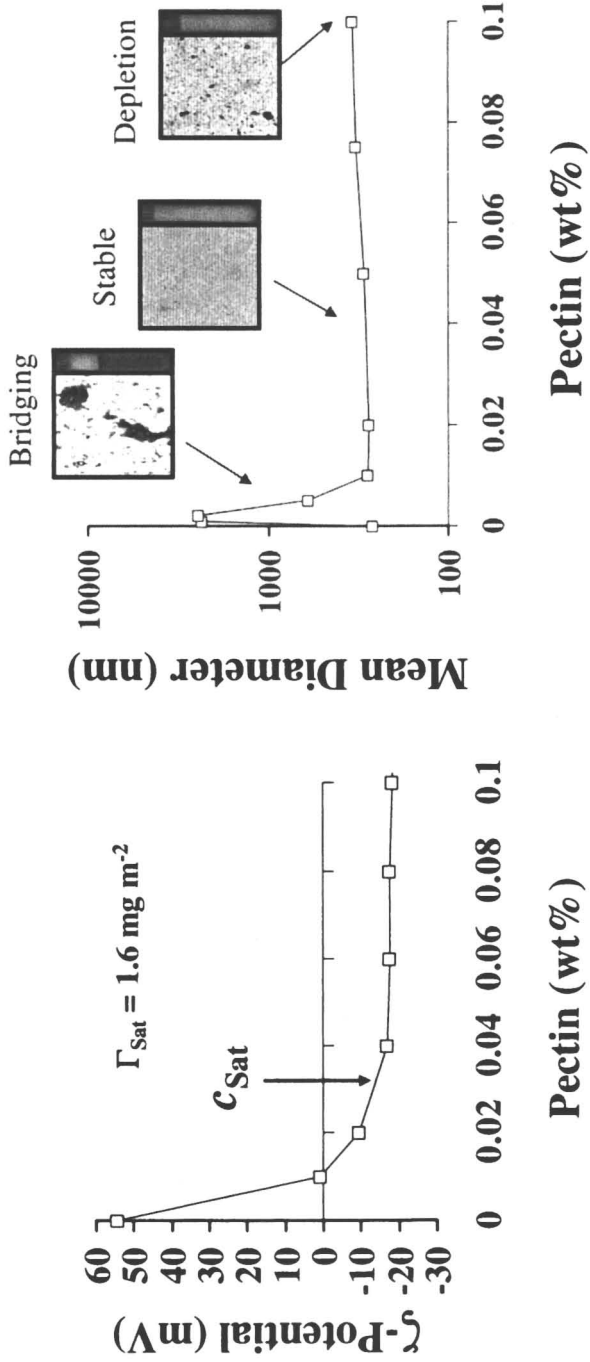

Figure 3. The formation of stable multilayer emulsions can be conveniently monitored using ζ-potential, particle size and microscopy measurements.

Improved Stability to Environmental Stresses

Food emulsions experience a variety of different environmental stresses during their manufacture, storage, transport and utilization, including pH extremes, high ionic strengths, thermal processing, freeze-thaw cycling, dehydration, and mechanical agitation [5]. Many of the emulsifiers currently available for utilization within the food industry provide limited stability to these environmental stresses. In this section, some of the recent work carried out in our laboratories on utilizing the *LbL*-electrostatic deposition technique to improve emulsion stability to various environmental stresses is reviewed.

pH

The influence of pH on the ζ-potential, mean particle diameter and creaming stability of primary (β-lactoglobulin) and secondary (β-lactoglobulin-pectin) emulsions was measured (Figure 4). The ζ-potential of the protein-coated droplets in the primary emulsions changed from negative to positive as the pH decreased from 7 to 3, with the point of zero charge being around pH 5 (Figure 4). This can be attributed to the fact that the isoelectric point of the adsorbed proteins is around pH 5. The ζ-potential of the β-lactoglobulin-pectin coated droplets in the secondary emulsions had a similar negative charge as the β-lactoglobulin-coated droplets in the primary emulsions at pH values > 6, which indicates that the anionic pectin molecules did not adsorb to the anonic droplets. When the pH was decreased below 6 the charge on the secondary emulsion droplets was more negative than that on the primary emulsion droplets, which indicated pectin adsorption. Indeed, the primary emulsion droplets were cationic at low pH, whereas the secondary emulsion droplets were anionic. This may be important for designing delivery systems that have tunable charge characteristics so that they can adsorb to specific charged sites, or to alter the mouthfeel of delivery systems.

The influence of pH on the mean particle diameter and creaming stability of the droplets in the primary and secondary emulsions is also shown in Figure 4. The primary emulsion was stable to flocculation and creaming at low and high pH due to the strong electrostatic repulsion between the droplets, but was unstable to flocculation and creaming around the isoelectric point of the adsorbed protein because of the relatively low net charge on the droplets. On the other hand, the secondary emulsions were stable across the whole pH range, which can be attributed to the increased electrostatic and steric repulsion between the droplets, and the decreased van der Waals attraction [11, 18, 19]. These results clearly show that the multilayer technique can be used to improve the pH stability of protein-coated droplets, which may be useful in developing delivery systems for lipophilic functional components that can be used in a wider range of products than is currently possible using only protein-coated droplets.

