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Impact of Electrostatic Interactions on Formation and Stability of Emulsions Containing Oil Droplets Coated by β-Lactoglobulin–Pectin Complexes

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Interfacial protein−polysaccharide complexes can be used to improve the physical stability of oil-in-water emulsions. The purpose of this study was to examine the impact of ionic strength on the formation and stability of oil-in-water emulsions containing polysaccharide−protein-coated droplets. Emulsions were prepared that contained 0.1 wt % corn oil, 0.05 wt % β-lactoglobulin, and 0.02 wt % pectin at pH 7. The emulsions were then adjusted to pH 4 to promote electrostatic deposition of the pectin molecules onto the surfaces of the protein-coated droplets. The salt concentration of the aqueous phase (0 or 50 mM NaCl) was adjusted either before or after deposition of the pectin molecules onto the droplet surfaces. We found that stable emulsions containing polysaccharide−protein-coated droplets could be formed when the salt was added after pectin adsorption but not when it was added before pectin adsorption. This phenomenon was attributed to the ability of NaCl to promote droplet flocculation in the protein-coated droplets so that the pectin molecules adsorbed onto the surfaces of flocs rather than individual droplets when salt was added before pectin adsorption. We also found that polysaccharide−protein-coated droplets had a much improved stability to salt-induced flocculation than protein-coated droplets with the same droplet charge (ζ-potential). Theoretical predictions indicated that this was due to the ability of the adsorbed polysaccharide layer to strongly diminish the van der Waals attraction between the droplets.

KEYWORDS: Emulsion; β-lactoglobulin; pectin; multilayer; stability; electrostatic

INTRODUCTION

The stability of oil-in-water emulsions containing protein-coated droplets can often be improved by adding a polysaccharide that adsorbs onto the droplet surfaces and forms a protective layer (*1*−*5*). This process is normally achieved by mixing a protein-stabilized emulsion and a polysaccharide solution under conditions where there is an attractive force between the polysaccharide molecules and the protein-coated droplet surfaces. The most commonly utilized attractive force is the electrostatic interaction between the charged groups on polysaccharide molecules and the oppositely charged groups on adsorbed protein molecules. The charge on the protein-coated droplets can be varied from positive to negative by adjusting the pH from below to above the isoelectric point (pI) of the adsorbed proteins (*6*). On the other hand, the charge characteristics of the polysaccharide molecules can be controlled by selecting polysaccharides with different numbers, distributions, and types of ionizable functional groups (*7*). Thus, anionic polysaccharides can be adsorbed onto the surfaces of protein-coated droplets at pH values where the droplets either have a net positive charge or have a significant amount of positively charged patches on their surfaces (*8*−*11*). Conversely, cationic polysaccharides can be adsorbed onto the surfaces of protein-coated droplets at pH values where the droplets have either a net negative charge or an appreciable amount of negatively charged patches on their surfaces (*12*, *13*). The resulting "multilayer emulsions" contain oil droplets coated by protein−polysaccharide interfacial complexes.

Previously, it has been shown that oil droplets coated by protein−polysaccharide complexes often have superior stability to environmental stresses than those coated by proteins alone because of the alteration in interfacial charge, structure, and thickness (*1*, *2*). For example, the stability of emulsions to pH, ionic strength, thermal processing, freezing, and dehydration can be improved using the multilayer approach (*1*, *9*, *11*, *14*−*18*). Although the multilayer 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. A significant problem reported in studies that have used this approach to create multilayer-coated droplets is the tendency for extensive aggregation of the droplets to occur (*19*). Different kinds of droplet aggregation may occur depending on the system conditions: (i) Bridging flocculation

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occurs if there is insufficient polyelectrolyte to completely coat all of the droplet surfaces when they collide, which may occur either because there is insufficient polyelectrolyte present or because it does not adsorb fast enough relative to droplet−droplet collisions; (ii) depletion flocculation occurs if there is too much excess polyelectrolyte present in the continuous phase; and (iii) flocculation may also occur if the repulsive interactions between the coated droplets are not strong enough to overcome the attractive interactions (*19*). Droplet aggregation therefore has to be retarded by carefully controlling preparation conditions, such as droplet characteristics (e.g., concentration, size, and charge), polyelectrolyte characteristics (e.g., concentration, molar mass, and charge density), solvent properties (e.g., ionic strength, pH, and dielectric constant), and/or the mixing method (e.g., order of addition, mixing speed, and flow profile).

The main driving force for polyelectrolyte adsorption to the droplet surfaces is electrostatic; hence, multilayer formation depends strongly on solution pH and ionic strength because these parameters influence the magnitude of the electrical charge on the droplets and polyelectrolyte molecules, as well as the range of the electrostatic interactions in the system (*20*, *21*). Previous studies have shown that the amount of polyelectrolyte adsorbed onto colloidal particles and the properties of the interfaces formed are strongly influenced by the ionic strength of the aqueous phase, as well as on whether the ionic strength was adjusted before or after polyelectrolyte adsorption (*21*−*26*). The purpose of this study was therefore to examine the influence of electrostatic interactions on the formation and properties of emulsions containing protein−polysaccharide-coated droplets.

In this study, we prepared oil-in-water emulsions containing droplets coated by an interfacial layer comprised of two natural polymers: a globular protein [β-lactoglobulin (β-Lg)] and an anionic polysaccharide (pectin). β-Lg was used because its molecular properties are well-known and it is the major component in whey protein, which is commonly used as an emulsifier in the food industry (*6*). The isoelectric point of β-Lg has been reported to be around 4.7−5.2 (*27*−*29*). Pectin was used because it is an anionic polysaccharide ($pK_a \approx 3.5$) that is already widely used as a functional ingredient by the food industry (*30*). The major objective of this study was to examine the influence of electrostatic interactions, modulated by changing the pH and ionic strength, on the formation and stability of oil-in-water emulsions containing lipid droplets coated by β-Lg−pectin layers. Electrostatic interactions would be expected to influence the adsorption of charged polysaccharide molecules onto the surfaces of oppositely charged lipid droplets, as well as influencing the strength of the electrostatic repulsion between oil droplets.

MATERIALS AND METHODS

Materials. Powdered β-Lg was obtained from Davisco Foods International (Lot #JE 001-1-922, Le Sueur, MN). The protein content was reported to be 98.3% (dry basis) by the supplier, with β-Lg making up 95.5% of the total protein. The moisture content of the protein powder was reported to be 4.9%. The fat, ash, and lactose contents of this product were reported to be 0.3 ± 0.1, 2.5 ± 0.2, and <0.5 wt %, respectively. Pectin extracted from citrus fruit was purchased from Sigma Chemical Co. (Lot #91K1420, St. Louis, MO). The degree of esterification of the pectin was reported to be 59% by the supplier. Corn oil was purchased from a local supermarket and used without further purification. Analytical grade hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from the Sigma Chemical Co.. Distilled and deionized water from a Nanopure water system (Nanopure Infinity, Barnstead International, IA) was used for the preparation of all solutions.

Solution Preparation. Emulsifier solution containing 0.05 wt % protein was prepared by dispersing powdered β-Lg into 5 mM sodium phosphate buffer (pH 7.0) and stirring for at least 2 h to ensure complete hydration. A pectin solution was prepared by dispersing weighed amounts of the powdered material into the same buffer (0.4%, pH 7.0) and stirring for at least 2 h to ensure complete hydration.

Emulsion Preparation. A primary emulsion was prepared by homogenizing 0.8 wt % corn oil with 99.2 wt % aqueous emulsifier solution (0.05 wt % β-Lg, pH 7.0) with a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland) followed by five passes through a two-stage high-pressure valve homogenizer (LAB 1000, APV-Gaulin, Wilmington, MA): 5000 psi the first stage and 500 psi the second stage. Secondary emulsions were prepared by adding pectin solution and/or NaCl solution either before or after pH adjustment.

Effect of NaCl Addition before or after pH Adjustment. In this set of experiments, we examined the influence of NaCl addition on emulsion stability, with the salt being added either before or after pectin adsorption.

(i) NaCl Adjusted before Pectin Adsorption. The 0.8 wt % corn oil-in-water primary emulsion was mixed with different ratios of pectin and salt solutions at pH 7.0 to form 0.2 wt % secondary emulsions. The secondary emulsions were then adjusted to pH 4.0 by addition of HCl to induce pectin adsorption and then diluted with pH 4.0 buffer solution.

(ii) NaCl Adjusted after Pectin Adsorption. The 0.8 wt % corn oil-in-water primary emulsion was mixed with different ratios of pectin and buffer solutions at pH 7.0 to form 0.2 wt % secondary emulsions. The secondary emulsions were then adjusted to pH 4.0 by addition of HCl to induce pectin adsorption and then diluted with pH 4.0 NaCl solution.

