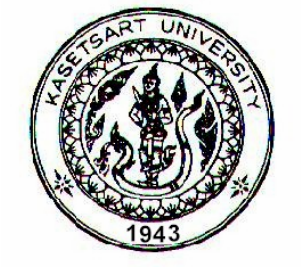

THESIS

PROTEIN-POLYSACCHARIDE INTERACTION AND ITS EFFECT ON THE STABILITY OF OIL-IN-WATER EMULSION

THEPKUNYA HARNSILAWAT

GRADUATE SCHOOL, KASETSART UNIVERSITY

2006

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THESIS

PROTEIN-POLYSACCHARIDE INTERACTION AND ITS EFFECT ON THE STABILITY OF OIL-IN-WATER EMULSION

THEPKUNYA HARNSILAWAT

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Proteins and polysaccharides are used as functional ingredients in many food products. Their interactions play an important role in the development of novel foods. Since the protein-polysaccharide interactions are sensitive to the molecular characteristics and solution conditions (pH, ionic strength and temperature), therefore the interaction between β-lactoglobulin (β-Lg) and sodium alginate (NaA) was selected and investigated in aqueous solutions and in oil-in-water emulsions. The effect of polysaccharide type and concentration, pH, ultrasound treatment, ionic strength and temperature on the formation, stability and properties of solutions and emulsions was determined using isothermal titration calorimetry, static and dynamic light scattering, ζ-potential, soluble protein, turbidity, creaming stability, and optical microscopy measurements. Initially, the influence of pH on the properties of NaA, β-Lg, and their mixtures in aqueous solutions was studied. The electrical charge of isolated β-Lg went from positive to negative as the pH increased from 3 to 7 with the isoelectric point being around pH 4.8, while the electrical charge of isolated NaA was negative at all pH values. Light scattering measurements indicated that isolated NaA was completely soluble from pH 3 to 7, but isolated β-Lg formed large complexes that scattered light at pH 4 and 5. When β-Lg and NaA were mixed together at pH 3 and 4 they formed large complexes, while at pH 5, β-Lg and NaA formed fairly soluble complexes. At pH 6 and 7, β-Lg and NaA did not form complexes due to electrostatic repulsion. When the influence of pH, sodium alginate and ultrasound treatment on the properties of 5 wt% palm oil-in-water emulsions stabilized by β-Lg was determined, anionic NaA adsorbed to the surfaces of β-Lg coated droplets at pH 3-5 due to electrostatic attraction. At pH 6 and 7, adsorption did not occur because of the strong electrostatic repulsion. Emulsion droplets coated by β-Lg-NaA complexes were prone to bridging flocculation which promoted creaming instability. However, high intensity ultrasound treatment was able to reduce the degree of droplet flocculation in these emulsions, thereby increasing creaming stability. The effect of ionic strength and mixing condition was examined in 0.1 wt% corn oil-in-water emulsions containing droplets stabilized by β-Lg-NaA interfaces. NaA adsorbed to the β-Lg-coated droplets from pH 3 to 6, which was attributed to electrostatic attraction between the anionic polymer and cationic patches on the droplet surfaces. Droplets coated by β-Lg-NaA had better stability to flocculation than those coated by β-Lg alone, especially around the isoelectric point of the adsorbed proteins and at low ionic strengths (< 100 mM NaCl). Mixing conditions had an impact on the formation and stability of secondary emulsions. More stable emulsions could be formed if the droplets and polymer were mixed at a pH where they had the same sign charge, and then the pH was adjusted to a value where they had different charges. Finally, the model beverage emulsions were selected as a food model. The emulsions consisted of 0.1 wt% corn oil droplets coated by β-Lg and β-Lg/alginate compared to those coated by β-Lg/ι-carrageenan, or β-Lg/gum arabic interfacial layers (pH 3 or 4). The emulsions were subjected to variations in pH (3 to 7), ionic strength (0 to 250 mM NaCl) and thermal processing (30 or 90°C) and the influence on their stability was determined. The emulsions containing alginate and carrageenan had the best stability to ionic strength and thermal processing. This study shows that the controlled formation of protein-polysaccharide complexes at droplet surfaces may be used to produce stable beverage emulsions, which may have important implications for industrial applications.

T. Harnsilawat — Student's signature

P. Pongsawatmanit — Thesis Advisor's signature

29 / 05 / 2006

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Thepkunya Harnsilawat

May 2006

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INTRODUCTION

Proteins and polysaccharides are used together as functional ingredients in many kinds of food products, including solutions, gels, emulsions and foams (Dickinson, 1992, 1993, 2003; Tolstoguzov, 1997, 2002, 2003a, 2003b). The control or manipulation of the macromolecular interactions between these two different kinds of biopolymers is a key factor in the development of novel food processes and products as well as in the formulation of fabricated food products (Grinberg and Tolstoguzov, 1997; Tolstoguzov, 1997, 2003a, 2003b; Weinbreck *et al.*, 2003). The functional properties of each kind of biopolymer are often modified by the presence of the other type of biopolymer. For example, the solubility, conformational stability, gel-forming ability, surface activity, emulsifying properties, and foaming properties of many proteins are strongly affected by their interactions with polysaccharides. Conversely, the ability of many polysaccharides to thicken solutions, form gels or stabilize emulsions is influenced by the presence of proteins.

Protein-polysaccharide interactions are particularly sensitive to the precise molecular characteristics of the protein and polysaccharide molecules involved, as well as to solution conditions that modulate the dominant intermolecular forces such as pH, ionic composition and temperature (Benichou *et al.*, 2002; de Kruif and Tuinier, 2001; de Kruif *et al.*, 2004; Dickinson, 2003; McClements, 2004c; Tolstoguzov, 1997). Aqueous solutions of mixed biopolymers are susceptible to phase separation by one of two alternative mechanisms - complex coacervation (associative phase separation) or thermodynamic incompatibility (segregative phase separation) (Dickinson and McClements, 1995; Tolstoguzov, 1997, 2002, 2003a, 2003b). Complex coacervation occurs when two biopolymers are strongly attracted to each other, which usually occurs because they have opposite electrical charges. This leads to the formation of a two-phase system that consists of a mixed biopolymer

complex phase suspended in a solvent phase depleted in both biopolymers. This complex may be either soluble or insoluble depending on the electrical characteristics of the biopolymers involved and the solution composition. On the other hand, thermodynamic incompatibility tends to occur when the biopolymers are uncharged or similarly charged so that there is a relatively strong steric or electrostatic repulsion between them. In this situation, the two biopolymers may co-exist in a single phase (miscibility) in domains in which they mutually exclude one another or, above a critical concentration, segregate into two different phases. Each of the phases formed is rich in one type of biopolymer and depleted in the other type.

In the emulsion system, proteins are used as emulsifiers in a variety of commercially important products, including foods, health care products, pharmaceuticals and personal care products (Dalgleish, 2004; Dickinson, 1992; Dickinson and Stainsby, 1982; Kabalnov, 1998; McClements, 2004a; Saether *et al.*, 2004). Proteins adsorb to the surfaces of the oil droplets created by homogenization of oil-water-protein mixtures, where they facilitate further droplet disruption by lowering the interfacial tension and retard droplet aggregation by forming protective membranes around the droplets (McClements, 2004a; Walstra, 2003; Wilde *et al.*, 2004). Proteins are particularly attractive as emulsifiers because they are natural, non-toxic, tasteless and widely available (McClements, 2004b; Wilde *et al.*, 2004).

Droplet flocculation is usually prevented in emulsions stabilized by adsorbed proteins due to the relatively strong electrostatic repulsion between the charged droplets (Dalgleish, 2004; Dickinson and Stainsby, 1982; Kabalnov, 1998; McClements, 2004a; Saether *et al.*, 2004). This means that protein-stabilized emulsions are particularly sensitive to pH and ionic strength effects, and will tend to flocculate at pH values close to the isoelectric point (pI) of the adsorbed proteins and when the ionic strength exceeds a certain level. Emulsions stabilized by globular proteins are also sensitive to thermal treatment, because these proteins tend to unfold when the temperature exceeds the thermal denaturation temperature (Tm) exposing reactive non-polar and sulfhydryl groups (McClements, 2004a). The sensitivity of

protein-stabilized emulsions to environmental stresses (such as pH, ionic strength, and temperature) limits their application in many types of commercial product.

Emulsions stabilized by polysaccharides, such as gum arabic and modified starch, are often more resistant to pH changes, high ionic strength and elevated temperatures than those stabilized by proteins. This has been attributed to the fact that polysaccharide-stabilized droplets are surrounded by a relatively thick porous polymer layer, which increases the steric repulsion and decreases the van der Waals attraction between droplets (McClements, 2004a). On the other hand, polysaccharides are usually much less effective at producing emulsions than proteins, because they are less surface active. Hence, they must be used in much higher concentrations than proteins to produce emulsions containing small droplets. It would therefore be advantageous to combine the beneficial attributes of proteins and polysaccharides to produce small emulsion droplets with good environmental stability (McClements, 2004a).

Recently, a novel interfacial engineering technology has been developed to increase the stability of emulsions to environmental stresses (Aoki *et al.*, 2005; Gu *et al.*, 2004a, 2004b, 2005a; Gu *et al.*, 2005c; Guzey *et al.*, 2004; Klinkesorn *et al.*, 2005a; McClements, 2004a; Moreau *et al.*, 2003; Ogawa *et al.*, 2003a, 2003b, 2004; Surh *et al.*, 2005). This technique is based on layer-by-layer deposition of polyelectrolytes onto oppositely charged surfaces due to electrostatic attraction, which results in the formation of droplets coated by multi-layered interfacial membranes (Decher, 1997; Schonhoff, 2003).

The objectives of this study are:

1. To investigate the interactions between β-lactoglobulin and sodium alginate in aqueous solutions using a variety of complementary techniques.
2. To investigate the interactions between β-lactoglobulin and sodium alginate in oil-in-water emulsions.

3. To determine the effect of ionic strength and mixing conditions of the protein-coated droplets and oil-in-water emulsion stability.

4. To determine whether model beverage emulsions can be created using a globular protein (β-lactoglobulin) and various charged polysaccharides (alginate, carrageenan, and gum arabic) that are stable to solution and environmental conditions normally found in practice (i.e., acid pH, salt, and thermal processing).

LITERATURE REVIEWS

Foods are multiphasic and multicomponent systems composed of proteins, polysaccharides, fats, water, and other minor nutrients and additives. Depending on their relative concentrations and solubility limits and limited thermodynamic compatibility in this complex milieu, the lipids and macromolecules generally exist as colloidally dispersed particles and aggregates. Most foods, such as milk, butter, margarine, spreads, salad dressings, frozen desserts, sausages, cakes, coconut milk, ice cream and many ready-to-use products, are emulsion and foam-type products (Chappat, 1994; Damodaran, 1997; Seow and Gwee, 1997).

The bulk physicochemical properties of these foods (appearance, flavor, rheology and stability) depend on colloidal properties, such as droplet concentration, size and physical state, colloidal interactions and interfacial properties. The diversity of food emulsions (low-viscosity white fluid of milk, viscoelastic gel of yogurt and semisolid of margarine) is the result of the different sorts of ingredients and processing conditions used to create each type of product. The manufacture of an emulsion based food product with specific quality attributes depends on the selection of the most appropriate raw materials and processing conditions (McClements, 2004a).

1. Protein-Polysaccharide Interactions

Two of the most important classes of functional ingredients in food colloids are proteins and polysaccharides (Dickinson, 1992; Dickinson and McClements, 1995; Dickinson and Stainsby, 1982). Both types of food macromolecules contribute to the structure, texture and stability of food through their thickening or gelling behaviour and surface properties (Doublier *et al.*, 2000). In addition, proteins are known specifically for their emulsifying and foaming behaviour and polysaccharides for their water holding and thickening properties (Dickinson and McClements, 1995). Attraction and repulsion are the two major types of interactions that occur between proteins and polysaccharides in the solution and can result in complex formation or immiscibility of the two biopolymers (thermodynamic incompatibility). Due to

polyelectrolyte interactions in solution, these interactions and their consequences on the mixture will be strongly influenced by pH, ionic strength, conformation, charge density and the concentration of the biopolymers (Benichou *et al.*, 2002).

1.1 The Mixing Behaviour of Biopolymer Solutions

Protein-polysaccharide interactions have been extensively studied since they appear in most food systems (Benichou *et al.*, 2002). A blend of two different biopolymers in aqueous solution behaves in mainly three ways: (i) incompatibility; (ii) complex coacervation; (iii) miscibility. In principle, it can be distinguished three possible equilibrium situations, as sketched in Figure 1.

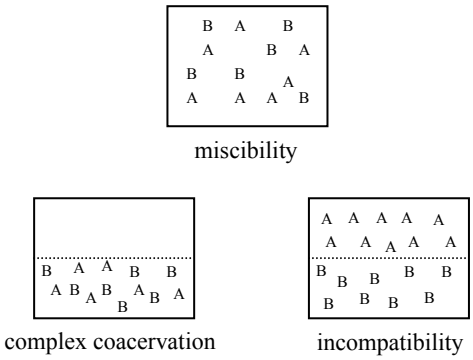


Figure 1. Classification of the mixing behaviour of ternary polymer solutions solvent + polymer A + polymer B systems.

Source: Syrbe *et al*. (1998).

On mixing two biopolymers in solution, for instance a polysaccharide and a protein solution, one may observe either one of the following possibilities as depicted in Figure 2 (de Kruif and Tuinier, 2001). The interaction of the two biopolymers maybe segregative (the biopolymers repel each other and are denoted as incompatible) or associative (the biopolymers attract one another).

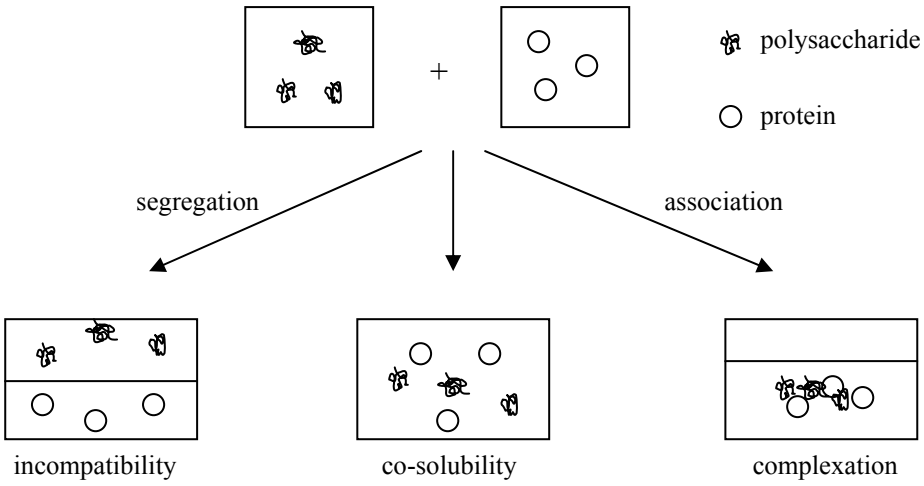


Figure 2. Main trends in the behavior of protein/polysaccharide mixtures. Source: de Kruif and Tuinier (2001).

1.1.1 Incompatibility

Interaction between different biopolymers is more repulsion than the averaged interaction between like biopolymers. Segregation of the biopolymers takes place. Two distinct immiscible aqueous phases are formed and each of them is mainly loaded with only one biopolymer species (Syrbe *et al.*, 1998). Molecular characteristics of biopolymers (molecular weight, conformation, charge density, etc.), factors affecting them (pH, ionic strength, solvent quality, etc.), mixing conditions (ratio, total concentration, etc.) and mixing procedures (heat treatment, pressure, shearing, etc.) must be considered as determining factors in thermodynamic incompatibility (Turgeon *et al.*, 2003). Incompatibility for mixed solutions of protein and polysaccharide takes place at high ionic strength and/or pH above the protein isoelectric point (Benichou *et al.*, 2002).

1.1.2 Complex Coacervation

Complex coacervation is a spontaneous separation of the system into coexisting solvent-rich and solvent-depleted phases, the latter consisting of a coprecipitate of the two different biopolymers (Dickinson, 1998; Dickinson and

McClements, 1995). In general, coacervates are formed reversibly. The word coacervates was used because the complex remained liquid, rather than precipitated. This is a phenomenon that occurs quite often in biopolymer mixtures in which the biopolymers are oppositely charged (de Kruif and Tuinier, 2001). In aqueous solution, complex coacervation takes place between two oppositely charged polymers due to electrostatic attraction. For instance, complexation between proteins and anionic polysaccharides occurs below the protein isoelectric point and at low ionic strengths (Benichou *et al.*, 2002).

1.1.3 Miscibility

If contact between different biopolymers is similar to contact between like biopolymer species, spontaneous mixing occurs. The system remains homogeneous, also at high polymer concentration. With increasing polymer molecular weight, however, the balancing between not too repulsive and not too attractive polymer interactions becomes difficult, as even slightly repulsive or attractive interaction potentials per polymer segment add up to considerable values per molecule. This means that high molecular weight polymers tend either to complex coacervation or incompatibility, but not to miscibility (Syrbe *et al.*, 1998).

1.2 Effect of pH and Ionic Strength on the Mixing Behaviour of Mixed Biopolymer Solutions

The interplay of pH and ionic strength dominates the mixing behaviour of ternary protein-polysaccharide solutions. The pH value relative to the respective isoelectric points (pI) determines the biopolymer net charge (Syrbe *et al.*, 1998). The role of excess electrolyte is threefold: (i) electrostatic attractive protein-polysaccharide interactions and protein-protein repulsions are shielded, which are the main factors preventing incompatibility; (ii) multivalent ion-biopolymer interactions (in dairy systems in particular calcium, citrate and phosphate) can selectively enhance the association of biopolymers and change the equilibrium conditions; (iii) counterion distribution effects are suppressed. In polyelectrolyte systems the number of

counterions is comparable to the number of polymer segments, but counterions do not experience the exclude volume effect. When they try to spread out over all the available volume in the mixture, they drag with them a part of their 'host' polymer molecules for reasons of electroneutrality. Cosolubility of the biopolymers is enhanced and the phase separation threshold rises. The counterion distribution effect becomes more pronounced under conditions of large charge density differences between protein and polysaccharide and low ionic strength (Syrbe *et al.*, 1998).

1.2.1 Protein-Nonionic Hydrocolloids

Ionic strength and pH only influence protein self-association (repulsion between protein molecules). Hydrocolloid self-association and protein-hydrocolloid cross-association play a minor role. Incompatibility is directly correlated to protein self-association, which is strongest at the pI due to attractive fluctuating dipole interactions and decreases towards acidic or alkaline pH because the increasing protein net charge. Hence, incompatibility decreases at extreme pHs and increases when electrolyte is added at pH values sufficiently above and below the $pI_{protein}$. Excess electrolyte also helps to suppress any counterion distribution effects, caused by charge density differences between protein and hydrocolloid at these pHs. In the pH range close to the $pI_{protein}$, however, incompatibility is linked to low ionic strengths (Syrbe *et al.*, 1998).

1.2.2 Protein-Anionic Hydrocolloids

Ionic hydrocolloids are in general carboxylated or sulphated and have isoelectric points around or below 3. In the pH range relevant to food, they can be regarded, to a good approximation, as anionic species with little tendency to self-association. In contrast to nonionic hydrocolloids, the Coulomb interaction[1] with the protein is important. Complex coacervation is observed for $pI_{hydrocolloid} < pH < pI_{protein}$,

[1] *Coulomb forces* are also called electrostatic forces, ion-ion bonds (if attractive), or charge-charge interaction. They always occur between charged particles, be they ions, protein molecules, or colloidal particles, and they are also quite strong (Walstra, 2003).

where the two biopolymers carry opposite charges. Complexation can take a non-equilibrium character when the electrostatic interaction becomes strong at low ionic strength and at pHs well in the inter-pI range (Tolstoguzov, 1996).

A number of studies have been investigated the interaction between proteins and polysaccharides. Casein and carrageenan interactions were studied by Langendorff *et al.* (2000). Casein micelles in milk are association colloids of four different caseins and calcium phosphate. The micelles are sterically stabilized by κ-casein. Casein micelles can be mixed with ι-carrageenan at high temperature. At low enough ι-carrageenan concentration depletion interaction does not yet lead to instability (Langendorff *et al.*, 2000). On cooling the mixture one observes a sudden increase in the apparent size of the casein micelles. This is explained by the fact that ι-carrageenan undergoes a coil to helix transition at about 47 °C. As a result of the helix formation the charge density along the ι-carrageenan chain increases and electrostatic interactions are strong enough for adsorption (Syrbe *et al.*, 1998). A similar behavior is observed with pectin/casein micelle mixtures (Maroziene and de Kruif, 2000). On increasing the pH the pectin desorbs and the apparent size of the casein micelles returns to its original value at neutral pH in absence of pectin. Both ι-carrageenan and pectin adsorption are found to be reversible.

In the acacia gum (gum arabic) and β-lactoglobulin system, if they are mixed at pH below pI = 4.6 complex coacervates are formed (Schmitt *et al.*, 1999). The increase of the total biopolymer concentration and the increase of the pH close to the β-lactoglobulin isoelectric point reduced the influence of pH and protein to polysaccharide ratio.

The influence of xanthan gum on physical characteristics of heat-denatured whey protein solutions and gels was investigated (Bryant and McClements, 2000). In the absence of added salt, xanthan promoted phase separation in heat-denatured (HD) whey protein isolate (WPI) solutions but no in native (N)-WPI solutions, which was attributed to the increased effective molecular weight of the heated proteins. In the presence of salt, xanthan caused a significant increase in gelation rate, shear modulus

and opacity of HD-WPI solutions gelled by adding 200 mM NaCl. Rheology and turbidity measurements suggested that HD-WPI gels containing xanthan gum formed water-in-water emulsions consisting of xanthan-rich regions surrounded by a protein-rich aqueous phase. These results indicated that the thermodynamic incompatibility of xanthan and heat denatured whey proteins can be utilized to create products that exhibit a variety of different physicochemical characteristics.

Recently, the characterization of β-lactoglobulin-chitosan interactions in aqueous solutions using a variety of complementary techniques was studied (Guzey and McClements, 2006a). This study showed that chitosan can interact with β-lactoglobulin to form either soluble or insoluble complexes depending on the pH. The characteristics of their complexes are determined by biopolymer type and concentration, as well as by preparation and environmental conditions.

Taken together, proteins and polysaccharides form two phase systems only under certain conditions which inhibit macromolecular interaction between different types and promote association between the same types. Conditions such as conformational transition of coil to helix form of the biopolymer favor self association and entanglement of macromolecules, and consequently increase incompatibility amongst the concerned biopolymers. For polysaccharides containing carboxyl groups, the compatibility decreases as the pH goes down below the isoelectric point of proteins, while for sulfated ones compatibility is entirely dependent on ionic strength. In all these cases, the thermodynamic compatibility decreases with increase in salt concentration and temperature (Samant *et al.*, 1993). Significant differences in compatibility of various proteins with the same polysaccharides are also observed, indicating a role of structure of proteins in compatibility. The compatibility of proteins with anionic polysaccharides decreases in the order, pectin > CMC > sodium alginate > gum arabic > dextran sulfate.

The use of protein-polysaccharide complexes as new ‘fat replacers’ is becoming increasingly important. Other uses of these complexes are in protein recovery (notably, dairy proteins and blood plasma proteins), protein fractionation and

purification (whey proteins), inhibition of protein precipitation (fruit-flavoured milk drinks), thermoplastic extrusion (soy protein isolates) and food preservation (enzymic inhibition) (Dickinson and McClements, 1995; Samant *et al.*, 1993). In non-food applications, the phenomenon of complex coacervation, usually involving gelatin as one of the polymers, is important in the technology of microencapsulation (Luckham, 1994).

2. Emulsions and Emulsion Stability

Emulsions are dispersed systems that consist of two immiscible liquids (usually oil and water), with one of the liquids dispersed as small spherical droplets in the other (McClements, 2004a). Emulsions can be conveniently classified according to the distribution of the oil and aqueous phases. Ordinary emulsions are traditionally classified as being of one of two morphological types. A system which consists of oil droplets dispersed in an aqueous phase is called an oil-in-water or O/W emulsion (e.g., mayonnaise, milk, cream, soups, sauces, and coconut milk). A systems consists water droplets dispersed in an oil phase is called a water-in-oil or W/O emulsion (e.g., margarine, butter, and spreads) (Figure 3) (Dickinson and McClements, 1995; McClements, 2004a; Seow and Gwee, 1997).

To produce an emulsion, new interfaces between the different phases have to be formed. To do this an agent has to initially facilitate interface production by lowering the internal free energy of the system, and then has to provide an absorbed layer between the aqueous phase and the oil. The interfacial behaviour of the agents is therefore crucial in the production and stability of the dispersed system (Hill, 1998).

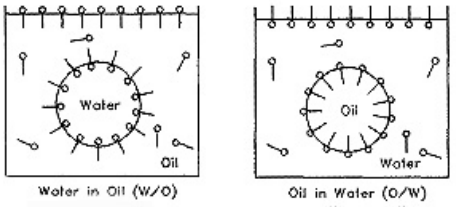


Figure 3. Schematic representation of both oil-in-water and water-in-oil emulsions. Source: modified from Chappat (1994).

