JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



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

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The possibility of producing stable oil-in-water (O/W) emulsions containing oil droplets surrounded by multiple layer interfacial membranes from food grade ingredients has been demonstrated. These emulsions were produced using a three stage process that relies on the adsorption of charged biopolymers to oppositely charged surfaces. Emulsions (0.5 wt % corn oil, 0.1 wt % lecithin, 0.0078 wt % chitosan, 0.02 wt % pectin, and 100 mM acetic acid, pH 3.0) containing oil droplets stabilized by lecithin−chitosan−pectin membranes were formed using this interfacial layer-by-layer deposition process. The droplets in these emulsions had good stability to aggregation over a wide range of pH values and salt concentrations (pH 4−8 at 0 mM NaCl and pH 3−8 at 100 mM NaCl). This technology could be extremely useful to the food industry for the creation of O/W emulsions with improved properties or novel applications, e.g., improved stability to environmental stresses, protection of labile substances, controlled release, and triggered release.

**KEYWORDS: Emulsion; stability; flocculation; ζ-potential; chitosan; lecithin; pectin**

## INTRODUCTION

The interfacial membrane surrounding the droplets in oil-in-water (O/W) emulsions largely determines their stability and physicochemical properties, e.g., shelf life, texture, appearance, and flavor (*1*−*7*). The interfacial membranes in most food emulsions are comprised of surface active molecules called emulsifiers, including small molecule surfactants, phospholipids, proteins, and polysaccharides (*1*, *3*, *8*, *9*). Normally, an emulsion is formed by homogenizing the oil and aqueous phases together in the presence of one or more emulsifiers (*1*−*3*). This usually leads to the formation of droplets coated by an interfacial membrane that consists of a single layer of surface active molecules.

Research in nonfood-related areas has shown that it is possible to coat charged surfaces and colloidal particles with interfacial membranes consisting of multiple layers of different materials (*10*−*16*). This technique is based on layer-by-layer (LbL) deposition of polyelectrolytes onto oppositely charged surfaces or colloidal particles due to electrostatic attraction. This LbL technology allows precise control over the thickness and properties of the interfacial membrane, which enables the creation of colloidal dispersions with improved or novel properties, such as encapsulation, protection, or delivery of functional components. The main purpose of the present study is to show that the same technology can be used to produce multilayered (O/W) emulsions from food grade ingredients. The LbL technique could then be used to create food emulsions with improved or novel properties.

In a previous study, we used the LbL method to prepare O/W emulsions containing cationic droplets coated with lecthin−chitosan bilayers (*17*, *18*). First, a primary emulsion containing anionic droplets coated with lecithin was produced using lecithin as an emulsifier. Lecithin was selected because it is a negatively charged food grade emulsifier that can produce small oil droplets during homogenization. Second, a secondary emulsion containing anionic droplets coated with lecithin−chitosan membranes was produced by mixing chitosan with the primary emulsion. Chitosan was chosen for this purpose because it had previously been shown to be capable of improving the stability of emulsions containing lecithin-coated lipid droplets (*19*, *20*). Secondary emulsions that were stable to flocculation could be produced by selecting appropriate environmental conditions (lecithin-to-chitosan ratio, pH, and ionic strength) and by disrupting any flocs formed by sonication (*17*). In a subsequent study, we showed that the secondary emulsions had better stability to freeze−thaw cycling, thermal processing, and high calcium concentrations than the primary emulsions (*18*). Nevertheless, the secondary emulsions could not be used at relatively high pH values (>5) because the chitosan lost its positive charge and desorbed from the droplet surfaces. In the current study, we aimed to determine whether it was possible to further improve the functional properties of O/W emulsions by creating interfacial membranes consisting of three layers of different materials (lecthin−chitosan−pectin). If multilayered membranes can be produced using food grade materials, then many of the unique functional properties developed in other colloidal systems

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10.1021/jf034436k CCC: $27.50 
Published on Web 05/04/2004

could be utilized in the food industry, e.g., encapsulation, protection, and delivery of functional food ingredients.