Finally, the emulsions had a composition of 0.1 wt % corn oil, 0.005 wt % β-lactoglobulin, 0−0.05 wt % pectin, and 0−300 mM NaCl. Emulsions were then stored at ambient temperature prior to analysis. The above experiments were also repeated using different final pH values (3.0−7.0).

Effect of NaCl on Emulsions with Similar Electrical Potentials. In this set of experiments, we wanted to distinguish between the effects of ionic strength and those of droplet charge (ζ-potential). Consequently, primary and secondary emulsions with the same initial ζ-potential were prepared by using different pH values. A primary emulsion was prepared at pH 5.7 where it had a ζ-potential ($\sim -30 \pm 1$ mV) that was approximately the same as that of a secondary emulsion at pH 4 ($\sim -33 \pm 1$ mV). These emulsions were then diluted with buffer and NaCl solution (of the appropriate pH) to have a final composition of 0.1% oil, 0.005% β-Lg, 0 or 0.02% pectin, and 0−300 mM NaCl. The salt was added to the secondary emulsions after the pectin had adsorbed to the droplet surfaces (i.e., at pH 4). Emulsions were then stored at ambient temperature prior to analysis.

Particle Charge and Size Measurements. The electrical charge (ζ-potential) and *z*-average diameter of the particles in the emulsions were determined using a commercial instrument capable of electrophoresis and dynamic light scattering measurements (Zetasizer NanoZS, Malvern Instruments, Worcs., United Kingdom). The emulsions were prepared and stored at room temperature for 24 h prior to analysis. It should be noted that particle size measurements made on flocculated emulsions should be treated with caution, since dilution and stirring may alter floc size, and the theory used by the instrument to determine the particle size distribution assumed that the particles were spheres.

Creaming Stability Measurements. Ten grams of each emulsion was transferred into test tubes (with 15 mm internal diameter and 125 mm height) and then stored for 7 days at room temperature. After storage, a number of emulsions separated into an optically opaque "cream" layer at the top and a transparent or turbid "serum" layer at the bottom. Digital images of the emulsions were taken after storage to observe creaming as an indication of long-term stability. Visible phase separation (formation of a distinct cream layer on top and a turbid or transparent serum layer at the bottom) was taken as evidence of poor creaming stability.

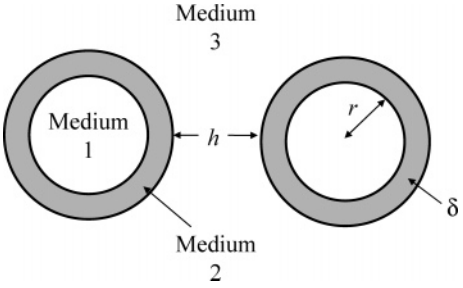

Figure 1. Schematic representation of two oil droplets of radius *r* coated with a polyelectrolyte interfacial layer of thickness δ, with a surface-to-surface separation of *h*.

COLLOIDAL INTERACTION POTENTIALS FOR NONCOATED AND COATED DROPLETS

A mathematical model was developed to estimate the colloidal interactions between a pair of spherical lipid droplets coated by a layer of biopolymer so as to interpret our experiments on the influence of an adsorbed polysaccharide layer on the stability of protein-coated lipid droplets. To a first approximation, the interaction potential between a pair of spherical particles can be described using the DLVO theory (*31*):

$$w(h) = w_V(h) + w_E(h) \quad (1)$$

where $w(h)$, $w_V(h)$, and $w_E(h)$ are the overall, van der Waals, and electrostatic interaction potentials at a surface-to-surface of *h*. Expressions for these interactions between coated spherical particles are available in the literature (*31*):

$$w_V(h) = -\frac{r}{12}\left[\frac{A_{232}(h)}{h} + \frac{A_{131}(h)}{h+2\delta} - \frac{2A_{123}(h)}{h+\delta}\right] \quad (2)$$

$$w_E(h) = -2\pi\epsilon_0\epsilon_R(r+\delta)\Psi^2 \ln[1 - e^{-\kappa h}] \quad (3)$$

where *r* is the lipid droplet radius, δ is the thickness of the adsorbed layer, $A_{ijk}(h)$ is the Hamaker function, and the subscripts 1, 2, and 3 refer to the disperse phase, biopolymer layer, and continuous phase (**Figure 1**). Mathematical formulas that can be used to calculate the Hamaker functions are given elsewhere in Appendix I. In addition, ϵ_0 is the permittivity of free space, ϵ_R is the relative dielectric constant of the continuous phase, Ψ is the surface potential of the particles (in V), and κ^{-1} is the Debye screening length (m). For aqueous solutions at room temperatures, $\kappa^{-1} \approx 0.304/\sqrt{I}$ nm, where *I* is the ionic strength expressed in moles per liter (*32*).

The above approach assumes that the free polymer concentration in the continuous phase is zero, so that the depletion attraction can be ignored (*31*). This would mean that there were just enough polymer molecules present to completely saturate the surfaces of all of the droplets. In addition, it is assumed that there is an extremely strong steric repulsion between the particles when the interfacial layers overlap with each other. Thus, the steric repulsion would be infinitely large when the distance between the outer edges of the particle surfaces was less than zero but would be zero otherwise (*31*). It is also assumed that the electrical charge is located on the outer edge of the adsorbed layer, which is a reasonable assumption since theoretical calculations with multilayer systems have shown that the charge is localized at the outer edge (*33*).

RESULTS AND DISCUSSION

Determination of Adsorbed Layer Characteristics. The purpose of this set of experiments was to determine the optimum amount of pectin required to produce stable secondary emulsions containing oil droplets coated by polysaccharide−protein layers, as well as to obtain some quantitative information about the physical characteristics of the adsorbed polysaccharide layer. The ζ-potential and *z*-average diameter of emulsions (0.1 wt % corn oil, 0.005 wt % β-Lg, and 5 mM phosphate buffer, pH 4.0) containing different pectin concentrations (0−0.05 wt %) and NaCl concentrations (0 or 50 mM) were measured 24 h after preparation (**Figure 2a**,**b**). The creaming stability of the emulsions was also observed after 7 days of storage at room temperature (data not shown). In these experiments, the NaCl was added to the emulsions before pectin adsorption to the droplet surfaces (i.e., at pH 7).

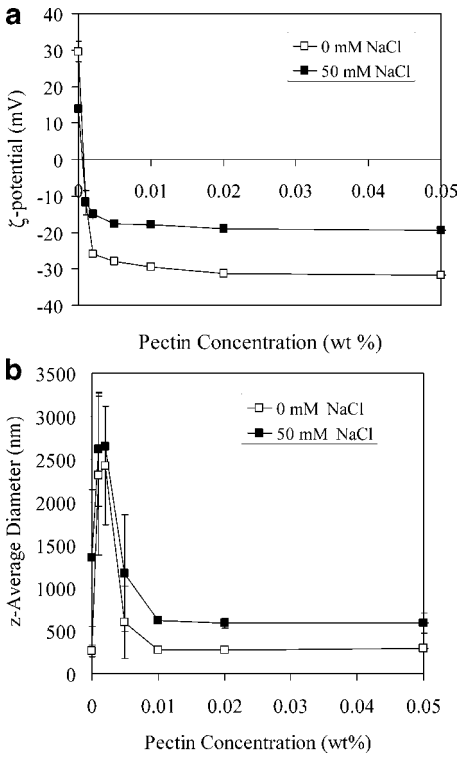

Figure 2. (**a**) Dependence of droplet ζ-potential of protein-stabilized oil-in-water emulsions on pectin concentration: 0.1 wt % corn oil, 0.005 wt % β-Lg, 5 mM sodium phosphate buffer, pH 4.0, and 0 and 50 mM NaCl (added before pH adjustment from 7 to 4). (**b**) Dependence of *z*-average particle diameter of protein-stabilized oil-in-water emulsions on pectin concentration: 0.1 wt % corn oil, 0.005 wt % β-Lg, 5 mM sodium phosphate buffer, pH 4.0, and 0 and 50 mM NaCl.

ζ-Potential Measurements. In both the absence and the presence of added salt, the ζ-potential changed from positive to negative as the pectin concentration was increased from 0 to 0.05 wt % (**Figure 2a**). There was a steep decrease in ζ-potential from 0 to 0.001 wt % pectin, after which a plateau value was attained somewhere above 0.002 wt % pectin. This data showed that anionic pectin molecules adsorbed to the surface of cationic protein-coated droplets until a critical pectin concentration was reached, after which the droplet surfaces were presumably covered with pectin molecules. Further pectin adsorption may have been prevented for other reasons: All of the available cationic binding sites on the protein-coated droplets were saturated with anionic groups from the pectin molecules, or there was an electrostatic repulsion between adsorbed and nonadsorbed pectin molecules.