Many important foods related macromolecules have the ability to aid in the formation and then stabilize these phase separated systems. Food allowed materials used in the preparation of emulsions and foams include: mono- and di-glycerides, phospholipids (e.g., lecithin), fatty acids and their esters, polysaccharides and proteins. Of major importance is the structure that the molecules can adopt at the interface between the two phases. Proteins are the major macromolecules used as the emulsifier or foaming agents, but polysaccharides are often used to improve stabilization. Protein can undergo a range of modifications to improve their functionality. Typically in foods and sometimes in model systems, mixtures of compounds are used to provide the functionality required. Competition and co-operation effects between surface active agents alter the properties of the disperse system (Hill, 1998).

Despite the adsorption of an entangled layer of macromolecules, all emulsions are inherently unstable. The energy of a dispersed system is higher than the energy of the separated phases because of the energy associated with the interface. In the food industry some dispersed systems need only a short lifetime (e.g., ice-cream mix, cake batter, margarine premix) whilst others must be stable for a very long time, even several years prior to consumption (e.g., mayonnaise, salad cream, cream liqueurs). With emulsions the instability can be divided into four categories and diagrams of these processes are shown in Figure 4 (Hill, 1998).

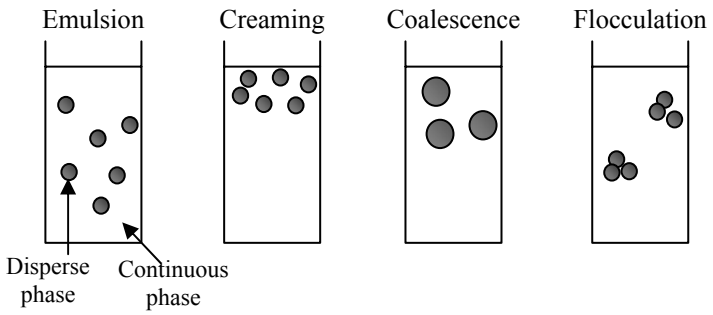


Figure 4. Schematic of the states of an emulsion during destabilization.
Source: Hill (1998).

The term “emulsion stability” refers to the ability of an emulsion to resist changes in its properties over time: the more stable the emulsion, the more slowly its properties change (McClements, 2004a).

2.1 Creaming

Creaming is the process by which buoyant emulsion droplets tend to rise to the top of a container. It is the same process as sedimentation, but in the opposite direction. In many food emulsions, the creaming behaviour is dominated by the flocculation of droplets, and both aspects are considered in this article (Robins, 2000). The densities of most edible oil (in their liquid state) are lower than that of water, and so there is a tendency for oil to accumulate at the top of an emulsion and water at the bottom. Thus, droplets in an oil-in-water emulsion tend to cream, whereas those in a water-in-oil emulsion tend to sediment (McClements, 2004a).

2.2 Flocculation

Flocculation is the process whereby two or more droplets come together to form an aggregate in which the droplets retain their individual integrity. Droplet flocculation may be either advantageous or detrimental to emulsion quality depending on the nature of the food product (McClements, 2004a). The types of flocs that arise in emulsions will depend on the volume fraction and the strength of the interactions between the droplets. Creaming is likely to occur if large aggregates are formed in diluted emulsions. If the phase volume is high then rather than seeing movement of the floc (as in creaming) syneresis is apparent as the continuous phase separates from the floc. If the phase volume is high and weak aggregation occurs, then the emulsion is likely to show thixotropic rheological properties as the floc will break down with shear. Strongly flocculated systems will show definite elastic properties (Hill, 1998).

2.3 Coalescence

Coalescence is the process whereby two or more liquid droplets merge together to form a single larger droplet (McClements, 2004a). Coalescence stability frequently affects processing or shelf life of oil-in-water emulsions. Foaming of whippable emulsions such as ice cream and whipping cream requires coalescence of fat globules (Goff and Jordan, 1989). However, coalescence can induce oiling-off and accelerate creaming upon storage of fluid emulsions (Halling, 1981). In water-in-oil emulsions, it leads to the accumulation of water at the bottom of the material (McClements, 2004a).

2.4 Phase Inversion

Phase inversion is the process whereby a system changes from an oil-in-water emulsion to a water-in-oil emulsion or vice versa. Phase inversion is an essential step in the manufacture of a number of important food products, including butter and margarine. In other foods, phase inversion is undesirable because it has an adverse effect on their appearance, texture, stability, and taste (McClements, 2004a).

The rate at which an emulsion breaks down, and the mechanism by which this process occurs, depends on its composition and microstructure, as well as on the environmental conditions it experiences during its life time (e.g., temperature variations, mechanical agitation, and storage conditions) (McClements, 2004a). Most food oil-in-water emulsions are primarily stabilized by an adsorbed layer of protein around the droplets; any polysaccharide (if present) often gives only secondary stabilization through a thickening or structuring of the aqueous continuous phase. In addition, depending on the chemical nature of the biopolymers involved and on the solvent conditions, an interfacial protein-polysaccharide complex may be formed which may either increase or reduce the stability with respect to creaming, flocculation or coalescence (Dickinson and McClements, 1995).

3. Multilayer Emulsions

Traditionally, oil-in-water emulsions are produced by homogenizing oil and aqueous phases together in the presence of one or more emulsifiers (Friberg *et al.*, 2004; McClements, 2004a). An emulsifier is a surface-active molecule that adsorbs to the surface of the emulsion droplets during homogenization to form a protective membrane that prevent them from aggregating (flocculating and/or coalescing) with one another by generating repulsive interactions. In addition, an emulsifier reduces the oil-water interfacial tension, thereby facilitating the disruption of emulsion droplets during homogenization (Walstra, 2003). The most common emulsifiers used in the food industry are proteins, polysaccharides, phospholipids, and small molecule surfactants (Charlambous and Doxastakis, 1989; McClements, 2004a). Emulsifiers vary greatly in their effectiveness at producing small oil droplets during homogenization, and in their ability to prevent droplet aggregation under different environmental stresses, such as pH, ionic strength, heating, and freezing (Garti and Reichman, 1993; McClements, 2004a). Food emulsifiers also differ in their cost, reliability, ease of use, ingredient compatibility, label friendliness, and legal status (Krog and Sparso, 2004; Stauffer, 1999).

On the other hand, emulsions stabilized by polysaccharides, such as gum arabic and modified starch, are often more resistant to pH changes, high ionic strength and elevated temperatures than those stabilized by proteins. This has been attributed to the fact that polysaccharide-stabilized droplets are surrounded by a relatively thick porous polymer layer, which increases the steric repulsion and decreases the van der Waals attraction between droplets (McClements, 2004a). Furthermore, polysaccharides are usually much less effective at producing emulsions than proteins, because they are less surface active. Hence, they must be used in much higher concentrations than proteins to produce emulsions containing small droplets. It would therefore be advantageous to combine the beneficial attributes of proteins and polysaccharides to produce small emulsion droplets with good environmental stability (McClements, 2004a).

One strategy has been to create an interfacial layer around oil droplets that consists of multiple layers of emulsifiers and/or polyelectrolytes using a layer-by-layer (LBL) electrostatic deposition technique (Decher, 1997; Schonhoff, 2003), which has been developed to increase the stability of emulsions to environmental stresses (e.g., pH, salt, heating, dehydration, freezing, and chilling) (Aoki *et al.*, 2005; Gu *et al.*, 2004a, 2004b, 2005a; Gu *et al.*, 2005c; Guzey *et al.*, 2004; Klinkesorn *et al.*, 2005a; Moreau *et al.*, 2003; Ogawa *et al.*, 2003a, 2003b, 2004; Surh *et al.*, 2005). In this approach, an ionic emulsifier that rapidly adsorbs to the surface of lipid droplets during homogenization is used to produced a *primary* emulsion containing small droplets, then an oppositely charged polyelectrolyte is added to the system that adsorbs to the droplet surfaces and produces a *secondary* emulsion containing droplets coated with a two-layer interface (Figure 5). This latter procedure can be repeated to form oil droplets coated by interfaces containing three or more layers (Caruso, 2001; Ogawa *et al.*, 2004). Under certain circumstances, emulsions containing oil droplets surrounded by multi-layer interfaces have been found to have better stability to environmental stress than conventional oil-in-water emulsions with single-layer interfaces.

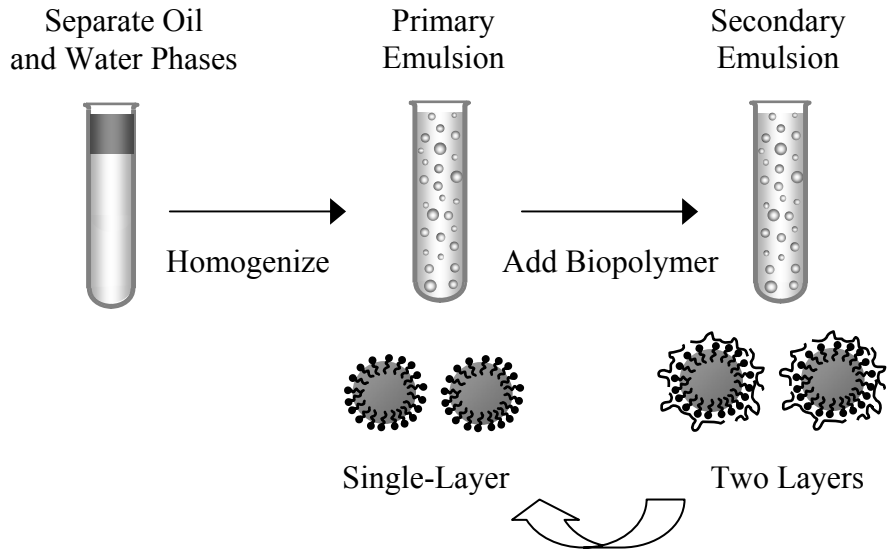


Figure 5. Utilization of LBL technique for the production of oil-in-water emulsions.

4. Factors Effecting Stability of Multilayer Emulsions

The main problem with using the LBL technique to prepare multilayer emulsions is the tendency for extensive flocculation of the particles to occur, even under conditions where they should be saturated with polyelectrolyte (McClements, 2004a; McClements, 2005). It is sometimes possible to disrupt the flocs formed by application of mechanical agitation, such as sonication, blending, or homogenization (Ogawa *et al.*, 2004). Alternatively, particle flocculation can be retarded by carefully controlling the preparation conditions, such as the characteristics of the colloidal particles (e.g., concentration, size and charge), the characteristics of the polymer (e.g., concentration, molecular weight, charge density, conformation), the properties of solvent (e.g, ionic strength, pH, and dielectric constant), and/or the mixing method (e.g., order of addition, mixing speed, flow profile) (McClements, 2005).

Two mechanisms have been proposed to account for the flocculation observed during the preparation of multilayer-coated particles: (i) bridging flocculation occurs when particle collisions take place more rapidly than the time required for the polymers to saturate the particle surfaces completely, and (ii) depletion flocculation occurs when the free polymer concentration exceeds a particular value (McClements, 2005). To minimize or avoid droplet aggregation, it is important to carefully control solution composition and preparation conditions: (i) there should be sufficient polyelectrolyte present to saturate all of the droplet surfaces; (ii) the polyelectrolyte molecules should adsorb to the droplet surfaces more rapidly than droplet-droplet collisions occur; (iii) there should not be too much polyelectrolyte present to promote depletion flocculation; (iv) the repulsive interactions between the coated droplets should be strong enough to prevent droplet aggregation (Guzey and McClements, 2006b).

5. Application of Multilayer Emulsions

Food emulsions experience a variety of different environmental stresses during their manufacture, storage, transport and utilization, including pH extremes, high ionic strength, thermal processing, freeze-thaw cycling, drying, and mechanical agitation (Guzey and McClements, 2006b; McClements, 2004b). Recent experiments (Table 1) have shown that droplets coated by multilayered interfacial membranes have improved stability to environmental stresses than those stabilized by single-layered membranes because of the increase in interfacial thickness and rheology (McClements, 2004b).

Table 1. Overview of the multilayer emulsion systems which improved stability against environmental stresses.

Multilayer systems	**Stability against Environmental stresses**	**References**
Lecithin-chitosan	pH (≤ 5), NaCl (< 500 mM), $CaCl_2$ (≤ 500 mM), thermal treatments (30-90 ºC for 30 min), freeze-thaw cycling (-10 ºC for 22 h/30 ºC for 2 h), oxidative stability	Ogawa *et al.* (2003a, 2003b) Klinkesorn *et al.* (2005a)
Lecithin-chitosan-pectin	pH 4-8 at 0 mM NaCl, pH 3-8 at 100 mM NaCl	Ogawa *et al.* (2004)
β-Lg-pectin	pH (3), NaCl (≤ 500 mM)	Moreau *et al.* (2003) Guzey *et al.* (2004)
β-Lg-ι-carrageenan	NaCl (≤ 500 mM), $CaCl_2$ (≤ 2 mM), thermal treatments (≤ 60 ºC for 20 min)	Gu *et al.* (2005c)
SDS-fish gelatin	pH (3-8), NaCl (≤ 100 mM), thermal treatments (30-90 ºC for 30 min)	Surh *et al.* (2005)
SDS-chitosan-pectin	pH (3-8), NaCl (≤ 500 mM), thermal treatments (30-90 ºC for 20 min), freeze-thaw cycling (-20 ºC for 22 h/30 ºC for 2 h)	Aoki *et al.* (2005)

5.1 pH

The aqueous solution surrounding the oil droplets in food oil-in-water emulsions may vary from acidic (e.g., pH 2.5 - 4 in some soft drinks) to alkaline (e.g., pH 7 - 8 in some dairy products) depending on the nature of the product. In addition, the aqueous phase pH may vary during the production, storage or utilization of the product. It is important to ensure that the oil droplets do not aggregate when they are exposed to variations in pH. The influence of pH on the tendency of oil droplets to aggregate is normally determined by how the various repulsive forces generated by the interfacial layer vary with pH, e.g., electrostatic and steric repulsion. Multilayer interfaces are normally produced using weak polyelectrolytes, and so their thickness, structure and electrical characteristics are strongly dependent on solution pH. By manipulating the type of polyelectrolytes used to prepare multilayer emulsions it is therefore possible to control the influence of pH on droplet aggregation (Guzey and McClements, 2006b). There are many studies that have been carried out on the influence of solution pH on the adsorption of polyelectrolytes to charged oil droplets, and on its impact on the stability of the multilayer emulsions formed to droplet aggregation.

The influence of pH on the ζ-potential of primary (sodium dodecyl sulphate, SDS), secondary (SDS-chitosan) and tertiary (SDS-chitosan-pectin) emulsions containing 0.2 wt% corn oil has been studied (Aoki *et al.*, 2005). The ζ-potential of the SDS stabilized droplets in the primary emulsions was negative at all pH values due to the presence of the adsorbed anionic surfactant. The ζ-potential of the SDS-chitosan stabilized droplets in the secondary emulsions was positive at low pH (< pH 6), but became negative at higher values (pH 7 and 8). The charge reversal on the emulsion droplets probably occurred because chitosan was desorbed from the droplet surfaces when the pH was increased above 6. The cationic groups on chitosan typically have a pK_a value around 6.3-7, hence the chitosan loses its charge around this pH (Ogawa *et al.*, 2004). The ζ-potential of the SDS-chitosan-pectin stabilized droplets in the tertiary emulsion was negative at all pH, which suggested that the chitosan layer did not desorb from the surface of the emulsion droplets at higher pH

values even though it did in the secondary emulsions. This may have been because the pK_a value of the positively charged groups on the chitosan molecules was increased appreciably when the chitosan was sandwiched between two negatively charged polyelectrolyte layers, as has been reported for other polyelectrolytes (Burke and Barrett, 2003a, 2003b). The increase in the magnitude of the negative charge with increasing pH, was probably because of the increase in the negative charge on the pectin molecules ($pK_a \sim 4.5$) and decrease in positive charge on the chitosan molecules ($pK_a \sim 6.5$) as the pH increased. Multilayer emulsions initially stabilized by lecithin, rather than SDS, exhibit similar characteristics (Klinkesorn *et al.*, 2005a; Ogawa *et al.*, 2003a, 2003b).

SDS and fish gelatin were selected as an anionic surfactant and a cationic biopolymer for preparation of two-layered interfacial membranes (Surh *et al.*, 2005). Fish gelatin has been shown to adsorb to the surfaces of SDS-coated droplets from pH 3 to 7. Under these conditions the SDS-stabilized droplets are negatively charged and the fish gelatin is either positively charged or slightly negatively charged (pI ~ 7) and had a value close to zero somewhere between pH 5 and 6. The charge reversal on the emulsion droplets suggests that either the gelatine lost some of its positive charge when the pH was increased or that it was (partially) desorbed from the droplet surfaces. The ζ-potential of droplets in SDS-gelatin stabilized emulsions was slightly negative even at pH values below the pI, due to the negative charge on the SDS molecules outweighing the positive charge on the gelatin molecules. Fish gelatin would be expected to lose its net charge and be detached from the droplet surfaces around its pI value. This result suggested that the adsorption of gelatin greatly improved the stability of the emulsions to droplet aggregation and oiling off at all pH values.

A series of experiments have examined the influence of solution pH on the adsorption of carrageenan to the surfaces of β-lactoglobulin stabilized droplets (Gu *et al.*, 2004b, 2005a, 2005b). ι-Carrageenan has been shown to adsorb to the surface of protein-coated droplets from pH 3 to 6, but not at pH 7. The primary emulsions which oil droplets stabilized by β-lactoglobulin are positively charged below the pI of

the adsorbed proteins (pI ~ 5), but negatively charged above, whereas the ι-carrageenan is strongly negatively charged across the whole pH range. The adsorption of carrageenan at pH 3 and 4 can therefore be attributed to a strong electrostatic attraction between the anionic carrageenan and the cationic protein-coated droplets. On the other hand, the adsorption of carrageenan at pH 5 and 6 can be attributed to binding of segments of carrageenan molecules to positive patches on the surfaces of the β-lactoglobulin molecules. The adsorption of carrageenan to the droplet surfaces improved the stability of the emulsions to droplet aggregation at pH 5 and 6, but promoted extensive droplet flocculation at pH 3 and 4. This latter effect was attributed to charge neutralization and polymer bridging effects (Gu *et al.*, 2005a; Surh *et al.*, 2005). The influence of carrageenan on the pH stability of protein-coated droplets also depended on the conformation and electrical characteristics of the carrageenan molecules used. Studies have shown that ι-carrageenan adsorbs more strongly and provides better stability than κ– or λ-carrageenan, which was attributed to its high charge density and helical structure (Gu *et al.*, 2005a).

Pectin has also been shown to adsorb strongly to β-lactoglobulin-stabilized droplets at pH values from 3 to 5 (Guzey *et al.*, 2004; Moreau *et al.*, 2003), which was attributed to the electrostatic attraction between the anionic polysaccharides and the cationic droplets. The presence of the pectin improved the stability of the emulsions to droplet aggregation at pH 4 and 5, but not at pH 3. This was attributed to the fact that pectin starts to lose its negative charge around this pH (pK ~ 3.5), so the magnitude of the charge on the emulsion droplets decreases. It is interesting to note, and of considerable practical importance, that the stability of multilayer emulsions is strongly dependent on the pH at which the polysaccharide and protein-coated droplets are mixed (Guzey *et al.*, 2004). If a multilayer emulsion is formed by mixing pectin and β-lactoglobulin-coated droplets together at pH 7 (where they are both anionic) and then reducing the pH to 4 (where the protein is cationic and the polysaccharide is anionic), the overall stability to droplet aggregation is better than directly mixing them together at pH 4. Presumably, this is because the polysaccharide is evenly distributed around the droplets when the latter gain their positive charge, which facilitates adsorption.

Overall, the stable multilayer emulsions can be formed at a particular pH, it is necessary to use preparation conditions that do not promote droplet flocculation, and to select a polysaccharide/emulsifier combination that provides a strong enough electrostatic and/or steric repulsion between the droplets.

5.2 Salt

The type and concentration of ions in a food emulsion may vary considerably depending on the nature of the food, the purity of the functional ingredients, and the hardness of the water used to prepare the emulsion. In many situations it is important to ensure that the presence of ions does not promote emulsion instability, e.g., by screening electrostatic interactions or by binding to oppositely charged groups. The presence of salts can alter interfacial and emulsion properties through a variety of physicochemical mechanisms, including changing the amount of polyelectrolyte adsorbed, altering the structure of the interfacial layer, or modulating the strength and range of the various colloidal interactions between the droplets (Guzey and McClements, 2006b).

A number of studies have shown that emulsions containing multilayer-coated droplets are more stable to high salt concentrations than those containing single layer-coated droplets (Aoki *et al.*, 2005; Gu *et al.*, 2005c; Guzey *et al.*, 2004; Moreau *et al.*, 2003; Ogawa *et al.*, 2003a; Surh *et al.*, 2005).

The ζ-potential of the SDS-stabilized droplets in the primary emulsions was negative at all ionic strengths (0-500 mM NaCl), but the magnitude of the ζ-potential decreased as the NaCl concentration was increased which can be attributed to electrostatic screening. The ζ-potential of the SDS-chitosan stabilized droplets in the secondary emulsions was positive at all ionic strengths but the magnitude of the ζ-potential decreased as the NaCl concentration was increased which can also be attributed to electrostatic screening. It is also possible that some of the decrease of charge on the emulsions droplets in the secondary emulsions was due to desorption of chitosan from the droplet surfaces when the ionic strength increased. The ζ-potential

of the SDS-chitosan-pectin stabilized droplets in the tertiary emulsion was negative at all ionic strengths. From this result, the secondary and tertiary emulsions were relatively stable to droplet aggregation at all ionic strengths (0-500 mM NaCl) because the relatively thick interfacial membranes provided good steric stabilization (Aoki *et al.*, 2005).

Primary (β-lactoglobulin coated) and secondary (β-lactoglobulin-carrageenan coated) emulsions were prepared at pH 6, and then different amounts of NaCl were added to the aqueous phase (Gu *et al.*, 2005c). The primary emulsions showed evidence of droplet aggregation and creaming at ≥ 100 mM NaCl, whereas the secondary emulsions were stable at all salt levels studied (≤ 500 mM NaCl). The origin of this effect was attributed to the fact that the steric repulsion is longer range due to the thicker interfacial membranes in the secondary emulsions. Similar results were also found with β-lactoglobulin-pectin coated droplets at pH 3 (Moreau *et al.*, 2003).

Other studies have shown that emulsions containing droplets coated by interfacial layers formed from an anionic surfactant and cationic biopolymer have better stability to high salt concentrations than those coated by anionic surfactants alone, e.g., lecithin-chitosan (Ogawa *et al.*, 2003a, 2003b) and SDS-gelatin (Surh *et al.*, 2005). For example, lecithin-chitosan coated droplets (pH 3) were stable to droplet aggregation at ≤ 500 mM $CaCl_2$, whereas lecithin coated droplets were unstable at ≥ 3 mM (Ogawa *et al.*, 2003a). The most likely reason is that the multivalent Ca^{2+} ions are counterions for the anionic droplets in the primary emulsion, whereas the monovalent Cl^- ions are counterions for the cationic droplets in the secondary emulsion. Multivalent ions are known to be much more effective at binding and screening than monovalent ions (Israelachvili, 1992; McClements, 2004a).

5.3 Temperature

Many food emulsions undergo some form of thermal processing, during their production, storage or utilization, e.g., pasteurization, sterilization or cooking (McClements, 2004a). They also can be chilled or frozen during storage and then warmed prior to use in the food industry, e.g., dairy products, desserts, sauces, and ice cream (McClements, 2004a). It is important that an emulsion is capable of withstanding these treatments without breaking down due to droplet flocculation or coalescence (Guzey and McClements, 2006b). Studies have shown that multilayer emulsions may be able to improve the stability to thermal treatments or freezing.

Multilayer emulsions containing droplets coated by an anionic surfactant and a cationic polyelectrolyte have been shown to be stable to thermal processing from 30 to 90 °C, e.g., SDS-chitosan (Aoki *et al.*, 2005), lecithin-chitosan (Ogawa *et al.*, 2003a) and SDS-gelatin (Surh *et al.*, 2005). In some cases, the stability of the secondary emulsions was better than that of the primary emulsion, which was partly attributed to the increased steric repulsion between the droplets. Multilayer emulsions containing droplets coated by protein-polysaccharide complexes have also been shown to have better stability to thermal processing than those stabilized by proteins alone, e.g., β-lactoglobulin-ι-carrageenan (Gu *et al.*, 2005c).