## 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 per g; peroxide value, 0.9 mequiv per kg; moisture, 0.77 wt %) was obtained from ADM-Lecithin (Decatur, IL). Powder pectin (TIC PRETESTED, prehydrated pectin, high methoxyl, rapid set powder) was obtained from TIC GUMS (Belcamp, MD). Corn oil (Mazola, ACH Food Companies, Inc.) was purchased from a local supermarket. Analytical grade sodium chloride, hydrochloric acid, sodium hydroxide, and acetic acid were purchased from the Sigma Chemical Company (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 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. A pectin solution was prepared by dispersing 0.4 wt % powdered pectin into stock buffer solution. An emulsifier solution was prepared by dispersing 1.0 wt % lecithin powder into stock buffer solution. The emulsifier solution was sonicated for 30 s at a frequency of 20 kHz, an amplitude of 40%, and a 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 a 5 wt % corn oil with a 95 wt % aqueous emulsifier solution in a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland) followed by two passes at 4000 psi through a two stage high-pressure valve homogenizer (LAB 1000, APV-Gaulin, Wilmington, MA). A secondary emulsion was prepared by mixing the primary emulsion with appropriate amounts of chitosan solution and buffer solution to obtain a final concentration of 1 wt % corn oil, 0.2 wt % lecithin, 0.0155 wt % chitosan, and 100 mM acetic acid (pH 3.0). These systems were stirred for 1 h using a magnetic stirrer at ambient temperature. The flocs formed in this emulsion were disrupted by passing it twice through a high-pressure valve homogenizer at a pressure of 4000 psi, as described previously (*11*, *12*). Tertiary emulsions were formed by diluting the secondary emulsion with aqueous pectin solutions to produce a series of emulsions with different pectin concentrations: 0.5 wt % corn oil, 0.1 wt % lecithin, 0.0078 wt % chitosan, 100 mM acetic acid, and 0−0.02 wt % pectin (pH 3.0). These systems were stirred for 1 h using a magnetic stirrer at ambient temperature. The tertiary emulsions were stored at room temperature for 24 h before they were analyzed.

**Particle Size Measurements.** Emulsions were diluted to a droplet concentration of approximately 0.005 wt % using buffer solution to avoid multiple scattering effects. The particle size distribution of the emulsions was measured using a laser light scattering instrument (Horiba LA-900, Irvine, CA). This instrument measures the angular dependence of the intensity of laser light ($\lambda = 632.8$ nm) scattered by a dilute emulsion and then finds the particle size distribution that gives the best agreement between theoretical predictions and experimental measurements. A refractive index ratio of 1.08 was used in the calculations of the particle size distribution. The theory used to calculate the particle size distribution assumes that the particles are spherical and homogeneous; therefore, the data obtained on emulsions that contained flocs should be treated with caution because they are nonspherical and nonhomogeneous. Mean particle diameters were calculated as the average of measurements made on at least two samples, with standard deviations being less than 10%.

**ζ-Potential Measurements.** Emulsions were diluted to a droplet concentration of approximately 0.005 wt % using buffer solution to avoid multiple scattering effects. Diluted emulsions were injected directly into the measurement chamber of a particle electrophoresis instrument (ZEM5003, Zetamaster, Malvern Instruments, Worcester, U.K.). The ζ-potential was then determined by measuring the direction and velocity of the droplet movement in a well-defined electric field. The ζ-potential measurements are reported as the mean and standard deviation of two separate injections, with five readings made per injection.

