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Production and Characterization of O/W Emulsions Containing Cationic Droplets Stabilized by Lecithin−Chitosan Membranes

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Oil-in-water emulsions containing cationic droplets stabilized by lecithin−chitosan membranes were produced using a two-stage process. A primary emulsion was prepared by homogenizing 5 wt % corn oil with 95 wt % aqueous solution (1 wt % lecithin, 100 mM acetic acid, pH 3.0) using a high-pressure valve homogenizer. This emulsion was diluted with aqueous chitosan solutions to form secondary emulsions with varying compositions: 1 wt % corn oil, 0.2 wt % lecithin, 100 mM acetic acid, and 0−0.04 wt % chitosan (pH 3.0). The particle size distribution, particle charge, and creaming stability of the primary and secondary emulsions were measured. The electrical charge on the droplets increased from −49 to +54 mV as the chitosan concentration was increased from 0 to 0.04 wt %, which indicated that chitosan adsorbed to the droplet surfaces. The mean particle diameter of the emulsions increased dramatically and the emulsions became unstable to creaming when the chitosan concentration exceeded 0.008 wt %, which was attributed to charge neutralization and bridging flocculation effects. Sonication, blending, or homogenization could be used to disrupt flocs formed in secondary emulsions containing droplets with high positive charges, leading to the production of emulsions with relatively small particle diameters (~1 μm). These emulsions had good stability to droplet aggregation at low pH (≤5) and ionic strengths (<500 mM). The interfacial engineering technology utilized in this study could lead to the creation of food emulsions with improved stability to environmental stresses.

KEYWORDS: Emulsion; chitosan; lecithin; stability; ζ-potential

INTRODUCTION

Oil-in-water emulsions that consist of small lipid droplets dispersed in an aqueous medium form the basis of many kinds of foods, e.g., milk, cream, beverages, dressings, dips, sauces, batters, and desserts (*1*−*3*). Ideally, manufacturers of food emulsions want to economically produce a high-quality product with consistent sensory and physiochemical properties and a sufficiently long shelf life. Unfortunately, emulsions are thermodynamically unstable systems because of the unfavorable contact between oil and water phases and because the oil and water phases have different densities; hence, they will always breakdown over time (*2*−*6*). Emulsion destabilization may occur through a variety of different physiochemical processes, including gravitational separation, flocculation, coalescence, and Ostwald ripening (*2*). For a particular emulsion-based product, the relative importance of these processes depends on the type of ingredients it contains, the way it was produced, and the environmental conditions it experiences during its manufacture, storage, and utilization.

One of the most important and widely used methods of improving the stability of oil-in-water emulsions is to utilize emulsifiers (*3*, *4*). Emulsifiers are surface active ingredients that adsorb to the surface of freshly formed lipid droplets during homogenization (*2*, *7*). Once adsorbed, they facilitate further droplet disruption by lowering the interfacial tension, thereby reducing the size of the droplets produced during homogenization. Emulsifiers also reduce the tendency for droplets to aggregate by forming protective membranes and/or by generating repulsive forces between the droplets. A good emulsifier should rapidly adsorb to the surface of the lipid droplets formed during homogenization, rapidly lower the interfacial tension by a significant amount, and protect the droplets against aggregation during emulsion processing, storage, and utilization (*2*, *4*, *7*). A wide variety of different kinds of synthetic and natural emulsifiers can be legally used in food emulsions, including small-molecule surfactants, phospholipids, proteins, and polysaccharides (*3*, *8*, *9*). Emulsifiers vary considerably in their ability to form and stabilize emulsions, as well as in their cost, ease of utilization, ingredient compatibility, and environmental sensitivity. Consequently, there is not a single emulsifier that is ideal for use in every food product. Instead, the development of each new food product depends on the rational selection of the most appropriate emulsifier for that particular system. This selection depends on the composition and structure of the food matrix, as well as on the changes in environmental conditions that the emulsifier experiences during processing, storage, and utilization, such as mechanical agitation, temperature, and pressure. At present there are few natural emulsifiers that can be used in

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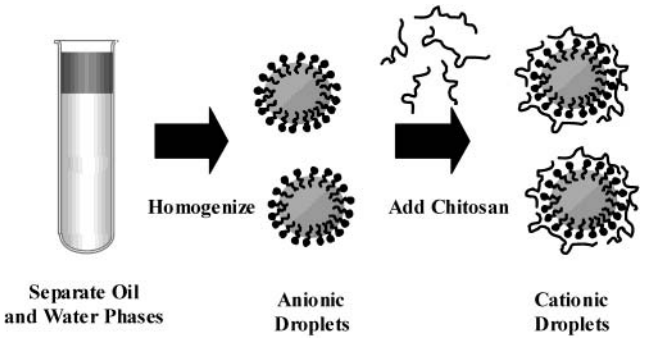

Figure 1. Two-stage mechanism for producing emulsion droplets coated by a two-layer interfacial membrane. First, a *primary* emulsion containing small droplets coated with an emulsifier membrane is formed by homogenizing oil, water, and lecithin together. Second, a *secondary* emulsion is formed by mixing the primary emulsion with a chitosan solution to form droplets that are coated with a lecithin–chitosan membrane.

foods that are capable of providing good emulsion stability to freeze−thaw cycling, thermal processing, and high mineral contents.