The critical polysaccharide concentration where the droplet surfaces became saturated with pectin was established by modeling the ζ-potential vs polysaccharide concentration curves

Table 1. Electrical Characteristics of Interfaces in 0.1 wt % Oil-in-Water Emulsions Coated by Protein or Polysaccharide–Protein Layers at pH 4

parameter	0 mM NaCl	50 mM NaCl
ionic strength (mM)	23	73
κ^{-1} (nm)	2.0	1.1
ζ_0 (mV)	29.6	13.9
ζ_{Sat} (mV)	–31.2	–19.5
$\Delta\zeta$ (mV)	–60.7	–33.4
σ_0 (C m^{-2})	0.010	0.0088
σ_{Sat} (C m^{-2})	–0.011	–0.012
$\Delta\sigma$ (C m^{-2})	–0.021	–0.021
n_0 (nm^{-2})	0.065	0.055
n_{Sat} (nm^{-2})	–0.069	–0.077
Δn (nm^{-2})	–0.134	–0.131

using the following empirical equation:

$$\frac{\Delta\zeta(c)}{\Delta\zeta_{Sat}} = \frac{\zeta(c) - \zeta_{Sat}}{\zeta_0 - \zeta_{Sat}} \approx \exp\left(-\frac{c}{c^*}\right) \approx \exp\left(-\frac{c}{3c_{Sat}}\right) \quad (4)$$

where ζ_0, ζ(c), and ζ_{Sat} are the ζ-potentials of the emulsion droplets in the absence of polysaccharide, at polysaccharide concentration c, and when they are saturated with polysaccharide. The variable c^* is the polysaccharide concentration where the change in ζ-potential is $1/e$ of the total change in ζ-potential at saturation: $\Delta\zeta = \Delta\zeta_{Sat}/e$. The variable c_{Sat} provides an estimate of the minimum polysaccharide concentration required to cover the droplet surfaces, which can be estimated from these curves by assuming that the surface is saturated when the overall change in ζ-potential is 95% of $\Delta\zeta$: $c_{Sat} = -c^*\ln(0.05)$ or $c_{Sat} \approx 3c^*$. This value of 95% change was selected because it was close to full saturation (100%), while enabling an analytical expression to be obtained for c_{Sat}. The binding of a polysaccharide to the droplet surfaces can therefore be characterized by $\Delta\zeta_{Sat}$ and c_{Sat}. Values for $\Delta\zeta_{Sat}$ and c_{Sat} were calculated for the two different salt concentrations by fitting the above equation to the experimental ζ-potential vs pectin concentration curves (**Table 1**). The saturation concentration was found to be 0.003 wt % pectin regardless of the NaCl concentration in the range 0−50 mM. The saturation concentration depends on the size and concentration of emulsion droplets used; therefore, it is useful to determine a more system-independent quantity for the amount of adsorbed pectin. The surface load at saturation can be calculated using the following expression:

$$\Gamma_{Sat} = \frac{c_{Sat}\, d_{32}}{6\phi} \quad (5)$$

Here, c_{Sat} is the mass of material adsorbed to the surface of the droplets per unit volume of emulsion (C_a/kg m^{-3}), d_{32} is the volume−surface mean droplet diameter, and ϕ is the droplet volume fraction. For this study, $d_{32} = 0.32$ μm, $\phi = 0.001$ (0.1%), and $C_a \approx 0.03$ kg m^{-3} (0.003 wt %) at both 0 and 50 mM NaCl; hence, $\Gamma_{Sat} = 1.6$ mg m^{-2}. This value is in the range typically found for the surface loads of biopolymers adsorbed to the surfaces of colloidal particles (*34*). It is insightful to compare this value with that calculated from knowledge of the molecular characteristics of pectin molecules in solution. If it is assumed that a single pectin molecule adsorbs onto a surface with a cross-sectional area similar to that in solution, then its surface load would be given by:

$$\Gamma_{Sat} \approx \frac{m_P}{A_P} \approx \frac{M_P}{N_A \pi r_P^{\,2}} \quad (6)$$

Here, m_P is the mass of an individual polyelectrolyte molecule, A_P ($\approx \pi r_P^2$) is the cross-sectional area of the polyelectrolyte at the surface after adsorption, r_P is the effective radius of the polyelectrolyte molecule in solution, M_P is the molar mass of the polyelectrolyte, and N_A is Avogadro's number. The effective radius of polymers in solution is usually related to the molecular mass by the relation: $r_P^2 \propto M_P$ (*35*); hence, the above equation should not be particularly sensitive to molar mass. The molar mass and radius of gyration of a range of different pectins have been determined as follows: M_P from 30 to 300 kDa and r_g from 14 to 40 nm (*36*). Calculations of the surface load from these values give $\Gamma_{Sat} \approx 0.09 \pm 0.03$ mg m^{-2}, which is more than an order of magnitude less than the value determined from the ζ-potential measurements. We must therefore conclude that numerous pectin molecules adsorb on top of each other at the surface or that the cross-sectional area occupied by an adsorbed pectin molecule is much less than that in solution. Studies of the adsorption of well-defined synthetic polyelectrolytes onto oppositely charged surfaces indicate that the adsorbed molecules are highly interpenetrating at the surface (*21*, *37*). It would therefore be possible for multiple anionic pectin molecules to adsorb on top of each other to counterbalance the positive charges associated with the adsorbed protein layer below.

A number of useful electrical characteristics of the droplets can also be derived from the ζ-potential measurements (**Table 1**). The ζ-potentials of protein-coated (ζ_0) and polysaccharide−protein-coated (ζ_{Sat}) droplets were determined directly from the measured change in ζ-potential with pectin concentration in the absence and presence of added NaCl at pH 4.0 (**Figure 2a**). Surface charge density values were then calculated from the ζ-potential measurements using the following relationship, which assumes that the surface potential is relatively low and there is no surface charge regulation (Appendix II):

$$\sigma \approx 0.233\sqrt{I}\,\zeta \quad (7)$$

To utilize this relationship, it is necessary to know the ionic strength (I) of the aqueous solution surrounding the oil droplets. There will be a "background" ionic strength (I_b) due to ions arising from a variety of sources, including the buffer solution, acids, or bases used to adjust the pH, minerals associated with the protein or pectin, etc. It is difficult to directly determine the ionic strength of such a compositionally complex system, so we used an indirect approach based on ζ-potential measurements at two different salt concentrations, which is described in Appendix II (along with possible limitations of this approach). This approach led to a value of $I_b = 23$ mM for the background ionic strength across the whole pH range studied. The surface charge densities in the absence ($I = 23$ mM) and presence ($I = 73$ mM) of added salt were then determined using eq 7 and the ζ-potential measurements for 0 and 50 mM NaCl. The number of electrical charges per unit area (n) was determined from the surface charge density measurements: $n = \sigma/e$, where e is the unit of elementary charge ($=1.60 \times 10^{-19}$ C).

It is possible to use these experimentally determined values to obtain some valuable insights into the electrical properties of the adsorbed polysaccharide−protein layer. The surface charge density of an interface can be related to the surface load by the following relationship:

$$\sigma = \frac{\Gamma_{Sat} N_A z_P e}{M_P} \quad (8)$$

Here z_P is the effective valancy of the adsorbed pectin molecules. An estimate of z_P can be obtained from the above equation by

using the experimentally determined values of Γ_{Sat} and σ and assuming a molar mass of 100 kDa: $z = -14$. This value is much smaller than the number of carboxyl groups per pectin molecule and the fraction that should be ionized at the solution pH: $z_{full} \approx -170$, assuming $M_P = 100$ kDa, DE = 59%, and $pK_a = 3.5$. Consequently, many of the charged carboxyl groups on the pectin molecules must be associated with counterions (such as H^+ or Na^+) that reduce the net overall change in charge. The condensation of counterions onto pectin molecules in aqueous solution has previously been observed by NMR studies (*38*).

The magnitude of the ζ-potential was higher at 0 than at 50 mM NaCl for both the protein-coated droplets and the polysaccharide−protein-coated droplets, as was $\Delta\zeta$, which can be attributed to electrostatic screening effects (*32*, *39*).