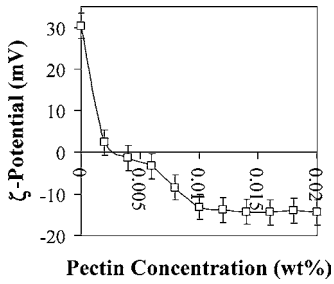


**Figure 1.** Dependence of particle electrical charge (ξ-potential) on pectin concentration for tertiary emulsions (0.5 wt % corn oil, 0.1 wt % lecithin, 0.0078 wt % chitosan, and 100 mM acetic acid, pH 3.0).

**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 turbidity (at 600 nm) of the emulsions was then measured 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. 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.

## RESULTS AND DISCUSSION

**Influence of Pectin Concentration on Droplet Characteristics.** The electrical charge and mean droplet diameter of tertiary emulsions (0.5 wt % corn oil, 0.1 wt % lecithin, 0.0078 wt % chitosan, and 100 mM acetic acid, pH 3.0) containing different pectin concentrations (0−0.02 wt %) were measured. In the absence of chitosan, the electrical charge on the secondary emulsion droplets was +30 mV (**Figure 1**), indicating that the lecithin−chitosan membrane had a relatively high positive charge at pH 3. The electrical charge on the droplets became increasingly less positive and eventually changed from positive to negative as the pectin concentration in the emulsions was increased (**Figure 1**). There was no net charge on the droplets when the pectin concentration was around 0.003 wt %, indicating that a sufficient amount of pectin had adsorbed to neutralize the charge on the original droplets. The negative charge on the droplets reached a constant value of −14 mV when the pectin concentration exceeded about 0.01 wt %.

The mean particle diameter of tertiary emulsions was measured 24 h after pectin was mixed with the secondary emulsions (**Figure 2**). The emulsion was stable to droplet aggregation in the absence of pectin. At pectin concentrations from 0.002 to 0.006 wt %, extensive droplet aggregation was observed in the emulsions. Droplet aggregation was so extensive that we were unable to make reliable particle sizing measurements using the laser diffraction technique, either because the aggregates were too large or because their concentration was too small to give a scattering pattern that was sufficiently different from that of the background. Individual particles within these samples could be distinctly observed by eye, which suggested that they were at least 100 $\mu$m in diameter. The origin of the extensive droplet flocculation in these emulsions is 2-fold. First, the magnitude of the net electrical charge on the droplets

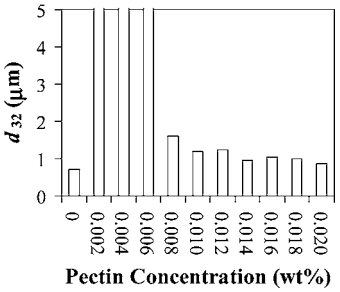


**Figure 2.** Dependence of mean particle diameter ($d_{32}$) on pectin concentration for tertiary emulsions (0.5 wt % corn oil, 0.1 wt % lecithin, 0.0078 wt % chitosan, and 100 mM acetic acid, pH 3.0).

is relatively small (<5 mV); therefore, the electrostatic repulsion between the droplets would not be sufficient to prevent aggregation (*21*, *22*). Second, it is possible that pectin molecules adsorbed to the surface of more than one emulsion droplet during the formation of the tertiary emulsions, thus acting as polymeric bridges that held the droplets together (*23*−*26*). At pectin concentrations from ≥0.008 to 0.02 wt %, the particle diameter ($d_{32} \sim 1.6-0.9$ μm) was significantly higher than that observed in the absence of chitosan ($d_{32} \sim 0.7$ μm), indicating that there was a limited degree of droplet aggregation. Nevertheless, it was much less than that observed from 0.002 to 0.006 wt % pectin. A previous study has shown that flocs in multilayered emulsions can be disrupted by application of mechanical agitation (*17*, *18*). In this study, we found that passing tertiary emulsions containing 0.02 wt % pectin twice through a high-pressure valve homogenizer at 4000 psi reduced the mean particle diameter ($d_{32} \sim 0.7$ μm) close to that found in the absence of pectin, suggesting that the flocs could be disrupted by application of mechanical agitation.