Each type of emulsifier has its own particular advantages and disadvantages. For example, some emulsifiers are highly effective at generating small emulsion droplets during homogenization because of their rapid adsorption rates, but are poor at providing long-term stability against droplet aggregation because they do not provide strong enough droplet−droplet repulsive interactions, e.g., some small molecule surfactants (*2*, *10*). On the other hand, some emulsifiers are highly effective at imparting long-term stability to emulsions, but are inefficient at creating emulsions with small droplet sizes during homogenization, e.g., some polysaccharides and proteins (*2*, *10*).

In this study, we intend to utilize a technology that will enable us to combine the beneficial attributes of different kinds of emulsifiers to create emulsions with improved stability (*11*−*13*). An anionic emulsifier (lecithin) that rapidly adsorbs to the surface of lipid droplets during homogenization will be used to produce a *primary* emulsion with small droplet sizes, then a cationic biopolymer (chitosan) will be added to the system to produce *secondary* emulsions containing droplets coated with an emulsifier−biopolymer membrane (**Figure 1**). The cationic biopolymer adsorbs to the surface of the anionic droplets due to electrostatic attraction. Under certain environmental conditions (lecithin-to-chitosan ratio, pH, ionic strength), the emulsions become unstable to flocculation due to charge neutralization and bridging flocculation (*14*−*17*). However, when sufficient chitosan is added to the emulsions, the net droplet charge switches from negative to positive, and kinetically stable emulsions can be produced (*13*). The production of cationic droplets has a number of important potential advantages for many applications in the food industry. For example, positively charged droplets are much less susceptible to destabilization by multivalent cations, such as calcium and iron (*18*, *19*). In addition, the lipids in positively charged droplets are much less susceptible to iron-catalyzed oxidation because of the electrostatic repulsion between the droplet surface and the iron (*20*, *21*). Finally, cationic droplets coated with lecithin−chitosan membranes have also been shown to have better stability against flocculation and coalescence than droplets coated with lecithin alone (*11*−*13*). The objective of this study is to identify a cost-effective process that utilizes food ingredients for producing stable emulsions containing cationic droplets and to test the influence of solution conditions (pH and ionic strength) on emulsion stability.

Chitosan was selected as a potential stabilizer of food emulsions because of its unique functional attributes, natural abundance, and underutilization (*22*−*25*). Chitosan is the partially deacetylated form of chitin and has a chemical structure that consists of 2-acetamido-2-deoxy-β-D-glucose monomers attached via a β1−4 linkage. Chitosan has a positive charge in acidic solutions due to the presence of protonated amino groups along its backbone that have pK_a values between 6.3 and 7.0. The functional attributes of chitosan depend on its molecular weight and degree of deacetylation, which can be controlled during the manufacturing process (*26*, *27*). Chitosan recently received "generally recognized as safe" (GRAS) status within the United States for general application in foods and beverages (FDA, 2001). The fact that chitosan can now be legally incorporated into food products means that novel chitosan-based technologies developed in other industries can be applied to foods.

MATERIALS AND METHODS

Materials. Powdered chitosan (medium molecular weight; deacetylation, 81%; viscosity of 1 wt % solution in 1 wt % acetic acid, 286 Cps; moisture 4.6 wt %; ash, 0.5 wt %) was obtained from Aldrich Chemical Co. (St. Louis, MO). Powdered lecithin (Ultralec P; acetone insolubles, 97.5%; acid value, 27.9 mg/g; peroxide value, 0.9 meqiuv/kg; moisture, 0.77 wt %) was donated by ADM-Lecithin (Decatur, IL). Analytical grade sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium azide (NaN_3) were purchased from the Sigma Chemical Co. (St. Louis, MO). Distilled and deionized water was used for the preparation of all solutions.

Solution Preparation. A stock buffer solution was prepared by dispersing 100 mM acetic acid in water (containing 0.02 wt % sodium azide as an antimicrobial agent) and then adjusting the pH to 3.0 using 1 M HCl. A chitosan solution was prepared by dispersing 0.2 wt % powdered chitosan into stock buffer solution. An emulsifier solution was prepared by dispersing 1.0 wt % lecithin powder into buffer solution. The emulsifier solution was sonicated for 30 s at a frequency of 20 kHz, amplitude of 40%, and duty cycle of 0.5 s (Model 500, sonic disembrator, Fisher Scientific, Pittsburgh, PA) to disperse the lecithin. The pH of the solution was adjusted back to 3.0 using HCl, and then the solution was stirred for about 1 h to ensure complete dissolution of the lecithin.

Emulsion Preparation. A primary emulsion was prepared by homogenizing 5 wt % corn oil with 95 wt % aqueous emulsifier solution in a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland) followed by one pass at 5000 psi through a two-stage high-pressure valve homogenizer (LAB 1000, APV-Gaulin, Wilmington, MA). This emulsion was diluted with aqueous chitosan solutions to form secondary emulsions with varying compositions: 1 wt % corn oil, 0.2 wt % lecithin, 100 mM acetic acid, and 0−0.04 wt % chitosan (pH 3.0). These "concentrated" emulsions (i.e. emulsions that could not be analyzed by light scattering without dilution) were stored at room temperature before being analyzed. In some experiments, for direct particle size and ζ-potential analysis, we used "diluted" emulsions that were prepared by diluting the concentrated emulsions to a final droplet concentration of 0.005 wt % using aqueous solutions of varying pH (3−8) and NaCl concentration (0−1000 mM). When there was any change in the pH of the aqueous solutions upon introduction of the emulsion droplets, we adjusted the pH back to the required value using HCl or NaOH solutions.