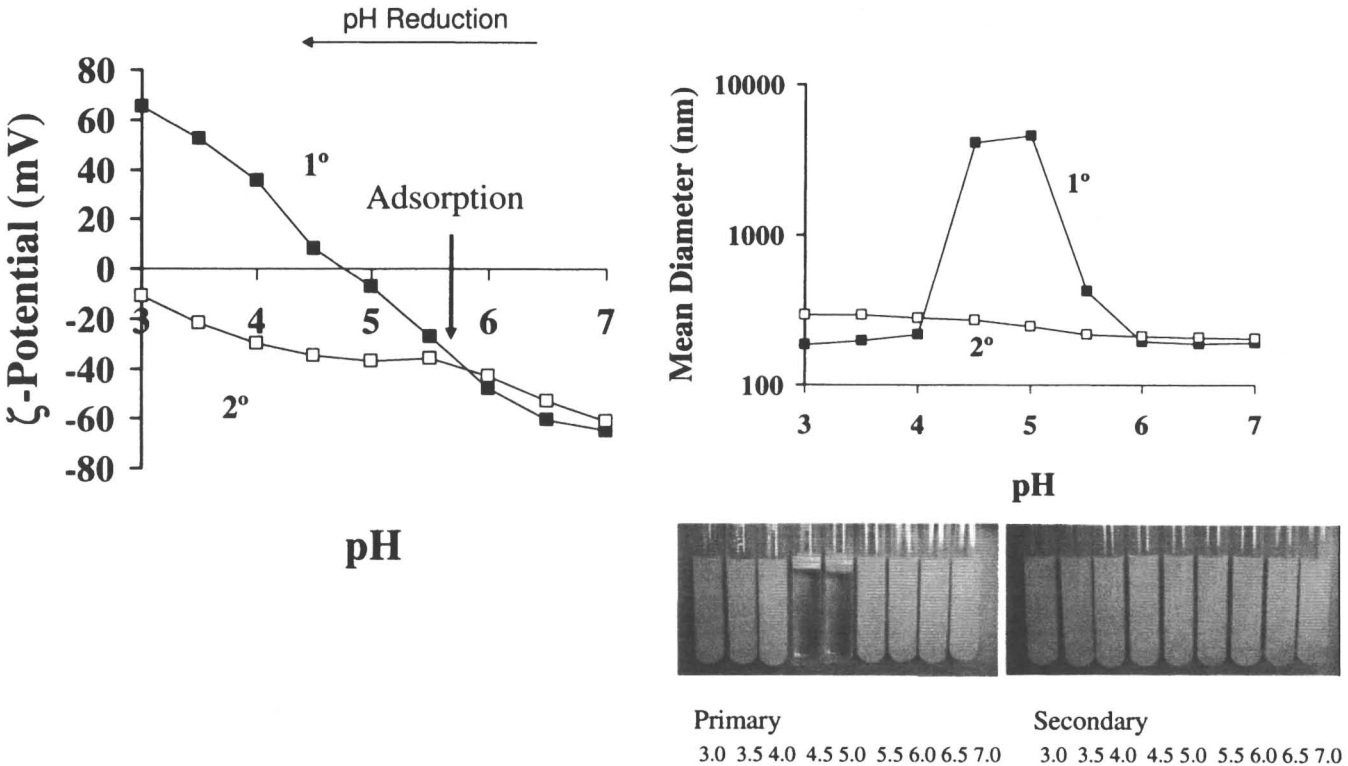

Figure 4. The pH-dependence of the ζ-potential, mean particle diameter and creaming stability of primary (β-lactoglobulin) and secondary (βlactoglobulin-pectin) emulsions.

Huang et al.; Micro/Nanoencapsulation of Active Food Ingredients
ACS Symposium Series; American Chemical Society: Washington, DC, 2009.

NaCl

Many food systems contain significant amounts of mineral ions in them, which can negatively impact the physical stability of emulsion-based delivery systems. The influence of NaCl concentration (0 to 300 mM) on the mean particle diameter and creaming stability of diluted primary and secondary emulsions at pH 3.5 has been measured [24]. The primary emulsions were unstable to droplet aggregation and creaming when the salt concentration was ≥ 50 mM (Figure 5), which can be attributed to screening of the electrostatic repulsion between the droplets. On the other hand, the secondary emulsions were relatively stable to droplet aggregation up to 200 mM NaCl (Figure 5), which can be attributed to the increased electrostatic and steric repulsion, and decreased van der Waals attraction [11, 18, 19, 24]. These results show that the multilayer technique can be used to improve the salt stability of protein-coated lipid droplets, thus extending the range of food matrices that this kind of delivery system could be used in.

Thermal Processing

The influence of thermal processing on the stability of primary (β-lacto-globulin) and secondary (β-lactoglobulin-carrageenan) emulsions has been studied at pH 6 [8, 10]. These emulsions were held isothermally at temperatures ranging form 30 to 90 °C, cooled to room temperature, and then stored for 24 hours. The ζ-potential, mean particle diameter and creaming stability of the emulsions were then measured (data not shown). In the absence of added salt, there was no significant change in the ζ-potential or mean particle diameter of the secondary emulsions upon heating, and there was no evidence of creaming, which indicated that they were stable to thermal processing in the temperature range used. Nevertheless, at 150 mM NaCl, there was evidence of desorption of carrageenan from the droplet surfaces at temperatures exceeding the thermal denaturation of the adsorbed protein molecules [8, 10]. This suggested that the conformational change of the adsorbed globular protein caused by heating weakened the attraction between the carrageenan and β-lactoglobulin molecules leading to polysaccharide desorption. The emulsions where the carrageenan molecules became detatched from the droplet surfaces were more unstable to flocculation after heating.

Freeze-Thaw Cycling

Primary (β-lactoglobulin) and secondary (β-lactoglobulin-pectin) emulsion samples (2 wt% oil, pH 3.5) were transferred into cryogenic test tubes and incubated in a –20 °C freezer for 22 hours. After incubation the emulsion samples were thawed by incubating them in a water bath at 30 °C for 2 hours.