Dynamic Light Scattering and Creaming Measurements. The influence of pectin concentration on the stability of the emulsions to droplet aggregation was measured by dynamic light scattering (**Figure 2b**) and visual observation of creaming (data not shown). At 0 mM NaCl, the emulsions were highly unstable to droplet aggregation and creaming at pectin concentrations less than 0.005 wt %. Possible physicochemical phenomena that can account for this observation include the following: (i) The ζ-potential was not large enough to generate a strong electrostatic repulsion between the droplets; (ii) there was insufficient pectin present to completely saturate the droplet surfaces so bridging flocculation occurred; and (iii) the pectin did not adsorb to the droplet surfaces fast enough before collisions occurred so bridging flocculation occurred (*19*). In the absence of pectin, mechanism (i) was probably the reason for the observed emulsion instability because the pH was close to the isoelectric point of the adsorbed proteins so that the ζ-potential was low. At pectin concentrations between 0 and 0.003 wt %, mechanism (ii) was probably responsible for droplet aggregation since there was insufficient pectin to completely cover the droplet surfaces (**Figure 2a**). At pectin concentrations from 0.003 to 0.005 wt %, mechanism (iii) was probably responsible for droplet aggregation since there should have been sufficient pectin to cover the droplet surfaces (**Figure 2a**), but the concentration may not have been high enough to favor pectin adsorption over droplet collisions. The presence of 50 mM NaCl in the emulsions increased the degree of droplet aggregation and creaming observed in the emulsions at all pectin concentrations from 0 to 0.1 wt %. This observation suggests that the droplets in the primary emulsions were only marginally stable to droplet flocculation and that even a small increase in the amount of salt was sufficient to screen the electrostatic repulsion and promote some aggregation (see later).

The dynamic light scattering measurements were also used to estimate the effective thickness of the pectin layer adsorbed to the droplet surfaces: $\delta = (d_{Sat} - d_0)/2$. The mean diameter of the particles in the emulsions containing no added salt (where no droplet flocculation was observed) was measured before (d_0) and after (d_{Sat}) pectin was added. The mean particle diameter increased from about $d_0 = 200 \pm 9$ nm in the protein-coated droplets (pH 7) to about $d_{Sat} = 290 \pm 10$ nm for the protein−polysaccharide-coated droplets (pH 4, 0.02 wt % pectin), which suggested that the hydrodynamic thickness of the adsorbed polysaccharide layer was about $\delta = 45 \pm 14$ nm. This value is fairly similar to the reported values for the diameter of pectin molecules in solution: 28−80 nm (*36*). In addition, it is fairly close to the value of 90 nm reported for the adsorption of pectin layers onto the surfaces of latex particles (*34*). The differences in the values may have been due to the different molecular characteristics of the pectins used in the two studies or differences in solution conditions. It should be mentioned that the thickness of the layers formed by synthetic polyelectrolytes at relatively low ionic strength tends to be much thinner than this value (a few nanometers) (*26*). It has been proposed that pectin consists of a negatively charged backbone, with fairly neutral side chains attached (*30*). It is therefore possible that the anionic backbone of pectin lies flat against the cationic adsorbed protein layer, whereas the neutral chains extend into the surrounding aqueous phase, thereby increasing the layer thickness.

Knowledge of the surface load (Γ_S) and the thickness of the adsorbed pectin layer (δ) can be used to estimate the packing of the pectin molecules within the adsorbed layer:

$$\Phi_P = \frac{V_P}{V_I} = \frac{\Gamma_S}{\delta \rho_P} \tag{9}$$

where V_P ($\approx m/\rho_P$) is the volume occupied by a mass m of polyelectrolyte chains within an interfacial layer of area A, V_I ($\approx A \times \delta$) is the volume of the overall interface in the same area, m/A ($=\Gamma_S$) is the mass of polyelectrolyte adsorbed per unit surface area, and ρ_P is the density of the polysaccharide chain ($\approx$1600 kg m^{-3}). Using the values for the surface load and interfacial thickness determined experimentally ($\Gamma_S = 1.6$ mg m^{-2}, $\delta = 45$ nm), we calculated that the volume fraction of the interface occupied by the polymer molecules was about 2.2%. This value is much higher than the value of about 0.09% calculated for free polymer molecules in solution ($\approx V_P/V_{sphere} = 3M_P/4\pi r_P^3 \rho_P N_A$), which again suggests that many pectin molecules adsorb on top of each other at the droplet surfaces.

In the remainder of this study, a pectin concentration of 0.02 wt % was used to prepare the secondary emulsions because this was high enough to completely saturate the droplet surfaces (**Figure 2a**) and to avoid droplet flocculation (**Figure 2b**) at 0 mM NaCl.

Effect of Salt Added before or after Pectin Adsorption. The purpose of these experiments was to examine the influence of salt, added either before or after the pectin had adsorbed to the surfaces of the protein-coated droplets, on the formation and stability of secondary emulsions.

Figure 3a shows the change in ζ-potential of secondary emulsions with salt concentration (0−100 mM NaCl) adjusted either before or after pectin adsorption to the droplet surfaces. The droplet ζ-potential decreased in magnitude (became less negative) as the NaCl concentration was increased, which can be attributed to electrostatic screening (*32*, *39*). Whether the salt was added before or after pectin adsorption had little effect on the dependence of ζ-potential on NaCl concentration (**Figure 3a**). This result implies that the amount and structure of the pectin molecules adsorbed to the droplet surfaces was independent of when the salt was added to the emulsions. On the other hand, the time when the salt was added to the secondary emulsions did have a major impact on their stability to droplet aggregation and creaming.

At all NaCl concentrations, the emulsions with salt added before pectin adsorption had larger particle sizes (**Figure 3b**) and were more susceptible to creaming (visible phase separation) than those with salt added after pectin adsorption. Indeed, the emulsions to which salt was added after pectin adsorption were stable at salt concentrations $\leq$100 mM NaCl, whereas those to which salt was added before pectin adsorption were only stable at $\leq$10 mM NaCl. These results indicate that the time when salt is added has a major impact on the formation and stability

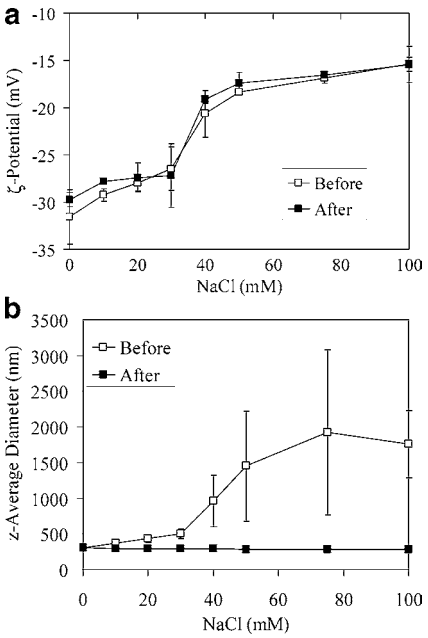

Figure 3. (**a**) Dependence of droplet ζ-potential of polysaccharide–protein-stabilized oil-in-water emulsions on NaCl concentration when the salt was added either *before* or *after* pectin adsorption: 0.1 wt % corn oil, 0.005 wt % β-Lg, 0.02 wt % pectin, and 5 mM sodium phosphate buffer, pH 4.0. (**b**) Dependence of *z*-average particle diameter of polysaccharide–protein-stabilized oil-in-water emulsions on NaCl concentration when the salt was added either *before* or *after* pectin adsorption: 0.1 wt % corn oil, 0.005 wt % β-Lg, 0.02 wt % pectin, and 5 mM sodium phosphate buffer, pH 4.0.

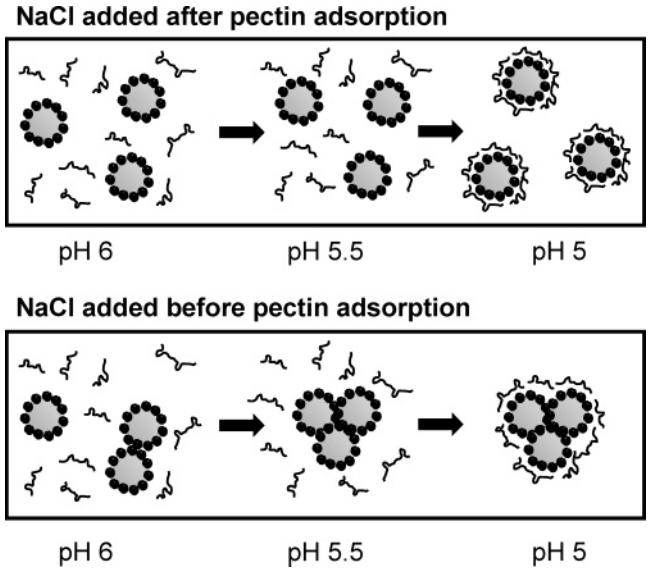

Figure 4. Schematic diagram of the influence of salt addition on the formation of stable secondary emulsions. If salt is absent during pectin adsorption, then pectin will adsorb to the surfaces of individual droplets. If salt is present during pectin adsorption, the droplets aggregate before the pectin can adsorb.

of secondary emulsions. The following mechanism was proposed to account for the observed phenomenon (**Figure 4**).