It should be noted that the tendency for polyelectrolytes to adsorb to the surfaces of emulsifier-coated droplets may be altered by changes in temperature. This is illustrated in recent studies have shown the adsorption of carrageenan to the surfaces of β-lactoglobulin coated droplets was strongly influenced by thermal processing and salt concentration (Gu *et al.*, 2005c). Secondary emulsions containing β-lactoglobulin-ι-carrageenan coated droplets (pH 6) were prepared at room temperature and then subjected to an isothermal heat treatment (30 - 90 °C for 20 min), before being cooled down to room temperature and analyzed. In the absence of salt, carrageenan remained adsorbed to the β-lactoglobulin coated droplets after all thermal treatments, which was attributed to the strong electrostatic attraction between the two kinds of molecules at low ionic strengths. On the other hand, in the presence

of 150 mM NaCl, the carrageenan molecules desorbed from the β-lactoglobulin coated droplet surfaces at temperatures of 60 °C and higher. This was attributed to the weakening of the electrostatic attraction by the presence of the salt, in combination with an alteration in the conformation of the adsorbed β-lactoglobulin molecules upon heating (e.g., due to a change in the charge density of the positive patches on the proteins surface). Consequently, the carrageenan layer was able to prevent droplet flocculation at relatively low temperatures (≤ 50 °C) where it remained adsorbed to the protein-coated droplet surfaces, but not at higher temperatures because it became desorbed (Gu *et al.*, 2005c).

The SDS-chitosan-pectin system (Aoki *et al.*, 2005) and the lecithin-chitosan system (Ogawa *et al.*, 2003a) can be able to improve the stability of the emulsions to cold storage. For example, SDS-chitosan-pectin coated droplets (pH 3) remained stable after 6 freeze-thaw cycles (-20 °C for 22 h/ 30 °C for 2 h), but the primary (SDS coated) and the secondary (SDS-chitosan coated) were highly unstable to freeze-thaw cycling (Aoki *et al.*, 2005).

Studies have also been shown that multilayer emulsions may be able to improve the stability of the encapsulated lipids to oxidation (Klinkesorn *et al.*, 2005a; Ogawa *et al.*, 2003a) and to freeze drying and spray drying (Klinkesorn *et al.*, 2005a, 2005b).

Multilayer emulsions may have a number of potential applications in the food industry which improve the stability to environmental stresses under certain circumstances. Thick highly charged multilayer interfaces may be useful in protecting droplets against aggregation or in preventing lipid oxidation. On the other hand, multilayer interfaces that change their properties in a controlled fashion in response to some environmental conditions could be use for controlled or triggered release of active ingredients (Guzey and McClements, 2006b).

MATERIALS AND METHODS

Materials

Powdered β-lactoglobulin (β-Lg) was obtained from Davisco Foods International (lot no. JE 001-3-922, Le Sueur, MN). The manufacturer reported that this product contained 95% β-Lg and 4.9% moisture.

Food grade sodium alginate was kindly donated by TIC gums (TIC Pretested® Colloid 488T, lot no. 6724). The supplier reported that this product had an M:G ratio of about 55:45, was of "medium" viscosity, and had a molecular mass of 216 kDa. Gum arabic (lot no. 8475) (food grade) and ι-carrageenan (lot no. 10325050) were donated by TIC gums and FMC BioPolymer (Philadelphia, PA), respectively. These biopolymers were used without further purification.

The hydrogenated palm oil (Cebes® 27-50) with a Wiley melting point of 33-35 °C was provided by Aarhus Inc. (New Jersey, USA). The corn oil was bought from local supermarket. The oils were used without further purification.

Analytical grade hydrochloric acid, sodium hydroxide, sodium azide, and sodium phosphate were obtained from Sigma-Aldrich (St. Louis, MO). Purified water from a Nanopure water system (Nanopure Infinity, Barnstead International, IA) was used for the preparation of all solutions.

Methods

1. Characterization of β-Lactoglobulin-Sodium Alginate Interactions in Aqueous Solutions

1.1 Solution Preparation

A stock buffer solution was prepared by dispersing 5 mM phosphate buffer in water containing 0.04 wt% sodium azide (using as an antimicrobial) and then adjusting to pH 7 using HCl or NaOH. Biopolymer stock solutions were prepared by dissolving either 1.0 wt% β-Lg or 1.0 wt% sodium alginate in the stock buffer solution, stirring for at least 2 h to ensure complete dispersion, and then storing overnight at 5 °C. Biopolymer mixtures containing β-Lg (0.1 wt%) and sodium alginate (0 to 0.1 wt%) were prepared by mixing different ratios of the stock solutions with buffer solution at the desired pH (3 to 7). The pH of each of the solutions was adjusted to the appropriate value prior to mixing. It should be noted that there would be a slight difference in the ionic strength of solutions at different pH values due to the different amounts of acid or base they contained.

1.2 Isothermal Titration Calorimetry (ITC)

An isothermal titration calorimeter (VP-ITC, Microcal Inc., Northampton, MA) was used to measure the enthalpy changes resulting from β-Lg-sodium alginate interactions. Aliquots of 20 μL sodium alginate solution (0.1 wt%) were injected sequentially into a 1.45-mL titration cell initially containing either 5 mM phosphate buffer solution or 0.1 wt% β-Lg in 5 mM phosphate buffer. Each injection lasted 40 s and there was an interval of 600 s between successive injections. The temperature of the solution in the titration cell was 30 ± 0.1 °C and the solution was stirred at 315 rpm throughout the experiments. Measurements were carried out in duplicate and the results are reported as the mean and standard deviation. Corrected enthalpy changes were calculated by subtracting the enthalpy change when sodium alginate was titrated into buffer solution from that obtained when sodium alginate was titrated into β-Lg

solution. The results are reported as the change in enthalpy per mole of sodium alginate (216 kDa) injected into the reaction cell (kcal mol^{-1}).

1.3 Soluble Protein and Turbidity Measurements

Aliquots (200 μL) of 0.1 wt% sodium alginate solution were injected into a glass test tube initially containing 14.5 mL of 0.1 wt% β-Lg solution. The resulting solutions were mixed for approximately 1 min using a vortex mixer, and then stored at room temperature for 24 h prior to analysis. The absorbance of the resulting solutions was measured at 280 nm (soluble protein) and 600 nm (turbidity) using a UV-visible spectrophotometer (UV-2101PC, Shimadzu Corporation, Tokyo, Japan) in 1 cm path length optical cells against distilled water. For the soluble protein measurements, the solutions were first centrifuged to remove any insoluble complexes and then only the supernatant was analyzed. Measurements were carried out in duplicate and the results are reported as the mean and standard deviation.

1.4 ζ-Potential and Particle Size Measurements

The electrical charge (ζ-potential) and mean (volume) diameter (d_V) of individual biopolymers or biopolymer complexes in aqueous solutions were determined using a commercial instrument capable of electrophoresis and dynamic light scattering measurements (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). Biopolymer solutions were prepared and stored at room temperature for 24 h prior to analysis. Measurements were carried out in duplicate and the results are reported as the mean and standard deviation.

2. Influence of Alginate, pH, and Ultrasound Treatment on Palm Oil-in-Water Emulsions Stabilized by β-Lactoglobulin

2.1 Solution Preparation

An emulsifier solution was prepared by dispersing 1.0 wt% β-Lg in 5 mM phosphate buffer (pH 7.0) containing 0.04 wt% sodium azide (as an antimicrobial agent) and stirring for at least 2 h. Sodium alginate solution was prepared by dispersing 1.0 wt% powdered sodium alginate in deionized and distilled water and stirring for at least 2 h. Sodium azide (0.04 wt%) was also added to this solution to prevent microbial growth. Both protein and polysaccharide dispersions were then kept overnight at 5 ºC to ensure complete hydration of biopolymers.

2.2 Emulsion Preparation

2.2.1 Primary Emulsion

A coarse primary emulsion was prepared by homogenizing 10 wt% hydrogenated palm oil with 90 wt% aqueous emulsifier solution (1 wt% β-Lg in 5 mM phosphate buffer, pH 7) using a high-speed blender (M133/1281-0, Biospec Products, Inc., Switzerland) at room temperature. This coarse emulsion (about 1000 g) was passed through a high-pressure valve homogenizer (APV-Gaulin, Model Rannie Mini-Lab 8.30 H, Wilmington, MA) three times at 4000 ± 500 psi to reduce the mean droplet diameter (d_{32}) to 0.30 ± 0.05 μm. All of these procedures were carried out ca. 40 ºC to prevent the crystallization of hydrogenated palm oil during emulsion preparation. The emulsion obtained from this procedure was called the "primary" emulsion. The pH of the primary emulsion was adjusted to values ranging from 3 to 7 using 0.1, 0.5 or 1 N HCl or NaOH solutions.

2.2.2 Secondary Emulsions

Secondary emulsions were prepared by diluting primary emulsions with different ratios of 1 wt% sodium alginate solution and 5 mM phosphate buffer solution, all at the same pH. The final composition of the secondary emulsions formed was: 5 wt% hydrogenated palm oil, 0.45 wt% β-Lg and 0 to 0.5 wt% sodium alginate at pH values from 3 to 7. These emulsions were stored at room temperature for 24 h prior to further analysis.

2.2.3 Ultrasound Treatment

In some experiments the emulsions were treated with high intensity ultrasonic waves to try to disrupt any flocculated droplets. Sonication was applied immediately after the secondary emulsions had been prepared by mixing primary emulsion with alginate solution. Sonication was conducted for 1 min at a frequency of 20 kHz, amplitude of 70% and duty cycle of 0.5 s (Model 500, Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA). These secondary emulsions were also kept for 24 h at room temperature prior to analysis.

2.3 Emulsion Characterization

The particle size distribution of diluted emulsions was measured using a laser light scattering instrument (Mastersizer, Malvern Instruments, Worcestershire, UK). Measurements are reported as the volume-surface mean diameter: $d_{32} = \Sigma n_i d_{i3} / \Sigma n_i d_{i2}$, where n_i is the number of droplets of diameter d_i. The ζ-potential of the emulsions was measured using a particle electrophoresis instrument (ZEM5003, Zetamaster, Malvern Instruments, Worcestershire, UK). For particle size and ζ-potential measurements the emulsions were diluted with phosphate buffer solution at the same pH as the emulsion to avoid multiple scattering effects.

The creaming stability was measured by visual observation of the emulsions (10 g) after they had been stored in glass test tubes (125 mm height, 15 mm

i.d.) at room temperature (25 ± 3 °C) for 24 h. The extent of creaming was characterized by a creaming index = $(H_S/H_E) \times 100$, where H_E is the total height of the emulsion and H_S is the height of the serum layer.

The microstructure of the emulsions was determined by optical microscopy (Nikon microscope Eclipse E400, Nikon Corporation, Japan) of emulsion samples placed on a microscope slide.

3. Influence of Ionic Strength and Mixing Condition on Formation and Stability of Emulsions Containing Oil Droplets Coated by β-Lactoglobulin-Alginate Interfaces at Different pH

3.1 Solution Preparation

A stock buffer solution was prepared by dispersing 5 mM phosphate buffer in water containing 0.02 wt% sodium azide (using as an antimicrobial) and then adjusting to pH 7.0 using 1 M HCl or 1 N NaOH. Biopolymer stock solutions were prepared by dissolving either 0.1 wt% β-Lg (to form primary emulsion) or 0.1 wt% sodium alginate (to form secondary emulsion) in stock buffer solution, stirring for at least 2 h to ensure complete dispersion, and then storing overnight at 5 °C. The pH of these two solutions was then adjusted to pH 7.0 using 1 M HCl or 1 N NaOH.

3.2 Emulsion Preparation

In this study, the term “primary emulsion” is used to refer to the emulsion created using the protein as the emulsifier, while the term “secondary emulsion” is used to refer to the primary emulsion to which the polysaccharide has also been added.

A coarse emulsion was prepared by blending 1.0 wt% corn oil with 99 wt% aqueous emulsifier solution (0.091 wt% β-Lg, pH 7) using a high-speed blender (M133/1281-0, ESGE, Switzerland) for 2 min. This coarse emulsion was then passed through a two-stage high-pressure valve homogenizer (LAB 1000, APV-Gaulin,

Wilmington, MA) three times: 4500 psi first stage / 500 psi second stage. Finally, 0.02 wt% sodium azide (NaN_3) was added to the stock emulsion as an antimicrobial agent to prevent deterioration during storage.

3.2.1 Influence of Ionic Strength on Formation and Stability of Emulsions at Different pH

In some experiments, the influence of solution pH and ionic strength on the stability and properties of emulsions was investigated. Dilute primary and secondary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg, 5 mM phosphate buffer, pH 7) were prepared by mixing the stock emulsion with sodium alginate solution (0.1 wt%, 5 mM phosphate, pH 7), NaCl solution (1 M, 5 mM phosphate, pH 7) and/or pure buffer solution (5 mM phosphate, pH 7). The resulting emulsions contained either 0 wt% (primary) or 0.004 wt% (secondary) sodium alginate, and 0 to 250 mM NaCl. The emulsions were stirred using magnetic stirrer for 30 min. The pH of the emulsions was then adjusted to 3.0, 4.0, 5.0, 6.0, and 7.0 using HCl or NaOH solution. These emulsions were then stored overnight at room temperature before being analyzed.

3.2.2 Influence of Mixing Conditions on Formation and Stability of Emulsions at Different pH

In some experiments, the influence of mixing conditions on the stability and properties of secondary emulsions was investigated by preparing these emulsions in two different ways;

3.2.2.1 One-Step Method

Stock primary emulsions and sodium alginate solutions were prepared at pH 7.0, and then their pH was adjusted to final values ranging from 3 to 7 (± 0.1) using HCl solutions. Secondary emulsions were then prepared by diluting the stock primary emulsions with sodium alginate solutions of the same pH.

Under these conditions the droplets and polymer are mixed together at a pH where they have opposite charges.

3.2.2.2 Two-Step Method

Secondary emulsions were prepared at pH 7.0 by mixing the stock primary emulsion (pH 7) with sodium alginate solution (pH 7), and then the final pH was adjusted to values ranging from 3 to 7. Under these conditions the droplets and polymer are initially mixed together at a pH where they have similar charges, and then the pH is adjusted to a value where they have opposite charges. The emulsions were then stirred for 30 min, and the pH of these emulsions was checked and re-adjusted to the appropriate values using HCl or NaOH solutions if required. The emulsions were then stored overnight at room temperature before being analyzed.

3.3 Particle Charge and Size Measurements

The electrical charge (ζ-potential) and size of the particles in the emulsions were determined using a commercial instrument capable of electrophoresis and dynamic light scattering measurements (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). The emulsions were prepared and stored at room temperature for 24 h prior to analysis.

3.4 Creaming Stability Measurements

Approximately 3.5 g samples of emulsion were transferred into 10-mm path length plastic spectrophotometer cuvettes and then stored at 30 °C for 7 days. The change in turbidity at 600 nm of the emulsions was measured with storage time using a UV-visible spectrophotometer (UV-2101PC, Shimadzu Corporation, Tokyo, Japan), with distilled water as a reference. The light beam passed through the emulsions at a height that was about 15 mm from the cuvette bottom, i.e., about 42% of the emulsion's height. The oil droplets in the emulsions moved upward due to gravity, which led to the formation of a relatively clear droplet-depleted serum layer

at the bottom of the cuvette. The rate at which this serum layer moved upward provides an indication of the creaming stability of the emulsions: the faster the rate, the more unstable the emulsions (Ogawa *et al.*, 2003b). An appreciable decrease in emulsion turbidity was therefore an indication of the fact that the serum layer had risen to at least 42% of the emulsion's height. It should also be noted that the turbidity of an emulsion also depends on particle size, so an observed change in turbidity may also reflect droplet aggregation as well as creaming.

3.5 Spectro-Turbidity Measurements

An indication of droplet aggregation in the emulsions was also obtained from measurements of the turbidity versus wavelength, since the turbidity spectrum of a colloidal dispersion depends on the size of the particles it contains (McClements, 2002). Approximately 1.5 g samples of emulsion were transferred into 5-mm path length plastic spectrophotometer cuvettes. The emulsions were inverted a number of times prior to measurements to ensure that they were homogeneous so as to avoid any changes in turbidity due to droplet creaming. The change in absorbance of the emulsions was recorded when the wavelength changed from 800 nm to 400 nm measured using a UV-visible spectrophotometer (UV-2101PC, Shimadzu Corporation, Tokyo, Japan), with distilled water as a reference. The emulsions were prepared and stored at room temperature for 24 h prior to analysis.

4. Stabilization of Model Beverage Emulsions Using Protein-Polysaccharide Electrostatic Complexes Formed at the Oil-Water Interfaces

4.1 Solution Preparation

An emulsifier solution was prepared by dispersing 0.1 wt% β-Lg in 5 mM phosphate buffer (pH 7.0) and stirring for at least 2 h. Sodium alginate, gum arabic and ι-carrageenan solutions were prepared by dispersing the appropriate amount of powdered polysaccharide into 5 mM phosphate buffer (pH 7.0) and stirring for at least 2 h. In the case of ι-carrageenan, the solution was then heated in a water bath at 70 °C

for 20 min to facilitate dispersion and dissolution (Gu *et al.*, 2005a). Sodium azide (0.02 wt%) was added to each of the solutions to prevent microbial growth. After preparation, protein and polysaccharide solutions were stored overnight at 5 °C to allow complete hydration of the biopolymers.

4.2 Emulsion Preparation

In this study, the term "primary emulsion" is used to refer to the emulsion created using only the protein as the emulsifier, while the term "secondary emulsion" is used to refer to the primary emulsion to which a polysaccharide has also been added. It should be noted, that the polysaccharide may or may not be adsorbed to the droplet surfaces in the secondary emulsions depending on solution conditions (e.g., pH and ionic strength).

A corn oil-in-water emulsion was prepared by blending 1 wt% corn oil and 99 wt% aqueous emulsifier solution (0.091 wt% β-Lg in 5 mM phosphate buffer, pH 7) for 2 min at room temperature using a high-speed blender (M133/1281-0, Biospec Products, Inc., Switzerland). This coarse emulsion was then passed through a two-stage high-pressure homogenizer (LAB 1000, APV-Gaulin, Wilmington, MA) three times to reduce the mean particle diameter: 4500 psi at the first stage and 500 psi at the second stage. The resulting emulsion was then diluted with phosphate buffer and sodium azide solution to obtain a dilute emulsion (0.2 wt% oil, 0.018 wt% β-Lg, pH 7.0). Finally, this dilute emulsion was diluted with different ratios of polysaccharide stock solutions (sodium alginate, ι-carrageenan, or gum arabic) and phosphate buffer solution to yield primary and secondary emulsions with the following compositions: 0.1 wt% corn oil, 0.009 wt% β-Lg, 0 to 0.012 wt% sodium alginate, or 0 to 0.012 wt% ι-carrageenan, or 0 to 0.05 wt% gum arabic (pH 7.0, 5 mM phosphate buffer). The primary and secondary emulsions were then stirred at room temperature for 30 min, and adjusted to either pH 3 or 4 by adding 0.1 or 1 M HCl. Emulsions were then stored at room temperature before being analyzed.

4.3 Emulsion Characterization

4.3.1 Particle Charge Measurements

The electrical charge of polysaccharide molecules in aqueous solutions was determined using a commercial instrument capable of electrophoresis measurements (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). The electrical charge of the droplets in oil-in-water emulsions was determined using another commercial electrophoresis instrument (ZEM5003, Zetamaster, Malvern Instruments, Worcestershire, UK). These instruments measure the direction and velocity of molecular or particle movement in an applied electric field, and then converts the calculated electrophoretic mobility into a ζ-potential value. The aqueous solutions and emulsions were prepared and stored at room temperature for 24 h prior to analysis.

4.3.2 Particle Size Measurements

The mean particle size of the emulsions was determined using a commercial dynamic light scattering instrument (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). This instrument infers the size of the particles from measurements of their diffusion coefficients. The emulsions were prepared and stored at room temperature for 24 h prior to analysis.

4.3.3 Spectro-Turbidity Measurements

An indication of droplet aggregation in the emulsions was obtained from measurements of the turbidity versus wavelength since the turbidity spectrum of a colloidal dispersion depends on the size of the particles it contains (McClements, 2002). Approximately 1.5 g samples of emulsion were transferred into 5-mm path length plastic spectrophotometer cuvettes. The emulsions were inverted a number of times prior to measurements to ensure that they were homogeneous so as to avoid any changes in turbidity due to droplet creaming. The change in absorbance of the

emulsions was recorded when the wavelength changed from 400 nm to 800 nm using a UV-visible spectrophotometer (UV-2101PC, Shimadzu Corporation, Tokyo, Japan), using distilled water as a reference. There was an appreciable increase in emulsion turbidity at 800 nm in those emulsions where droplet aggregation occurred. It can therefore be used the turbidity measurements at this wavelength to provide an indication of the degree of droplet aggregation in the emulsions. The emulsions were prepared and stored at room temperature for 24 h prior to analysis.

4.3.4 Creaming Stability Measurements

Approximately 3.5 g samples of emulsion were transferred into 10-mm path length plastic spectrophotometer cuvettes and then stored at 30 °C for 7 days. The change in turbidity (τ) at 600 nm of undisturbed emulsions was measured during storage using a UV-visible spectrophotometer (UV-2101PC, Shimadzu Corporation, Tokyo, Japan) with distilled water being used as a reference. The light beam passed through the emulsions at a height that was about 15 mm from the bottom of the cuvette, i.e., about 42% of the emulsion's height. The oil droplets in the emulsions tended to move upward with time due to gravity, which led to the formation of a relatively clear droplet-depleted serum layer at the bottom of the cuvette. The rate at which this serum layer moved upwards provided an indication of the creaming stability of the emulsions: the faster the rate, the more unstable the emulsions (Ogawa *et al.*, 2003b). An appreciable decrease in emulsion turbidity was therefore an indication of the fact that the serum layer had risen to at least 42% of the emulsion's height. The creaming stability was quantified in terms of the following expression: Creaming stability (%) = $100 \times \tau_{(7\ days)}/\tau_{(0\ days)}$, where $\tau_{(7\ days)}$ and $\tau_{(0\ days)}$ are the turbidity measurements made at day 0 and day 7, respectively. A value of 100% therefore indicates no evidence of droplet creaming during 7 days storage, whereas a value of 0% indicates that there was rapid creaming (i.e., all the droplets have moved above the measurement point). It should also be noted that the turbidity of an emulsion depends on particle size as well as droplet concentration, so an observed change in creaming stability may also reflect changes in droplet aggregation as well as creaming.

5. Statistical Analysis

Each of the measurements described above was carried out using at least two freshly prepared samples, and the results are reported as the mean and standard deviation.

RESULTS AND DISCUSSION

1. Characterization of β-Lactoglobulin-Sodium Alginate Interactions in Aqueous Solutions

1.1 Characterization of Individual Biopolymers in Solution

1.1.1 ζ-Potential Measurements

Electrostatic forces are usually the major driving force for the interaction of charged biopolymers in aqueous solutions (Ducel *et al.*, 2004), and so it was important to determine the electrical characteristics of the biopolymers used in this study. The pH dependence of the ζ-potential of the two biopolymers was determined from their movement in an electrical field using electrophoresis (Figure 6). The ζ-potential of β-Lg (0.1 wt%) went from positive (+19.6 mV) to negative (-27.7 mV) as the pH was increased from 2 to 8. The isoelectric point (pI) of the protein was estimated to be around pH 4.8 from Figure 6, which is within the range of 4.7 to 5.2 reported in the literature by other workers (Bromley *et al.*, 2005; Das and Kinsella, 1989; Sawyer and Kontopidis, 2000).

The ζ-potential of sodium alginate (0.1 wt%) changed from -8.7 mV to -68.4 mV as the pH was increased from 2 to 8 (Figure 6). The magnitude of the negative charge on the sodium alginate molecules was appreciably lower in the pH range 2 to 4 than at higher pH values, which can be attributed to the fact that the anionic carboxylic ($–COO^-$) groups on the mannuronic and guluronic acid groups became partially protonated (–COOH) in this pH range ($pK \approx 3.5$) (Draget, 2000).

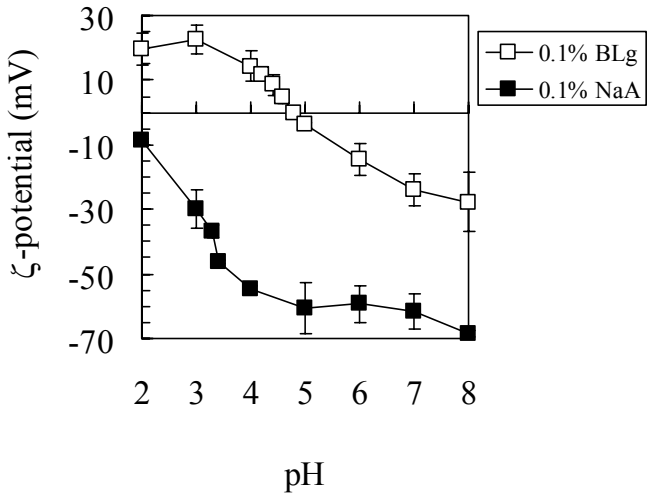


Figure 6. pH-dependence of ζ-potential for 0.1 wt% β-Lg and 0.1 wt% sodium alginate in 5 mM phosphate buffer.