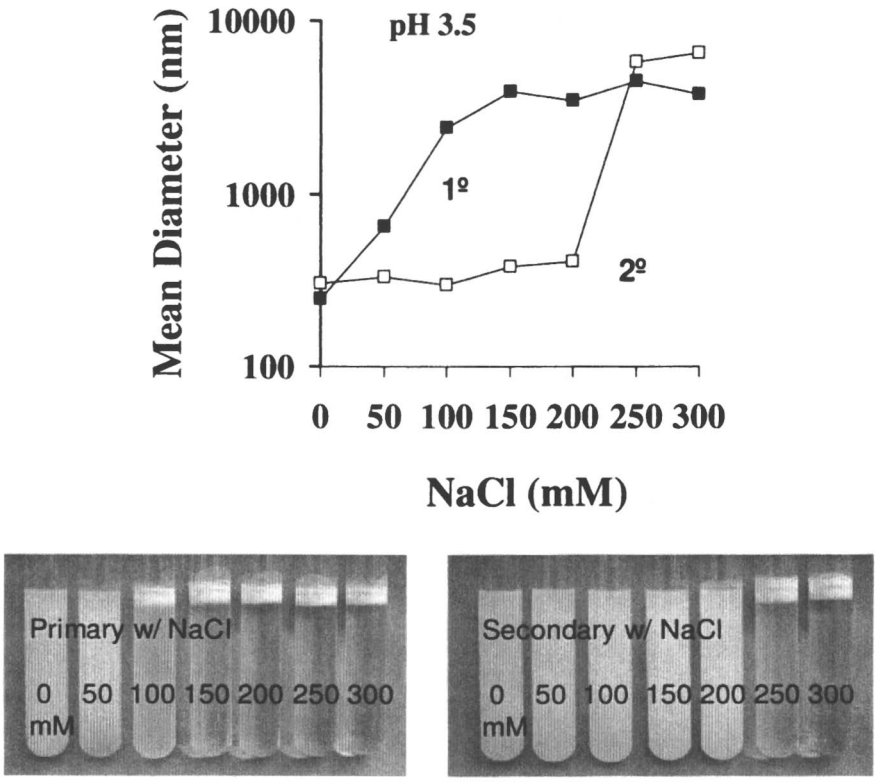

Figure 5. The salt-dependence of the mean particle diameter and creaming stability of primary (β-lactoglobulin) and secondary (β-lactoglobulin-pectin) emulsions. The primary emulsion flocculates at lower salt concentrations than the secondary emulsion.

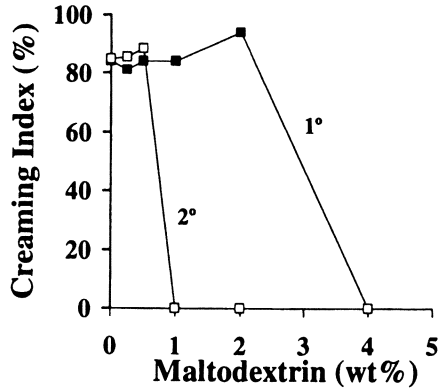

Figure 6. Impact of added maltodextrin on the creaming stability of primary (β-lactoglobulin) and secondary (β-lactoglobulin-pectin) emulsions after freezing and thawing (3 cycles). Less maltodextrin is required to stabilize the secondary emulsion than the primary emulsion.

This freeze-thaw cycle was repeated two times and its influence on emulsion stability (creaming index) was measured (Figure 6). The emulsions were all unstable to droplet aggregation in the absence of maltodextrin. We therefore carried out experiments to determine the minimum amount of maltodextrin required to stabilize the primary and secondary emulsions against droplet aggregegation during freezing and thawing. We found that secondary emulsions containing pectin required only 1 wt% maltodextrin to stabilize them against aggregation, whereas the primary emulsions required 4 wt% maltodextrin. We also found that there were differences between polysaccharides, with pectin being more effective than carrageenan. Hence, the multilayer technique may be useful for reducing the amount of sugars that are needed in frozen products to protect lipid droplets against aggregation.

Freeze Drying

We have recently carried out preliminary experiments comparing the stability of primary (β-lactoglobulin) and secondary (β-lactoglobulin-pectin) emulsions to freeze-drying at pH 3.5 [25]. Emulsion samples (30 mL) were transferred into Petri dishes and frozen by placing them overnight in a –40 °C freezer. A laboratory scale freeze-drying device (Virtis, the Virtis Company, Gardiner, NY) was used to dry the frozen emulsions. After finishing the drying process the dried products were ground using a mechanical device (Handy Chopper, Black & Decker Inc., Shelton, CT). The secondary emulsions had a much better stability to droplet aggregation than the primary emulsions after

freeze-drying and reconstitution in buffer solution, especially when maltodextrin was incorporated into the emulsions. For example, we found that secondary emulsions containing pectin were stable to aggregation at maltodextrin concentrations of 2 wt% and higher, whereas more than 8 wt% maltodextrin was required to stabilize primary emulsions. The multilayer technique may therefore prove useful for increasing the lipid load of dehydrated emulsions that could be used as powdered delivery systems.

Lipid Oxidation

We have recently compared the oxidative stability of primary (β-lactoglobulin) and secondary (β-lactoglobulin-pectin) emulsions at pH 3.5. The primary emulsion droplets were cationic, whereas the secondary emulsion droplets were anionic, so we would have expected the primary emulsions to have been more oxidatively stable due to repulsion of the positively charged iron ions from the positively charged protein-coated droplets [26]. Nevertheless, we found that secondary emulsions containing lipid drolets coated by citrus pectin actually had slightly better oxidative stability (data not shown). This suggests that the relatively thick polysaccharide layer may have been able to prevent the iron ions from reaching the lipid surfaces.

Other Systems

In addition to the systems described above we have examined the suitability of other types of emulsifier and biopolymer combinations for preparing stable oil-in-water emulsions containing droplets surrounded by multi-layered interfacial layers. We have shown that laminated lipid droplets can be formed using a variety of different emulsifiers (lecithin, SDS, β-lactoglobulin, caseinate) and biopolymers (chitosan, gelatin, pectin, carrageenan, alginate, gum arabic) [8, 9, 11, 15-20, 27-31]. Recently we have used a similar technique to prepare "colloidosomes", which consist of large oil droplets surrounded by a layer of small oil droplets [32].