Particle Size Measurements. Concentrated emulsions were diluted to a droplet concentration of approximately 0.005 wt % using buffer solution (pH 3) to avoid multiple scattering effects prior to analysis, whereas diluted emulsions were analyzed directly. The particle size distribution of the emulsions was then measured using a laser light scattering instrument (Horiba LA-900, Irvine, CA). This instrument measures the angular dependence of the intensity of laser light (λ = 632.8 nm) scattered by a dilute emulsion and then finds the particle size distribution that gives the best agreement between theoretical

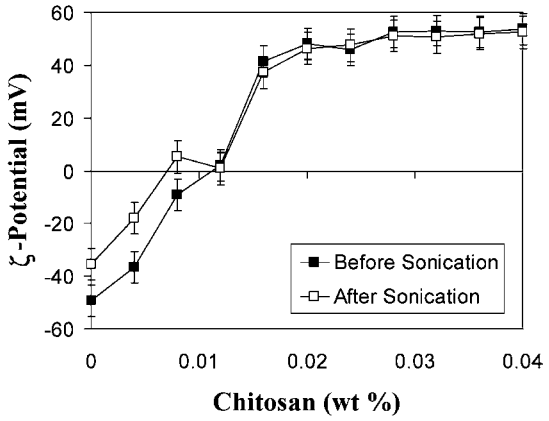

Figure 2. Dependence of electrical charge on emulsion droplets (ξ-potential) on chitosan concentration for secondary emulsions (1 wt % corn oil, 0.2 wt % lecithin, 100 mM acetic acid, pH 3.0) after dilution with buffer solution.

predictions and experimental measurements. A refractive index ratio of 1.08 was used in the calculations of the particle size distribution. Particle size measurements are reported as weight-average mean diameters, d_{43} ($=\sum n_i d_i^4/\sum n_i d_i^3$, where n_i is the number of particles with diameter d_i). Mean particle diameters were calculated as the average of measurements made on at least two freshly prepared samples, with standard deviations being less than 10%.

ζ-Potential Measurements. Concentrated emulsions were diluted to a droplet concentration of approximately 0.005 wt % using buffer solution (pH 3) prior to analysis, whereas diluted emulsions were analyzed directly. Emulsions were then injected into the measurement chamber of a particle electrophoresis instrument (ZEM5003, Zetamaster, Malvern Instruments, Worcs., UK), and the ζ-potential was determined by measuring the direction and velocity of the droplets in the applied electric field. The ζ-potential measurements are reported as the average and standard deviation of measurements made on at least two freshly prepared samples, with 10 instrument readings taken per sample.

Creaming Stability Measurements. Approximately 3.5 g samples of diluted emulsion (0.005 wt % oil) were transferred into 1-cm 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 an UV−visible spectrophotometer (Spectronic 21D, Milton Roy, Rochester, NY). The light beam passed through the emulsions at a height that was about 10 mm from the cuvette bottom, i.e., about 30% 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. An appreciable decrease in emulsion turbidity was therefore an indication of the fact that the serum layer had risen to at least 30% of the emulsion's height. Creaming stability measurements were carried out on two separate freshly prepared samples.

RESULTS AND DISCUSSIONS

Influence of Chitosan Concentration on Droplet Characteristics. The electrical charge and mean particle diameter of secondary emulsions (1 wt % corn oil, 0.2 wt % lecithin, 100 mM acetic acid, pH 3.0) containing different chitosan concentrations (0 to 0.04 wt %) was measured after dilution (**Figures 2** and **3**). In the absence of chitosan, the electrical charge on the emulsion droplets was around −49 mV, because the lecithin emulsifier used to stabilize the droplets has a net negative charge at this pH. The electrical charge on the droplets became less negative and eventually changed from negative to positive, as the chitosan concentration in the emulsions was increased (**Figure 2**). This change suggests that cationic chitosan molecules adsorbed to the surface of the anionic lecithin-coated emulsion droplets. There was no net charge on the droplets when the chitosan concentration was around 0.01 wt %, indicating that droplet charge neutralization occurred at a mass ratio (R) of about 0.05 g of chitosan per gram of lecithin. When the chitosan concentration was increased further, the positive charge on the droplets continued to increase, until it reached a constant value when the chitosan concentration exceeded about 0.02 wt % ($R = 0.1$ g/g) (**Figure 2**). The ability of charged polyelectrolytes to adsorb to the surface of oppositely charged colloidal particles and cause charge reversal ("overcharging") is well-established in the literature (*28*−*33*). Overcharging occurs because only a fraction of the charged groups on a polymer are required to neutralize the oppositely charged groups on the surface of a colloidal particle. The remainder of the charged polymer groups may protrude into the aqueous solution or may be in contact with uncharged regions on the particle surface (*28*−*33*).

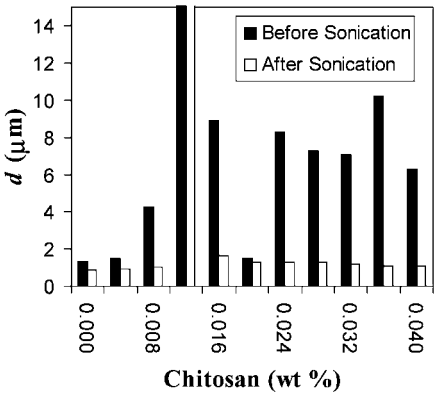

Figure 3. Dependence of mean particle diameter on chitosan concentration for secondary emulsions (1 wt % corn oil, 0.2 wt % lecithin, 100 mM acetic acid, pH 3.0) after dilution with buffer solution.