**Influence of pH and Ionic Strength on Properties of Primary, Secondary, and Tertiary Emulsions.** A tertiary emulsion was prepared with a composition of 0.5 wt % corn oil, 0.1 wt % lecithin, 0.0078 wt % chitosan, 0.02 wt % pectin, and 100 mM acetic acid (pH 3.0). Prior to utilization, any flocs formed in this emulsion were disrupted by passing it twice through a high-pressure value homogenizer at 4000 psi (*17*, *18*). A series of dilute emulsions (∼0.005 wt % corn oil) with different pH values (3−8) and ionic strengths (0 or 100 mM NaCl) were formed by diluting primary, secondary, and tertiary emulsions with distilled water or NaCl solutions and then adjusting the pH with HCl or NaOH. These emulsions could be analyzed directly by laser diffraction, particle electrophoresis, and turbidity techniques without the need for further dilution. The diluted primary, secondary, and tertiary emulsions were then stored for 1 week at room temperature, and their electrical charge, mean droplet diameter, and creaming stability were measured (**Figures 3**−**5**).

*Effect on Droplet Charge.* The ζ-potential of the droplets in the primary emulsions was negative at all pH values but was appreciably more negative at high rather than at low pH (**Figure 3**). The droplet charge was probably less negative at low pH because a smaller fraction of the adsorbed lecithin molecules was ionized, since the p$K_a$ value of the anionic phosphate groups on lecithin is around pH 1.5 (*21*, *22*). The magnitude of the electrical charge on the droplets in the primary emulsions decreased upon the addition of salt, e.g., the ζ-potential changed from −42 to −13 mV at pH 3 when the NaCl was increased from 0 to 100 mM (**Figure 3a**,**b**). This reduction can be attributed to electrostatic screening effects, which cause a reduction in the surface charge potential of colloidal particles with increasing ionic strength (*21*,*22*).

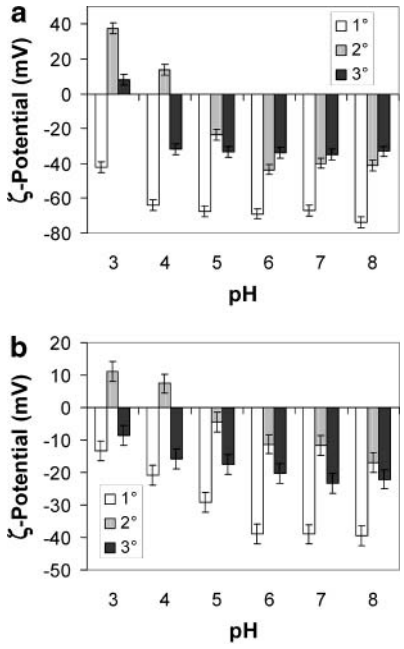


**Figure 3.** Dependence of particle electrical charge (ξ-potential) on pH for dilute tertiary emulsions (0.5 wt % corn oil, 0.1 wt % lecithin, 0.0078 wt % chitosan, 0.02 wt % pectin, and 100 mM acetic acid, pH 3.0); (**a**) 0 mM NaCl and (**b**) 100 mM NaCl.