Nano-laminated Coatings on Macroscopic Objects

Applications of Laminated Edible Coatings

Potentially, the LbL deposition technology can also be used to form multifunctional laminated coatings on macroscopic objects, such as fruit, vegetables, meat and fish. For example, there is currently a need for high-performance edible coatings for application on fresh-cut fruits & vegetables that

are capable of exhibiting a variety of different functions, *e.g.,* control of moisture or gas migration; anti-microbial, anti-oxidant and anti-browning activity; prevention of textural degradation; encapsulation of nutraceuticals, colors or flavors; controlled or triggered release of active components [33-36]. Conventional technologies used by the food industry have limited scope for precise engineering of novel functionalities into edible coatings. The LbL deposition technique could be used to design and fabricate laminated edible coatings with greatly improved functional properties. These coatings could be created from food-grade ingredients (*e.g.,* proteins, polysaccharides and lipids) using simple and inexpensive processing operations (*e.g.,* dipping, spraying and washing). In addition, they could be designed to have a range of functional attributes that are difficult to achieve using conventional coating methods, such as selective permeability to water and other volatile components; encapsulation of active components (such as antioxidants, antimicrobials, anti-browning agents, colors, flavors or nutraceuticals); and texture stabilization.

Formation of Laminated Edible Coatings

The principle of using the LbL technique to coat macroscopic objects is highlighted in Figure 7. The object to be coated is dipped sequentially into a series of solutions containing substances that adsorb to its surface. (Alternatively, the solutions containing the adsorbing substances could be sprayed onto the surface of the object). Between each dipping step it may be necessary to have a washing and/or drying step to remove the excess solution attached to the surface prior to introduction of the object into the next dipping solution. The composition, thickness, structure and properties of the laminated coating formed around the object could be controlled in a number of ways, including: (i) changing the type of adsorbing substances in the dipping solutions; (ii) changing the total number of dipping steps used; (iii) changing the order that the object is introduced into the various dipping solutions; (iv) changing the solution and environmental conditions used, such as pH, ionic strength, solvent, temperature, dipping time, stirring speed *etc.* The driving force for adsorption of a substance to a surface would depend on the nature of the surface and the nature of the adsorbing substance, and could be electrostatic, hydrogen bonding, hydrophobic interactions, *etc.* Nevertheless, the major driving force utilized by the LbL deposition method is **electrostatic attraction** between electrically charged substances.

In general, a variety of different adsorbing substances could be used to create the different layers (Figure 8), including:

- *Natural Polyelectrolytes.* Any food-grade polyelectrolyte that is capable of adsorbing to the exposed surface of the object could be used, such as proteins (*e.g.,* whey, casein, soy) or polysaccharides (*e.g.,* pectin, alginate, xanthan, carrageenan, chitosan).

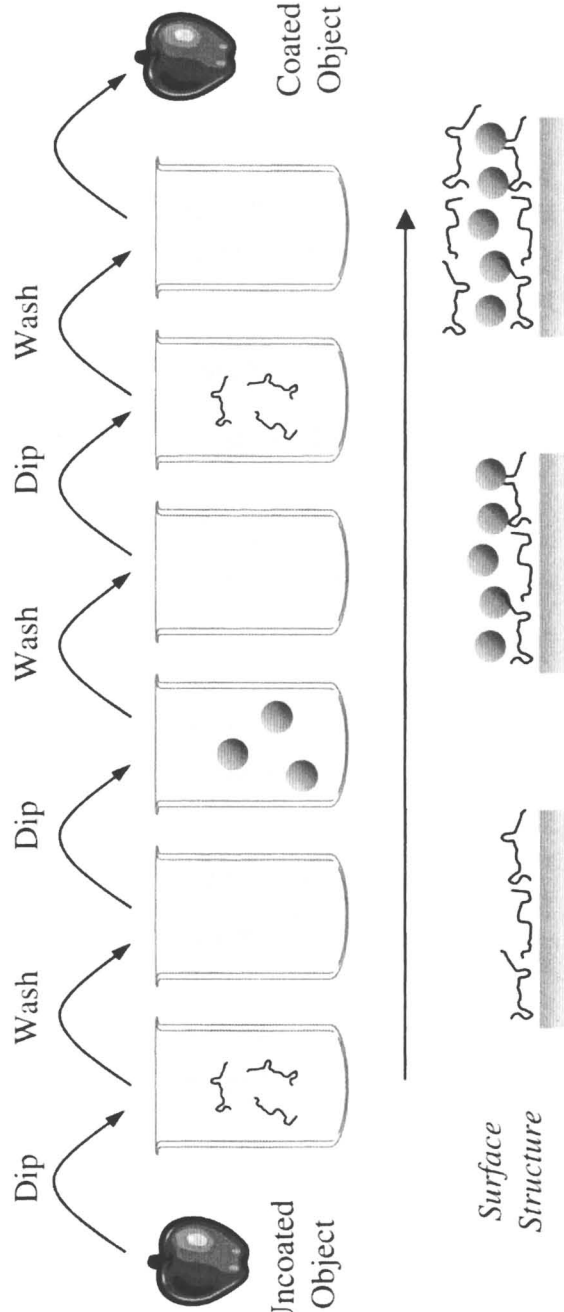

Figure 7. Schematic representation of coating a fruit with multiple layers using a successive dipping and washing procedure. Each dipping solution contains a component that will adsorb to the surface of the material to form a laminated coating around the fruit.