(i) NaCl Adjusted before Pectin Adsorption. The addition of salt to the emulsions at pH 7 reduced the electrostatic repulsion between the protein-coated droplets, which promoted some droplet flocculation (see next section). When the pH was reduced, the pectin molecules adsorbed onto the surfaces of flocs, rather than individual protein-coated droplets, which meant that the final emulsions contained relatively large particles that creamed rapidly.

(ii) NaCl Adjusted after Pectin Adsorption. In the absence of salt in the emulsions at pH 7, there was a relatively strong electrostatic repulsion between different protein-coated droplets, so that little droplet flocculation occurred. When the pH was decreased, the pectin molecules adsorbed onto the surfaces of individual protein-coated droplets, which accounts for the smaller mean particle diameter and better creaming stability.

Addition of increasing levels of salt to the secondary emulsions after pectin adsorption caused a slight decrease in the thickness of the adsorbed pectin layer, since the mean particle diameter decreased by about 13.9 ± 1.3 nm when the NaCl concentration was increased from 0 to 100 mM. This suggested that the interfacial thickness decreased by about 7.0 ± 0.7 nm. A possible explanation for this phenomenon is the weakening of the electrostatic repulsion between pectin molecules in the adsorbed layer, which allows them to pack more closely. This process is analogous to the decrease in the radius of hydration of charged polyelectrolytes in aqueous solution when salt is added (*35*).

Influence of pH on the Formation of Secondary Emulsions. To provide some additional experimental support for the mechanism proposed in **Figure 4**, the combined influence of solution pH and NaCl on the properties of primary and secondary emulsions to which salt was added *after* adjusting the pH was investigated. The pH dependencies of droplet ζ-potential, mean particle diameter, and creaming stability are shown in **Figure 5**.

At pH 7, the droplets in the primary emulsion had a high negative ζ-potential because the adsorbed protein was well above its isoelectric point (pI). The droplets in the secondary emulsion had a similar ζ-potential as those in the primary emulsion at this pH because both the protein-coated droplets and the polysaccharide molecules were strongly negatively charged, so the pectin would not have been expected to adsorb to the droplet surfaces. When the pH was reduced, pectin adsorbed to the protein-coated droplets (as demonstrated by a deviation in ζ-potential between the primary and secondary emulsions) starting at pH 5.5 and below (**Figure 5a**). The fact that pectin adsorbed to the droplet surfaces at pH values slightly above the pI (≈pH 5) of the protein molecules suggested that there was an electrostatic attraction between anionic groups on the polysaccharide and positive patches on the protein surfaces, as has been reported previously (*8*, *9*). It is interesting to note that pectin adsorption occurred at fairly similar pH values (around pH 5.5) for the emulsions containing 0 and 50 mM NaCl, which suggested that this level of salt did not alter the tendency for the pectin molecules to adsorb to the droplet surfaces. The reason that the magnitude of the ζ-potential was less for the emulsions containing 50 mM NaCl than for the ones containing 0 mM NaCl at all pH values can be attributed to electrostatic screening effects (*32*, *39*).

The influence of pH and NaCl on the stability of primary and secondary emulsions to droplet aggregation was assessed using dynamic light scattering (**Figure 5b**) and creaming stability (data not shown) measurements. In the absence of salt, extensive droplet aggregation and rapid creaming occurred in the primary emulsions over a range of intermediate pH values (pH 4.5 and 5.0). The origin of this droplet aggregation can be attributed to the relatively weak electrostatic repulsion between the droplets due to the low droplet ζ-potential near the pI of the adsorbed proteins (*31*, *40*). In the presence of 50 mM NaCl, the range of intermediate pH values where the primary emulsion

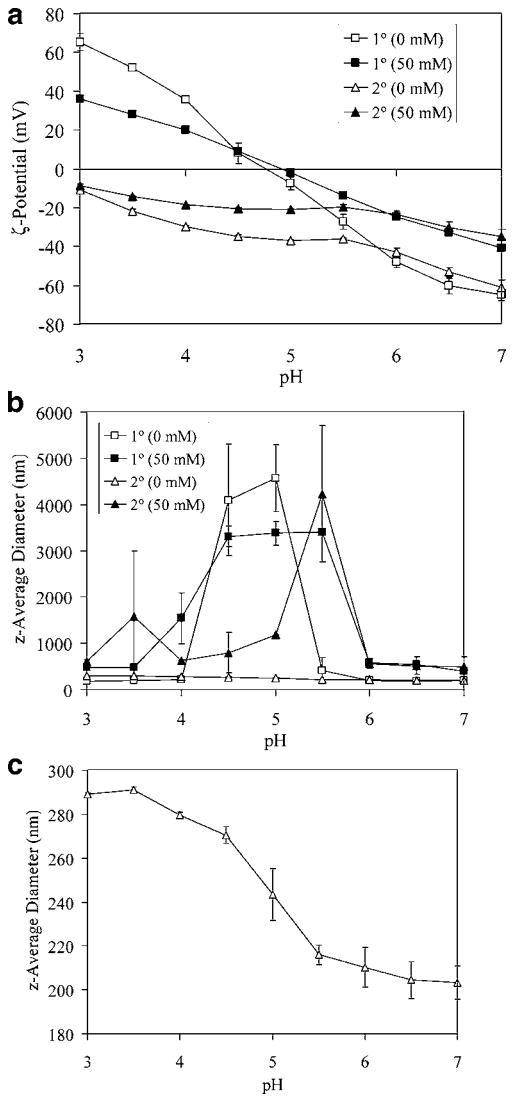

Figure 5. (**a**) Dependence of droplet ζ-potential on pH for primary and secondary emulsions: 0.1 wt % corn oil, 0.005 wt % β-Lg, 0 or 0.02 wt % pectin, 5 mM sodium phosphate buffer, and 0 or 50 mM NaCl. Any salt was added after pectin adsorption had occurred. (**b**) Dependence of *z*-average particle diameter on pH for primary and secondary emulsions: 0.1 wt % corn oil, 0.005 wt % β-Lg, 0 or 0.02 wt % pectin, 5 mM sodium phosphate buffer, and 0 or 50 mM NaCl. Any salt was added after pectin adsorption had occurred. (**c**) Dependence of *z*-average particle diameter on pH for secondary emulsions (0.1 wt % corn oil, 0.005 wt % β-Lg, 0 or 0.02 wt % pectin, 5 mM sodium phosphate buffer, and 0 mM NaCl). This figure suggests adsorption of pectin molecules onto droplet surfaces as the pH is decreased.

was highly unstable to droplet aggregation broadened (pH 4.0 to 5.5), which can be attributed to the fact that the salt further depressed the electrostatic repulsion between the droplets (*31*). In particular, appreciable droplet aggregation was observed at pH 5.5, which is the pH where the pectin first started to adsorb to the droplet surfaces when the pH was reduced from 7 (**Figure 5a**). There was also a limited amount of droplet aggregation in the primary emulsions containing 50 mM NaCl at pH 3.5 and at pH 6.0−7.0 (as indicated by an increase in mean particle diameter), which indicated that this level of salt promoted some flocculation even at pH values away from the pI. Previous studies with oil-in-water emulsions have shown that β-Lg-coated droplets are unstable to droplet aggregation when surface denaturation of the adsorbed protein molecules occurs (*41*−*43*).

In the secondary emulsion containing 0 mM NaCl, the mean particle diameter remained relatively low (d < 300 nm) and there was no visible evidence of creaming when the pH was reduced from 7.0 to 3.0 (**Figure 5b**). At the higher pH values (pH 5.5−7.0), this would have been because of the strong electrostatic repulsion between the protein-coated droplets, but at the lower pH range (pH 5.5 to 3.0), it would have been due to the stabilizing influence of the adsorbed pectin layer. The adsorption of the pectin molecules onto the droplet surfaces when the pH was reduced from 7.0 to 5.0 can be seen in **Figure 5c**, which shows the pH dependence of the mean particle diameter in the secondary emulsions containing no added salt.

There was an appreciable difference in the pH stability of the secondary emulsions containing 50 mM NaCl (**Figure 5b**). The mean particle diameter was similar to that in the primary emulsion from pH 5.5 to 7.0, presumably because the pectin did not adsorb under these conditions. Below pH 5.5, there was evidence of droplet aggregation and creaming instability (visual phase separation) in the secondary emulsions but less so than in the primary emulsions, suggesting that the adsorption of pectin did have some stabilizing effects. It is possible that the instability observed in the secondary emulsions containing salt at pH < 5.5 was because protein-coated droplets aggregated with each other before the pectin molecules could adsorb to the droplet surfaces (**Figure 4**). This would therefore explain the difference between the stability of emulsions to which NaCl was added before or after pectin adsorption discussed in the previous section.