The ζ-potential of the secondary emulsions was highly positive (∼38 mV) at pH 3 due to adsorption of cationic chitosan molecules onto the surface of the anionic lecithin-coated droplets (*17*). As the pH was increased, the electrical charge on the droplets became less positive (pH 4), and eventually, it became negative (pH ≥ 5). The reduction in the positive charge on the droplets with increasing pH is probably the result of deprotonation of the $-NH_3^+$ groups on the chitosan. These groups have a p*K* value around 6.3−7 (*27*); hence, as the pH is increased, the chitosan becomes less positively charged. As the chitosan loses its positive charge, the electrostatic attraction between the anionic lecithin molecules and the cationic chitosan molecules decreases. Consequently, it is possible that the chitosan molecules may have desorbed from the droplet surfaces at higher pH, although this is not necessary to explain the observed effects. It is interesting to note that the negative charge on the droplets in the secondary emulsion was significantly lower than that on the droplets in the primary emulsion at high pH (pH 6−8). A possible explanation of this phenomenon is that there is some chitosan adsorbed to the droplet surfaces that still has a residual positive charge. Alternatively, the presence of adsorbed chitosan at the droplet surface may increase the thickness of the Stern layer, which would reduce the measured ζ-potential (*21*). The magnitude of the ζ-potential in the secondary emulsions decreased as the NaCl concentration was increased from 0 to 100 mM, presumably due to the electrostatic screening effects mentioned earlier.

At pH 3, the ζ-potential in the tertiary emulsions was slightly positive (+8 mV) in the absence of salt, which suggests that the negative charge on the adsorbed pectin molecules was insufficient to overcome the high positive charge on the lecithin−chitosan-coated droplets (+38 mV). The p$K_a$ value of the carboxylic groups on pectin is usually around pH 4−5 (*28*); hence, pectin has a smaller negative charge at low pH rather than at high pH. Consequently, its effectiveness at decreasing the positive charge on the lecitin−chitosan-coated droplets would have been reduced at this low pH. Interestingly, when 100 mM NaCl was present at pH 3, the charge on the tertiary

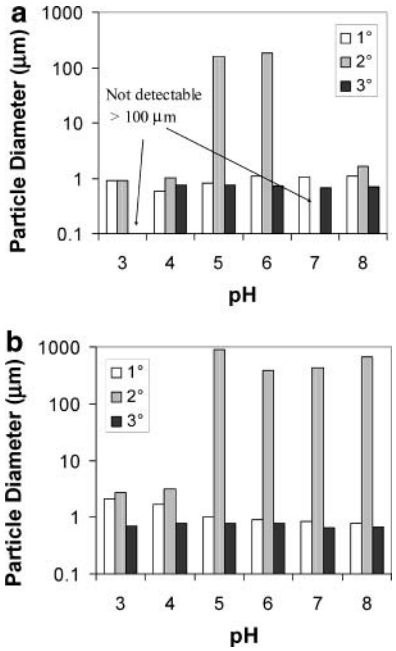


**Figure 4.** Dependence of mean particle diameter ($d_{32}$) on pH for dilute tertiary emulsions (0.5 wt % corn oil, 0.1 wt % lecithin, 0.0078 wt % chitosan, 0.02 wt % pectin, and 100 mM acetic acid, pH 3.0); (**a**) 0 mM NaCl and (**b**) 100 mM NaCl.

emulsions was negative (−9 mV), which suggests that the negative charge on the adsorbed pectin was sufficient to overcome the much reduced positive charge (+11 mV) on the lecithin−chitosan-coated droplets in the presence of salt. At pH ≥ 4, the tertiary emulsions were anionic in the presence and absence of salt, which suggested that the negative charge on the adsorbed pectin molecules was more than sufficient to balance the positive charge on the lecithin−chitosan-coated droplets. At high pH values (pH 6−8), the ζ-potential of the tertiary emulsions was less negative than in the primary emulsions in the presence and absence of salt. However, the ζ-potential of the tertiary emulsions was less negative than that of the secondary emulsions in the absence of salt but more negative in the presence of salt. The precise reason for these differences is currently unclear but is probably associated with changes in the composition and structure of the interfacial membranes in the primary, secondary, and tertiary emulsions with pH and ionic strength. The magnitude of the ζ-potential decreased when the salt concentration was increased from 0 to 100 mM NaCl, presumably due to electrostatic screening effects.

