Influence of pH and Ionic Strength on Formation and Stability of Emulsions Containing Oil Droplets Coated by β-Lactoglobulin−Alginate Interfaces

Thepkunya Harnsilawat,[†] Rungnaphar Pongsawatmanit,[†] and David J. McClements*[,‡]

Department of Product Development, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand, 10900, and Department of Food Science, University of Massachusetts, Amherst, Massachusetts 01003

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Emulsions of 0.1 wt % corn oil-in-water containing oil droplets coated by β-lactoglobulin (0.009 wt % β-Lg, 5 mM phosphate buffer, pH 7.0) were prepared in the absence and presence of sodium alginate (0 or 0.004 wt %). The pH (3−7) and ionic strength (0−250 mM NaCl) of these emulsions were adjusted, and the particle charge, particle size, and creaming stability were measured. 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.

Introduction

Proteins are used as emulsifiers in a variety of commercially important products, including foods, health care products, pharmaceuticals, and personal care products.[1−6] 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.[7−9] Proteins are particularly attractive as emulsifiers because they are natural, nontoxic, tasteless, and widely available.[7,8]

Droplet flocculation is usually prevented in emulsions stabilized by adsorbed proteins due to the relatively strong electrostatic repulsion between the charged droplets.[1−5] 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 (T_m) exposing reactive nonpolar and sulfhydryl groups.[3] 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.[3] 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.[3] Recently, a novel interfacial engineering technology has been developed to increase the stability of emulsions to environmental stresses.[10−21] 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 multilayered interfacial membranes.[22,23]

Previously, it has been shown that oil droplets coated by multilayered interfacial membranes often have improved stability to environmental stresses than those coated by single-layered membranes because of the alteration in interfacial charge and thickness.[10−21] Although this interfacial engineering technology provides scientists with a powerful method to improve the resistance of emulsions to environmental stresses, it is still essential to select the most appropriate system composition and preparation conditions to create stable multilayer emulsions with the desired physicochemical characteristics.

In this study, we intend to prepare oil-in-water emulsions containing droplets coated by an interfacial membrane comprised of two natural polymers: a globular protein (β-lactoglobulin) and an anionic polysaccharide (sodium alginate). β-Lactoglobulin (β-Lg) is obtained from cow's milk, where it is the major protein in the whey fraction. Structurally, it is a compact globular protein (molecular mass = 18.3 kDa) containing 162 amino acid residues with one thiol group and two disulfide bonds. The isoelectric point of β-Lg has been reported to be around 4.7−5.2.[24−26] Alginates are a group of acidic polysaccharides that occur naturally as the major structural polysaccharides of brown marine algae (*Phaeophyceae*) and as extracellular mucilages secreted by certain species of bacteria.[27] They are a family of unbranched binary copolymers of (1 → 4) linked β-D-mannu-

* To whom correspondence should be addressed. Phone: (413) 545-1019. Fax: (413) 545-1262. E-mail: mcclements@foodsci.umass.edu.
[†] Kasetsart University.
[‡] University of Massachusetts.

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ronic acid and α-L-guluronic acid residues.[28] The dissociation constants for mannuronic acid (M) and guluronic acid (G) monomers are 3.38 and 3.65, respectively.[28,29] The β-Lg will be used as an emulsifier to form an oil-in-water emulsion containing small droplets, then the sodium alginate will be added to produce emulsions containing droplets coated with protein−polysaccharide membranes. The objective of this study is to establish the major factors (pH, ionic strength, and mixing conditions) that influence the adsorption of the polysaccharide molecules onto the protein-coated droplets and to investigate the influence of these factors on emulsion stability. In addition, we intend to compare the findings of this study with those of earlier studies using polysaccharides with different molecular characteristics so that we can distinguish system-specific from more general features of this phenomenon. Ultimately, we would like to understand the influence of droplet characteristics (e.g., concentration, size, net charge, and charge distribution), polyelectrolyte characteristics (e.g., molecular weight, chain flexibility, net charge, and charge distribution), and solution conditions (e.g., ionic strength, pH, temperature, and mixing) on the formation, stability, and properties of multilayer emulsions. This knowledge could be used to design and fabricate emulsions with novel or improved properties in a more rational and systematic fashion.

Experimental Section

Materials. 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 and a molecular mass of 216 kDa. Powdered 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. 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.

Solution Preparation. A stock buffer solution was prepared by dispersing 5 mM phosphate buffer in water containing 0.02 wt % NaN_3 (used 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 the primary emulsion) or 0.1 wt % sodium alginate (to form the 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.

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.

Preparation of Stock Emulsion. A stock emulsion was prepared using the following procedure. 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.