Influence of Ionic Strength on the Properties of Emulsions with the Same Droplet Electrical Potential. The purpose of this series of experiments was to distinguish the effects of ionic strength from the effects of droplet electrical potential. This was achieved by preparing primary (−30 mV, pH 5.7) and secondary emulsions (−33 mV, pH 4.0) with the same initial ζ-potential by using different pH values. The salt was added to the secondary emulsions after the pectin had adsorbed to the droplet surfaces. The ζ-potential, particle diameter, and creaming stability of these primary and secondary emulsions were then measured with increasing ionic strength (**Figure 6**).

Figure 6a shows that the salt dependence of the ζ-potential was fairly similar for the primary and secondary emulsions, starting from ∼−30 mV and decreasing in magnitude as the NaCl concentration increased. This is to be expected because of electrostatic screening effects. **Figure 6b** shows the particle diameter of emulsions after the addition of salt. In the absence of salt, the primary emulsion was stable to droplet aggregation, but it showed extensive droplet aggregation and creaming (visual phase separation) at 50 mM NaCl and higher. On the other hand, the secondary emulsion was stable to droplet aggregation from 0 to 200 mM NaCl, after which there was an appreciable increase in particle diameter and visible creaming. This instability could have been because of a reduction in the electrostatic repulsion between the droplets and/or because pectin molecules (partially) desorbed from the droplet surfaces at these high salt concentrations due to the weakening of the electrostatic attraction between the polymer and droplets. These experiments show that secondary emulsions are more stable to salt than primary emulsions with the same ζ-potential.

Numerical Calculations. In this section, we use the equations for the van der Waals and electrostatic interaction potentials described in eqs 1−3 to explore the influence of an adsorbed polymer layer on the aggregation stability of oil droplets. In all of the calculations, it was assumed that the interactions occurred between two oil droplets (r = 0.1 μm) coated by an interfacial

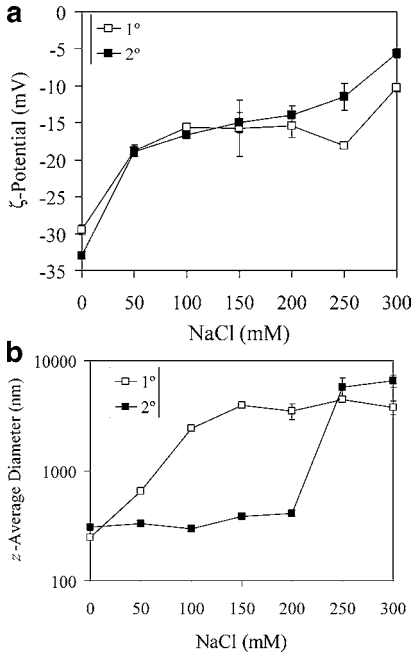

Figure 6. (**a**) Dependence of droplet ζ-potential of primary and secondary emulsions on NaCl concentration: 0.1 wt % corn oil, 0.005 wt % β-Lg, 0 or 0.02 wt % pectin, and 5 mM sodium phosphate buffer. The pH was controlled so that both emulsions had approximately the same initial ζ-potential: pH 5.7 for primary emulsion and pH 4.0 for secondary emulsion. Any salt was added after pectin adsorption had occurred. (**b**) Dependence of *z*-average particle diameter of primary and secondary emulsions on NaCl concentration: 0.1 wt % corn oil, 0.005 wt % β-Lg, 0 or 0.02 wt % pectin, and 5 mM sodium phosphate buffer. The pH was controlled so that both emulsions had approximately the same initial ζ-potential: pH 5.7 for primary emulsion and pH 4.0 for secondary emulsion. Any salt was added after pectin adsorption had occurred.

layer ($\delta = 45$ nm, $\Phi = 0.02$) suspended in an aqueous solution at ambient temperature ($T = 30$ °C). The values of the droplet radius (r), interfacial thickness (δ), and interfacial packing (Φ) used in the calculations were selected to be similar as those determined in our experiments at pH 4. The physicochemical parameters used to calculate the droplet interactions were $\epsilon_R = 80$, 2 and $5\Phi + 80(1 - \Phi)$; $n = 1.333$, 1.433, and $1.56\Phi + 1.333(1 - \Phi)$; and $\upsilon_e = 3.0$, 2.9, and 3.0×10^{15} s^{-1} for the water, oil, and interfacial regions, respectively (*31*). Here, Φ is the fraction of the polymer in the interfacial layer, which varies from 0 for pure water to 1 for pure polymer.

In this section, the term "noncoated" refers to bare oil droplets, whereas the term "coated" means oil droplets covered with a homogeneous interfacial layer. The noncoated droplets should therefore represent the particles in the primary emulsion used in this study (oil droplets coated with a thin protein layer), whereas the coated droplets represent the particles in the secondary emulsion (oil droplets coated by a thick protein−polysaccharide layer).

The electrostatic, van der Waals, and total interaction potentials for noncoated and coated droplets with the same electrical potential ($\Psi = 15$ mV) and ionic strength ($I = 50$ mM) were calculated (**Figure 7**). For the noncoated droplets, the van der Waals interaction becomes increasingly attractive with decreasing separation, whereas the electrostatic interaction becomes increasingly repulsive (**Figure 7a**). The electrostatic repulsion dominated at large droplet separations, whereas the van der Waals attraction dominated at close droplet separations.

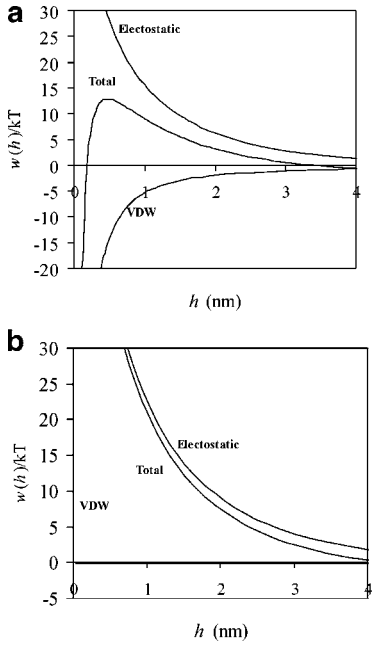

Figure 7. Comparison of the van der Waals, electrostatic, and total interaction potentials for (**a**) noncoated and (**b**) coated droplets. It was assumed that $\Psi = 15$ mV, $I = 50$ mM, $r = 0.1$ μm, $\delta = 45$ nm, $\Phi = 0.02$, and $T = 30$ °C.

At intermediate droplet separations, there was a maximum in the interaction potential, which is usually referred to as the repulsive energy barrier, w_{Max}. If this energy barrier is sufficiently high, it will effectively prevent the droplets from coming close enough together to aggregate on a reasonable time scale. Predictions have shown that about 50% of the droplets in an emulsion will flocculate in 1 h for $w_{Max}/kT \sim 10$, in about 1 day for $w_{Max}/kT \sim 15$, and in about 3 years for $w_{Max}/kT \sim 20$ (*44*). To prevent the particles in an electrostatically stabilized system from aggregating, it is therefore necessary to ensure that this energy barrier is sufficiently high. The value of w_{Max}/kT decreases with increasing ionic strength; for example, for the noncoated system shown in **Figure 7a**, $w_{Max}/kT = 27$, 21, 13, 8, and 3 for $I = 0.01$, 0.02, 0.05, 0.1, and 0.2 M. Consequently, one would expect the emulsion to breakdown somewhere around 50−100 mM NaCl, which is what was observed in the experimental study.

For the coated droplets, the electrostatic interaction was fairly similar to that in the noncoated droplets, but the van der Waals interaction was almost completely absent at all separations (**Figure 7b**). This great reduction in the strength of the van der Waals attraction can be attributed to the fact that the interfacial layer is largely water (≈98%). Thus, when the outer surfaces of the coated droplets are touching, there has already been a substantial reduction in the strength of the van der Waals attraction between the two oil droplets inside. As a consequence, the total interaction potential is dominated by the electrostatic repulsion for coated droplets. This suggests that coated droplets should be more stable to NaCl-induced aggregation than noncoated droplets with the same electrical potential or that a lower ζ-potential is needed to ensure emulsion stability for a coated droplet than for a noncoated droplet at the same ionic strength. This prediction would therefore account for the experimental observation that secondary emulsions were more stable than primary emulsions to NaCl-induced aggregation when the droplets had the same ζ-potential (**Figure 3**) and that at the same ionic strength secondary emulsions were more stable than primary emulsions with higher ζ-potentials (**Figure 5**).