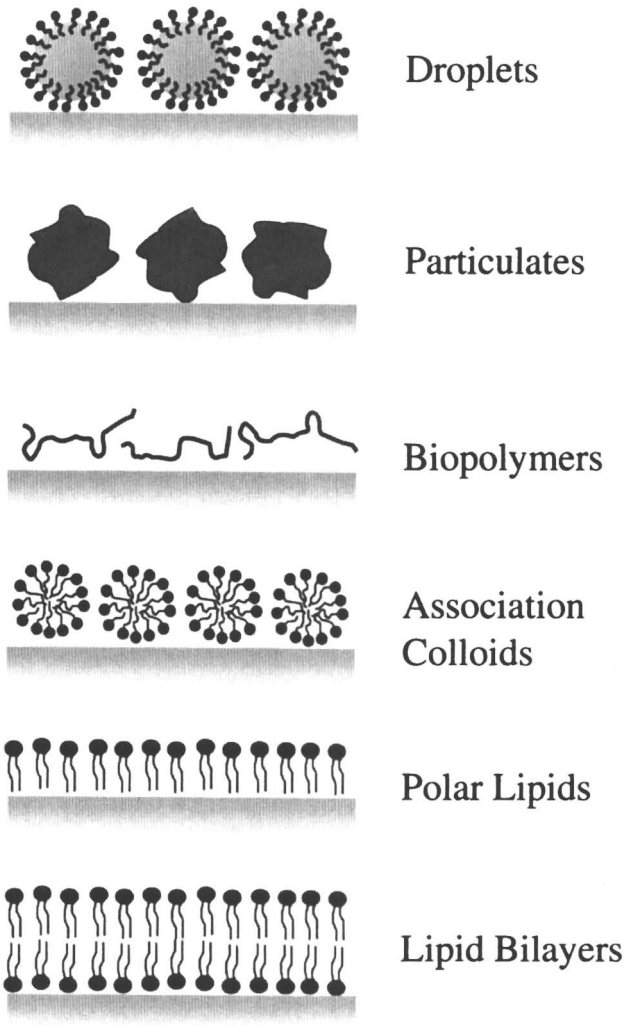

Figure 8. Possible components that could be used to assemble multilayered edible films or coatings

- *Surface-Active Lipids.* Any food-grade lipid that is capable of adsorbing to the exposed surface of the object, *e.g.* phospholipids and small molecule surfactants. These surface active lipids could form single layers, bi-layers, multiple layers, micelles, vesicles or other association colloids at the surface.
- *Lipid Droplets.* Any food-grade lipid droplet that is capable of adsorbing to the exposed surface on the object. The emulsion droplet would usually consist of a liquid oil droplet coated by a food grade emulsifier, but it could also be a partly or fully crystallized oil droplet, or an oil droplet containing small water droplets or other material.

The choice of the type of adsorbing substances used to create each layer, the total number of layers incorporated into the overall coating, the sequence of the different layers, and the preparation conditions used to prepare each layer will determine the functional performance of the final coatings: permeability (*e.g.,* to gasses, organic substances, minerals or water); mechanical properties (*e.g.,* rigidity, flexibility, brittleness); swelling and wetting characteristics; environmental sensitivity (*e.g.,* to pH, ionic strength and temperature). In addition, the above procedure enables one to encapsulate various hydrophilic, amphiphilic or lipophilic substances within the coatings, *e.g.,* non-polar substances could be incorporated in micelles or lipid droplets, while polar substances could be incorporated in biopolymer layers. Thus, it would be possible to incorporate active functional agents such as antimicrobials, anti-browning agents, antioxidants, enzymes, flavors, colors and nutraceuticals into the coatings. These functional agents could be used to increase the shelf-life and quality of the coated fresh-cut fruit and vegetables. An example of a possible multi-component, multi-layered coating is shown in Figure 9.

Preliminary Studies

Recently, we have carried out studies that have shown that the electrostatic layer-by-layer technique can be used to form laminated coatings on planar hydrogels (agar-pectin) that were designed to mimic cut-fruit surfaces (which contain a significant amount of pectin) [37]. However, we have also observed some interesting physicochemical phenomena that need to be considered when forming these coatings on macroscopic objects. An anionic hydrogel was prepared in a Petri dish and then cationic protein-coated droplets were brought into contact with the hydrogel for a specified period. The contact emulsion was then removed and the plate was washed with buffer solution (Figure 10). The turbidity of the plates was measured to monitor the adsorption of droplets to the hydrogel surfaces (Figure 11), and the charge and size of the droplets in the contact emulsion removed from the hydrogel surfaces were measured (Figure 12). The turbidity measurements indicated that the lipid droplets did adsorb to the droplet surfaces fairly rapidly, but the results were not what would be

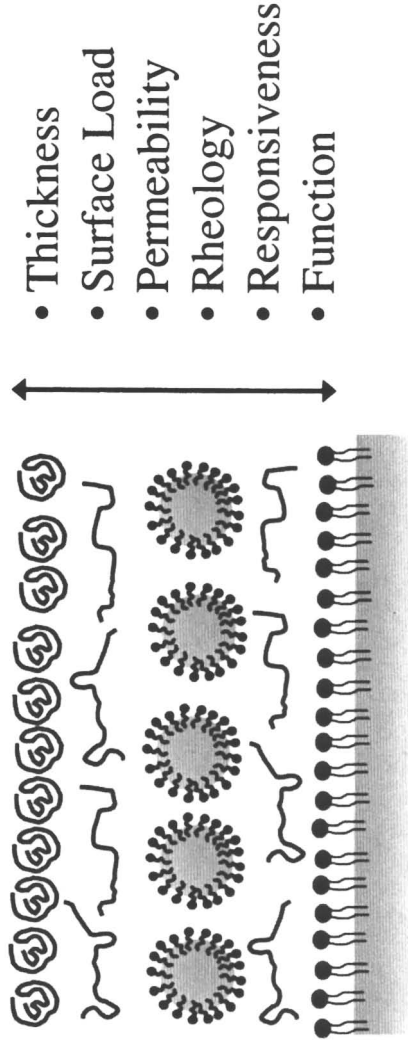

Figure 9. Example of possible multilayered edible film containing different functional layers.