Influence of pH and Ionic Strength. In some experiments, we investigated the influence of solution pH and ionic strength on the stability and properties of emulsions. 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 0−250 mM NaCl. The emulsions were stirred using a 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.

Influence of Mixing Conditions. In some experiments, we investigated the influence of mixing conditions on the stability and properties of secondary emulsions. We prepared these emulsions in two different ways.

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.

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 readjusted to the appropriate values using HCl or NaOH solutions if required. The emulsions were then stored overnight at room temperature before being analyzed.

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, Worcs., U.K.). The emulsions were prepared and stored at room temperature for 24 h prior to analysis.

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−vis 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.[19] 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.

Spectroturbidity 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.[30] 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 to 400 nm measured using a UV−vis 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.

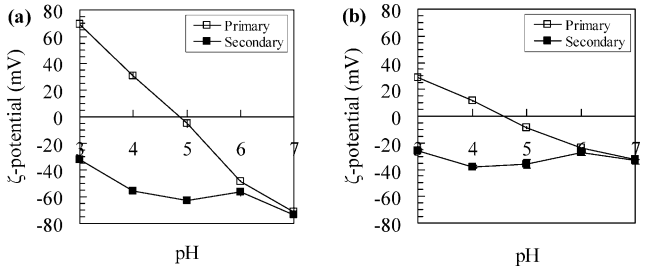

Figure 1. Influence of pH and ionic strength on the electrical charge (ζ-potential) of emulsions in primary and secondary emulsions (0.1 wt % corn oil, 0.009 wt % β-Lg, 0.004 wt % sodium alginate in 5 mM phosphate buffer) after being stored for 24 h: (a) 0 mM NaCl, (b) 100 mM NaCl.

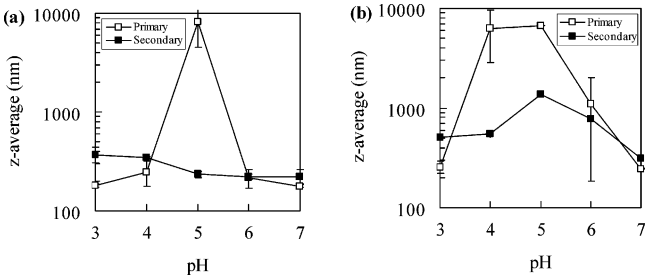

Figure 2. Influence of pH and ionic strength on the particle size of emulsions in primary and secondary emulsions (0.1 wt % corn oil, 0.009 wt % β-Lg, 0.004 wt % sodium alginate in 5 mM phosphate buffer) after being stored for 24 h: (a) 0 mM NaCl, (b) 100 mM NaCl.

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

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 (Figure 1, parts a and b, and Figure 2, parts a and b).

Adsorption of Alginate. Initially, we examined the influence of pH on the adsorption of alginate molecules to the emulsion droplet surfaces. 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 1a), 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 $\approx$ 4.7−5.2).[24−26] On the other hand, the ζ-potential of the droplets in the secondary emulsions was negative at all pH values (Figure 1a). 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-Lg-coated droplets.[13,21] 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 1b), which can be attributed to electrostatic screening effects.[19,31,32] 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 3−5, which suggested that the alginate molecules did not appreciably desorb from the droplet surfaces at this salt concentration.[31]

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 1), 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[16] and ι- and λ-carrageenan[17] onto the surfaces of β-Lg-coated droplets and for the adsorption of LM- and HM-pectin onto the surfaces of caseinate-coated droplets.[33] 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.[34] 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.[34]

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 2), 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 3 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.[30] On the other hand, at pH 5, the turbidity of the emulsion only decreased slightly

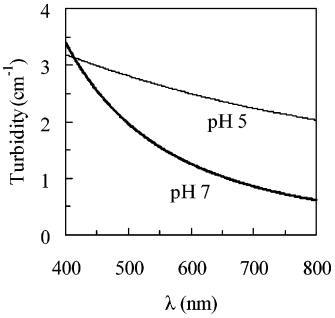

Figure 3. Influence of pH on the turbidity spectra of primary emulsions (0.1 wt % corn oil, 0.009 wt % β-Lg in 5 mM phosphate buffer) after being stored for 24 h.