The mean particle diameter (**Figure 3**) of secondary emulsions was measured 24 h after chitosan was mixed with the primary emulsions. At the lowest chitosan concentrations (≤0.004 wt %), there was no evidence of droplet aggregation, suggesting that the negative charge on the emulsion droplets was still sufficient to prevent droplet flocculation through electrostatic repulsion. At chitosan concentrations >0.004 wt % there was a large increase in mean particle diameter, which was attributed to extensive droplet aggregation. The maximum amount of droplet aggregation occurred at chitosan concentrations around 0.012 wt %, which corresponded to the emulsions where charge neutralization of the droplets occurred (**Figure 2**). Extensive droplet aggregation was even observed in emulsions that contained droplets with relatively high positive charges, i.e., chitosan concentrations >0.02 wt %. One might have expected that these systems would have been stable to droplet aggregation, because of relatively strong electrostatic repulsive interactions between the droplets (*2*). Observation of the emulsions by optical microscopy indicated that the droplets were highly flocculated (data not shown). It is likely that the droplets were held together by chitosan molecules that were adsorbed to the surface of more than one droplet (*14*−*17*). This is not surprising, since charged polymers are known to induce bridging flocculation of oppositely charged colloidal particles (*1*, *4*, *33*). During the initial stages of mixing of the chitosan solution with the primary emulsion, there would have been many droplets present with completely negative surfaces; hence, it is likely that chitosan molecules could adsorb to the surface of two or more of these droplets simultaneously.

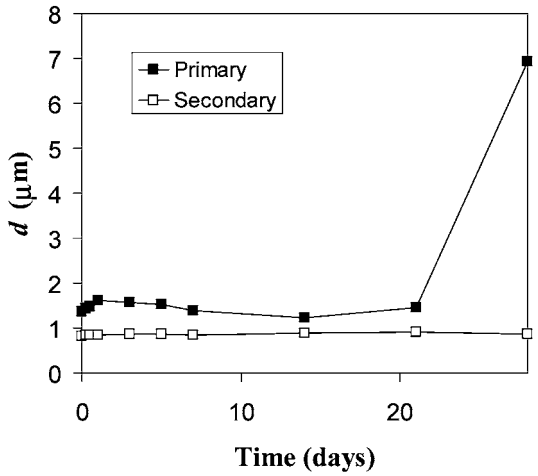

Figure 4. Influence of storage time on the stability of primary ($R = 0$) and secondary ($R = 0.18$) emulsions (1 wt % corn oil, 0.2 wt % lecithin, 100 mM acetic acid, pH 3.0).

To create emulsions with improved stability to droplet aggregation, we decided to determine whether sonication could be used to disrupt the flocs. Small samples of emulsions (~2 g) were sonicated for 75 s at a frequency of 20 kHz, an amplitude of 20%, and a duty cycle of 0.5 s (Model 500, Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA), and then the mean ζ-potential and particle diameter of the droplets were measured (**Figures 2** and **3**). Sonication caused a slight decrease in the negative charge on the emulsion droplets at relatively low chitosan concentrations (<0.008 wt %) but had little effect at higher concentrations. On the other hand, sonication caused a pronounced decrease in the mean particle diameter of all the emulsions (**Figure 3**), which suggests that it was capable of generating forces sufficiently large to either physically cleave chitosan molecules or to cause them to become detached from all but one of the droplets. Despite the application of sonication, emulsions containing droplets with low net charges still exhibited extensive aggregation (0.012 wt % chitosan), presumably because the electrostatic repulsion between the droplets was insufficient to prevent flocculation. At relatively high chitosan concentrations (≥0.02 wt %), where the droplets had relatively high positive charges (>+40 mV), the measured mean particle diameters were similar to those of the primary emulsion. In these systems, the electrical charge on the droplets must have been large enough to prevent them from coming back into close contact after the flocs had been disrupted by sonication. These experiments showed that emulsions with nonaggregated droplets could be produced by applying sonication to secondary emulsions containing sufficiently high levels of chitosan.

The long-term stability of primary ($R = 0$; 0 wt % chitosan) and secondary ($R = 0.18$; 0.036 wt % chitosan) emulsions (1 wt % corn oil, 0.2 wt % lecithin, 100 mM acetic acid, pH 3.0) to droplet aggregation was compared by measuring the change in their mean particle size with time (**Figure 4**). The flocs in the secondary emulsion were disrupted by application of sonication prior to storage. The primary emulsion exhibited extensive droplet aggregation somewhere between 22 and 28 days, whereas the secondary emulsions remained stable for over 3 months (later data not shown). Hence, emulsion stability could be significantly improved by coating the primary emulsion droplets with chitosan.