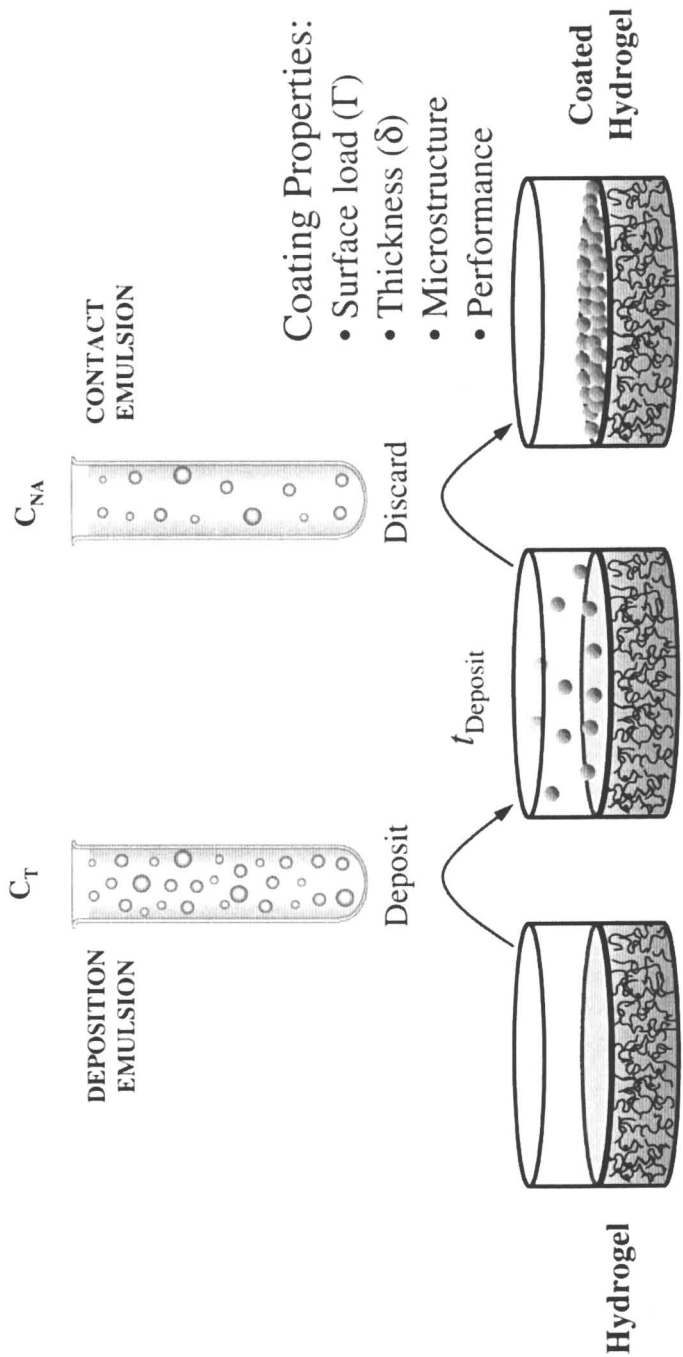

Figure 10. Schematic representation of coating a hydrogel surface with lipid droplets.

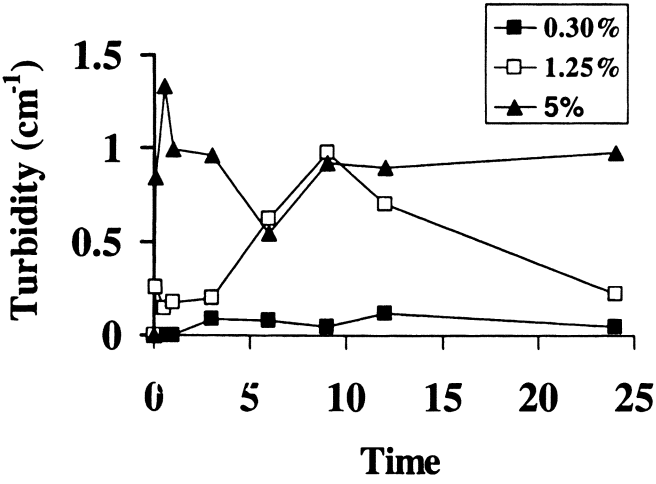

Figure 11. Turbidity of emulsions over time.

expected for a simple adsorption process (*e.g.,* a Langmuir isotherm). For example, we observed increases and then decreases in plate turbidity over time, and no clear dependence of plate turbidity on the initial droplet concentration in the deposition emulsion. In addition, the droplets in the contact emulsion removed from the hydrogel plates became increasingly negative and aggregated over time, with the effect being more pronounced in the more dilute emulsions (Figure 12). We postulate that anionic pectin molecules diffused out of the hydrogels and adsorbed onto the surfaces of the cationic protein-coated droplets, which made them become more negative and aggregate. Indeed, spectrophotometry measurements indicated that the pectin molecules did diffuse out of the hydrogels over time. The diffusion of biopolymers out of macroscopic surfaces may be an important consideration when forming laminated coatings on fruits and vegetables.

In other preliminary studies, we have examined the impact of biopolymer type, pH, contact time, stirring speed, salt concentration and surfactants on the formation of edible coatings on hydrogel surfaces. For example, Figure 13 shows the turbidity of anionic hydrogels (carrageenan/agar) when they have been brought into contact with emulsions containing whey protein coated droplets at different pH values. The droplets only stick to the hydrogel surfaces when the droplets are positively charged (pH < pI), *i.e.,* they have opposite charges to the hydrogel surfaces. Recently, we have shown that laminated coatings (chitosan and/or eugenol droplets) can be formed on fruit (strawberry and cantaloupe) and vegetable (sweet potatoes) surfaces, and that these provide protection against microbial growth.