1.1.2 Solution Turbidity and Mean Particle Diameter Measurements

Information about the size of the individual protein and polysaccharide molecules in solution and about the formation of any insoluble aggregates was obtained using dynamic light scattering and turbidity measurements (Figure 7). It was not possible to obtain reliable measurements of the mean diameter of the sodium alginate molecules in solution using the dynamic light scattering technique, although the turbidity measurements showed that the solutions were completely transparent across the entire pH range studied, indicating that there were no large aggregates formed. At pH 3, 6 and 7, the β-Lg was present as small particles of diameter around 4 to 5 nm. These dimensions are consistent with the formation of dimers, since previous light and X-ray scattering measurements have shown that β-Lg dimers have a diameter of 4.8 nm (Baldini, 1999). Presumably, the relatively strong electrostatic repulsion between the protein molecules at pH values sufficiently far from the isoelectric point prevents them from forming any larger aggregates. At pH 4 and 5, the β-Lg was present as relatively large particles of diameter greater than 200 nm, indicating that extensive protein aggregation had occurred (Cayot and Lorient, 1997; Verheul *et al.*, 1999). The driving force for protein aggregation around the isoelectric point is probably a combination of hydrophobic attraction, van der Waals

attraction and some electrostatic attraction between positive groups on one protein and negative groups on another. The formation of these large aggregates around the isoelectric point of β-Lg accounts for the large increase in solution turbidity that was observed at pH 4 and 5 (Figure 7). The fact that the turbidity was higher at pH 5 than 4, even though the aggregates had a similar size, suggests that there was a higher concentration of aggregates near the pI.

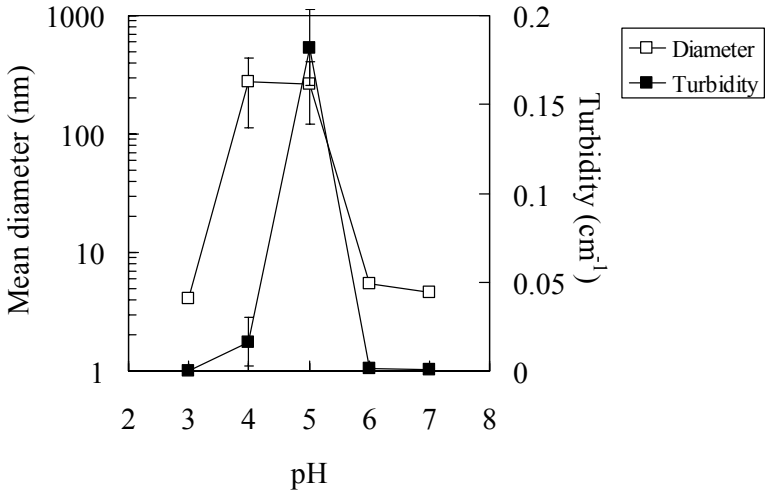


Figure 7. pH-dependence of the mean diameter (d_v) and turbidity of 0.1 wt% β-Lg in 5 mM phosphate buffer.

1.2 Characterization of Biopolymer Mixtures in Solution

The influence of pH on the interactions of β-Lg and sodium alginate in aqueous solutions was studied using isothermal titration calorimetry (ITC), light scattering, ζ-potential and soluble protein measurements (Figures 8 to 14). ITC was used to provide information about the enthalpy changes associated with the protein-polysaccharide interactions. Light scattering and ζ-potential measurements were used to provide information about the size and electrical charge of any protein-polysaccharide complexes formed. Soluble protein measurements were used to provide some insight into the composition of the complexes formed.

1.2.1 Enthalpy Measurements

The influence of pH on the enthalpy changes associated with protein-polysaccharide interactions was determined using ITC when sodium alginate solution was titrated into β-Lg solution (30.0 °C, 5 mM phosphate buffer, pH 3 to 7). The dependence of the enthalpy change per mole of sodium alginate injected into the reaction cell on the total concentration of sodium alginate in the reaction cell after an injection was measured (Figure 8). In addition, the enthalpy change associated with the first injection of the sodium alginate into the measurement cell ΔH_1 versus pH was plotted in order to provide some general indication of the pH-dependence of the interactions (Figure 9). These measurements indicate that the dependence of the enthalpy change on sodium alginate concentration was highly dependent on solution pH:

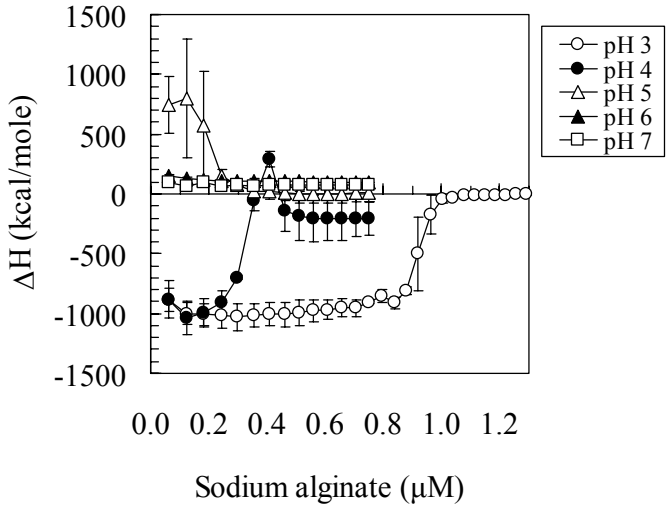


Figure 8. Dependence of corrected enthalpy change per mole of sodium alginate (ΔH) on sodium alginate concentration in the reaction cell. Values were calculated from measurements made when 0.1 wt% sodium alginate was injected into a reaction cell containing either buffer solution or 0.1 wt% β-Lg in buffer solution (5 mM phosphate buffer, 30 °C).

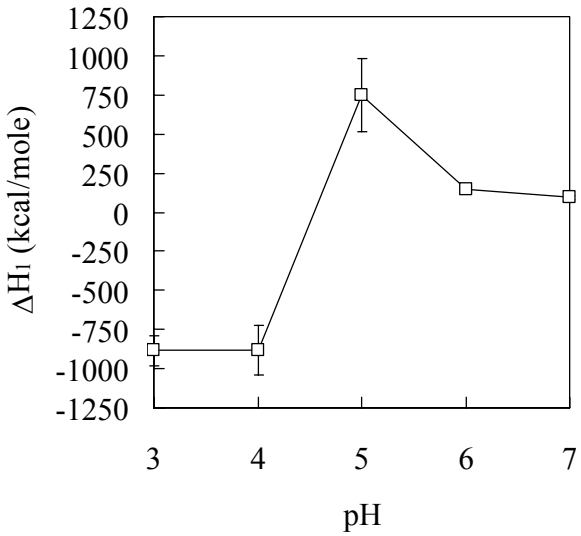


Figure 9. pH-dependence of the enthalpy change per mole of sodium alginate (ΔH_1) when 0.0046 mM sodium alginate was injected into a reaction cell containing 0.1 wt% β-Lg solution (5 mM phosphate buffer, 30 °C).

At pH 3, a relatively high exothermic enthalpy change was observed up to 0.9 μM sodium alginate, after which the enthalpy change rapidly fell to a value close to zero.

At pH 4, the dependence of the enthalpy change on sodium alginate concentration was quite complex. A relatively high exothermic enthalpy change was observed up to 0.35 μM sodium alginate, followed by a small endothermic peak at 0.41 μM, followed by a fairly small exothermic enthalpy change at higher sodium alginate concentrations.

At pH 5, a relatively high endothermic enthalpy change was observed below 0.24 μM sodium alginate, which rapidly fell to a value close to zero at higher sodium alginate concentrations.

At pH 6 and 7, the observed enthalpy change was relatively small across the entire range of sodium alginate concentrations studied.

In general, it is difficult to assign precise molecular events to enthalpy changes measured in calorimetry measurements because many different physicochemical phenomena contribute to the overall measured signal, e.g., various kinds of association-disassociation processes and conformational changes. Nevertheless, the fact that the enthalpy changes observed in the pH range from 3 to 5 were endothermic under some circumstances but exothermic under other circumstances suggests that at least two different physicochemical phenomena occurred in this pH range. Based on the light scattering and soluble protein measurements discussed later, the endothermic enthalpy changes observed at pH 4 and 5 were primarily due to dissolution of β-Lg aggregates when sodium alginate was titrated into the measurement cell. On the other hand, the exothermic enthalpy changes observed at pH 3 and 4 were due to binding of β-Lg molecules to the sodium alginate through electrostatic attraction.

At pH 3, the ITC data suggests that there is a strong interaction between the protein and polysaccharide up to about 0.9 μM sodium alginate. At higher sodium alginate concentrations (> 1 μM) the observed enthalpy change was relatively low because there was no more free β-Lg remaining in the reaction cell (see later) and so any additional sodium alginate added to the system had nothing to react with. These results suggest that there was approximately 0.9 μM of sodium alginate bound to 0.1 wt% of β-Lg at pH 3.

At pH 4, the ITC data suggests that there was a binding interaction between the protein and polysaccharide up to 0.35 μM of sodium alginate, after which all of the protein in the reaction cell had been used up. The fact that the minimum concentration of sodium alginate required to interact with all of the β-Lg molecules present in the reaction cell was appreciably lower at pH 4 than at pH 3 suggests that the number of protein molecules bound per polysaccharide molecule was greater at pH 4 than at pH 3. This can be attributed to the fact that the negative charge on the sodium alginate molecules is greater at pH 4 than at pH 3 (Figure 6), hence there are more binding sites available for the positively charged protein molecules. The molecular origin of the small endothermic peak observed at pH 4 around 0.41 μM

sodium alginate is currently unknown, but it may be caused by some different kind of binding mechanism, or by some rearrangement of the proteins and polysaccharides within the complexes formed.

At pH 5, there appeared to be a relatively strong endothermic interaction between the protein and the polysaccharide up to 0.21 μM sodium alginate, after which no more interaction occurred. At this pH individual sodium alginate molecules are highly negatively charged and are completely soluble, whereas individual β-Lg molecules have little net charge, are partially insoluble, and tend to form large aggregates (Section 1.1.1). It can be postulated that when sodium alginate was titrated into the reaction cell some of the β-Lg molecules become dissociated from the large protein aggregates and interacted with the polysaccharide molecules. The driving force for this interaction would be the electrostatic attraction between the negatively charged side groups on the sodium alginate and the positively charged patches on the surface of the β-Lg (de Vries *et al.*, 2003; Dubin *et al.*, 1994; Weinbreck *et al.*, 2003; Wen and Dubin, 1997). This electrostatic attraction is a combination of changes in the Coulombic interactions between the charged groups, and entropy of mixing effects associated with the release of counter-ions when the two biopolymers interact.

At pH 6 and 7, the fact that only a small enthalpy change was observed when sodium alginate was mixed with β-lactoglobulin suggested that there was little interaction between the polysaccharide and protein. This is not surprising since both biopolymers are negatively charged at these pH values (Figure 6) and therefore there would be a relatively strong electrostatic repulsion between them.

1.2.2 Solution Turbidity and Mean Particle Diameter Measurements

Turbidity and mean particle diameter (d_V) measurements were used to provide information about the formation of soluble or insoluble complexes. The turbidity of mixed biopolymer solutions was measured as 0.1 wt% sodium alginate solution was titrated into a test tube initially containing 14.5 mL of 0.1 wt% β-Lg in

phosphate buffer (Figure 10). The turbidity and mean particle diameter were also measured for 0.1 wt% β-Lg solutions containing 0.75 μM sodium alginate, since this amount of the polysaccharide was found to be sufficient to saturate the protein at pH values where strong interactions occurred (Figures 11 and 12).

The turbidity and mean particle diameter of β-Lg - sodium alginate solutions was highly dependent on solution pH. At pH 3, the turbidity of mixed solutions increased almost linearly with increasing sodium alginate concentration from 0 to 0.8 μM, after which it reached a plateau value (Figure 10). For solutions containing 0.1 wt% β-Lg and 0.75 μM sodium alginate, the mean particle diameter was greater than 1,000 nm and the turbidity exceeded 1.0, indicating the presence of large particles that scattered light (Figures 11 and 12). At pH 4, the turbidity of the mixed solutions increased steeply from 0 to 0.35 μM sodium alginate, after which it reached a plateau value (Figure 10). For solutions containing 0.1 wt% β-Lg and 0.75 μM sodium alginate, the mean particle diameter was greater than 1,000 nm and the turbidity exceeded 1.0, indicating the presence of large particles that scattered light (Figures 11 and 12). At pH 5, the turbidity of the mixed solutions increased steeply from 0 to 0.12 μM sodium alginate, then fell sharply from 0.12 to 0.3 μM sodium alginate, and then remained fairly low at higher sodium alginate concentrations (Figure 10). For solutions containing 0.1 wt% β-Lg and 0.75 μM sodium alginate, the mean particle diameter and turbidity ($d_V \approx 70$ nm; $\tau \approx 0.13$ cm^{-1}) were appreciably less than that measured in the isolated β-Lg solution ($d_V \approx 260$ nm; $\tau \approx 0.13$ cm^{-1}) (Figures 11 and 12). At pH 6 and 7, the solutions were completely transparent across the whole range of sodium alginate concentrations studied (Figure 10). For solutions containing 0.1 wt% β-Lg and 0.75 μM sodium alginate, the mean particle diameter and turbidity ($d_V \approx$ 4-5 nm; $\tau < 0.006$ cm^{-1}) were approximately the same as those measured in isolated β-Lg solutions ($d_V \approx$ 4-5 nm; $\tau < 0.002$ cm^{-1}) (Figures 11 and 12).

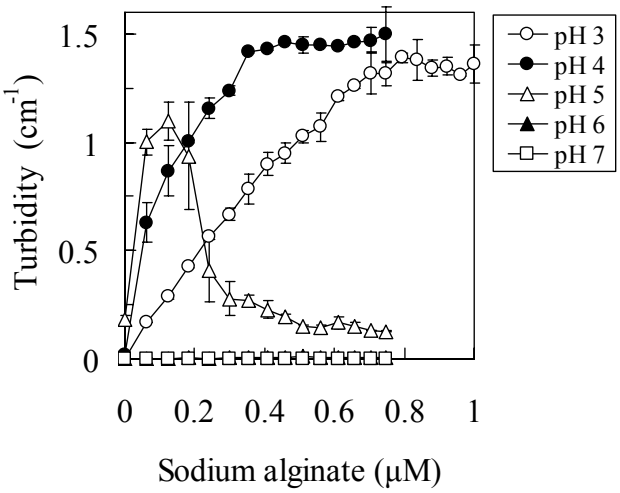


Figure 10. Dependence of the turbidity (at 600 nm) of mixed biopolymer solutions on sodium alginate concentration when 0.1 wt% sodium alginate was titrated into 0.1 wt% β-Lg solution (5 mM phosphate buffer, pH 3-7).

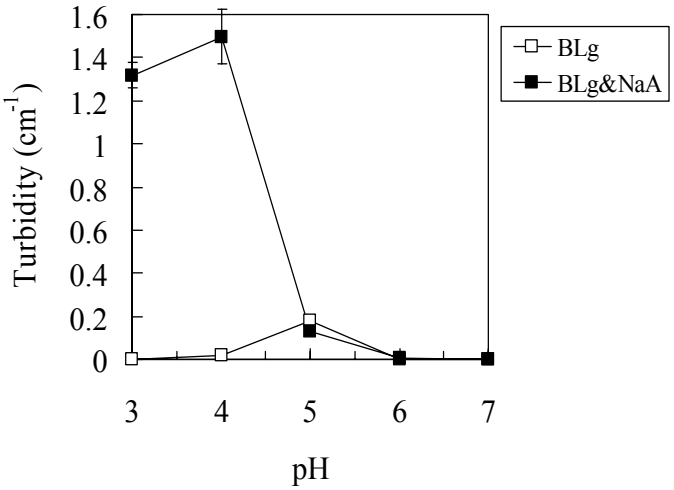


Figure 11. pH-dependence of the turbidity (at 600 nm) of mixed biopolymer solutions containing 0.1 wt% β-Lg and 0.75 μM sodium alginate (5 mM phosphate buffer).

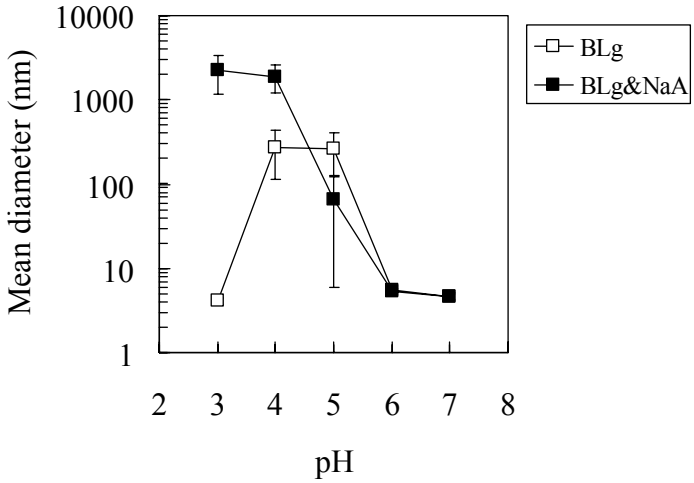


Figure 12. pH-dependence of the mean particle diameter of biopolymer solutions containing either (i) 0.1 wt% β-Lg or (ii) 0.1 wt% β-Lg + 0.75 μM sodium alginate (5 mM phosphate buffer).

The turbidity measurements at pH 3 and 4 indicated that an increasing amount of insoluble protein-polysaccharide complex was formed as the sodium alginate concentration was increased, until eventually no further changes in complex formation occurred (Figure 10). This can be attributed to the fact that there was no more protein present in the reaction cell to react with the additional sodium alginate added to the system (see later). The driving force for formation of these insoluble complexes is electrostatic attraction between the negatively charged sodium alginate and the positively charged protein (de Kruif and Tuinier, 2001). The fact that more sodium alginate had to be added to the protein solution in order to reach the plateau turbidity value at pH 3 (≈ 0.75 μM) than at pH 4 (≈ 0.35 μM) can be attributed to the fact that the alginate is less highly charged at pH 3 and can therefore bind less protein molecules per polysaccharide molecule (Figure 6).

The turbidity measurements at pH 5 showed a more complex dependence on sodium alginate concentration (Figure 10). In the absence of sodium alginate, the β-Lg solutions were slightly turbid due to the presence of some insoluble protein near the isoelectric point. The relatively sharp increase in turbidity from 0 to 0.12 μM sodium alginate suggests that there was an increase in the amount of

insoluble particulate material formed in the solution. It is possible that some of the sodium alginate molecules acted as cross-links between the protein aggregates leading to the formation of bigger particles. The relatively sharp decrease in turbidity from 0.12 to 0.3 μM sodium alginate suggests that the insoluble complexes partially dissociated and became more soluble. This may have occurred because the net negative charge on the protein-polysaccharide complexes increased with increasing sodium alginate concentration, hence there was a stronger electrostatic repulsion between them causing some dissociation. The fact that the solutions remained slightly turbid at higher sodium alginate concentrations suggests that the protein and polysaccharide still formed a complex, but that there was either a smaller amount of material within it or the complex had a smaller size.

The fact that the solutions remained transparent at all sodium alginate concentrations at pH 6 and 7 indicated that there was no insoluble complex formation between the protein and the polysaccharide, which is to be expected because of the relatively strong electrostatic repulsion between the two biopolymers when they are both negatively charged (Weinbreck *et al.*, 2003). This data is therefore consistent with the ITC data, which also indicated that there was no strong interaction between the two biopolymers at pH 6 and 7.

For the soluble protein measurements, indirect information about the influence of pH on the composition of insoluble complexes was obtained by measuring the amount of soluble protein remaining in the aqueous phase after any insoluble complexes were removed by centrifugation. The amount of soluble protein present was determined by measuring the absorbance of transparent solutions at 280 nm (Chang, 2003). Mixed biopolymer solutions were prepared by titrating 200 μL aliquots of 0.1 wt% sodium alginate solution into a test tube initially containing 14.5 mL of 0.1 wt% β-Lg in phosphate buffer.

At pH 3 and 4, there was an appreciable decrease in free protein with increasing sodium alginate concentration (Figure 13), which suggested that progressively more protein was incorporated into the insoluble complexes. No

soluble protein remained in the biopolymer mixtures when the sodium alginate concentration exceeded 0.8 μM at pH 3 and 0.35 μM at pH 4. This data therefore supports the ITC and turbidity measurements, which also indicated that a larger amount of sodium alginate was required to interact with all of the protein initially present in the reaction cell at pH 3 than at pH 4. As mentioned earlier, this is due to the fact that the sodium alginate has a smaller negative charge at pH 3 and therefore can bind fewer positively charged protein molecules.

At pH 5, there was a sharp decrease in the amount of soluble protein remaining in the solutions from 0 to 0.2 μM sodium alginate, which was followed by a marked increase in protein solubility at higher polysaccharide concentrations. This result also supports the turbidity measurements reported earlier, which showed an initial increase in insoluble complex up to 0.2 μM sodium alginate followed by a decrease. At pH 6 and 7, the soluble protein concentration remained relatively high and was independent of sodium alginate concentration, suggesting that the protein was not incorporated into an insoluble complex due to the strong electrostatic repulsion between the biopolymer molecules.

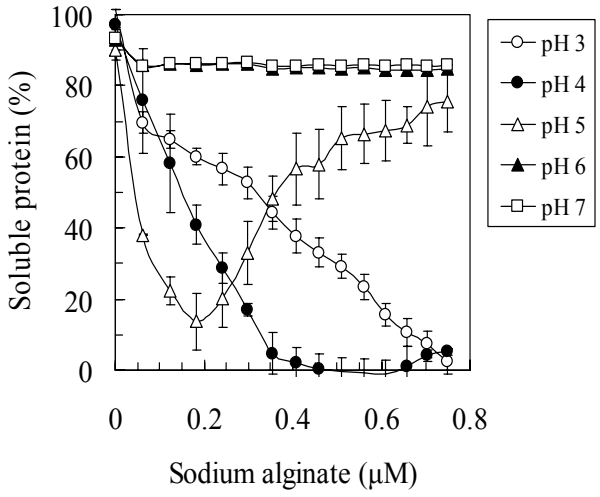


Figure 13. Dependence of free protein (at 280 nm) on sodium alginate concentration for 0.1 wt% sodium alginate titrated into 0.1 wt% β-Lg solution (5 mM phosphate buffer).

1.2.3 ζ-Potential Measurements

The ζ-potential of biopolymer mixtures containing 0.1 wt% β-Lg and 0.75 μM sodium alginate was measured as a function of pH (Figure 14). This solution composition was chosen since the ITC, turbidity and soluble protein measurements at lower pH values (where there was a strong interaction between the protein and polysaccharide) indicated that sodium alginate was saturated with β-Lg molecules at this concentration. At pH 3, it was not possible to measure the ζ-potential because the insoluble complexes formed were too big to place in the measurement cell. The measured ζ-potential of the β-Lg-sodium alginate solutions was negative at all pH values studied and became more negative as the pH was increased from 4 to 7 (Figure 14). It is important to note that ζ-potential measurements made on mixed biopolymer solutions should be treated with caution, since the measured ζ-potential represents an average of the different types of molecular species present in solution e.g., free and bound proteins, or free and bound polysaccharides (Mattison *et al.*, 1995). The magnitude of this average value depends on the relative concentration, charge and scattering intensity of the different molecular species. At pH 4 and 5, there were relatively large insoluble complexes in the mixed biopolymer solutions that would be expected to scatter light much more strongly than any soluble biopolymers. It can therefore be fairly confident that the ζ-potential measurements made at these pH values reflect the charges of the insoluble protein-polysaccharide complexes. These results suggest that the insoluble complexes formed at pH 4 and 5 had a net negative charged, indicating that the negative charge from the polysaccharide dominated the charge from the protein.

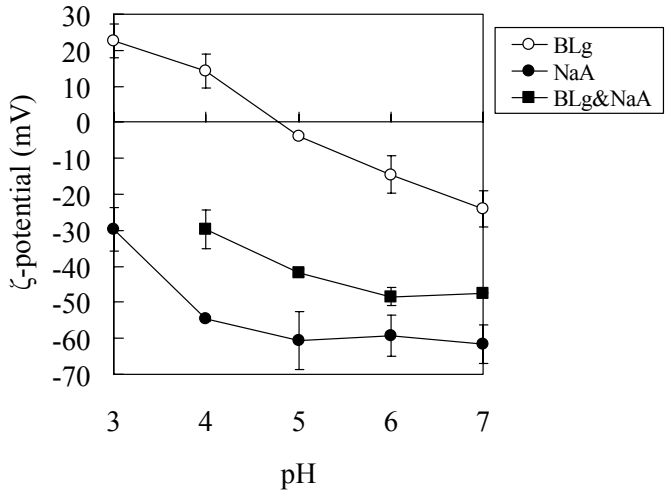


Figure 14. pH-dependence of ζ-potential of biopolymer solutions containing either (i) 0.1 wt% β-Lg; (ii) 0.1 wt% β-Lg + 0.75 μM sodium alginate ; or (iii) 0.75 μM sodium alginate (5 mM phosphate buffer).

This study showed that alginate and β-Lg formed insoluble complexes in solution at pH values where the polysaccharide and protein had high opposite charges (e.g., pH 3-4), formed soluble complexes at pH values where the polysaccharide was negatively charged but the protein had a low net charge (e.g., pH 5), and did not form complexes at pH values where the polysaccharide and protein had similar charges (e.g., pH 6 and 7).