Influence of Mechanical Agitation on Particle Disruption. From a practical standpoint, it may not be feasible to use high-intensity sonication for commercial preparation of chitosan−lecithin-coated emulsion droplets. We therefore examined the effectiveness of a high-speed blender and a high-pressure valve homogenizer at breaking down flocs in secondary emulsions and compared their effectiveness with that of a sonicator. These experiments were carried out using secondary emulsions consisting of 1 wt % corn oil, 0.2 wt % lecithin, 0.036 wt % chitosan, and 100 mM acetic acid (pH 3.0). This chitosan concentration was chosen because it is sufficiently high to produce strongly positively charged droplets (**Figure 2**). All three devices were able to reduce the mean particle diameter to values similar to or less than those of the untreated primary emulsion, i.e., 1.2 μm (Table 1). For the sonicator, the mean particle diameter decreased with increasing sonication time, with the majority of flocs being effectively disrupted when the samples were sonicated for ≥1 min. For the high-speed blender, the mean particle diameter decreased with increasing mixing time, with the majority of flocs being disrupted when the emulsions were blended for ≥2 min. For the high-pressure valve homogenizer, the mean particle diameter decreased with increasing homogenization pressure, with the majority of flocs being disrupted when the emulsions were homogenized at pressures ≥1000 Pa. It should be noted that the homogenization pressures we used to disrupt the aggregates were below those required to create the primary emulsion (5000 psi), so that little disruption of individual droplets would have been expected. Nevertheless, we did see some decrease in the particle size of the secondary emulsions at the higher pressures, which suggested that either some individual droplets were broken down or that some flocs were disrupted. These experiments showed that simple blending or homogenization of the secondary emulsion was sufficient to produce nonaggregated chitosan−lecithin-coated droplets.

Table 1. Influence of Mechanical Agitation Conditions on Mean Particle Diameter of Emulsions Consisting of 1 wt % Corn Oil, 0.2 wt % Lecithin, 100 mM Acetic Acid (pH 3.0), and 0 wt % Chitosan (primary) or 0.036 wt % Chitosan (secondary)

	mean particle diameter (μm)	
mechanical agitation	primary emulsion	secondary emulsion
sonication (40% power)		
0	1.23	12.4
30 s	1.15	1.85
1 min	1.12	1.31
2 min	1.06	1.15
3 min	1.02	1.07
blending		
1 min	1.31	1.68
2 min	1.26	1.34
4 min	1.15	1.20
8 min	1.10	1.06
high-pressure homogenization		
500 psi	1.23	1.32
1000 psi	0.76	1.02
2000 psi	0.65	0.76
4000 psi	0.26	0.59

It should be noted that there was a significant decrease in the mean particle size of the primary emulsions with increasing sonication and blending times and an appreciable decrease in the mean particle size of the primary emulsions with increasing homogenization pressure, which suggests that the mechanical forces applied to these emulsions were sufficient to disrupt the lecithin-stabilized droplets.

Influence of Solution Conditions on Emulsion Stability. The purpose of these experiments was to examine the influence of pH and ionic strength on the stability of both lecithin (primary

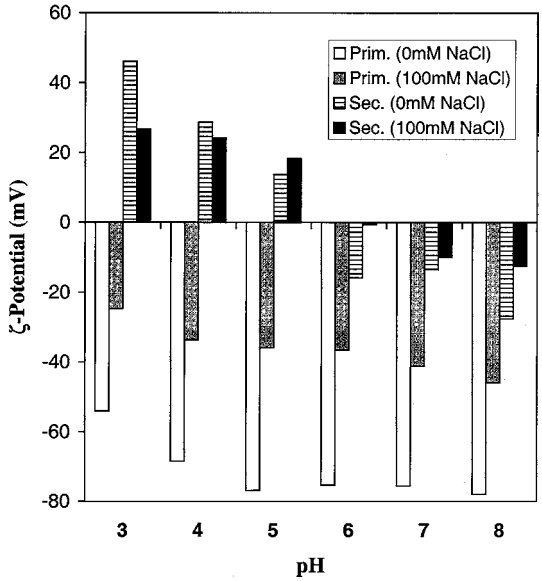

Figure 5. Influence of pH and ionic strength on electrical charge of emulsion droplets (ξ-potential) in diluted primary ($R = 0$) and secondary ($R = 0.18$) emulsions.

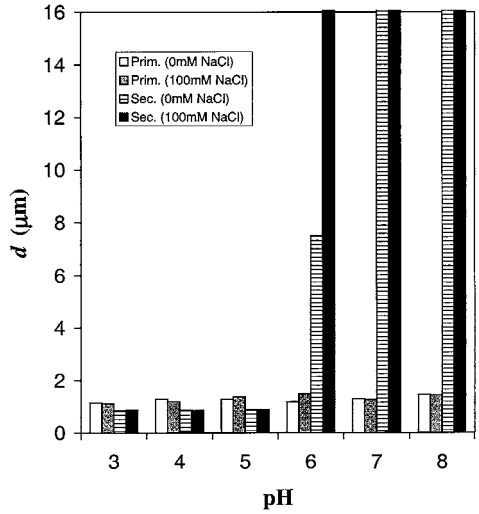

Figure 6. Influence of pH and ionic strength on mean diameter of particles in diluted primary ($R = 0$) and secondary ($R = 0.18$) emulsions.

emulsion) and chitosan−lecithin (secondary emulsion) coated droplets. A secondary emulsion was prepared containing highly positively charged droplets using the procedure described above: 1 wt % corn oil, 0.2 wt % lecithin, 0.036 wt % chitosan, and 100 mM acetic acid (pH 3.0). The flocs in this emulsion were disrupted by passing the emulsion once through a high-pressure valve homogenizer at a pressure of 4000 psi. A series of dilute emulsions was then prepared by dispersing the nonflocculated secondary emulsion (to a final droplet concentration of 0.005 wt %) in a variety of solutions with different pH (3−8) and ionic strength (0−1000 mM NaCl). The emulsions were then stored at room temperature and the mean particle diameter, electrical charge, and creaming stability were measured periodically. The properties of secondary emulsions were compared with those of primary emulsions prepared under the same solution conditions.