It should be noted that we used relatively dilute oil-in-water emulsions (0.1 wt %) in this study to avoid bridging flocculation effects and that at high droplet concentrations (>5 wt %) there may be problems with preparing stable multilayer emulsions (*45*).

In conclusion, in this manuscript, the influence of electrostatic interactions on the formation of stable multilayer emulsions based on the electrostatic deposition of anionic polysaccharide molecules onto the surfaces of protein-coated droplets was examined. At relatively high pH values, the protein-coated droplets and polysaccharide molecules were both strongly negatively charged and so no adsorption occurred. When the pH was decreased, positive patches developed on the surface of the protein-coated droplets, which promoted adsorption of the negatively charged pectin molecules. In the absence of salt, the protein-coated droplets had a strong electrostatic repulsion between them that prevented flocculation. Consequently, the pectin molecules adsorbed onto the surfaces of individual droplets leading to the formation of stable multilayer emulsions. On the other hand, in the presence of salt, the electrostatic repulsion between the protein-coated droplets was reduced, which promoted flocculation. In this case, the pectin molecules adsorbed onto the surface of flocs, rather than individual droplets. This study indicates that one must therefore be careful to ensure that the primary emulsion is stable prior to adsorption of the next polyelectrolyte layer. In future studies, it would be useful to systematically identify and quantify the key molecular characteristics of pectin required to provide a good protective layer around protein-coated droplets, e.g., molecular weight, charge density (DE), and structure.

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APPENDIX I

The Hamaker functions appropriate for a system consisting of droplets (material 1) coated by interfacial membranes (material 2) that are suspended in a continuous phase (material 3) can be calculated using the equations given below (**Figure 1**) (*46*). The Hamaker function can be divided into a "zero-frequency" and a "frequency-dependent" contribution due to differences in the origin of the various van der Waal interactions (*46*):

$$A_{ijk} = A_{ijk,\upsilon=0} + A_{ijk,\upsilon>0} \tag{I.1}$$

where

$$A_{ijk,\upsilon=0} = \frac{3}{4} kT\left(\frac{\epsilon_i - \epsilon_j}{\epsilon_i + \epsilon_j}\right)\left(\frac{\epsilon_k - \epsilon_j}{\epsilon_k + \epsilon_j}\right) \tag{I.2}$$

$$A_{ijk,\upsilon>0} = \frac{3h\upsilon_e}{8\sqrt{2}} \frac{(n_i^2 - n_j^2)(n_k^2 - n_j^2)}{(n_i^2 + n_j^2)^{1/2}(n_k^2 + n_j^2)^{1/2}\{(n_i^2 + n_j^2)^{1/2} + (n_k^2 + n_j^2)^{1/2}\}} \tag{I.3}$$

The $A_{ijk,\upsilon=0}$ term represents the nonscreened "zero-frequency" contribution, and the $A_{ijk,\upsilon>0}$ term represents the nonretarded "frequency-dependent" contribution (*46*). Here, k is Boltzmann's constant (1.381×10^{-23} J K^{-1}), T is the absolute temperature, h is Planck's constant (6.626×10^{-34} J s), ϵ is the static relative dielectric constant, n is the refractive index, and υ_e is the major electronic absorption frequency in the ultraviolet region of the electromagnetic spectrum.

The influence of electrostatic screening and retardation can be taken into account using the following expression (*41*, *46*):

$$A_{ijk} = [e^{-2\kappa h}]A_{ijk,\upsilon=0} + \left[1 - 5.32 \times 10^7 h \ln\left(1 + \frac{1}{5.32 \times 10^7 h}\right)\right]A_{ijk,\upsilon>0} \tag{I.4}$$

The prefactors in the square brackets take into account the influence of electrostatic screening on $A_{\upsilon=0}$ and retardation on $A_{\upsilon>0}$, respectively. Here, κ^{-1} is the Debye screening length (see text).

APPENDIX II

When the electrostatic interaction between a charged surface and the counterions is relatively weak and there is no surface charge regulation, the surface charge density is simply related to the surface potential (*39*):

$$\sigma = \epsilon_R \epsilon_0 \kappa \Psi_\delta \tag{II.1}$$

For aqueous solutions at room temperatures, $\kappa \approx 3.29 \times 10^9 \sqrt{I}$ nm^{-1} and $\epsilon_R \approx 80$ where I is the ionic strength expressed in moles per liter (*31*). Experimentally, one can usually replace the surface potential with the ζ-potential, i.e., $\Psi_\delta \approx \zeta$. Hence, the above equation can be approximated by:

$$\sigma \approx 0.233\sqrt{I}\,\zeta \tag{II.2}$$

If one assumes that the surface charge density is unaffected by salt, which is usually the case for relatively low concentrations of indifferent ions (*39*), then a simple relationship can be developed between the ζ-potential values measured in the absence ($\zeta[0]$) and presence ($\zeta[m]$) of a known amount of added salt (m):

$$\zeta(0)\sqrt{I_b} = \zeta(m)\sqrt{I_b + m} \tag{II.3}$$

This equation can be rearranged to give an expression for the background ionic strength:

$$I_b = m\left[\left(\frac{\zeta(0)}{\zeta(m)}\right) - 1\right]^{-1} \tag{II.4}$$

This equation was used to predict the background ionic strength of primary and secondary emulsions containing 0 or 50 mM as a function of pH. Over the pH range 3−7, we found that I_b was equal to 23 ± 5 mM for the primary emulsion (ignoring pH 4.0 and 4.5) and 23 ± 4 mM for the secondary emulsion (ignoring pH 3.0 and 3.5). Some values were not included in the calculations because the magnitude of the ζ-potential was relatively small (<20 mV), which would have caused large errors.

The calculated values (~23 mM) appear to be somewhat higher than what might be expected from knowledge of the solution composition. The ionic strength of the 5 mM phosphate buffer solution used in this study is ~9 mM. Some mineral impurities would come from the protein and polysaccharide used to prepare the emulsions; however, the biopolymer levels used were so small (<0.1 wt %) that this mineral contribution would have been expected to be small (<1 mM). In addition, there may have been some additional ions coming from the acid or

base solutions used to adjust pH, but again, this contribution would have been expected to be relatively small. The difference between the I_b values calculated using the above approach and those expected from the solution composition may have been because some of the assumptions underlying the above theory were not applicable. First, the linearized form of the Poisson−Boltzmann equation is assumed in derivation of eq II.1, which is only valid for relatively low surface potentials (<25 mV); however, many of our ζ-potentials measurements were somewhat above this value. Second, the above approach assumes that the surface charge density is unaffected by salt (i.e., there is no charge regulation), which may not be the case if counterions are bound or released to the biopolymers in the adsorbed layer when the ionic strength is changed. Despite the limitations mentioned above, this approach does appear to provide a useful first approximation that provides some valuable insights into the electrical characteristics of the adsorbed layers. In addition, the above approach could be further developed using more sophisticated mathematical models to increase its range of applicability.

LITERATURE CITED

(1) Guzey, D.; Kim, H. J.; McClements, D. J. Factors influencing the production of O/W emulsions stabilized by beta-lactoglobulin-pectin membranes. *Food Hydrocolloids* **2004**, *18* (6), 967−975.

(2) Moreau, L.; Kim, H. J.; Decker, E. A.; McClements, D. J. Production and characterization of oil-in-water emulsions containing droplets stabilized by beta-lactoglobulin-pectin membranes. *J. Agric. Food Chem.* **2003**, *51* (22), 6612−6617.

(3) Dickinson, E. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids* **2003**, *17* (1), 25−39.

(4) Dickinson, E.; Pawlowsky, K. Effect of l-carrageenan on flocculation, creaming, and rheology of a protein-stabilized emulsion. *J. Agric. Food Chem.* **1997**, *45* (10), 3799−3806.

(5) Dickinson, E.; Pawlowsky, K. Influence of kappa-carrageenan on the properties of a protein-stabilized emulsion. *Food Hydrocolloids* **1998**, *12* (4), 417−423.

(6) Damodaran, S. Amino acids, peptides and proteins. In *Food Chemistry*, 3rd ed.; Fennema, O. R., Ed.; Marcel Dekker: New York, 1996; pp 321−429.

(7) Cui, S. W. *Food Carbohydrates: Chemistry, Physical Properties and Applications*; Taylor and Francis: Boca Raton, FL, 2005.

(8) Gu, Y. S.; Decker, A. E.; McClements, D. J. Production and characterization of oil-in-water emulsions containing droplets stabilized by multilayer membranes consisting of beta-lactoglobulin, iota-carrageenan and gelatin. *Langmuir* **2005**, *21* (13), 5752−5760.