*Effect on Droplet Aggregation.* The droplets in the primary emulsions were relatively stable to extensive droplet aggregation at all pH and NaCl values (**Figure 4**). Nevertheless, the particles in the emulsions stored at low pH values (pH 3 and 4) in the presence of salt (**Figure 4b**) were significantly larger than those in the emulsions stored in the absence of salt (**Figure 4a**). For example, at pH 3, $d_{32} = 2.1 \pm 0.2$ $\mu$m at 100 mM NaCl and $0.91 \pm 0.09$ $\mu$m at 0 mM NaCl. Droplet aggregation at low pH and high salt may have been because the reduced charge on the lecithin molecules combined with the increased electrostatic screening caused a reduction in the electrostatic repulsion between the droplets. In addition, salt reduces the curvature of phospholipid membranes by reducing the effective headgroup size of the polar lipids, which favors droplet coalescence in emulsions (*22*).

In the absence of added NaCl, the droplets in the secondary emulsions were relatively stable to droplet aggregation at low (pH 3 and 4) and high (pH 8) pH values but were highly unstable at intermediate pH values (**Figure 4a**). The droplets were probably stable to droplet aggregation at pH 3 because the high positive charge on the droplets led to strong electrostatic repulsion between the droplets. As the pH was increased, the chitosan molecules began to lose their positive charge ($pK_a \sim 6.3-7$); hence, the charge on the droplets decreased. In addition, the chitosan molecules would be less strongly held to the surface of the lecithin-coated droplets because the electrostatic attraction between the cationic chitosan and the anionic lecithin molecules would be reduced. Consequently, some of the chitosan molecules may have been completely or partly displaced from the surface of the emulsion droplets. These chitosan molecules could then act as polymeric bridges that held the negatively charged lecithin-coated droplets together (*23*−*26*). Bridging flocculation may therefore have been responsible for the high degree of droplet aggregation observed at intermediate (pH 5−7) pH values. The emulsion may have become more stable to droplet aggregation at pH 8 because the chitosan molecules had lost most of the positive charge and were therefore not as effective at inducing bridging flocculation. In the presence of 100 mM NaCl, the emulsions were still relatively stable to flocculation at low pH values (pH 3 and 4) but were unstable at all higher values. Aggregation may have occurred in the pH 8 emulsion when salt was added because the electrostatic repulsion between the droplets was sufficiently screened.

The droplets in the tertiary emulsions were stable to droplet aggregation at all pH values in the absence and presence of salt, with the exception of the pH 3 emulsion at 0 mM NaCl (**Figure 4a**). Aggregation probably occurred in this emulsion because the droplets had a small ζ-potential (**Figure 3a**) so that the electrostatic repulsion between them was relatively weak. In addition, there may have been bridging flocculation between the negatively charged pectin molecules in the aqueous phase and the positively charged droplets. These results indicate that emulsions with good stability against droplet aggregation can be produced using lecithin−chitosan−pectin membranes. It is interesting to note that the chitosan layer did not appear to desorb from the droplet surfaces in the tertiary emulsions at high pH values, as occurred 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 biopolymer layers, as has been reported for other polyelectrolytes (*12*, *13*).

*Effect on Creaming Stability.* The creaming stability of the diluted emulsions was determined by measuring the turbidity at 30% of their height after 1 week of storage (**Figure 5**). If extensive droplet aggregation occurred, then we would expect the turbidity to be reduced because flocs cream more rapidly than individual droplets. All emulsions were more stable to creaming in the absence of salt than in its presence. This can primarily be attributed to the greater screening of electrostatic repulsive interactions between electrically charged droplets at higher salt concentrations. The primary emulsions were relatively stable to creaming at all pH values in the absence of salt but exhibited some creaming at low pH values (pH 3−5) in the presence of salt. The secondary emulsions were highly susceptible to creaming at intermediate pH values (pH 4−7) in the absence of salt and at all pH values in the presence of salt. The droplets in the tertiary emulsions were relatively stable to creaming at all pH values in the absence and presence of salt, with the exception of the pH 3 emulsion at 0 mM NaCl. The creaming stability measurements therefore largely supported the