The influence of pH and NaCl concentration (0 or 100 mM) on the ζ-potential, mean particle diameter, and creaming stability of primary and secondary emulsions was measured during storage at 30 °C for 1 week (**Figures 5**−**7**). The ζ-potential of the lecithin-stabilized droplets in the primary emulsions was negative at all pH values (**Figure 5**). The magnitude of the ζ-potential decreased when the pH was decreased from 8 to 3 and when the NaCl concentration was increased from 0 to 100 mM. The droplets became less negatively charged when the pH was decreased, presumably because a smaller fraction of the anionic groups on the lecithin molecules were charged at lower pH, since the pK_a value of the anionic phosphate groups on lecithin are typically around pH 1.5 (*34*). The ζ-potential of the droplets in the primary emulsion decreased with increasing ionic strength due to electrostatic screening effects (*35*). The ζ-potential of the chitosan−lecithin-stabilized droplets in the secondary emulsions was positive at low pH values (<pH 6) but became negative at higher values (**Figure 5**). The reversal of charge on the emulsion droplets suggests that the chitosan was desorbed from the emulsion droplet surfaces when the pH was increased. The cationic groups on chitosan typically have pK_a values around pH 6.3−7; hence, the chitosan loses its charge around this pH (*22*). Consequently, there is no longer an electrostatic attraction between the lecithin and the chitosan, leading to a release of some or all of the adsorbed cationic biopolymer. The fact that the ζ-potential of the particles was less negative in the secondary emulsions than in the primary emulsions at the same high pH values suggests that some of the chitosan remained adsorbed to the droplet surfaces. At low and high pH values, the presence of 100 mM NaCl decreased the magnitude of the ζ-potential of the chitosan−lecithin-stabilized droplets, presumably through electrostatic screening (*35*). At pH 5, the positive charge on the secondary emulsion droplets was actually higher in the presence of salt than in its absence, which cannot be explained by electrostatic screening effects.

The particle size measurements indicated that the primary emulsions were stable to droplet aggregation from pH 3 to 8 at both 0 and 100 mM NaCl concentrations (**Figure 6**). The turbidity measurements indicated that the primary emulsions containing 0 mM NaCl were stable to creaming at all pH values (**Figure 7a**), but the ones containing 100 mM NaCl showed some instability to creaming (**Figure 7b**). The secondary emulsions were stable to droplet aggregation from pH 3 to 5, but highly unstable at higher pH values, as deduced from the large increase in mean particle diameter (**Figure 6**) and rapid decrease in creaming stability (**Figure 7c**) observed in these emulsions. The instability of the secondary emulsions at higher pH values was probably because the decrease in droplet charge (**Figure 5**) reduced the electrostatic repulsion between the droplets, thus leading to extensive droplet flocculation. The secondary emulsions containing 100 mM NaCl were slightly less stable to creaming than the ones containing 0 mM NaCl (**Figure 7d**).

The influence of NaCl concentration (0 or 1000 mM) on the ζ-potential, mean particle diameter, and creaming stability of diluted primary and secondary emulsions at pH 3 was measured during storage at 30 °C for 1 week (**Figures 8 and 9**). The ζ-potential of the lecithin-stabilized droplets in the primary emulsions was negative at all ionic strengths, but the magnitude of the ζ-potential decreased as the NaCl concentration was increased (**Figure 8**), which can be attributed to electrostatic screening (*35*). Interestingly, the ζ-potential of the chitosan-lecithin stabilized droplets in the secondary emulsions was positive at low ionic strengths, but became negative somewhere between 500 and 1000 mM NaCl (**Figure 8**). The reversal of charge on the emulsion droplets suggested that chitosan was desorbed from the emulsion droplet surfaces when the ionic

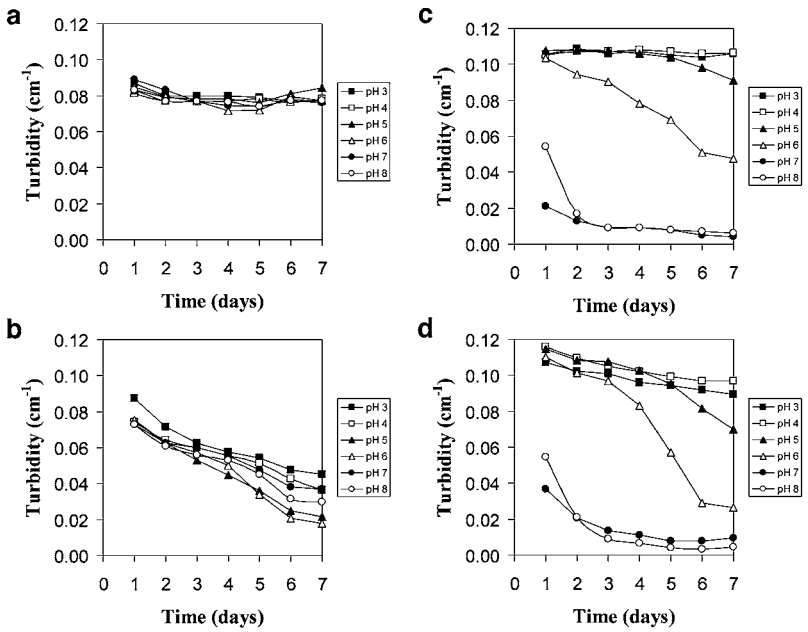

Figure 7. Influence of pH and ionic strength on creaming stability of diluted primary ($R = 0$) and secondary ($R = 0.18$) emulsions. [a] Primary emulsion at 0 mM NaCl. [b] Primary emulsion at 100 mM NaCl. [c] Secondary emulsion at 0 mM NaCl. [d] Secondary emulsion at 100 mM NaCl.