(9) Gu, Y. S.; Decker, E. A.; McClements, D. J. Influence of pH and carrageenan type on properties of beta-lactoglobulin stabilized oil-in-water emulsions. *Food Hydrocolloids* **2005**, *19* (1), 83−91.

(10) Gu, Y. S.; Regnier, L.; McClements, D. J. Influence of environmental stresses on stability of oil-in-water emulsions containing droplets stabilized by beta-lactoglobulin-iota-carrageenan membranes. *J. Colloid Interface Sci.* **2005**, *286* (2), 551−558.

(11) Harnsilawat, T.; Pongsawatmanit, R.; McClements, D. J. Characterization of [beta]-lactoglobulin-sodium alginate interactions in aqueous solutions: A calorimetry, light scattering, electrophoretic mobility and solubility study. *Food Hydrocolloids* **2006**, *20* (5), 577−585.

(12) Laplante, S.; Turgeon, S. L.; Paquin, P. Emulsion stabilizing properties of various chitosans in the presence of whey protein isolate. *Carbohydr. Polym.* **2005**, *59* (4), 425−434.

(13) Guzey, D.; McClements, D. J. Influence of environmental stresses on O/W emulsions stabilized by β-lactoglobulin−pectin and β-lactoglobulin−pectin−chitosan membranes produced by the electrostatic layer-by-layer deposition technique. *Food Biophys.* **2006**, *1* (1), 30−40.

(14) Aoki, T.; Decker, E. A.; McClements, D. J. Influence of environmental stresses on stability of O/W emulsions containing droplets stabilized by multilayered membranes produced by a layer-by-layer electrostatic deposition technique. *Food Hydrocolloids* **2005**, *19* (2), 209−220.

(15) Ogawa, S.; Decker, E. A.; McClements, D. J. Influence of environmental conditions on the stability of oil in water emulsions containing droplets stabilized by lecithin-chitosan membranes. *J. Agric. Food Chem.* **2003**, *51* (18), 5522−5527.

(16) Klinkesorn, U.; Sophanodora, P.; Chinachoti, P.; McClements, D. J.; Decker, E. A. Increasing the oxidative stability of liquid and dried tuna oil-in-water emulsions with electrostatic layer-by-layer deposition technology. *J. Agric. Food Chem.* **2005**, *53* (11), 4561−4566.

(17) Klinkesorn, U.; Sophanodora, P.; Chinachoti, P.; McClements, D. J.; Decker, E. A. Stability of spray-dried tuna oil emulsions encapsulated with two-layered interfacial membranes. *J. Agric. Food Chem.* **2005**, *53* (21), 8365−8371.

(18) Gu, Y. S.; Decker, E. A.; McClements, D. J. Influence of iota-carrageenan on droplet flocculation of beta-lactoglobulin-stabilized oil-in-water emulsions during thermal processing. *Langmuir* **2004**, *20* (22), 9565−9570.

(19) McClements, D. J. Theoretical analysis of factors affecting the formation and stability of multilayered colloidal dispersions. *Langmuir* **2005**, *21* (21), 9777−9785.

(20) Caruso, F.; Lichtenfeld, H.; Donath, E.; Mohwald, H. Investigation of electrostatic interactions in polyelectrolyte multilayer films: Binding of anionic fluorescent probes to layers assembled onto colloids. *Macromolecules* **1999**, *32* (7), 2317−2328.

(21) Schonhoff, M. Self-assembled polyelectrolyte multilayers. *Curr. Opin. Colloid Interface Sci.* **2003**, *8* (1), 86−95.

(22) Caruso, F.; Caruso, R. A.; Donath, E.; Mohwald, H.; Sukhorukov, G. B. Fabrication of multilayer-coated particles and hollow shells via electrostatic self-assembly of nanocomposite multilayers on decomposable colloid templates. 2002.

(23) Caruso, F.; Mohwald, H. Protein multilayer formation on colloids through a stepwise self-assembly technique. *J. Am. Chem. Soc.* **1999**, *121* (25), 6039−6046.

(24) Caruso, F.; Schuler, C. Enzyme multilayers on colloid particles: Assembly, stability, and enzymatic activity. *Langmuir* **2000**, *16* (24), 9595−9603.

(25) Decher, G. Fuzzy nanoassemblies: Toward layered polymeric multicomposited. *Science* **1997**, *277*, 1232−1237.

(26) Decher, G.; Schlenoff, J. B. *Multilayer Thin Films: Sequential Assembly of Nanocomposite Materials*; Wiley-VCH: Weinheim, 2003; pp xix, 524.

(27) Sawyer, L.; Kontopidis, G. The core lipocalin, bovine beta-lactoglobulin. *Biochim. Biophys. Acta* **2000**, *1482* (1−2), 136−148.

(28) Bromley, E. H. C.; Krebs, M. R. H.; Donald, A. M. Aggregation across the length-scales in beta-lactoglobulin. *Faraday Discuss.* **2005**, *128*, 13.

(29) Magdassi, S.; Bach, U.; Mumcuoglu, K. Y. Formation of positively charged microcapsules based on chitosan-lecithin interactions. *J. Microencapsulation* **1997**, *14* (2), 189−195.

(30) Willats, W. G. T.; Knox, J. P.; Mikkelsen, D. Pectin: New insights into an old polymer are starting to gel. *Trends Food Sci. Technol.* **2006**, *17*, 97−104.

(31) McClements, D. J. *Food Emulsions: Principles, Practice, and Techniques*, 2nd ed.; CRC Press: Boca Raton, 2004.

(32) Israelachvili, J. N. *Intermolacular and Surface Forces*, 2nd ed.; Academic Press Inc.: San Diego, CA, 1992; p 450.

(33) Caruso, F. S.; Sukhorukov, G. B. In *Multilayer Thin Films: Sequential Assembly of Nanocomposite Materials*; Decher, G.

S., Schlenoff, J. B., Eds.; Wiley-VCH: Weinheim, Germany, 2003; pp 331−362.
(34) Tuinier, R.; Rolin, C.; de Kruif, C. G. Electrosorption of pectin onto casein micelles. *Biomacromolecules* **2002**, *3* (3), 632−638.
(35) Teraoka, I. *Polymer Solutions: Introduction to Physical Properties*; John Wiley & Sons: New York, 2002.
(36) Fishman, M. L.; Chau, H. K.; Kolpak, F.; Brady, J. Solvent effects on the molecular properties of pectins. *J. Agric. Food Chem.* **2001**, *49* (9), 4494−4501.
(37) Schönhoff, M. Layered polyelectrolyte complexes: Physics of formation and molecular properties. *J. Phys.: Condens. Matter* **2003**, *15*, R1781−R1808.
(38) Catoire, L.; Derouet, C.; Redon, A. M.; Goldberg, R.; duPenhoat, C. H. An NMR study of the dynamic single-stranded conformation of sodium pectate. *Carbohydr. Res.* **1997**, *300* (1), 19−29.
(39) Hunter, R. J. *Foundations of Colloid Science*; Oxford University Press: Oxford, 1986; Vol. 1.
(40) McClements, D. J. Protein-stabilized emulsions. *Curr. Opin. Colloid Interface Sci.* **2004**, *9* (5), 305−313.
(41) Kim, H. J.; Decker, E. A.; McClements, D. J. Role of postadsorption conformation changes of beta-lactoglobulin on its ability to stabilize oil droplets against flocculation during heating at neutral pH. *Langmuir* **2002**, *18* (20), 7577−7583.
(42) Kim, H. J.; Decker, E. A.; McClements, D. J. Influence of protein concentration and order of addition on thermal stability of beta-lactoglobulin stabilized n-hexadecane oil-in-water emulsions at neutral pH. *Langmuir* **2005**, *21* (1), 134−139.
(43) Kim, H. J.; Decker, E. A.; McClements, D. J. Influence of free protein on flocculation stability of beta-lactoglobulin stabilized oil-in-water emulsions at neutral pH and ambient temperature. *Langmuir* **2004**, *20* (24), 10394−10398.
(44) Friberg, S.; Larsson, K.; Sjoblom, J. *Food Emulsions*, 4th ed.; Marcel Dekker: New York, 2004.
(45) Gu, Y. S.; Decker, E. A.; McClements, D. J. Influence of pH and carrageenan type on properties of β-lactoglobulin stabilized oil-in-water emulsions. *Food Hydrocolloids* **2005**, *19* (1), 83.
(46) Vold, M. J. Effect of adsorption on Van Der Waals interaction of spherical colloidal particles. *J. Colloid Sci.* **1961**, *16* (1), 1.

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