However, an understanding of the interfacial interactions between polysaccharides and proteins-coated oil droplets would facilitate the rational design and production of emulsion-based food products. The next study would therefore be investigated the possibility of forming stable oil-in-water emulsions containing droplets surrounded by protein-polysaccharide interfacial complexes consisting of β-Lg and alginate.

2. Influence of Alginate, pH and Ultrasound Treatment on Palm Oil-in-Water Emulsions Stabilized by β-Lactoglobulin.

2.1 Interaction of Alginate Molecules with β-Lg-Coated Droplets

The interaction between alginate molecules and β-Lg-coated droplet surfaces was monitored by measuring the electrical charge on the emulsion droplets (the ζ-potential). Emulsions were prepared (5 wt% hydrogenated palm oil, 0.45 wt% β-Lg, 5 mM phosphate buffer) with different sodium alginate concentrations (0 to 0.5 wt%) and pH values (3 to 7). The ζ-potential of the droplets in the primary emulsions (0 wt% sodium alginate) changed from +61 mV to -69 mV as the pH was increased from 3 to 7, with the point of zero net charge being around pH 5 (Figure 15). The pH-dependence of the droplet ζ-potential can be attributed to the electrical properties of the adsorbed β-Lg molecules, which change from positively charged to negatively charged as the pH is increased from below to above their isoelectric point (pI = 5.2). The ζ-potential of the droplets in secondary emulsions (0.5 wt% sodium alginate) changed from -29 mV to -65 mV as the pH was increased from 3 to 7 (Figure 15).

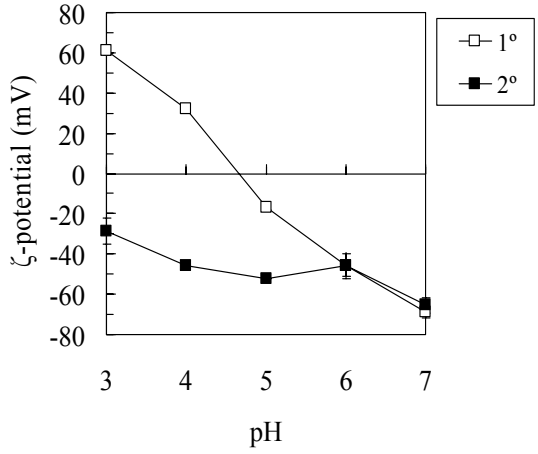


Figure 15. Dependence of particle charge (ζ-potential) on pH for primary (0 wt% alginate) and secondary emulsions (0.5 wt% alginate) containing 5 wt% hydrogenated palm oil (0.45 wt% β-Lg, 5 mM phosphate buffer).

The droplet charge in the secondary emulsions was much more negative than that in the primary emulsions at pH 3, 4 and 5, which indicated that alginate

molecules adsorbed to the surfaces of the β-Lg-coated droplets. On the other hand, the droplet charge of the primary and secondary emulsions was similar at pH 6 and 7, indicating that no alginate adsorption occurred. At pH 3 and 4, the alginate is anionic and the β-Lg is cationic so there is a strong electrostatic attraction between the polysaccharide and protein that drives adsorption. At pH 5, the alginate is anionic and the β-Lg has a low net charge, but adsorption still occurs because the polysaccharide can adsorb to cationic patches ($-NH_3^+$) on the protein surface. At pH 6 and 7, the alginate and the β-Lg are both strongly anionic and therefore there is a strong electrostatic repulsion between them that opposes adsorption. The results from this study on the interactions between alginate and β-Lg molecules adsorbed on to droplet surfaces are therefore similar to the previous study on the interactions between alginate and β-Lg molecules in aqueous solutions, which also showed that binding occurred at pH 3, 4 and 5, but not at pH 6 and 7 (Harnsilawat *et al.*, 2006).

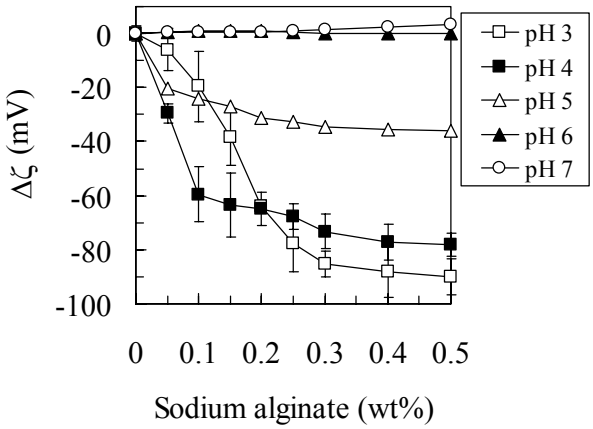


Figure 16. pH-dependence of the change in droplet charge with sodium alginate concentration for 5 wt% hydrogenated palm oil (0.45 wt% β-Lg, 5 mM phosphate buffer).

The combined influence of pH and alginate concentration on the droplet charge in the secondary emulsions is shown in Figure 16. The extent of alginate adsorption to the droplet surfaces is characterized in terms of the change in droplet charge when alginate was added to the primary emulsion: $\Delta\zeta = \zeta_2 - \zeta_1$, where ζ_1 and ζ_2 are the ζ-potentials of the primary and secondary emulsions, respectively.

At pH 3 and 4, there was an increase in the negative charge on the droplets as the sodium alginate concentration was increased until a plateau level was reached, which suggested that alginate molecules adsorbed to the droplet surfaces until they became saturated. The initial change in $\Delta\zeta$ with sodium alginate concentration was steeper at pH 4 than at pH 3, and the plateau level was reached at a lower alginate concentration (0.15% at pH 4 compared to 0.3% at pH 3). These results suggest that the driving force for the adsorption of the polysaccharide molecules to the droplet surfaces was greater at pH 4 than at pH 3. This may be due to the fact that the pKa values of the carboxyl groups on alginate are close to pH 3.5 so that the alginate molecules are appreciably less negatively charged at pH 3 than at pH 4 (Harnsilawat *et al.*, 2006). On the other hand, the positive charge on the emulsion droplets is greater at pH 3 than pH 4 (Figure 15). Overall, it seems that the reduction of negative charge on the polysaccharide molecules is more important than the increase of positive charge on the adsorbed protein molecules in determining the driving force for adsorption. At saturation, the overall change in droplet charge ($\Delta\zeta_{SAT}$) was slightly greater at pH 3 than at pH 4, which may have been due to the fact that there were a greater number of positive charges on the droplet surfaces for the alginate molecules to interact with and neutralize at the lower pH. At pH 5, the negative charge on the droplets also increased as the sodium alginate concentration was increased, but the magnitude of the increase was much less than at pH 3 and 4, which suggests that the amount of alginate molecules adsorbed to the droplet surfaces at pH 5 was less. The most likely reason for this phenomenon is that the droplet surfaces have fewer positively charged groups for the alginate molecules to attach to, as well as more negatively charged groups that will repel the alginate molecules away from the droplet surface. At pH 6 and 7, there was no change in the ζ-potential with increasing sodium alginate concentration, suggesting that alginate molecules did not adsorb to the droplet surfaces due to the relatively strong electrostatic repulsion between the anionic polysaccharide and the adsorbed anionic protein molecules.

The ability of charged polyelectrolytes (such as alginate) to adsorb to the surface of oppositely charged colloidal particles (such as β-Lg-coated fat droplets) and cause charge reversal is usually referred to as “overcharging” (Chodanowski and

Stoll, 2001; Dobrynin, 2001; Jonsson and Linse, 2001; Kong and Muthukumar, 1998; Netz and Joanny, 1999; von Goeler and Muthukumar, 1994). Overcharging occurs because only a fraction of the charged groups on a polyelectrolyte are required to neutralize the oppositely charged groups on the surface of a colloidal particle. The remainder of the charged polyelectrolyte groups may protrude into the aqueous solution or may be in contact with uncharged regions on the particle surface (Chodanowski and Stoll, 2001; Dobrynin, 2001; Jonsson and Linse, 2001; Kong and Muthukumar, 1998; Netz and Joanny, 1999; von Goeler and Muthukumar, 1994).

2.2 Influence of Alginate on Stability of Secondary Emulsions

The purpose of these experiments was to examine the influence of pH and alginate concentration on the stability of oil-in-water emulsions containing β-Lg-coated droplets. Secondary emulsions were prepared (5 wt% hydrogenated palm oil, 0.45 wt% β-Lg, 5 mM phosphate buffer) with different sodium alginate concentrations (0 to 0.5 wt%) and pH values (3 to 7). The stability of the emulsions to droplet aggregation was then determined using light scattering, microscopy and creaming stability measurements (Figures 17 to 19).

At pH 3 and 4, there was a pronounced increase in mean particle diameter with increasing alginate concentration until a plateau region was reached (Figure 17), which can be attributed to bridging flocculation of the positively charged droplets by the negatively charged polysaccharide molecules. In addition, there was evidence of accelerated creaming in some of these emulsions, particularly at intermediate alginate concentrations (Figure 18), which can be associated with the increase in mean particle size caused by droplet flocculation. Nevertheless, the emulsion at pH 4 appeared stable to creaming at high alginate concentrations, which suggests that the bridging flocculation was so extensive that a network of aggregated droplets was formed in the emulsion that prevented droplet movement (McClements, 2004a). The optical microscopy measurements of emulsion microstructure confirmed that extensive droplet flocculation occurred in the secondary emulsions at pH 3 and 4 (Figure 19).

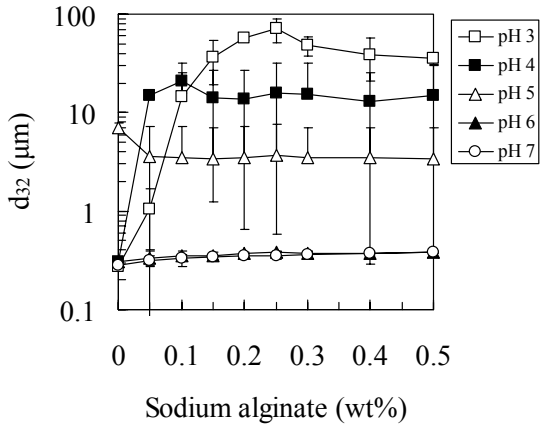


Figure 17. Dependence of mean particle diameter (d_{32}) on pH and sodium alginate concentration for secondary emulsions (5 wt% hydrogenated palm oil, 0.45 wt% β-Lg, 0 to 0.5 wt% sodium alginate, 5 mM phosphate buffer).

At pH 5, the mean particle diameter of the emulsions was relatively high in the absence of alginate, and decreased slightly when alginate was added (Figure 17). In addition, a clear serum layer was formed in the emulsions after storage, whose height decreased with increasing alginate concentration (Figure 18). Finally, the emulsions had a fairly coarse microstructure at all alginate concentrations (0 to 0.5 wt%) when observed by optical microscopy (Figure 19). These measurements indicated that the primary emulsion was unstable to droplet flocculation prior to addition of alginate, which is due to the fact that the β-Lg-coated droplets have a low net charge near the isoelectric point of the adsorbed proteins, so the electrostatic repulsion between the droplets is not strong enough to prevent their aggregation (McClements, 2004a). The addition of sodium alginate to the emulsions caused a slight improvement in their stability to droplet flocculation (lower d_{32} and creaming index), probably because the alginate molecules adsorbed to the droplet surfaces and increased the electrostatic and steric repulsion between them.

At pH 6 and 7, the light scattering measurements indicated that there was no change in the mean particle diameter of the emulsions with increasing sodium alginate concentration (Figure 17), which suggested that the droplets were not aggregated in the measurement cell of the particle size analyzer. On the other hand,

the creaming stability measurements indicated that rapid creaming occurred in the emulsions when 0.05 wt% sodium alginate was present, and that the extent of creaming decreased with a further increase in alginate concentration (Figure 18). Finally, the optical microscopy measurements clearly indicated that extensive droplet flocculation occurred in the emulsions in the presence of alginate (Figure 19). Taken together, these measurements indicated that the alginate promoted depletion flocculation in the emulsions. Droplet aggregation was not observed in the light scattering experiments because the emulsions had to be extensively diluted prior to analysis to avoid multiple scattering effects, which reduced the alginate concentration below that required to promote depletion flocculation. The reduction in the creaming index when the alginate concentration was increased from 0.05 wt% to 0.5 wt% can be attributed to the fact that the continuous phase viscosity increased and the depletion attraction between the droplets increased. An increase in continuous phase viscosity slows down the upward movement of the oil droplets. An increase in the depletion attraction causes the droplets to stick to each other more strongly, which leads to the formation of a fairly open network of aggregated emulsion droplets in the cream layer. Eventually, this network is so open that it fills the entire volume of the container and no creaming is observed (McClements, 2004a).

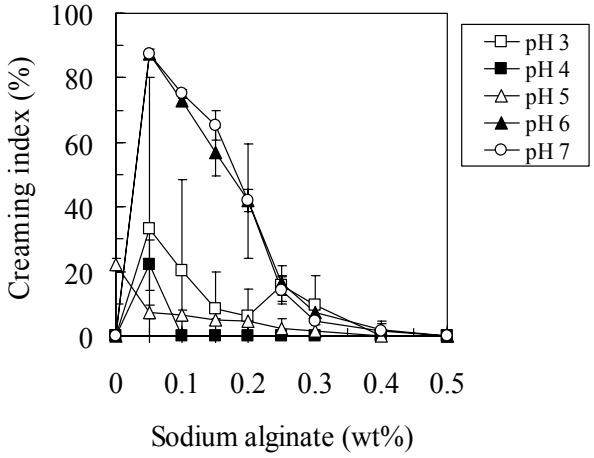


Figure 18. Dependence of creaming stability on pH and sodium alginate concentration for secondary emulsions (5 wt% hydrogenated palm oil, 0.45 wt% β-Lg, 0 to 0.5 wt% sodium alginate, 5 mM phosphate buffer).

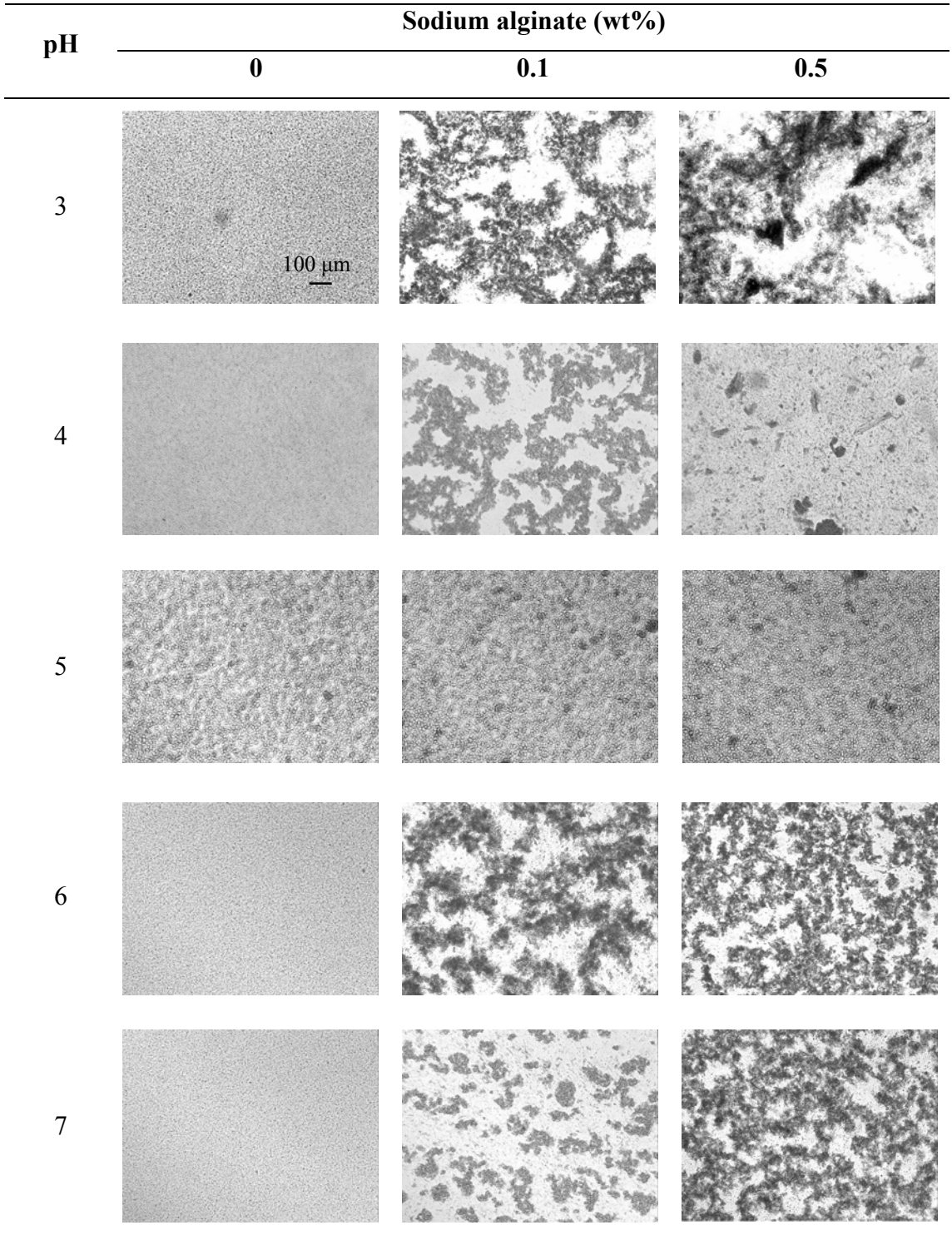


Figure 19. pH dependence of the microstructure of primary emulsions (0 wt% alginate) and secondary emulsions (0.1 and 0.5 wt% alginate) containing 5 wt% hydrogenated palm oil (0.45 wt% β-Lg, 5 mM phosphate buffer).

2.3 Influence of Sonication on Stability of Secondary Emulsions

Previous studies have shown that high intensity sonication is often effective at breaking down the flocs formed during the preparation of secondary emulsions (Guzey *et al.*, 2004; Moreau *et al.*, 2003; Ogawa *et al.*, 2003b). The purpose of this series of experiments was therefore to examine the influence of sonication on the stability of the secondary emulsions used in this study. Emulsions were prepared (5 wt% hydrogenated palm oil, 0.45 wt% β-Lg, 5 mM phosphate buffer) with different sodium alginate concentrations (0 to 0.5 wt%) and pH values (3 to 7). The emulsions were then sonicated and their stability to droplet aggregation was determined using light scattering, creaming stability and microscopy measurements (Figure 20 to 22).

At pH 3, extensive droplet aggregation and creaming were still observed in the emulsions at intermediate sodium alginate concentrations (0.05 to 0.3 wt%) after sonication, but sonication did improve emulsion stability at higher alginate concentrations. Droplet aggregation still occurred at intermediate alginate concentrations because there was either insufficient polymer to completely saturate the droplet surfaces leading to bridging flocculation and/or because the ζ-potential was not large enough to generate a sufficiently strong electrostatic repulsion between the droplets. Hence, even if the flocs present were disrupted by sonication, they would reform once the sonication was removed. At higher alginate concentrations, there was sufficient alginate present to adsorb to the surfaces of the individual droplets produced during sonication and completely cover them with alginate molecules thus preventing bridging flocculation. In addition, the electrical charge and thickness of the alginate layer surrounding the droplets was large enough to generate a strong electrostatic and steric repulsion between the individual droplets after the sonication was removed.

At pH 4, the effects of sonication on emulsion stability were more pronounced. After sonication, droplet aggregation and rapid creaming were only observed at 0.05 wt% sodium alginate, but the emulsions were stable at higher

alginate concentrations. The same arguments can be used to describe the ability of sonication to improve the stability of these emulsions as were used for the emulsions at pH 3. However, the range of alginate concentrations where the emulsion was unstable to droplet aggregation was much narrower because the alginate molecules adsorbed more strongly to the droplet surfaces (Figure 16), thereby saturating the droplet surfaces and generating a strong droplet charge at lower levels of alginate.

At pH 5, the stability of the primary emulsion to droplet aggregation could not be improved by sonication, which can be attributed to the fact that the droplets still had a low net charge so that the electrostatic repulsion was not strong enough to prevent flocculation after sonication. On the other hand, sonication caused a dramatic reduction in mean particle diameter (Figure 20) and creaming stability (Figure 21) for all the secondary emulsions containing alginate. It can be postulated that sonication disrupted the flocs formed in the primary emulsion, allowing the alginate molecules to adsorb to the surfaces of the individual droplets and form a highly charged and thick membrane that prevented further droplet flocculation.

At pH 6 and 7, sonication had little affect on the mean particle diameter, microstructure or creaming stability of the emulsions, which can be attributed to the fact that there was still the same amount of alginate present in the continuous phase to promote depletion flocculation.

It should be noted that sonication is unlikely to be used for the large scale industrial disruption of flocculated droplets in commercial food emulsions. Instead, it is more likely that flocculated emulsions would be passed through a second homogenization stage to break up any flocs formed. This second homogenization stage would be carried out at a lower homogenization pressure than that required to form the initial primary emulsions so that it disrupted flocs, but not individual droplets.

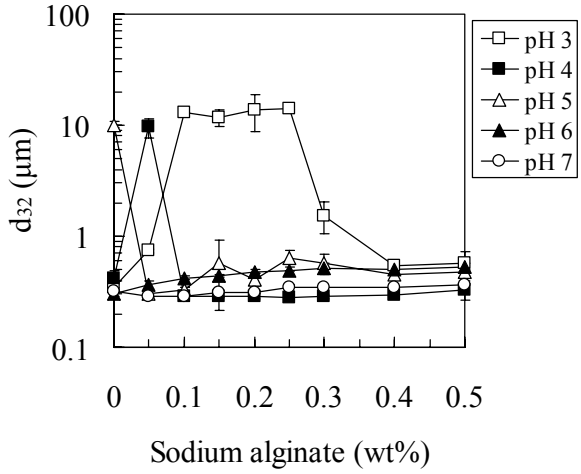


Figure 20. Dependence of mean particle diameter (d_{32}) on pH and sodium alginate concentration for secondary emulsions (5 wt% hydrogenated palm oil, 0.45 wt% β-Lg, 0 to 0.5 wt% sodium alginate, 5 mM phosphate buffer). Emulsions were sonicated after preparation.

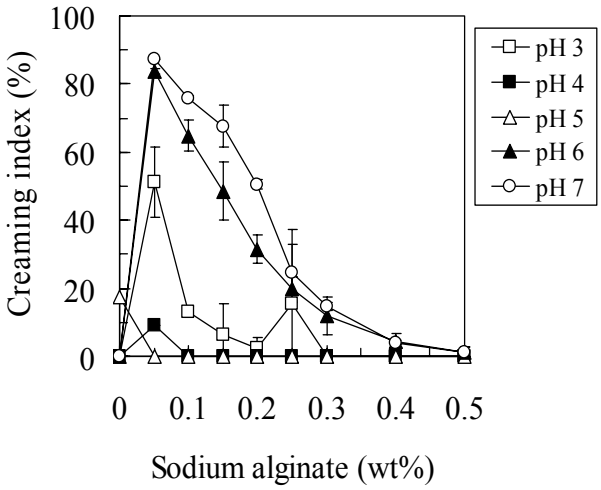


Figure 21. Dependence of creaming stability on pH and sodium alginate concentration for secondary emulsions (5 wt% hydrogenated palm oil, 0.45 wt% β-Lg, 0 to 0.5 wt% sodium alginate, 5 mM phosphate buffer). Emulsions were sonicated after preparation.