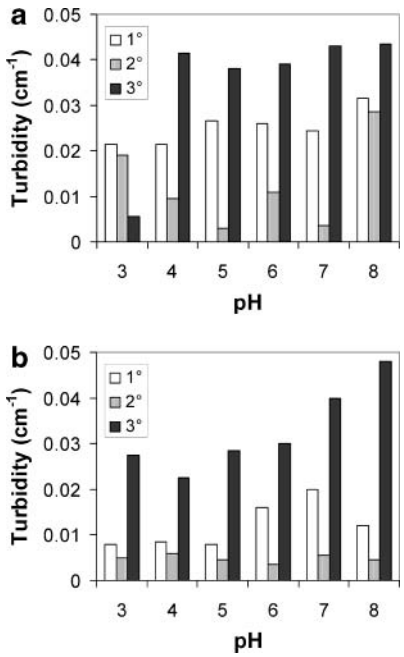


**Figure 5.** Dependence of emulsion creaming stability on pH for dilute tertiary emulsions (0.5 wt % corn oil, 0.1 wt % lecithin, 0.0078 wt % chitosan, 0.02 wt % pectin, and 100 mM acetic acid, pH 3.0); (**a**) 0 mM NaCl and (**b**) 100 mM NaCl. The creaming stability was determined as turbidity (at 600 nm) measured at 30% of emulsion height after 7 days of storage; creaming instability is indicated by a low turbidity. Initial emulsion turbidities were around 0.4–0.45 $cm^{-1}$.

particle size measurements made using laser diffraction (**Figure 4**): Emulsions with relatively small particles tended to be more stable to creaming. The tertiary emulsions may have been stable to droplet aggregation because of the relatively strong electrostatic and steric repulsion associated with the relatively thick and electrically charged three layer interfacial membrane. In addition, the creaming stability may have been improved because the multiple layers increased the overall density of the droplets, thereby reducing the density contrast between the dispersed and the continuous phases and decreasing the driving force for gravitational separation (*1*−*3*).

## CONCLUSIONS

This study has shown that emulsions containing trilayer-coated lipid droplets can be prepared using a method that utilizes food grade ingredients (lecithin, chitosan, and pectin) and standard preparation procedures (homogenization and mixing). Initially, a primary emulsion containing small anionic droplets was produced by homogenization of oil, water, and lecithin. A secondary emulsion containing cationic droplets coated with a lecithin−chitosan membrane was then produced by mixing a chitosan solution with the primary emulsion and applying mechanical agitation to disrupt any flocs formed. A tertiary emulsion containing anionic droplets coated with a lecithin−chitosan−pectin membrane was then produced by mixing a pectin solution with the secondary emulsion and again applying mechanical agitation to disrupt any flocs formed. The droplets in the tertiary emulsions had good stability to droplet aggregation and creaming over a wide range of pH values (pH 4−8 at 0 mM NaCl and pH 3−8 at 100 mM NaCl). The tertiary emulsions may have a number of potential applications in the food industry for specialized applications. For example, the ability to have greater control over the thickness, structure, and composition of the interfacial membranes surrounding droplets may lead to novel methods of protecting labile lipid components, controlling or targeting ingredient release, or improving the stability of emulsions to environmental stresses, such as freezing and heating. We are currently examining potential applications for three-layered emulsion systems based on food grade ingredients.

## ACKNOWLEDGMENT

We thank the Tokyo University of Fisheries (Japan) for financial support of S.O. We also thank ADM for kindly donating the lecithin ingredient used in this study.

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**Received for review April 28, 2003. Revised manuscript received February 27, 2004. Accepted April 1, 2004. This material is based upon work supported by the National Research Initiative, Competitive Grants Program, United States Department of Agriculture, and the CREES, IFAFS Program, United States Department of Agriculture (Award No. 2002-35503-12296).**

JF034436K