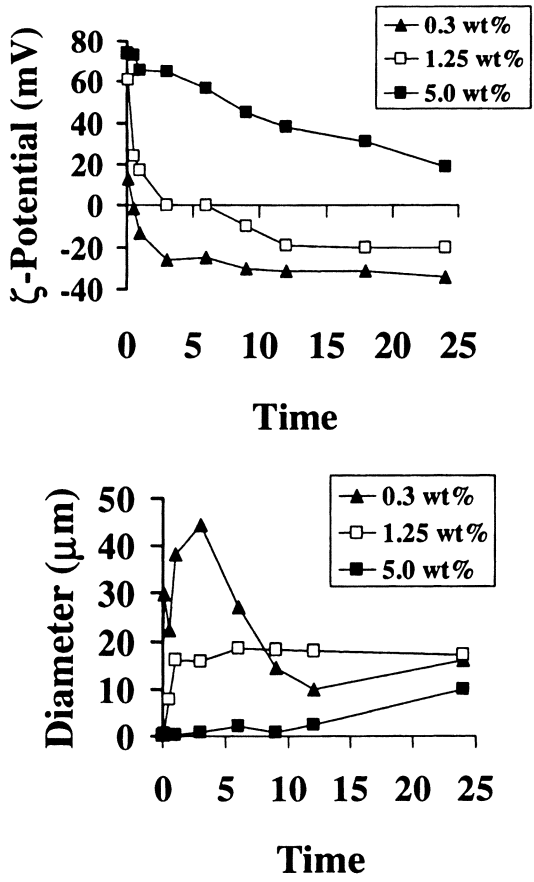

Figure 12. ζ-potential and mean particle diameter of droplets present in the contact emulsion removed from the surfaces of hydrogels. The droplets became more negative over time and aggregated, which can be attributed to diffusion of pectin out of the hydrogels and onto the droplet surfaces.

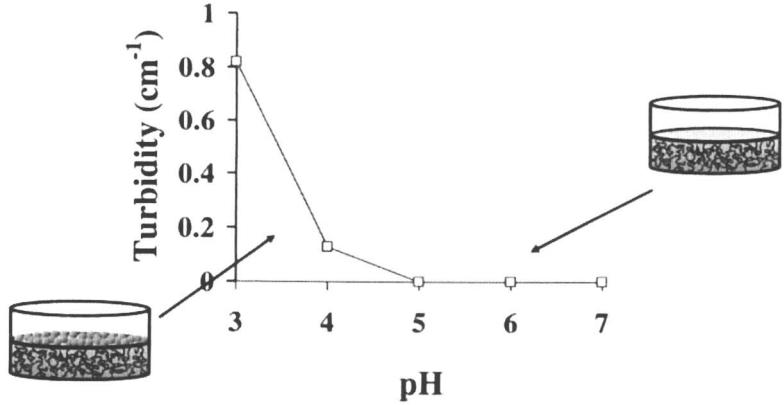

Figure 13. Turbidity of anionic hydrogels (carrageenan/agar) in contact with emulsions containing whey protein coated droplets at different pH values.

Conclusion

Our work so far has shown that stable emulsions containing multi-layered lipid droplets can be prepared using a simple cost-effective method and food grade ingredients. These multilayered emulsions have better stability to environmental stresses than conventional emulsions under certain conditions. More research is still needed to establish, at a fundamental level, the factors that influence the preparation of stable multilayered emulsions with specific functional attributes, including emulsifier characteristics (*e.g.,* sign and magnitude of droplet charge), biopolymer characteristics (*e.g.,* molecular weight, charge density and flexibility), mixing conditions (*e.g.,* order of addition, stirring speed) and washing solution composition (*e.g.,* ionic strength and pH). In addition, research needs to be carried out to establish where these multilayered emulsions can be practically used within the food industry as delivery systems. We have also carried out preliminary experiments showing that the LbL technique may be useful for coating macroscopic objects, such as meat, fish, fruit and vegetables.

References

1. Shefer, A. and S. Shefer, *Novel encapsulation system provides controlled release of ingredients.* Food Technology, 2003. **57**: p. 40-43.
2. Ubbink, J., *Flavor delivery systems: Trends, technologies and applications.* Abstracts of Papers of the American Chemical Society, 2002. **223**: p. U34-U34.

3. Ubbink, J. and J. Kruger, *Physical approaches for the delivery of active ingredients in foods.* Trends in Food Science & Technology, 2006. **17**(5): p. 244-254.
4. Chen, L.Y., G.E. Remondetto, and M. Subirade, *Food protein-based materials as nutraceutical delivery systems.* Trends in Food Science & Technology, 2006. **17**(5): p. 272-283.
5. McClements, D.J., *Food Emulsions: Principles, Practice, and Techniques.* 2nd ed. CRC series in contemporary food science. 2005, Boca Raton: CRC Press.
6. Friberg, S., K. Larsson, and J. Sjoblom, *Food Emulsions.* 4 ed. 2004, New York: Marcel Dekker.
7. Aoki, T., E.A. Decker, and D.J. McClements, *Influence of environmental stresses on stability of O/W emulsions containing droplets stabilized by multilayered membranes produced by a layer-by-layer electrostatic deposition technique.* Food Hydrocolloids, 2005. **19**(2): p. 209-220.
8. Gu, Y., E. Decker, and D. McClements, *Irreversible thermal denaturation of beta-lactoglobulin retards adsorption of carrageenan onto beta-lactoglobulin-coated droplets* Langmuir, 2006. **22** p. 7480-7486
9. Gu, Y.S., A.E. Decker, and D.J. McClements, *Production and characterization of oil-in-water emulsions containing droplets stabilized by multilayer membranes consisting of beta-lactoglobulin, iota-carrageenan and gelatin.* Langmuir, 2005. **21**(13): p. 5752-5760.
10. Gu, Y.S., E.A. Decker, and D.J. McClements, *Influence of iota-carrageenan on droplet flocculation of beta-lactoglobulin-stabilized oil-in-water emulsions during thermal processing.* Langmuir, 2004. **20**(22): p. 9565-9570.
11. Guzey, D., H.J. Kim, and D.J. McClements, *Factors influencing the production of O/W emulsions stabilized by beta-lactoglobulin-pectin membranes.* Food Hydrocolloids, 2004. **18**(6): p. 967-975.
12. Guzey, D. and D.J. McClements, *Influence of Environmental Stresses on O/W Emulsions Stabilized by β-Lactoglobulin–Pectin and β-Lactoglobulin–Pectin–Chitosan Membranes Produced by the Electrostatic Layer-by-Layer Deposition Technique.* Food Biophysics, 2006. **1**(1): p. 30-40.
13. Harnsilawat, T., R. Pongsawatmanit, and D. McClements, *Characterization of β-lactoglobulin–sodium alginate interactions in aqueous solutions: A calorimetry, light scattering, electrophoretic mobility and solubility study,* Food Hydrocolloids, 2006. **20** p. 577-585.
14. Harnsilawat, T., R. Pongsawatmanit, and D. McClements, *Stabilization of model beverage cloud emulsions using protein-polysaccharide electrostatic complexes formed at the oil-water interface* Journal of Agricultural and Food Chemistry, 2006. **54** p. 5540-5547
15. Klinkesorn, U., et al., *Encapsulation of emulsified tuna oil in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition.* Food Hydrocolloids, 2005. **19**(6): p. 1044-1053.