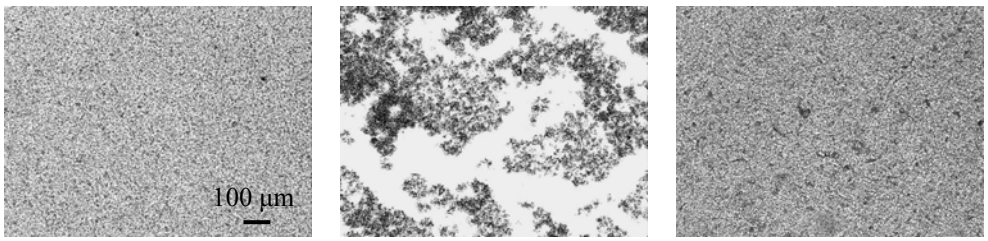
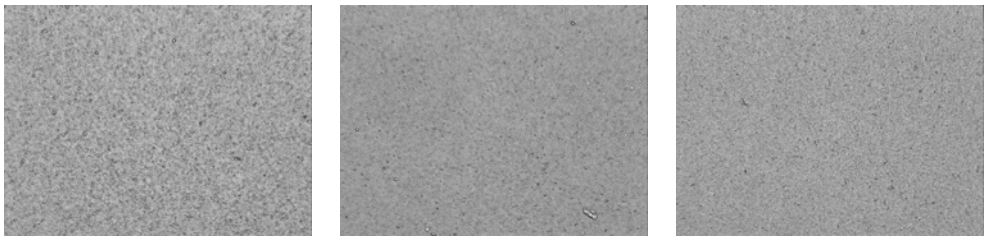
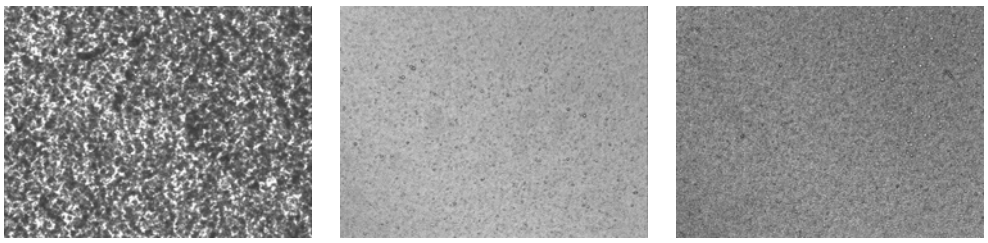
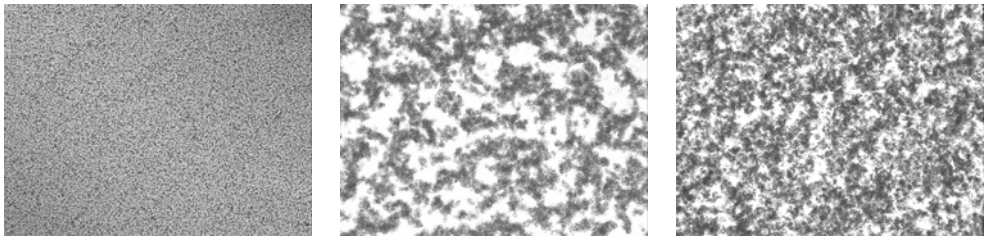
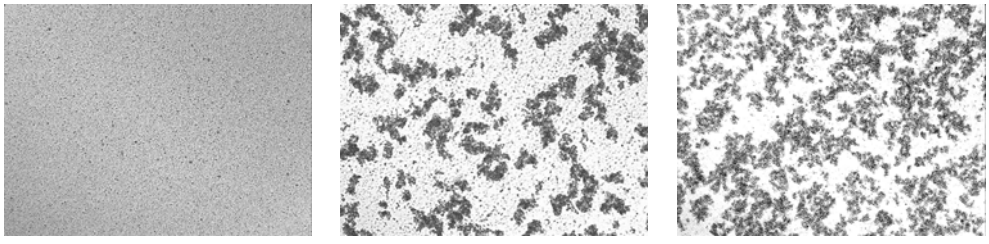
pH	Sodium alginate (wt%) with sonication		
	0	0.1	0.5
3			
4			
5			
6			
7			

Figure 22. pH dependence of the microstructure of primary emulsions (0 wt% alginate) and secondary emulsions (0.1 and 0.5 wt% alginate) containing 5 wt% hydrogenated palm oil (0.45 wt% β-Lg, 5 mM phosphate buffer). Emulsions were sonicated after preparation.

The results of this part of the study suggest that pH (3-7), sodium alginate (0-0.5 wt%) and high intensity sonication have an impact on the stability of emulsions containing β-Lg coated droplets. Stable emulsions containing droplets surrounded by β-Lg-alginate interfacial complexes can be produced at pH 4 and 5 by using ultrasound treatment to disrupt any flocs formed when the polysaccharide and emulsions are mixed together.

Since palm oil is easy to solidification at the ambient temperature (20-25 °C) and can be prevented by holding at 40 °C, therefore corn oil was used in the further study. Corn oil is derived from corn germ. It can play a major role in human nutrition (e.g., an excellent source of essential fatty acids (EFA) and vitamin E, an effective dietary oil for lowering blood cholesterol and elevated blood pressure levels and reducing the risk of coronary heart disease). Its utilization extended to many emulsion-based food products (e.g., margarines, mayonnaise, salad dressings, sauces) (Strecker *et al.*, 1996). Recently, there is also an increasing tendency toward removing or reducing the amounts of food constituents that have been associated with human health concerns (e.g., saturated fat, trans fatty acids, cholesterol, and salt) (McClements, 2004a). The next study related to the influence of ionic strength and mixing condition on formation and stability of emulsions containing 0.1 wt% oil droplets coated by β-Lg-alginate interfaces at different pH would be evaluated.

3. Influence of Ionic Strength and Mixing Condition on Formation and Stability of Emulsions Containing Oil Droplets Coated by β-Lactoglobulin-Alginate Interfaces at Different pH

3.1 Influence of pH on Emulsion Properties

The main purpose of these experiments was to establish the effect of the final pH on the formation and stability of secondary emulsions. In this experiment, the alginate molecules and emulsion droplets were initially mixed at a pH where they both had a negative charge (pH 7), and then the pH was adjusted to the final value (pH 3 to 7). The electrical charge (ζ-potential) and particle size of primary and

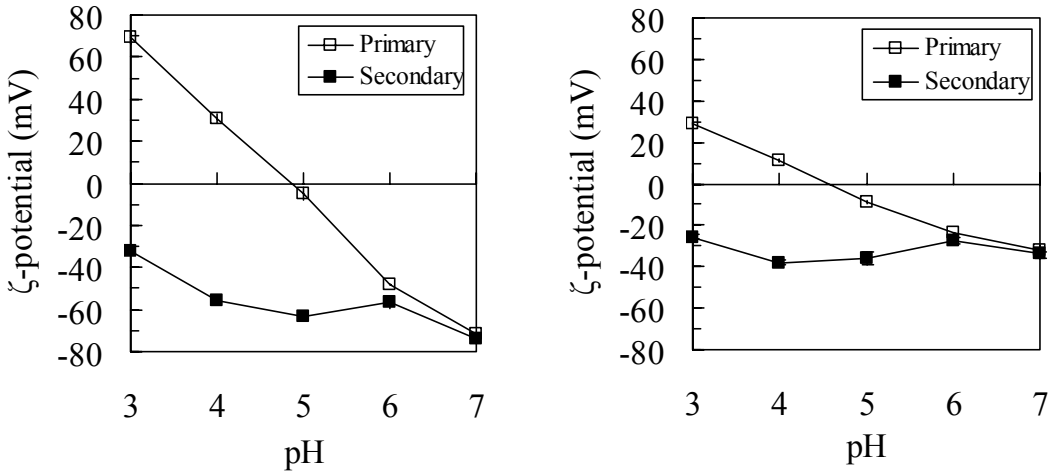
secondary emulsions containing different NaCl concentrations (0 and 100 mM NaCl) were then measured after they had been stored at room temperature for 24 h (Figures 23 and 24).

3.1.1 Adsorption of Alginate

Initially, the influence of pH on the adsorption of alginate molecules to the emulsion droplet surfaces was examined. In the absence of NaCl, the ζ-potential of the β-Lg-stabilized droplets in the primary emulsions went from being highly positive to highly negative as the pH was increased from 3 to 7 (Figure 23a), which is due to the change in the electrical charge of the adsorbed protein molecules as they move from below to above their isoelectric point (pI ≈ 4.7-5.2) (Bromley *et al.*, 2005; Das and Kinsella, 1989; Sawyer and Kontopidis, 2000). On the other hand, the ζ-potential of the droplets in the secondary emulsions was negative at all pH values (Figure 23a). At pH 6 and 7, the ζ-potential of the droplets in the primary and secondary emulsions was fairly similar, suggesting that there was little adsorption of alginate to the protein-coated droplet surfaces, which would be expected because of the relatively strong electrostatic repulsion between the anionic alginate and anionic droplets. A similar effect has been reported for the adsorption of other anionic polysaccharides to β-lactoglobulin-coated droplets (Gu *et al.*, 2005a; Moreau *et al.*, 2003). At pH 3, 4 and 5, the ζ-potential of the droplets in the secondary emulsions was much more negative than that in the primary emulsions, which can be attributed to adsorption of anionic alginate molecules to the droplet surfaces. At pH 3 and 4, the driving force for polymer adsorption is the electrostatic attraction between the anionic alginate molecules and the cationic droplets. At pH 5, polymer adsorption still occurs, even though both the alginate and droplets are negatively charged, because of the interaction of negative groups on the polysaccharide molecules with positive patches on the adsorbed proteins.

The magnitude of the ζ-potential on the droplets in the primary emulsions decreased when 100 mM NaCl was present in the aqueous phase (Figure 23b), which can be attributed to electrostatic screening effects (Hunter, 1986;

Kulmyrzaev and Schubert, 2004; Ogawa *et al.*, 2003b). The magnitude of the ζ-potential of the droplets in the secondary emulsions also decreased with increasing ionic strength. This decrease could be for two reasons: electrostatic screening effects (all pH) or desorption of alginate from the droplet surfaces (pH 3 to 5). There was no evidence of charge reversal in the emulsions at pH 3 to 5, which suggested that the alginate molecules did not appreciably desorb from the droplet surfaces at this salt concentration (Hunter, 1986).



(a) 0 mM NaCl (b) 100 mM NaCl

Figure 23. Influence of pH and ionic strength on electrical charge (ζ-potential) of emulsions in primary and secondary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg, 0.004 wt% sodium alginate in 5 mM phosphate buffer) after stored for 24 h; (a) 0 mM NaCl, (b) 100 mM NaCl.

A number of previous studies have also examined the influence of pH on the adsorption of charged polysaccharides onto the surfaces of protein-coated droplets. It is informative to compare the results obtained from this study with those obtained from these earlier studies so as to distinguish system-specific from more general phenomenon. Upon a reduction of the solution pH, the alginate molecules were first observed to attach to the droplet surfaces around pH 6 (Figure 23), which is a pH unit above the isoelectric point of β-Lg. This phenomenon can be attributed to attachment of anionic groups on the polysaccharide molecules to positive patches on

the exposed surfaces of the adsorbed β-Lg molecules. Similar results were also obtained for the adsorption of HM-pectin (Guzey *et al.*, 2004) and ι- and λ-carrageenan (Gu *et al.*, 2004b) onto the surfaces of β-Lg coated droplets, and for the adsorption of LM- and HM-pectin onto the surfaces of caseinate coated droplets (Surh *et al.*, 2005). The attachment of anionic polysaccharides to the surfaces of protein-coated droplets above the isoelectric point (pI) of the adsorbed protein therefore appears to be a fairly general phenomenon. It should be noted that a similar kind of behavior is also observed when proteins and anionic polysaccharides are mixed together in aqueous solutions (de Kruif *et al.*, 2004). In this case, the protein and polysaccharide form either soluble complexes or complex coacervates at pH values above pI (up to one unit or so), even though they are both negatively charged, which has been attributed to the attachment of negative groups on the polysaccharides to positive patches on the surfaces of the proteins (de Kruif *et al.*, 2004).

3.1.2 Droplet Aggregation

Information about the influence of the final pH on droplet aggregation was obtained from dynamic light scattering and turbidity measurements. The data obtained from the dynamic light scattering instrument was presented as the "z-average" particle diameter of the emulsions (Figure 24), which is the scattering intensity-weighted mean diameter. An increase in the z-average is therefore indicative of droplet aggregation. Turbidity versus wavelength measurements were also used to ascertain the extent of droplet aggregation in the emulsions. An example of the sensitivity of turbidity spectra to droplet aggregation is shown in Figure 25 for primary emulsions in the absence of salt at two pH values. At pH 7, the turbidity of the emulsions was relatively high at short wavelengths and decreased steeply with increasing wavelength, which is indicative of a system containing small particles (McClements, 2002). On the other hand, at pH 5, the turbidity of the emulsion only decreased slightly with increasing wavelength, which is indicative of a system containing large particles (McClements, 2002). At high wavelengths there is a large difference in the turbidity of the samples due to droplet aggregation. Hence, turbidity measurements at 800 nm were used as a convenient indicator of the extent of droplet

aggregation in the emulsions: the higher the turbidity, the more unstable to aggregation (Figure 26).

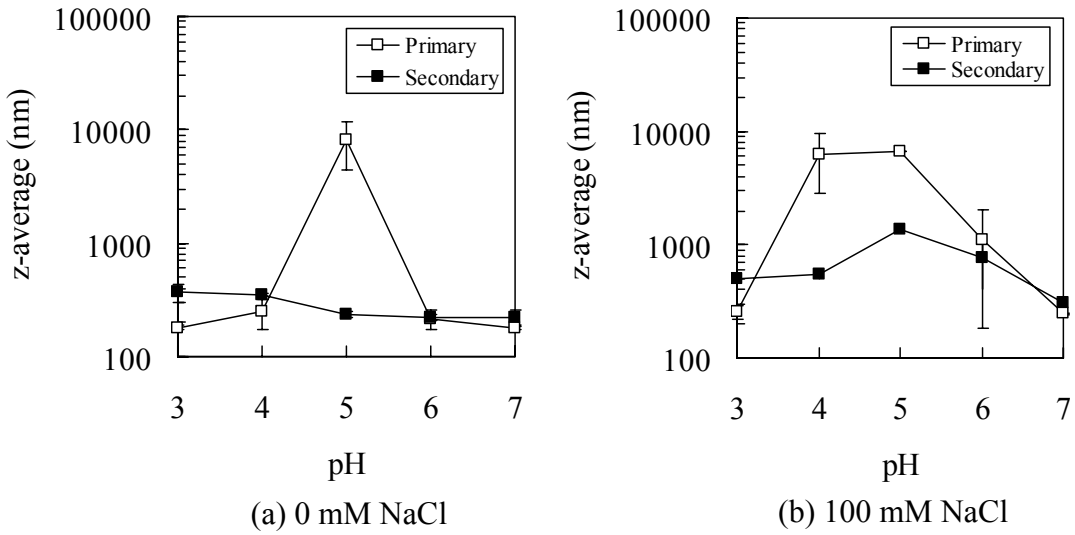


Figure 24. Influence of pH and ionic strength on particle size of emulsions in primary and secondary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg, 0.004 wt% sodium alginate in 5 mM phosphate buffer) after stored for 24 h; (a) 0 mM NaCl, (b) 100 mM NaCl.

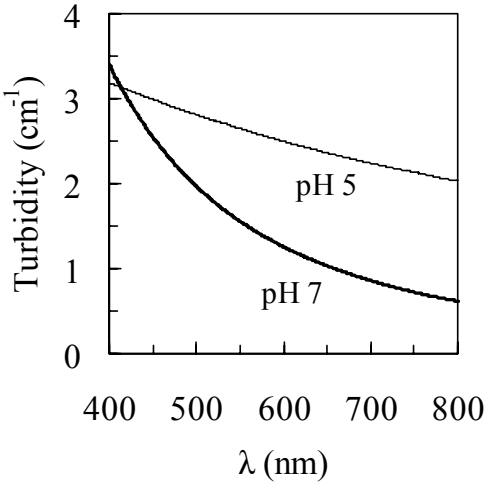
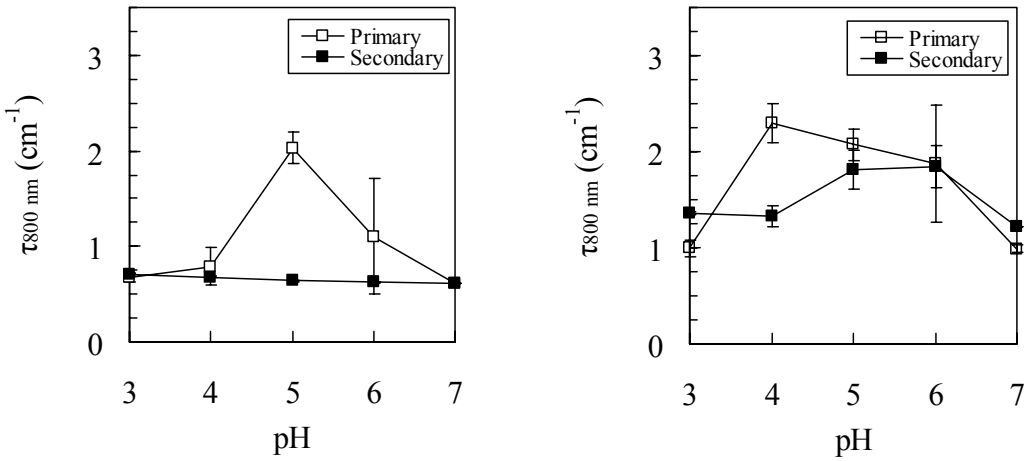


Figure 25. Influence of pH on turbidity spectra of primary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg in 5 mM phosphate buffer) after stored for 24 h.



(a) 0 mM NaCl (b) 100 mM NaCl

Figure 26. Influence of pH and ionic strength on turbidity of emulsions in primary and secondary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg, 0.004 wt% sodium alginate in 5 mM phosphate buffer) after stored for 24 h.

In the absence of NaCl, the particle size and turbidity measurements indicated that the primary emulsions were stable to droplet aggregation at all pH values except pH 5 (Figures 24a and 26a). The relatively large particle diameter and high turbidity observed at pH 5 can be attributed to extensive droplet aggregation close to the isoelectric point (pI) of the proteins. At the pI the number of negatively charged groups balances the number of positively charged groups on the adsorbed proteins so that the net charge on the droplets is zero (Figure 23a). Under these conditions the electrostatic repulsion between the droplets is no longer strong enough to overcome the attractive interactions (e.g., van der Waals), which results in extensive droplet flocculation (Kulmyrzaev and Schubert, 2004; McClements, 2004a). On the other hand, the mean particle size and turbidity remained small at all pH values for the secondary emulsions, indicating that the emulsions were stable to droplet aggregation (Figures 24a and 26a). This can be attributed to the fact that the magnitude of the droplet ζ-potential was relatively high at all pH values ($|\zeta| > 20$ mV) (Figure 23a), so that the electrostatic repulsion between the droplets would be sufficient to overcome the attractive droplet-droplet interactions (Friberg, 1997; McClements, 2004a). In addition, there would have been an increase in the thickness

of the interfacial membrane surrounding the droplets, which would have increased the steric repulsion between the droplets and possibly reduced the magnitude of the van der Waals attraction (McClements, 2004a).

In the presence of 100 mM NaCl, the primary emulsions were unstable to droplet aggregation over a wider range of pH values (pH 4 to 6) than in the absence of NaCl (Figures 24b and 26b). The most likely reason for this effect is that as the ionic strength increased, the electrostatic repulsion between the droplets was progressively screened by the counter-ions (Na^+ or Cl^-) surrounding the droplets, hence the magnitude of the electrostatic repulsion was reduced. The stability of the secondary emulsions to droplet aggregation decreased somewhat upon addition of salt, but they were still considerably more stable than the primary emulsions, which may have been because the interfacial membranes were more highly charged and thicker thereby increasing the overall repulsive interactions between the droplets.

The results of this study with those obtained in earlier studies on the influence of anionic polysaccharides on the stability of protein-coated droplets were compared. In this study, alginate caused a marked improvement in the aggregation stability of the droplets around the isoelectric point (pH 5) of the adsorbed proteins, i.e., the mean particle size was reduced considerably (Figure 24). Similar observations have been made for other systems consisting of protein-coated droplets to which anionic polysaccharides have been added, e.g., HM-pectin (Guzey *et al.*, 2004) and carrageenan (Gu *et al.*, 2004b) to β-lactoglobulin coated droplets, and LM- and HM-pectin onto caseinate coated droplets (Surh *et al.*, 2005). The ability of anionic polysaccharides to increase the electrostatic and steric repulsion between protein-coated droplets around the pI of the adsorbed protein, thereby improving emulsion stability, therefore appears to be a fairly general phenomenon.

3.1.3 Creaming Stability

The influence of final pH on the creaming stability of primary and secondary emulsions containing different amounts of NaCl (0 or 100 mM) was

determined by measuring the change in turbidity of undisturbed samples at a fixed sample height (Figures 27 and 28).

The principle of this method is given in Figure 27, which shows the change in turbidity (at 600 nm) of selected primary emulsions with storage time (0 mM NaCl). In an emulsion that is stable to creaming (pH 7), the droplets remain evenly distributed throughout the measurement cell and so the turbidity stays relatively constant with time. In an emulsion that is highly unstable to creaming (pH 5), the droplets rapidly move to the top of the measurement cell and so the turbidity measured at 42% of the sample height is close to zero because there are no more droplets left to scatter the light. In an emulsion that has an intermediate stability to creaming (pH 4), the aggregated droplets move more slowly to the top of the measurement cell. Hence, the turbidity measured at 42% of the sample height remains relatively high for a certain period then rapidly falls when the serum layer moves past the measurement position. The turbidity measurements made after 7 days storage was used to compare the influence of pH on the creaming stability of the emulsions: a high turbidity after 7 days storage indicated good stability to creaming (Figure 28).

The primary emulsions containing 0 mM NaCl were unstable to creaming at pH 5, while those containing 100 mM NaCl were unstable at pH 4 and 5 (Figure 24). The secondary emulsions containing 0 mM NaCl were stable to creaming at all pH values, while those containing 100 mM NaCl were only unstable at pH 5 (Figure 24). The results from the creaming stability measurements (Figure 28) therefore largely supported those from the droplet aggregation measurements (Figures 24 and 26), which should be expected since droplet aggregation leads to accelerated creaming in dilute emulsions (McClements, 2004a).

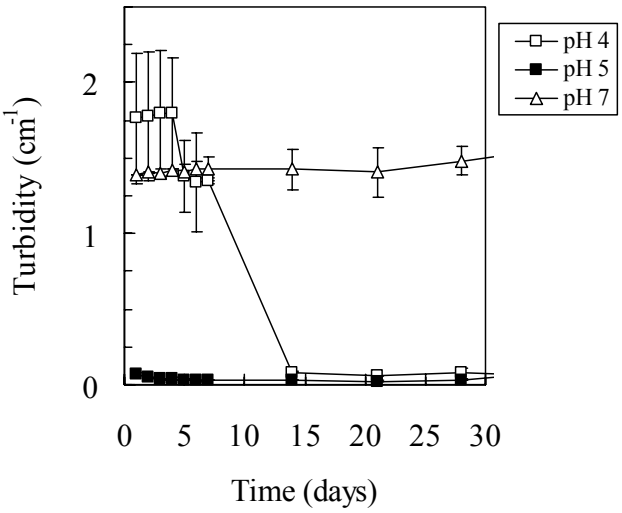


Figure 27. Influence of pH on creaming stability of emulsions in primary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg in 5 mM phosphate buffer) after stored for 28 days measured at 600 nm.

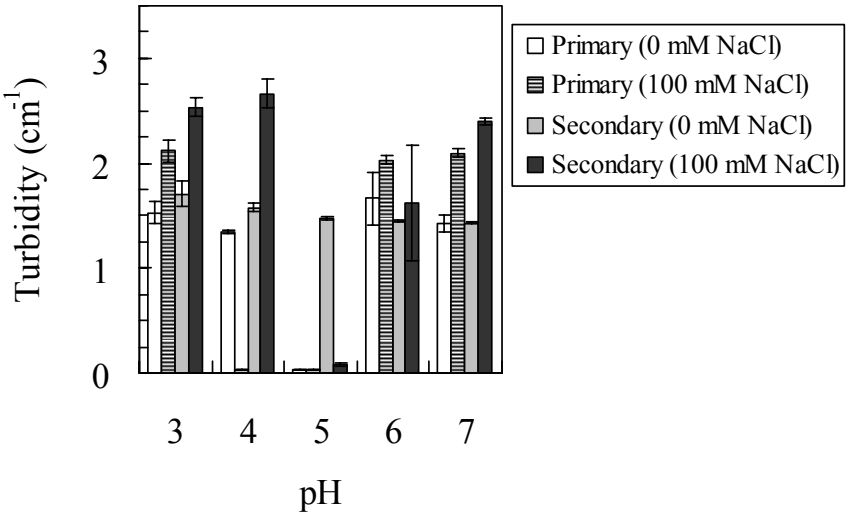


Figure 28. Influence of NaCl on creaming stability of emulsions in primary and secondary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg, 0.004 wt% sodium alginate in 5 mM phosphate buffer) after stored for 7 days.

3.2 Influence of Ionic Strength on the Properties of Primary and Secondary Emulsions

A number of studies have shown that secondary emulsions are more stable to droplet aggregation than primary emulsions in the presence of salt (Aoki *et al.*, 2005; Guzey *et al.*, 2004; Moreau *et al.*, 2003; Ogawa *et al.*, 2003a). The purpose of these experiments was therefore to examine the influence of NaCl on the stability of β-Lg–coated droplets (primary emulsions) and on the formation and stability of β-Lg-alginate–coated droplets (secondary emulsions). These experiments were carried out at pH 4 and 5 because stable secondary emulsions could be formed at these pH values (Figure 24). In addition, the alginate molecules would adsorb more strongly to β-Lg–coated droplets with an appreciable positive charge (pH 4) than with a slight negative charge (pH 5), hence the influence of NaCl might be different at these two pH values. The influence of NaCl concentration (0 to 250 mM) on the ζ-potential and mean particle size was measured after the emulsions had been stored at room temperature for 24 h, while the creaming stability was measured throughout storage at room temperature for 1 week (Figures 29 to 31).