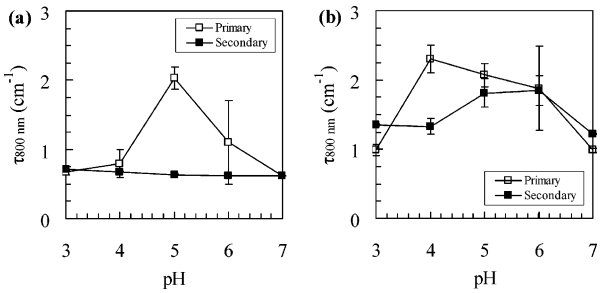

Figure 4. Influence of pH and ionic strength on the turbidity of emulsions in primary and secondary emulsions (0.1 wt % corn oil, 0.009 wt % β-Lg, 0.004 wt % sodium alginate in 5 mM phosphate buffer) after being stored for 24 h: (a) 0 mM NaCl, (b) 100 mM NaCl.

with increasing wavelength, which is indicative of a system containing large particles.[30] 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 4).

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 2a and 4a). 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 1a). 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.[3,32] 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 2a and 4a). 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 1a) so that the electrostatic repulsion between the droplets would be sufficient to overcome the attractive droplet−droplet interactions.[3,35] 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.[3]

In the presence of 100 mM NaCl, the primary emulsions were unstable to droplet aggregation over a wider range of pH values (pH 4−6) than in the absence of NaCl (Figures 2b and 4b). The most likely reason for this effect is that as the ionic strength

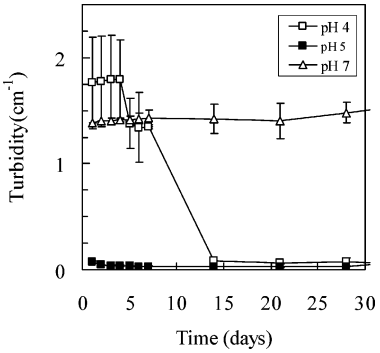

Figure 5. Influence of pH on the creaming stability of emulsions in primary emulsions (0.1 wt % corn oil, 0.009 wt % β-Lg in 5 mM phosphate buffer) after being stored for 28 days measured at 600 nm.

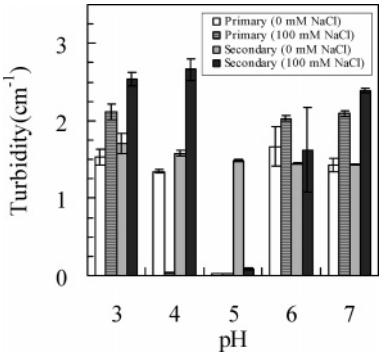

Figure 6. Influence of pH and ionic strength on the creaming stability of emulsions in primary and secondary emulsions (0.1 wt % corn oil, 0.009 wt % β-Lg, 0.004 wt % sodium alginate in 5 mM phosphate buffer) after being stored for 7 days.

increased, the electrostatic repulsion between the droplets was progressively screened by the counterions (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.

We now compare the results of this study with those obtained in earlier studies on the influence of anionic polysaccharides on the stability of protein-coated droplets. 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 2). Similar observations have been made for other systems consisting of protein-coated droplets to which anionic polysaccharides have been added, e.g., HM-pectin[16] and carrageenan[17] to β-Lg-coated droplets and LM- and HM-pectin onto caseinate-coated droplets.[33] 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.

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 5 and 6). The principle of this method is given in Figure 5, 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

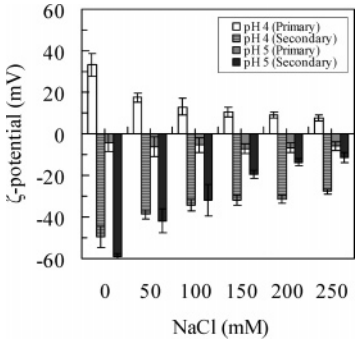

Figure 7. Influence of NaCl on the electrical charge (ζ-potential) of emulsion droplets in primary and secondary emulsions (0.1 wt % corn oil, 0.009 wt % β-Lg, 0.004 wt % sodium alginate in 5 mM phosphate buffer) after being stored for 24 h.

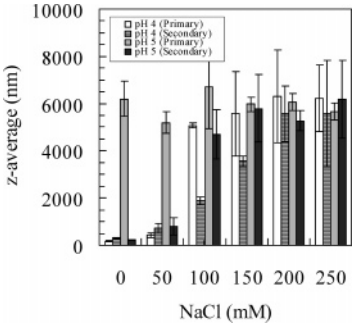

Figure 8. Influence of NaCl on the particle size of emulsion droplets in primary and secondary emulsions (0.1 wt % corn oil, 0.009 wt % β-Lg, 0.004 wt % sodium alginate in 5 mM phosphate buffer) after being stored for 24 h.

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. We used the turbidity measurements made after 7 days of storage to compare the influence of pH on the creaming stability of the emulsions: a high turbidity after 7 days of storage indicated good stability to creaming (Figure 6).