16. Klinkesorn, U., et al., *Stability of spray-dried tuna oil emulsions encapsulated with two-layered interfacial membranes.* Journal of Agricultural and Food Chemistry, 2005. **53**(21): p. 8365-8371.
17. Klinkesorn, U., et al., *Increasing the oxidative stability of liquid and dried tuna oil-in-water emulsions with electrostatic layer-by-layer deposition technology.* Journal of Agricultural and Food Chemistry, 2005. **53**(11): p. 4561-4566.
18. McClements, D.J., *Theoretical analysis of factors affecting the formation and stability of multilayered colloidal dispersions.* Langmuir, 2005. **21**(21): p. 9777-9785.
19. Moreau, L., et al., *Production and characterization of oil-in-water emulsions containing droplets stabilized by beta-lactoglobulin-pectin membranes.* Journal of Agricultural and Food Chemistry, 2003. **51**(22): p. 6612-6617.
20. Mun, S., E.A. Decker, and D.J. McClements, *Influence of droplet characteristics on the formation of oil-in-water emulsions stabilized by surfactant-chitosan layers.* Langmuir, 2005. **21**(14): p. 6228-6234.
21. Ogawa, S., E.A. Decker, and D.J. McClements, *Influence of environmental conditions on the stability of oil in water emulsions containing droplets stabilized by lecithin-chitosan membranes.* Journal of Agricultural and Food Chemistry, 2003. **51**(18): p. 5522-5527.
22. Ogawa, S., E.A. Decker, and D.J. McClements, *Production and characterization of O/W emulsions containing cationic droplets stabilized by lecithin-chitosan membranes.* Journal of Agricultural and Food Chemistry, 2003. **51**(9): p. 2806-2812.
23. Ogawa, S., E.A. Decker, and D.J. McClements, *Production and characterization of O/W emulsions containing droplets stabilized by lecithin-chitosan-pectin mutilayered membranes.* Journal of Agricultural and Food Chemistry, 2004. **52**(11): p. 3595-3600.
24. Guzey, D. and D.J. McClements, *Impact of electrostatic interactions on formation and stability of emulsions containing oil droplets coated by beta-lactoglobulin-pectin complexes.* Journal of Agricultural and Food Chemistry, 2007. **55**(2): p. 475-485.
25. Mun, S., E.A. Decker, and D.J. McClements, *Influence of Freeze-Thaw and Freeze-Dry Processes on Stability of Lipid Droplets Coated by Protein-Polysaccharide Layers* In preparation, 2007.
26. McClements, D.J. and E.A. Decker, *Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems.* Journal of Food Science, 2000. **65**(8): p. 1270-1282.
27. Gu, Y.S., E.A. Decker, and D.J. McClements, *Influence of pH and iota-carrageenan concentration on physicochemical properties and stability of beta-lactoglobulin-stabilized oil-in-water emulsions.* Journal of Agricultural and Food Chemistry, 2004. **52**(11): p. 3626-3632.

28. Gu, Y.S., L. Regnier, and D.J. McClements, *Influence of environmental stresses on stability of oil-in-water emulsions containing droplets stabilized by beta-lactoglobulin-iota-carrageenan membranes.* Journal of Colloid and Interface Science, 2005. **286**(2): p. 551-558.
29. Gu, Y.S., E.A. Decker, and D.J. McClements, *Influence of pH and carrageenan type on properties of beta-lactoglobulin stabilized oil-inwater emulsions.* Food Hydrocolloids, 2005. **19**(1): p. 83-91.
30. Harnsilawat, T., R. Pongsawatmanit, and D. McClements, *Influence of pH and ionic strength on formation and stability of emulsions containing oil droplets coated by beta-lactoglobulin-alginate interfaces.* Biomacromolecules, 2006. **7**: p. 2052-2058.
31. Mun, S.H. and D.J. McClements, *Influence of interfacial characteristics on Ostwald ripening in hydrocarbon oil-in-water emulsions.* Langmuir, 2006. **22**(4): p. 1551-1554.
32. Gu, Y.S., E.A. Decker, and D.J. McClements, *Formation of colloidosomes by adsorption of small charged oil droplets onto the surface of large oppositely charged oil droplets.* Food Hydrocolloids, 2006. **20**(1): p. In Press.
33. Krochta, J.M. and C. DeMulderJohnston, *Edible and biodegradable polymer films: Challenges and opportunities.* Food Technology, 1997. **51**(2): p. 61-74.
34. Olivas, G.I. and G.V. Barbosa-Canovas, *Edible coatings for fresh-cut fruits.* Critical Reviews in Food Science and Nutrition, 2005. **45**(7-8): p. 657-670.
35. Krochta, J.M. and C.L. Demulder, *Biodegradable Polymer-Films from Agricultural Products.* Abstracts of Papers of the American Chemical Society, 1995. **209**: p. 61-Btec.
36. Debeaufort, F., M. Martinpolo, and A. Voilley, *Polarity Homogeneity and Structure Affect Water-Vapor Permeability of Model Edible Films.* Journal of Food Science, 1993. **58**(2): p. 426.
37. Vargas, M., J. Weiss, and D.J. McClements, *Adsorption of Protein-Coated Lipid Droplets to Mixed Biopolymer Hydrogel Surfaces: Role of Biopolymer Diffusion.* In preparation, 2007.