At pH 4, the ζ-potential of the β-Lg-stabilized droplets in the primary emulsions remained positive when the NaCl concentration was increased, while the ζ-potential of the β-Lg-sodium alginate-stabilized droplets in the secondary emulsions remained negative at all ionic strengths (Figure 29). However, the magnitude of the ζ-potential decreased as the NaCl concentration was increased. In both the primary and secondary emulsions this affect can be attributed at least partly to electrostatic screening caused by the addition of salt (McClements, 2004a, 2004b). In the secondary emulsions it is also possible that there was a reduction in the negative charge on the droplets caused by desorption of alginate molecules from the droplet surfaces brought about by screening of the electrostatic attraction between the anionic alginate molecules and the cationic droplets. Nevertheless, the fact that the ζ-potential remained negative in the secondary emulsions at high ionic strengths suggests that at least some alginate molecules remained attached to the droplet surfaces. In addition, the magnitude of the ζ-potential decreased to about 23% of its

initial value when the salt concentration was increased from 0 to 250 mM NaCl in the primary emulsions, whereas it only decreased to about 56% of its initial value in the secondary emulsions. This suggests that the alginate molecules did not desorb from the droplet surfaces, and that there was some mechanism associated with the β-Lg-alginate interfaces that resisted a change in droplet ζ-potential with increasing salt concentration, e.g., charge regulation or a change in interfacial thickness (Hunter, 1986).

At pH 5, the ζ-potential of the β-Lg-stabilized droplets in the primary emulsions remained slightly negative at all ionic strengths. Nevertheless, the magnitude of the charge on the β-Lg-stabilized droplets was low (< 8 mV) due to the fact that the pH was close to the pI of the adsorbed protein (Bromley *et al.*, 2005; Das and Kinsella, 1989; Kulmyrzaev and Schubert, 2004; Sawyer and Kontopidis, 2000). The ζ-potential of the β-Lg-alginate-coated droplets in the secondary emulsions was also negative at all ionic strengths, but its magnitude decreased appreciably with increasing NaCl (Figure 29). The decrease in negative charge with increasing salt concentration was much greater at pH 5 than at pH 4. For example, the magnitude of the ζ-potential in the secondary emulsions was reduced to about 56% of its initial value when the salt concentration was increased from 0 to 250 mM NaCl at pH 4, but to about 20% at pH 5. This difference was attributed to partial desorption of anionic alginate molecules from the droplet surfaces. The most likely origin of this effect is that the electrostatic attraction between the anionic alginate molecules and positively charged patches on the β-Lg-coated droplets was weakened at higher ionic strengths due to electrostatic screening (Ogawa *et al.*, 2003b).

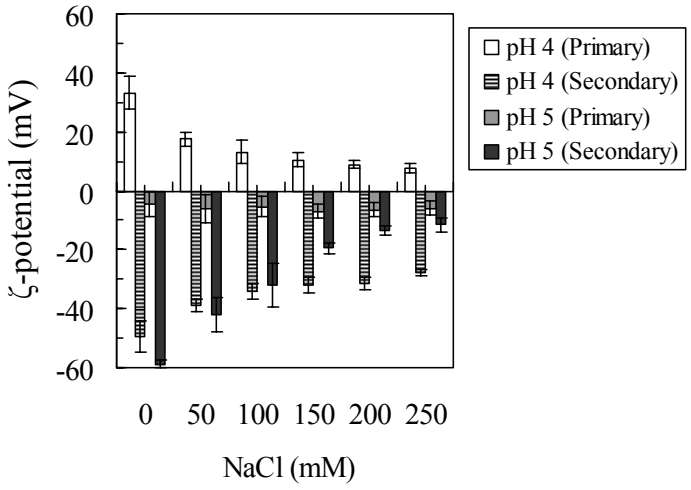


Figure 29. Influence of NaCl on electrical charge (ζ-potential) of emulsion droplets in primary and secondary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg, 0.004 wt% sodium alginate, 5 mM phosphate buffer) after stored for 24 h.

The particle size and creaming measurements indicated that both the primary and secondary emulsions were relatively stable to droplet aggregation at low ionic strengths (< 50 mM), but became strongly aggregated at higher ionic strengths (Figures 30 and 31). The secondary emulsions seemed to be somewhat more stable to droplet aggregation than the primary emulsions at 100 mM NaCl (Figure 30), but they were still unstable to creaming (Figure 31). This improved stability may have been due to the greater electrostatic and steric interactions between the droplets in the secondary emulsions. In the case of the primary emulsions, droplet aggregation was probably caused by the reduction of the electrostatic repulsion between the droplets due to electrostatic screening, while for the secondary emulsions it was probably a combination of electrostatic screening and desorption of alginate molecules from the droplet surfaces.

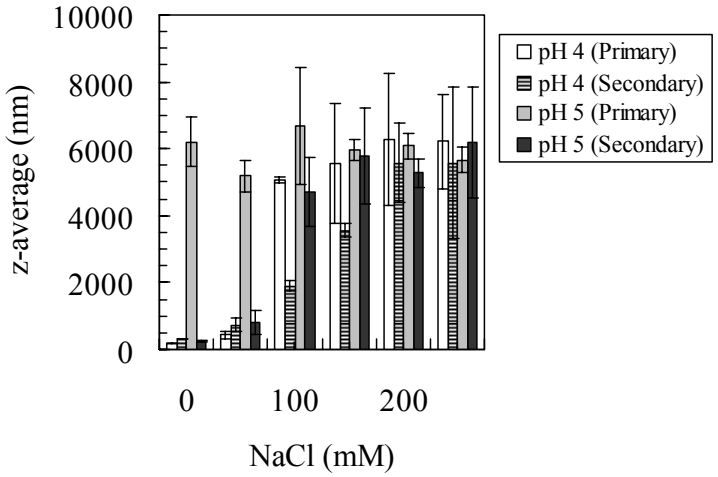


Figure 30. Influence of NaCl on particle size of emulsion droplets in primary and secondary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg, 0.004 wt% sodium alginate in 5 mM phosphate buffer) after stored for 24 h.

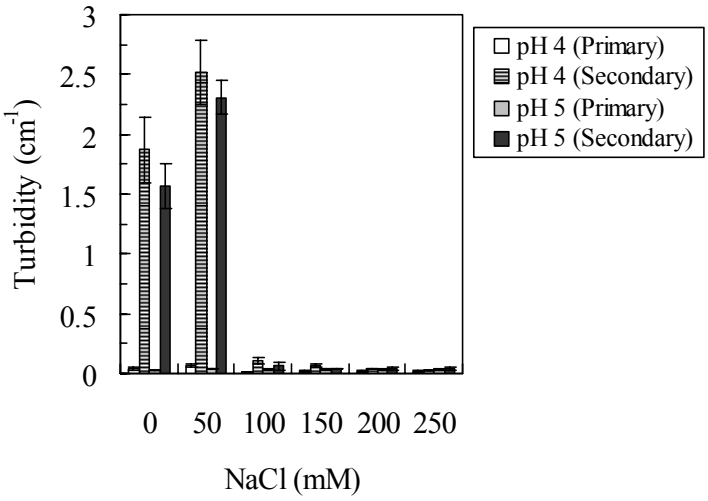


Figure 31. Influence of NaCl on creaming stability of emulsion droplets in primary and secondary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg, 0.004 wt% sodium alginate in 5 mM phosphate buffer) after stored for 7 days.

A number of previous studies have also examined the influence of salt on the stability of protein-coated and protein-polysaccharide-coated oil droplets. Emulsions containing droplets coated by ι-carrageenan-β-Lg membranes (at pH 6) were found to be stable up to 500 mM NaCl, whereas those coated by β-Lg alone were only stable up to 100 mM NaCl (Gu *et al.*, 2005c). The range of emulsion

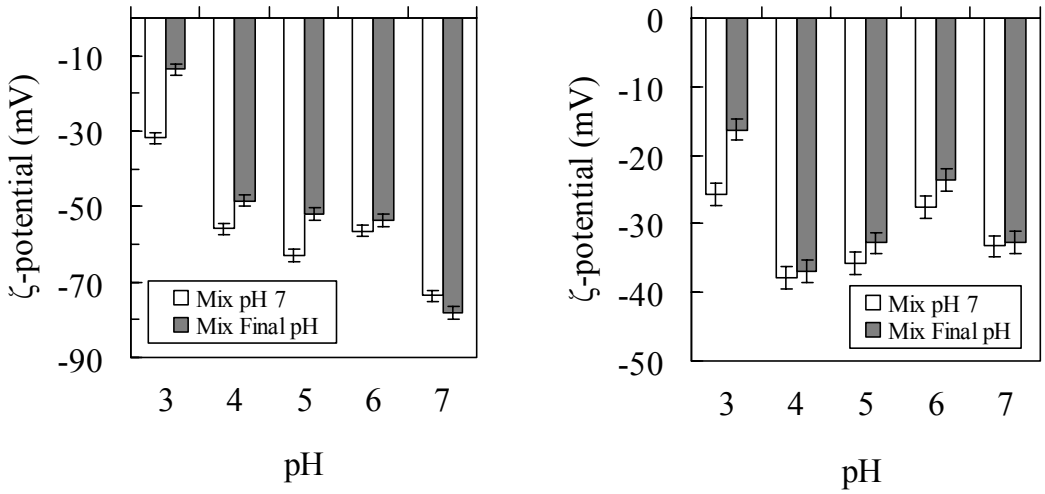
stability to NaCl-induced flocculation was therefore appreciably wider for carrageenan-β-Lg at pH 6 (<500 mM) (Gu *et al.*, 2005c), than for alginate-β-Lg at pH 4 or 5 (<100 mM - Figures 30 and 31). This result suggests that carrageenan is more effective at improving the stability of protein-stabilized emulsions against the effects of salt than alginate, possibly because of differences in the net charge, thickness or structure of the interfacial layers formed around the droplets.

3.3 Influence of Mixing Condition on the Properties of Secondary Emulsions

Previous studies suggest that the mixing method has an appreciable influence on the formation and stability of secondary emulsions (Guzey *et al.*, 2004). For example, mixing can be carried out using a one-step method where droplets and polymers are mixed directly at the final pH, or they can be prepared using a two-step method where droplets and polymers are mixed at a pH where they have the same electrical charge and then the solution pH is adjusted to the final value. In this section, the effect of mixing method on the properties of secondary emulsions was investigated by mixing β-Lg-coated droplets with sodium alginate: (i) mixing directly at final pH; (ii) mixing at pH 7, then adjusting to final pH. The dependence of the ζ-potential and mean particle size of these emulsions after storing for 24 h was measured (Figures 32 and 33).

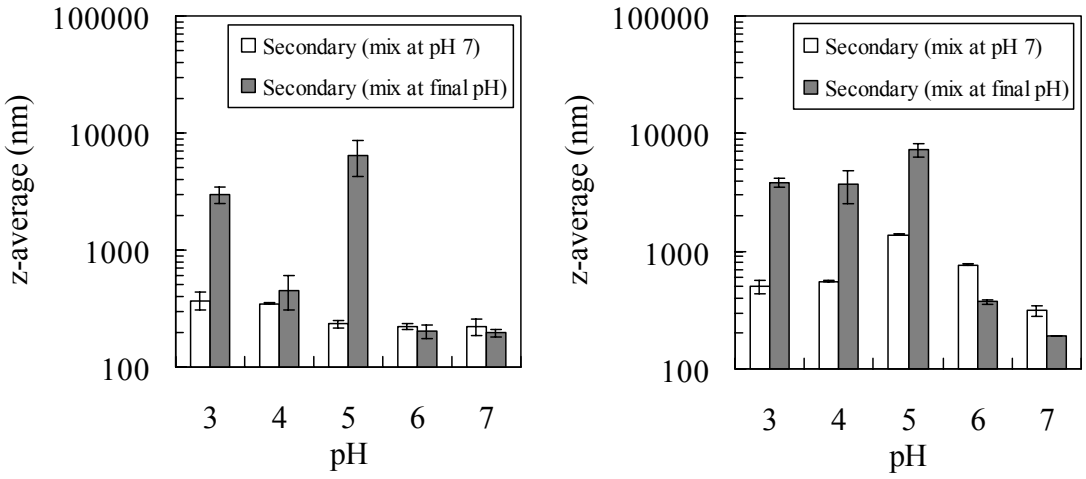
The mixing method had an appreciable influence on both the ζ-potential and aggregation of the droplets in the secondary emulsions. The ζ-potential was significantly lower ($p < 0.05$) in the secondary emulsions prepared by mixing directly at pH 3, than in the ones mixed at pH 7 then brought to pH 3. This would suggest that at this pH less alginate adsorbed to the droplet surfaces when the secondary emulsions were prepared using the one-step method than the two-step method. This may have occurred because the droplets rapidly aggregated and therefore there was less surface area available for the alginate molecules to adsorb to, or because the packing of the alginate molecules was less efficient.

At pH values where the alginate molecules adsorbed to the droplet surfaces (pH 3 to 5), there was considerably more droplet aggregation in the secondary emulsions prepared by the one-step method than the two-step method (Figure 33). Droplet aggregation was less extensive when the two-step method was used because the alginate molecules are already distributed uniformly throughout the aqueous solution surrounding the droplets before they start to adsorb to the droplet surfaces. Hence, polymer adsorption can occur more rapidly and uniformly when the pH is adjusted to a value where the polymer and droplets have opposite charges. On the other hand, in the one-step method the droplets and polymers initially start in different solutions which have to be mixed together and therefore there are local regions of high and low polymer and droplet concentrations. Hence, polymer adsorption is much less uniform and bridging flocculation is more likely to occur. The results of this study are therefore in agreement with those of an earlier study of pectin adsorption to the surfaces of β-Lg coated droplets, which also showed that the two-step mixing process gave less droplet flocculation than the one-step mixing process (Guzey *et al.*, 2004).



(a) 0 mM NaCl (b) 100 mM NaCl

Figure 32. Influence of pH and mixing pH on electrical charge (ζ-potential) of emulsion droplets in primary and secondary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg, 0.004 wt% sodium alginate in 5 mM phosphate buffer) after stored for 24 h; (a) 0 mM NaCl, (b) 100 mM NaCl.



(a) 0 mM NaCl (b) 100 mM NaCl

Figure 33. Influence of pH and mixing pH on particle size of emulsion droplets in primary and secondary emulsions (0.1 wt% corn oil, 0.009 wt% β-Lg, 0.004 wt% sodium alginate in 5 mM phosphate buffer) after stored for 24 h; (a) 0 mM NaCl, (b) 100 mM NaCl.

This study showed that alginate adsorbed to the β-Lg-coated droplets from pH 3 to 6, which was attributed to electrostatic attraction between the anionic polymer and cationic patches on the droplet surfaces. Droplets coated by β-Lg-alginate had better stability to flocculation than those coated by β-Lg alone, especially around the isoelectric point of the adsorbed proteins and at low ionic strengths (< 100 mM NaCl). At pH 5, alginate molecules desorbed from the droplet surfaces at high salt concentrations due to weakening of the electrostatic attraction.

Therefore, pH 7 was chosen as the preparation pH for the primary and secondary emulsions for the next study, since this mixing condition has an impact on the formation and stability of emulsions containing β-Lg-alginate coated droplets. In addition, the beverage emulsion consisting of low oil content was selected as a food model for further study.

4. Stabilization of Model Beverage Emulsions Using Protein-Polysaccharide Electrostatic Complexes Formed at the Oil-Water Interface

The term "beverage emulsion" normally refers to non-dairy based beverages that are drunken cold, such as fruit, soft and cola drinks (Tan, 1998, 2004). This group of products has a number of common manufacturing, compositional and physicochemical features. Beverage emulsions are normally prepared by homogenizing an oil and aqueous phase together to create a concentrated oil-in-water emulsion, which is later diluted with an aqueous solution to create the finished product (McClements, 2004a).

Beverage emulsion was selected as a food model because it is a thermodynamically unstable system that tends to breakdown during storage through a variety of physicochemical mechanisms, including creaming, flocculation, coalescence and Ostwald ripening (McClements, 2004a). The hypothesis of this study is to investigate that the interfacial engineering technology could be used by the food industry to create beverage emulsions with good physical stability at relatively low total emulsifier levels.

4.1 Formation of Interfacial Complexes at Oil Droplet Surfaces

The purpose of this set of experiments was to determine whether various polysaccharides would adsorb to the surface of protein-coated oil droplets, and to obtain some information about the electrical characteristics of the interfaces formed. Initially, β-Lg-stabilized emulsions at pH 7 were prepared in the absence (primary emulsions) and presence (secondary emulsions) of different types and concentration of polysaccharide. At pH 7, the protein and polysaccharides have similar electrical charges and therefore the polysaccharides would not have been expected to have adsorbed to the surfaces of the protein-coated droplets. The pH of the emulsions was then decreased from pH 7 to either pH 3 or 4 and measured the particle ζ-potential of the resulting emulsions after 1 day storage (Figure 34). At these pH values, the signs of the electrical charge on the protein (positive) and polysaccharides (negative) are

opposite, so that one would expect the anionic polysaccharides in the aqueous phase to be electrically attracted towards the cationic protein-coated droplets.

The electrical charge (ζ-potential) on the emulsion droplets was strongly dependent on final pH, polysaccharide type and polysaccharide concentration (Figure 34). In the absence of polysaccharide, the electrical charge on the protein-coated emulsion droplets was positive, because the adsorbed β-Lg was below its isoelectric point (pI ~ 5.0) (Bromley *et al.*, 2005; Das and Kinsella, 1989; Sawyer and Kontopidis, 2000). As the polysaccharide concentration in the aqueous phase of the emulsions was increased, the electrical charge on the droplets initially became less positive then it became more negative, until it finally reached a plateau value (ζ_{Sat}). Similar results have been observed in previous studies, where the change in ζ-potential was attributed to progressive adsorption of anionic polysaccharides onto the surfaces of cationic protein-coated droplets, until the droplet surfaces had become saturated (Gu *et al.*, 2004a, 2004b, 2005a; Gu *et al.*, 2005c; Guzey *et al.*, 2004; Moreau *et al.*, 2003). The steepness of the initial change in ζ-potential with increasing polysaccharide concentration and the saturation ζ-potential depended on polysaccharide type and pH.

The ζ-potential versus polysaccharide concentration curves were modeled in terms of the following empirical equation:

$$\frac{\zeta(c) - \zeta_{Sat}}{\zeta_0 - \zeta_{Sat}} = \exp\left(-\frac{c}{c^*}\right) \quad (1)$$

Where ζ(c) is the ζ-potential of the emulsion droplets at polysaccharide concentration c, ζ_0 is the ζ-potential in the absence of polysaccharide, ζ_{Sat} is the ζ-potential when the droplets are saturated with polysaccharide, and c* is a critical polysaccharide concentration. Mathematically, c* is the polysaccharide concentration where the change in ζ-potential is $1/e$ of the total change in ζ-potential for saturation: $\Delta\zeta = \Delta\zeta_{Sat}/e$. The value of c* is therefore a measure of the binding affinity of the polysaccharide for the droplet surface: the higher c*, the lower the binding affinity.

The binding of a polysaccharide to the droplet surface can therefore be characterized by ζ_{Sat} and c*. Values for ζ_0, ζ_{Sat} and c* are tabulated in Table 2 for the three different polysaccharides at pH 3 and 4. The values of ζ_0 and ζ_{Sat} were determined from the ζ-potential measurements in the absence of polysaccharide and at the highest polysaccharide concentration used (where saturation was assumed). The c* values were then obtained by finding the quantities that gave the best fit between Equation 1 and the experimental data (using the *Solver* routine in Excel, Microsoft Corp). There was good agreement between the experimental measurements and the ζ-potential values predicted for the secondary emulsions using Equation 1 and the parameters listed in Table 2 (Figure 34).

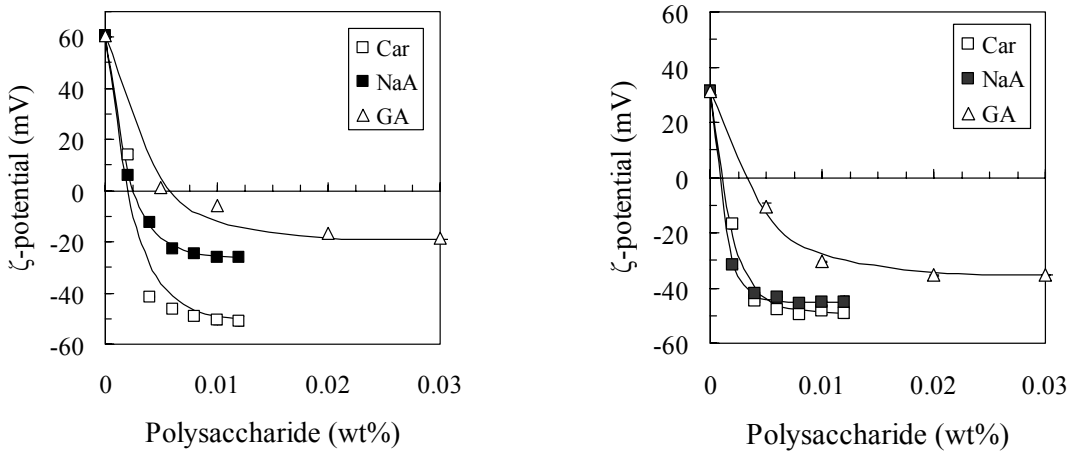


Figure 34. Dependence of droplet charge (ζ-potential) on polysaccharide concentration in 0.1 wt% corn oil-in-water emulsions containing different kinds of polysaccharide: (a) pH 3; (b) pH 4. The curves on predictions made using Equation 1 and the parameters in Table 2; ι-carrageenan (□ Car); sodium alginate (■ NaA); gum arabic (Δ GA).

Table 2. Parameters characterizing the binding of polysaccharides to protein-coated droplet surfaces determined from ζ-potential versus polysaccharide concentration measurements at pH 3 and 4 using Equation 1.

Parameter	ι-carrageenan		Sodium alginate		Gum arabic	
	pH 3	pH 4	pH 3	pH 4	pH 3	pH 4
ζ_0 (mV)	60.6 ± 0.7	31.4 ± 0.9	60.6 ± 0.7	31.4 ± 0.9	60.6 ± 0.7	31.4 ± 0.9
ζ_{Sat} (mV)	-51.1 ± 1.9	-49.2 ± 2.0	-26.2 ± 2.0	-45.1 ± 2.6	-19.2 ± 0.4	-35.4 ± 0.4
$\Delta\zeta_{Sat}$ (mV)	112 ± 2	80.6 ± 2.2	86.8 ± 2.1	76.5 ± 2.8	79.8 ± 0.8	66.8 ± 1.0
c* (wt%)	0.0025	0.0019	0.0021	0.0012	0.0042	0.0046

The binding affinity was dependent on polysaccharide type and solution pH (Table 2). At both pH 3 and 4, the c* values were appreciably lower for alginate and carrageenan than for gum arabic, which suggested that they had a stronger binding affinity for the droplet surfaces. For carrageenan and gum arabic the binding affinities were fairly similar at pH 3 and 4, but for alginate the binding affinity was considerably higher (lower c*) at pH 4 than at pH 3. The saturation value of the ζ-potential was also dependent on polysaccharide type and solution pH (Table 2). The protein/carrageenan-coated droplets had the highest negative charge and had similar ζ_{Sat} values at pH 3 and 4 ($\zeta_{Sat} \approx$ -50 mV). The protein/alginate-coated droplets had a high negative charge at pH 4 ($\zeta_{Sat} \approx$ -45 mV), but were appreciably less charged at pH 3 ($\zeta_{Sat} \approx$ -26 mV). The protein/gum arabic-coated droplets had the smallest negative charge at both pH values, but the negative charge was appreciably higher at pH 4 ($\zeta_{Sat} \approx$ -35 mV) than at pH 3 ($\zeta_{Sat} \approx$ -19 mV).

It can be postulated that the difference in the electrical characteristics of the protein/polysaccharide-coated droplets was due to differences in the electrical charge densities of the polysaccharide molecules. Consequently, the electrical characteristics (ζ-potential versus pH) of 0.1 wt% aqueous polysaccharide solutions were measured (Figure 35). These measurements show that the ζ-potential of the polysaccharide molecules (ζ_{PS}) follows the same trend as the ζ_{Sat} values of the emulsion droplets coated by protein/polysaccharide complexes: ζ_{PS} = -53, -30 and -9

mV at pH 3 and ζ_{PS} = -51, -55 and -23 mV at pH 4 for carrageenan, alginate and gum arabic, respectively (Figure 35). The electrical charge on the carrageenan molecules and protein/carrageenan-coated droplets is highly negative at both pH 3 and 4. The electrical charge on the alginate molecules and protein/alginate-coated droplets is highly negative at pH 4 but less so at pH 3. The electrical charge on the gum arabic molecules and protein/gum arabic-coated droplets is considerably less negative than for the other two polysaccharides, and is appreciably lower at pH 3 than 4. Thus, it appears that the electrical characteristics of the protein/polysaccharide-coated droplets are largely determined by the electrical characteristics of the polysaccharide molecules.