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 2a). 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 2a). The results from the creaming stability measurements (Figure 6) therefore largely supported those from the droplet aggregation measurements (Figures 2 and 4), which should be expected since droplet aggregation leads to accelerated creaming in dilute emulsions.[3]

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.[14,16,20,21] 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 2). In addition, we hypothesized that 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 (0−250 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 7−9).

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 7). 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.[3,8,31] 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.[31]

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.[24−26,32] 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 7). 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.[19]

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 8 and 9). The secondary emulsions seemed to be somewhat more

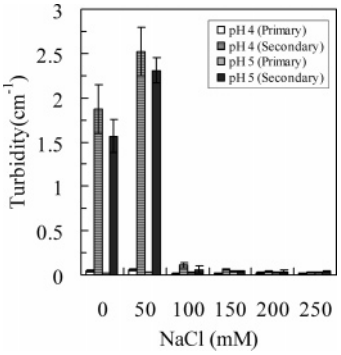

Figure 9. Influence of NaCl on the creaming stability of emulsion droplets in primary and secondary emulsions (0.1 wt % corn oil, 0.009 wt % β-Lg, 0.004 wt % sodium alginate in 5 mM phosphate buffer) after being stored for 7 days.

stable to droplet aggregation than the primary emulsions at 100 mM NaCl (Figure 8), but they were still unstable to creaming (Figure 9). 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.

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.[12] The range of emulsion stability to NaCl-induced flocculation was therefore appreciably wider for carrageenan−β-Lg at pH 6 (<500 mM)[12] than for alginate−β-Lg at pH 4 or 5 (<100 mM, Figures 8 and 9). This 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.

Influence of Mixing Method 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.[16] 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, we investigated the effect of mixing method on the properties of secondary emulsions prepared 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 10 and 11).

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

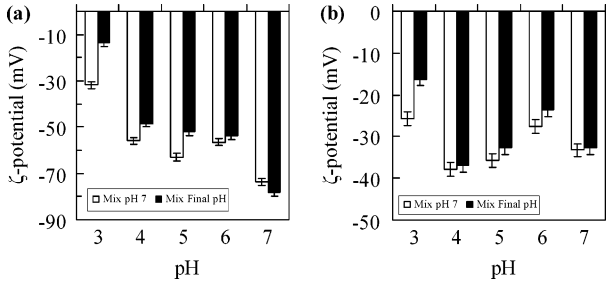

Figure 10. Influence of pH and mixing pH on the electrical charge (ζ-potential) of emulsion droplets in primary and secondary emulsions (0.1 wt % corn oil, 0.009 wt % β-Lg, 0.004 wt % sodium alginate in 5 mM phosphate buffer) after being stored for 24 h: (a) 0 mM NaCl, (b) 100 mM NaCl.

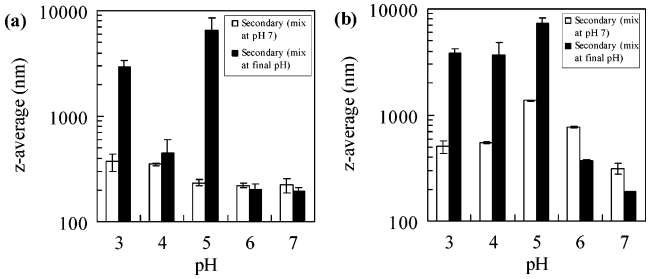

Figure 11. Influence of pH and mixing pH on the particle size of emulsion droplets in primary and secondary emulsions (0.1 wt % corn oil, 0.009 wt % β-Lg, 0.004 wt % sodium alginate in 5 mM phosphate buffer) after being stored for 24 h: (a) 0 mM NaCl, (b) 100 mM NaCl.

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 3−5), there was considerably more droplet aggregation in the secondary emulsions prepared by the one-step method than the two-step method (Figure 11). We propose that 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.[16]

Conclusions

The objective of this study was to examine the influence of preparation conditions on the formation and properties of secondary emulsions containing droplets coated by β-Lg−alginate interfaces. We have shown that 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 polysac-

charide. Finally, we have shown 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. Comparison of the results from this study with those from earlier studies indicates that various types of anionic polysaccharides (e.g., alginate, carrageenan, and pectin) will adsorb to the surfaces of protein-coated droplets (e.g., β-Lg- or β-casein-coated) at a pH where both the protein and polysaccharide have similar net negative charges and that this adsorption can prevent droplet flocculation around the isoelectric point of the adsorbed proteins. On the other hand, it appears that the salt stability of the polysaccharide−protein-coated droplets does depend strongly on polysaccharide type, with carrageenan being more effective than alginate at preventing NaCl-induced flocculation. It is clear that further research is needed to identify the precise role of the molecular characteristics of the proteins and polysaccharides on the formation and stability of multilayer emulsions.

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