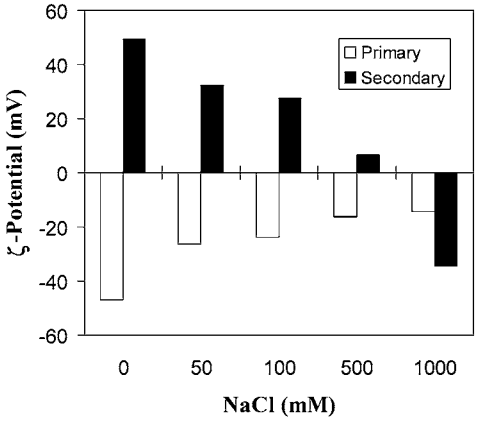

Figure 8. Influence of ionic strength on electrical charge of emulsion droplets (ξ-potential) in diluted primary ($R = 0$) and secondary ($R = 0.18$) emulsions at pH 3.0.

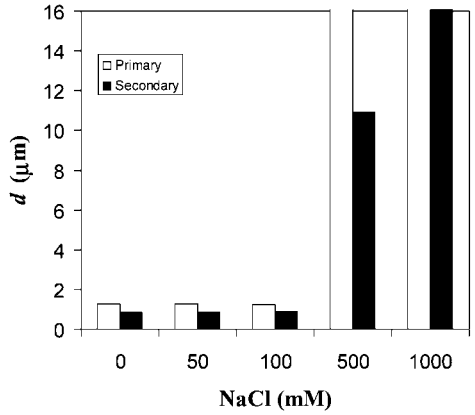

Figure 9. Influence of ionic strength on mean diameter of particles in diluted primary ($R = 0$) and secondary ($R = 0.18$) emulsions at pH 3.0.

strength was increased. The most likely origin of this effect is that the electrostatic attraction between the negatively charge lecithin molecules and the positively charged chitosan molecules was weakened at higher ionic strengths, due to electrostatic screening, thus allowing desorption of the cationic biopolymer.

The primary and secondary emulsions were relatively stable to droplet aggregation at low ionic strengths (<500 mM) but became strongly aggregated at higher ionic strengths (**Figure 9**). In the case of the primary emulsion, droplet aggregation was probably primarily caused by the reduction of repulsive interactions between the droplets due to electrostatic screening (*35*) but may also have been due to the ability of salt to change the optimum curvature of the interfacial membrane (*34*). In the case of the secondary emulsions, droplet aggregation was probably the result of a reduction in the electrostatic repulsion between the droplets caused by electrostatic screening and chitosan desorption from the droplet surfaces.

CONCLUSIONS

This study has shown that emulsions containing cationic lipid droplets can be prepared using a simple cost-effective method. Initially, a primary emulsion with small droplet sizes was produced by homogenization of oil, water, and a low-cost food-grade anionic emulsifier (lecithin). A secondary emulsion containing cationic lipid droplets coated with a lecithin−biopolymer membrane was then produced by mixing an edible cationic biopolymer (chitosan) with the primary emulsion and applying sonication, blending, or homogenization to disrupt any flocs formed. The secondary emulsions have good long-term stability at low pH (3−5) and low ionic strength (<500 mM). Secondary emulsions containing cationic droplets may have certain advantages over existing systems, e.g., improved stability to lipid oxidation, high multivalent mineral contents, thermal processing, and freeze−thaw cycling. This is the subject of current work in our laboratory. In addition, we are investigating the use of different emulsifier combinations to create emulsions containing droplets surrounded by multilayered membranes with novel properties.

Finally, we note that our experiments were carried out using fairly dilute model emulsions (1 wt % or less). These emulsions may be relatively good models for food products with low droplet concentrations, such as cloud or beverage emulsions, but they may not be realistic models for food products with higher droplet concentrations, such as salad dressings or mayonnaise. Indeed, it would be useful to examine the pos-

sibility of using a similar strategy for creating concentrated emulsions. One important potential application of the interfacial engineering technology utilized in this study is in the development of stable beverage emulsions, where gum Arabic could be replaced by a combination of lecithin and chitosan.