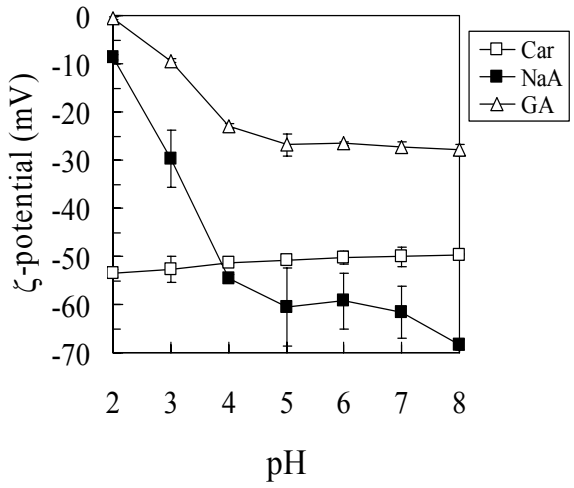


Figure 35. Dependence of the effective ζ-potential of polysaccharide molecules in aqueous solutions on pH; ι-carrageenan (□ Car); sodium alginate (■ NaA); gum arabic (Δ GA).

It is also insightful to examine the overall change in the ζ-potential when the protein-coated droplets are saturated with polysaccharide: $\Delta\zeta_{Sat} = \zeta_0 - \zeta_{Sat}$ (Table 2). For carrageenan, the overall change in ζ-potential is considerably higher at pH 3 ($\Delta\zeta_{Sat} \approx 112$ mV) than at pH 4 ($\Delta\zeta \approx 81$ mV), even though the final ζ_{Sat} values are fairly similar at both pH values ($\zeta_{Sat} \approx -50$ mV). The electrical charge on the carrageenan molecules was fairly similar at pH 3 and 4 (Figure 35), hence it can be postulated that more carrageenan molecules adsorbed to the droplet surfaces at pH 3 than at pH 4. A

possible explanation for this observation can be given in terms of the electrical interactions between a charged polysaccharide and a charged surface that it is approaching.

Studies of the adsorption of synthetic polyelectrolytes onto oppositely charge surfaces have reported that the final ζ-potential is largely independent of the charge density of the adsorbing polyelectrolyte, provided that its charge density is not too low (Schonhoff, 2003). This phenomenon was attributed to the fact that once the surface charge has reached a certain value there will be a strong electrostatic repulsion between the surface and similarly charged polyelectrolytes in the aqueous phase, which limits further adsorption of the polyelectrolyte. Hence, it can be postulated that the carrageenan molecules adsorbed to the protein-coated droplet surfaces until a certain ζ-potential was reached (≈ -50 mV) and then the electrostatic repulsion was strong enough to prevent further polymer adsorption.

4.2 Stability of Emulsions Stabilized by Interfacial Complexes

The purpose of these experiments was to examine the influence of polysaccharide type, polysaccharide concentration and pH on the stability of oil-in-water emulsions containing β-Lg-coated droplets. As explained above, β-Lg-stabilized emulsions at pH 7 were prepared in the absence (primary emulsions) and presence (secondary emulsions) of different types and concentration of polysaccharide, and then the pH was reduced to either 3 or 4 by adding acid. The stability of the emulsions to droplet aggregation and creaming was then determined using light scattering, turbidity and creaming stability measurements (Figures 36 to 38).

The stability of the emulsions to droplet aggregation and creaming was highly dependent on polysaccharide type, polysaccharide concentration and solution pH (Figures 36 to 38). In the absence of polysaccharide, the primary emulsions appeared stable to droplet aggregation (low z-average, low τ_{800}) after 24 hours storage at pH 3 and 4. Presumably, the positive charge on the protein-coated droplets was sufficiently high to prevent droplet aggregation by generating a strong inter-droplet

electrostatic repulsion (McClements, 2004a). The primary emulsion at pH 3 was also stable to creaming after 7 days storage at room temperature, which indicated that droplet aggregation did not occur. On the other hand, the primary emulsion at pH 4 was unstable to creaming after 7 days storage, which indicated that some droplet aggregation had occurred over time. The reason that the primary emulsion was unstable to creaming at pH 4 may have been because this pH is fairly close to the isoelectric point of the adsorbed β-Lg molecules, so that there may not have been a sufficiently strong electrostatic repulsion between the droplets to prevent aggregation during long-term storage.

At intermediate polysaccharide concentrations, the secondary emulsions were highly unstable to droplet aggregation (high z-average, high τ_{800}) and creaming. This phenomenon can be attributed to charge neutralization and bridging flocculation affects (Dickinson, 2003; Friberg *et al.*, 2004; von Goeler and Muthukumar, 1994). When there is insufficient polysaccharide present to completely cover the protein-coated droplets there will be regions of positive charge and regions of negative charge exposed at the droplets surfaces, which will promote bridging flocculation. In addition, the overall net charge on the droplets was relatively small ($|\zeta| < 15$ mV), so that the electrostatic repulsion between the droplets would have been insufficient to overcome the attractive interactions (e.g., van der Waals and hydrophobic). At high polysaccharide concentrations, the secondary emulsions were stable to droplet aggregation (low z-average, low τ_{800}) and creaming at both pH 3 and 4. This re-stabilization can be attributed to the fact that the droplet surfaces were completely covered with polysaccharide and the droplet charge was relatively high (Figure 34). In addition, the interfacial thickness will have increased due to the adsorption of the polysaccharide to the droplet surfaces. Hence, there would be a strong electrostatic and steric repulsion between the protein/polysaccharide-coated droplets that should oppose their aggregation.

The range of intermediate polysaccharide concentrations where the emulsions were unstable to droplet aggregation and creaming depended on polysaccharide type and pH (Figures 36 to 38). For example, emulsions containing

protein-coated droplets to which carrageenan was added were only unstable at 0.002 wt% at pH 3 and 4; those where alginate was added were unstable at 0.002 wt% at pH 4 but from 0.002 to 0.006 at pH 3; and, those where gum arabic was added were unstable from 0.002 to 0.006 wt% at pH 4 but from 0.002 to 0.01 wt% at pH 3. These differences in droplet aggregation behavior can be attributed to the differences in droplet charge (Figure 34). In general, the emulsions were stable to droplet aggregation provided the magnitude of the ζ-potential was high and the droplets were sufficiently covered with polysaccharide.

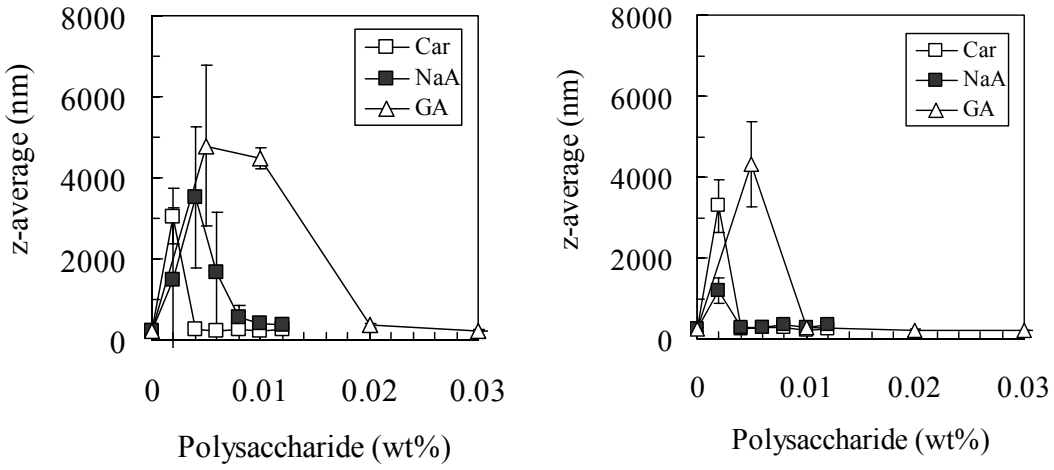


Figure 36. Dependence of the mean particle diameter on polysaccharide concentration in 0.1 wt% corn oil-in-water emulsions containing different kinds of polysaccharide: (a) pH 3; (b) pH 4 ; ι-carrageenan (□ Car); sodium alginate (■ NaA); gum arabic (Δ GA).

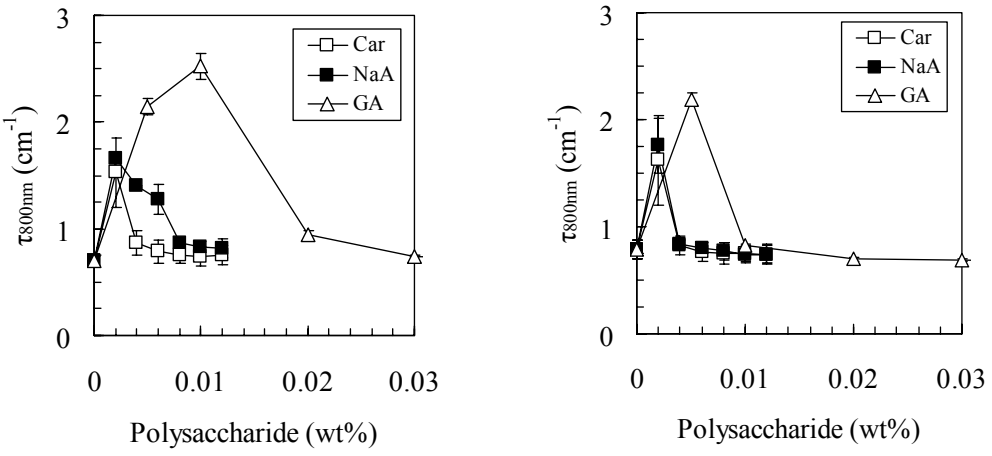


Figure 37. Dependence of the turbidity at 800 nm on polysaccharide concentration in 0.1 wt% corn oil-in-water emulsions containing different kinds of polysaccharide: (a) pH 3; (b) pH 4. An increase in turbidity is indicative of particle aggregation; ι-carrageenan (□ Car); sodium alginate (■ NaA); gum arabic (Δ GA).

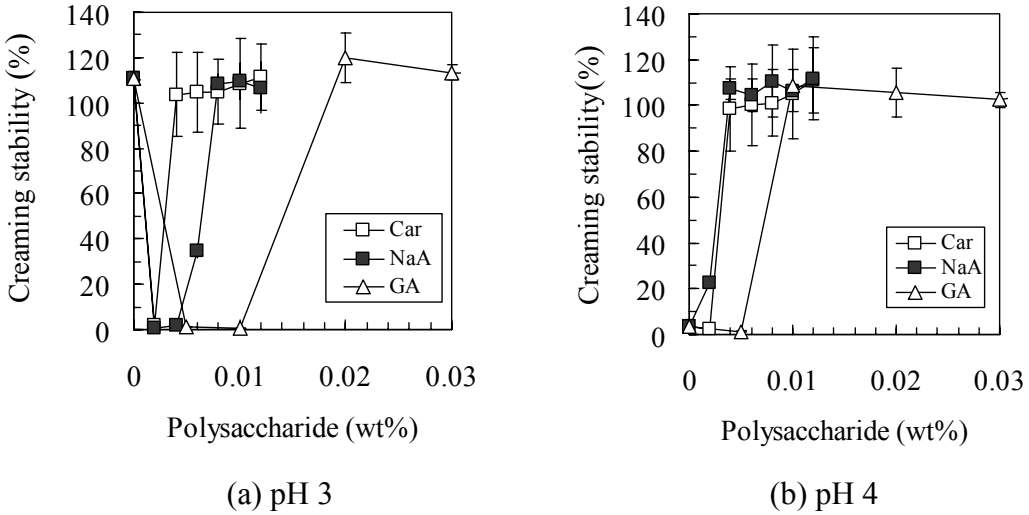


Figure 38. Dependence of the creaming stability on polysaccharide concentration in 0.1 wt% corn oil-in-water emulsions containing different kinds of polysaccharide: (a) pH 3; (b) pH 4. A decrease in creaming stability is indicative of particle aggregation; ι-carrageenan (□ Car); sodium alginate (■ NaA); gum arabic (Δ GA).

4.3 Stability of Emulsions to Environmental Stresses

The purpose of this series of experiments was to determine whether the secondary emulsions containing protein/polysaccharide-coated droplets had better stability to environmental stresses than the primary emulsions containing protein-coated droplets. The ζ-potential measurements were used to assess the interaction of the polysaccharides with the protein-coated droplets and creaming stability measurements were used to assess the overall stability of the emulsions. Primary and secondary emulsions (0.1 wt% corn oil-in-water emulsions, pH 4) with different salt concentrations (0, 50 or 100 mM NaCl), sugar concentrations (0 or 10 wt% sucrose) and heat treatments (30 or 90 ºC) were analyzed. The polysaccharide concentration in the secondary emulsions was selected so that: (i) it was sufficient to saturate the protein-coated droplet surfaces as determined from ζ-potential measurements (Figure 34); (ii) it was just above the minimum amount needed to produce secondary emulsions that were stable to droplet aggregation and creaming (Figures 36 to 38). For this reason, the secondary emulsions were prepared using 0.004 wt% carrageenan, 0.004 wt% alginate or 0.02 wt% gum arabic.

The influence of thermal processing (30 or 90 ºC for 30 minutes) on the stability of the emulsions is shown in Figure 39. Previous studies have shown that heating β-Lg stabilized emulsions to 90 ºC can promote droplet flocculation due to thermal denaturation of the adsorbed proteins (Kim *et al.*, 2003). The unheated and heated primary emulsions were both unstable to heating because the pH was fairly close to the isoelectric point of the adsorbed β-lactoglobulin so that there was not a sufficiently strong electrostatic repulsion between the droplets to prevent aggregation. On the other hand, all of the secondary emulsions were stable to heat treatment (Figure 39). It can be proposed that the polysaccharides adsorbed to the surfaces of the protein-coated droplets and increased the steric and electrostatic repulsion between the droplets by increasing the thickness and charge of the interfaces. These results suggest that heating did not cause the polysaccharides to be desorbed from the droplet surfaces otherwise the secondary emulsions would have become unstable to droplet aggregation like the primary emulsions. This hypothesis was confirmed by

the ζ-potential measurements, which showed that the electrical charge on the droplets in the secondary emulsions changed by less than ± 2 mV upon thermal processing (data not shown). Hence, there was no evidence of desorption of the polysaccharides from the droplet surfaces induced by heating.

The influence of salt addition (0, 50 or 100 mM NaCl) on the stability of the emulsions is shown in Figure 40. The primary emulsion was unstable at all salt concentrations for the reasons mentioned above. The secondary emulsions containing alginate and carrageenan were stable to creaming at 0 and 50 mM NaCl, but were unstable at 100 mM NaCl. On the other hand, the secondary emulsions containing gum arabic were highly unstable to creaming at 50 and 100 mM NaCl. The addition of salt to the emulsions may have adversely affected their creaming stability in a number of ways. First, salt screens the electrostatic repulsion between charged droplets, which can promote droplet aggregation when the strength of the repulsive colloidal interactions is no longer strong enough to overcome the attractive colloidal interactions (McClements, 2004a). Second, the presence of salt in the emulsions may have weakened the electrostatic attraction between the polysaccharides and the protein-coated oil droplets, which may have led to partial or full desorption of the polysaccharide molecules. The fact that the ζ-potential of these emulsions did not change appreciably with increasing salt concentration (see below), suggests that the carrageenan molecules were not fully desorbed from the droplet surfaces. Nevertheless, weakening of the attraction between the polysaccharides and the protein-coated droplet surfaces may have led to bridging flocculation due to adsorption of a polysaccharide onto more than one droplet. At pH 4, the protein/gum arabic-coated droplets have an appreciably lower ζ-potential than the protein/carrageenan- or protein/alginate-coated droplets, which means that the electrostatic repulsion between the droplets is weaker. This would account for the fact that a lower amount of NaCl was needed to promote droplet aggregation in the gum arabic emulsions. In addition, the binding affinity of the gum arabic for the droplet surfaces was less than that of the carrageenan and alginate (Table 2), so it is also possible that the NaCl may have desorbed the gum arabic more easily. Measurements of the droplet ζ-potential were used to provide further insight into the

physicochemical origin of the observed changes in emulsion stability with salt addition.

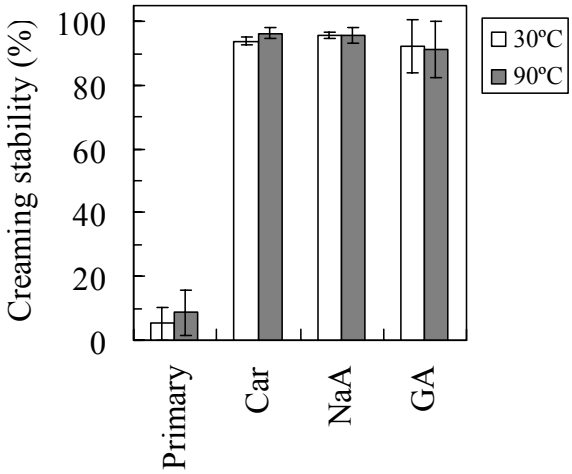


Figure 39. Influence of thermal processing on the stability of 0.1 wt% corn oil-in-water emulsions (pH 4) in the absence and presence of different kinds of polysaccharide.

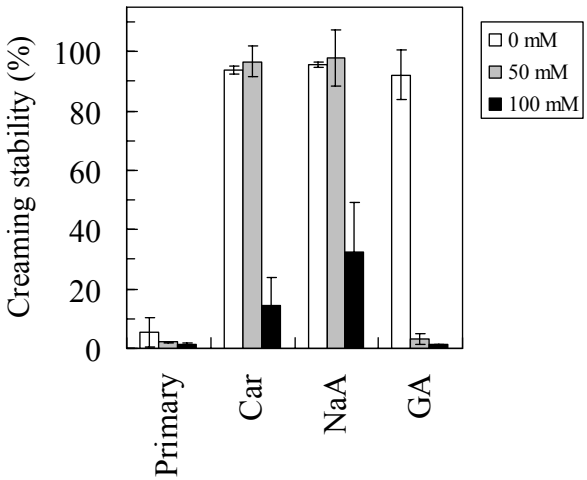


Figure 40. Influence of NaCl on the stability of 0.1 wt% corn oil-in-water emulsions (pH 4) in the absence and presence of different kinds of polysaccharide.

The influence of NaCl on the ζ-potential measurements was highly dependent on the polysaccharide type used to prepare the secondary emulsions (Figure 41). Normally, one would expect a progressive decrease in ζ-potential with increasing salt concentration due to electrostatic screening affects, since $\zeta \propto \kappa^{-1}$ (assuming constant surface charge density and no change in interfacial structure), where κ^{-1} is the Debye screening length (McClements, 2004a). For aqueous solutions at room temperature, the Debye screening length is related to the ionic strength through: $\kappa^{-1} \approx 0.304/\sqrt{I}$ nm, where I is the ionic strength of the solution expressed in moles per liter (McClements, 2004a). Hence, one would expect that the droplet potential should decrease with increasing salt concentration in the following manner: $\zeta \propto 1/\sqrt{I}$.

For the protein-coated droplets there was a progressive decrease in ζ-potential with increasing salt concentration (Figure 41), which can be attributed to electrostatic screening effects. On the other hand, for the protein/carrageenan- and protein/alginate-coated droplets the reduction in ζ-potential with increasing salt concentration was much less than expected. This type of behavior has also been observed for secondary emulsions containing β-lactoglobulin/pectin-coated droplets, where it was attributed to a change in the composition, thickness or structure of the interfacial membrane with salt concentration (Guzey *et al.*, 2004). Changes in these interfacial properties as a result of salt addition may arise due to a reduction in the electrostatic interactions between adsorbed and non-adsorbed polysaccharides (repulsive), between two or more adsorbed polysaccharides (repulsive), or between adsorbed polysaccharides and proteins (attractive). Finally, the protein/gum arabic-coated droplets showed a much larger decrease in ζ-potential with increasing salt concentration than the protein/alginate- or protein/carrageenan-coated droplets, which suggested that some of the gum arabic may have desorbed from the droplet surfaces, thereby promoting instability at a lower NaCl concentration through charge neutralization and polymer bridging effects. The different behavior of the three polysaccharides may have been because of their different chemical composition (functional groups) or their different molecular conformations. Carrageenan and

alginate molecules would be expected to be more extended in structure than gum arabic molecules.

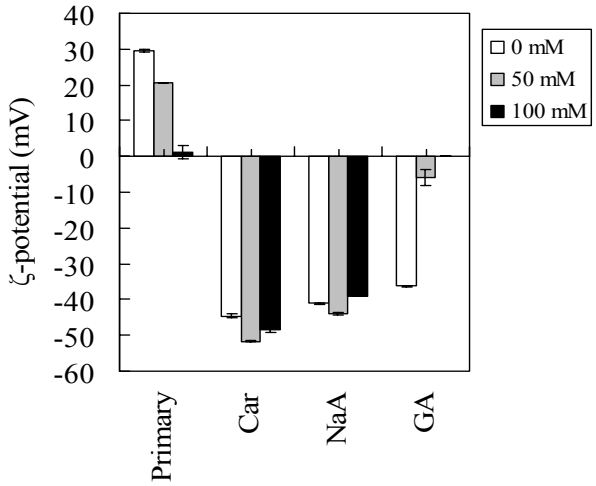


Figure 41. Influence of NaCl on the ζ-potential of 0.1 wt% corn oil-in-water emulsions (pH 4) in the absence and presence of different kinds of polysaccharide.

The influence of sugar addition (0 or 10 wt% sucrose) on the stability of the emulsions was also determined (data not shown). The change in droplet ζ-potential or creaming stability was not found in the absence or presence of sucrose, which indicated that sucrose had no affect on interfacial composition or emulsion stability.

This study has been shown that model beverage emulsions can be produced by engineering their interfacial membranes using the electrostatic interaction of proteins and polysaccharides at oil-water interfaces. These interfacial complexes were formed by mixing charged polysaccharides with oil-in-water emulsions containing oppositely charged protein-coated oil droplets. It showed that the properties of emulsions containing droplets stabilized by multilayer interfaces can be controlled by altering the electrostatic interactions in the system, which may have important implications for their industrial utilization.

CONCLUSION

The interactions between β-lactoglobulin and sodium alginate in aqueous solutions and in oil-in-water emulsions were investigated. The ITC, turbidity, particle size, soluble protein, and ζ-potential measurements provided useful quantitative information about the interactions of β-lactoglobulin and sodium alginate in aqueous solutions. β-Lg could interact with sodium alginate and form either soluble or insoluble complexes depending on the solution pH. Insoluble complexes were formed at pH values where the protein and polysaccharide had opposite electrical charges (pH 3 and 4) because of strong electrostatic attraction between the two biopolymers. There was a greater amount of protein bound per unit mass of polysaccharide at pH 4 than at pH 3 because the sodium alginate had a greater negative charge at the higher pH. Fairly soluble complexes were formed at a pH where the polysaccharide was negatively charged, but the protein had little net charge (pH 5), which was attributed to the binding of anionic sodium alginate to cationic patches on β-Lg's surface. No complexes were formed at pH values where the protein and polysaccharide had similar electrical charges (pH 6 and 7) due to strong electrostatic repulsion between the two biopolymers.

In the emulsion system, the stability of β-Lg-stabilized palm oil-in-water emulsions to droplet flocculation and creaming depended strongly on solution pH, sodium alginate concentration and sonication. There was an electrostatic attraction between alginate molecules and β-Lg coated droplets at pH 3, 4 and 5, which caused bridging flocculation and creaming to occur. The flocs formed could be broken down by sonication, provided that the repulsive interactions between the individual droplets after sonication were sufficient to prevent further flocculation. At pH 6 and 7, both biopolymers were negatively charged and so alginate molecules did not adsorb to the surfaces of the β-Lg coated droplets. Nevertheless, the presence of free alginate in the emulsions promoted droplet flocculation and creaming through a depletion mechanism. Secondary emulsions with little droplet aggregation and good creaming stability could be prepared at pH 4 and 5 by adding sodium alginate to β-Lg-stabilized emulsions and then applying high intensity sonication to disrupt any flocs formed.

The effect of ionic strength and mixing condition on formation and stability of emulsions at different pH was investigated. 0.1 wt% corn oil-in-water emulsions containing oil droplets coated by β-Lg were prepared in the absence and presence of sodium alginate (0 or 0.004 wt%). The stability of β-Lg-stabilized emulsions to droplet aggregation around the isoelectric point of the adsorbed protein can be greatly improved by coating the droplets with alginate. In addition, the stability of the protein-stabilized emulsions to droplet aggregation at high salt concentrations can also be improved somewhat by adding the polysaccharide. The results showed that mixing conditions have a major impact on the formation and stability of emulsions containing β-Lg-alginate coated droplets. More stable emulsions can be formed if the droplets and polymer are mixed at a pH where they have the same sign charge, and then the pH is adjusted to a value where they have different charges.

Model beverage emulsions can be produced by engineering their interfacial membranes using the electrostatic interaction of proteins and polysaccharides at oil-water interfaces. These interfacial complexes were formed by electrostatic deposition of anionic polysaccharides onto cationic protein-coated droplets. The electrical characteristics of the interfaces formed appeared to be mainly determined by the electrical charge of the polysaccharides, which was governed by solution pH and polysaccharide type. The secondary emulsions formed were stable to thermal processing (90 °C for 30 minutes), sugar (10 wt% sucrose) and salt (≤ 50 mM NaCl). These results suggest that this interfacial engineering technology could be used by the beverage industry to replace traditional polysaccharide emulsifiers such as gum arabic and modified starch. The main advantages of the protein/polysaccharide complexes over traditional polysaccharide emulsifiers are that they can be used at much lower levels, and that there may be less variation in price and quality in protein than in polysaccharide emulsifiers.

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