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LITERATURE CITED

(1) Friberg, S.; Larsson, K. *Food Emulsions*, 3rd ed.; Marcel Dekker: New York, 1997.
(2) McClements, D. J. *Food Emulsions: Principles, Practice and Techniques;* CRC Press: Boca Raton, FL, 1999.
(3) Stauffer, C. E. *Emulsifiers;* Eagen Press: St Paul, MN, 1999.
(4) Dickinson E. *An Introduction to Food Colloids;* Oxford Science Publishers: Oxford, England, 1992.
(5) Walstra, P. Emulsion stability. In *Encyclopedia of Emulsion Technology;* Becher, P.; Marcel Dekker: New York, 1996; Vol. 4, Chapter 1.
(6) Walstra, P. Disperse systems: Basic considerations. In *Food Chemistry*, 3rd ed.*;* Fennema, O. R., Ed.; Marcel Dekker: New York, 1996; Chapter 3.
(7) Walstra, P. Principles of emulsion formation. *Chem. Eng. Sci.* **1993**, *48*, 333−350.
(8) Charlambous, G.; Doxastakis, G. *Food Emulsifiers: Chemistry, Technology, Functional Properties and Applications;* Elsevier: Amsterdam, Holland, 1989.
(9) Krog, N. J. Food Emulsifiers. In *Food Emulsions*, 3rd ed.; Friberg, S.; Larsson, K., Eds.; Marcel Dekker: New York, 1997; p 141.
(10) Stang, M.; Karbstein, H.; Schubert, H. Adsorption kinetics of emulsifiers at oil−water interfaces and their effect on mechanical emulsification. *Chem. Eng. Process.* **1994**, *33*, 307.
(11) Faldt, P.; Bergenstahl, B.; Claesson, P. M. Stabilization by chitosan of soybean oil emulsions coated with phospholipid and glycholic acid. *Colloids Surf. A.* **1993**, *71*, 187−195.
(12) Calvo, P.; Remunan-Lopez, C.; Vila-Jato, J. L.; Alonso, M. J. Development of positively charged colloidal drug carriers: Chitosan−copolyester nanocapsules and submicron-emulsions. *Colloid Polym. Sci.* **1997**, *275*, 46−53.
(13) Magdassi, S.; Bach, U.; Mumcuoglu, K. Y. Formation of positively charged microcapsules based on chitosan−lecithin interactions. *J. Microencaps.* **1997**, *14*, 189−195.
(14) Pinotti, A.; Zaritzky, N. Effect of aluminum sulfate and cationic polyelectrolytes on the destabilization of emulsified wastes. *Waste Management* **2001**, *21*, 535−542.
(15) Pinotti, A.; Bevilacqua, A.; Zaritzky, N. Optimization of the flocculation stage in a model system of a food emulsion waste using chitosan as polyelectrolyte. *J. Food Eng.* **1997**, *32*, 69−81.
(16) Pinotti, A.; Bevilacqua, A.; Zaritzky, N. Treatment of anionic emulsion systems using chitosan, polyacrylamide, and aluminum sulfate. *Scanning* **1999**, *21*, 354−358.
(17) Pinotti, A.; Bevilacqua, A.; Zaritzky, N. Comparison of the performance of chitosan and a cationic polyacrylamide as flocculants of emulsion systems. *J. Surfactants Detergents* **2001**, *4*, 57−63.
(18) Silvestre, M. P. C.; Decker, E. A.; McClements, D. J. Influence of copper on the stability of whey protein stabilized emulsions. *Food Hydrocolloids* **1999**, *13*, 419−424.
(19) Kulmyrzaev, A.; Silvestre, M. P. C.; McClements, D. J. Rheology and stability of whey protein stabilized emulsions with high $CaCl_2$ concentrations. *Food Res. Int.* **2000**, *33*, 21−25.
(20) Mei, L.; Decker, E. A.; McClements, D. J. Evidence of iron association with emulsion droplets and its impact on lipid oxidation. *J. Agric. Food Chem.* **1998**, *46*, 5072−5077.
(21) McClements, D. J.; Decker, E. A. Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *J. Food Sci.* **2000**, *65*, 1270−1282.
(22) Koide, S. S. Chitin−chitosan: Properties, benefits and risks. *Nutr. Res.* **1998**, *18*, 1091−1101.
(23) Ravikumar, M. N. V. Chitin and chitosan fibres: A review. *B. Mater. Sci.* **1999**, *22*, 905−915.
(24) Jeon, Y. J.; Shahidi, F.; Kim, S. K. Preparation of chitin and chitosan oligomers and their applications in physiological functional foods. *Food Rev. Int.* **2000**, *16*, 159−176.
(25) Paul, W.; Sharma, C. P. Chitosan, a drug carrier for the 21st century: A review. *Stp. Pharma Sci.* **2000**, *10*, 5−22.
(26) Cho, Y. I.; No, H. K.; Meyers, S. P. Physiochemical characteristics and functional properties of various commercial chitin and chitosan products. *J. Agric. Food Chem.* **1989**, *37*, 3839−3843.
(27) Del Blanco, L. F.; Rodriguez, M. S.; Schulz, P. C.; Agullo, E. Influence of deacetylation degree on chitosan emulsification properties. *Colloid Polym. Sci.* **1999**, *277*, 1087−1092.
(28) Kong, K. Y.; Muthukumar, M. Monte Carlo study of adsorption of a polyelectrolyte onto charged spheres. *J. Chem. Phys.* **1998**, *109*, 1522−1527.
(29) Netz, R. R.; Joanny, J.-F. Complexation between a semiflexible polyelectrolyte and an oppositely charged sphere. *Macromolecules* **1999**, *32*, 9026−9040.
(30) Chodanowski, P.; Stoll, S. Polyelectrolyte adsorption on charged particles: Ionic concentration and particle size effects−A Monte Carlo approach. *J. Chem. Phys.* **2001**, *115*, 4951−4960.
(31) Dobrynin, A. V. Effect of solvent quality on polyelectrolyte adsorption at an oppositely charged surface. *J. Chem. Phys.* **2001**, *115*, 8145−8153.
(32) Jonsson, M.; Linse, P. Polyelectrolyte−macroion complexation: Effect of linear charge density and macroion charge. *J. Chem. Phys.* **2001**, *115*, 3406−3418.
(33) Von Goeler, F.; Muthukumar, M. Adsorption of polyelectrolytes onto curved spheres. *J. Chem. Phys.* **1994**, *100*, 7796−7803.
(34) Israelachvili, J. N. *Intermolecular and Surface Forces;* Academic Press: London, England, 1992.
(35) Hunter, R. J. *Foundations of Colloid Science;* Oxford University Press: Oxford, England, 1986; Vol. 1.

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