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Influence of Environmental Stresses on Stability of O/W Emulsions Containing Cationic Droplets Stabilized by SDS−Fish Gelatin Membranes

JEONGHEE SURH, YEUN SUK GU, ERIC A. DECKER, AND D. JULIAN MCCLEMENTS*

Biopolymers and Colloids Research Laboratory, Department of Food Science, University of Massachusetts, Amherst, Massachusetts 01003

Oil-in-water (O/W) emulsions containing small oil droplets ($d_{32} \approx 0.22\ \mu$m) stabilized by sodium dodecyl sulfate (SDS)−fish gelatin (FG) membranes were produced by an electrostatic deposition technique. A primary emulsion containing anionic SDS-coated droplets ($\zeta \approx -40$ mV) was prepared by homogenizing oil and emulsifier solution using a high-pressure valve homogenizer (20 wt % corn oil, 0.46 wt % SDS, 100 mM acetic acid, pH 3.0). A secondary emulsion containing cationic SDS−FG-coated droplets ($\zeta \approx +30$ mV) was formed by diluting the primary emulsion with an aqueous fish gelatin solution (10 wt % corn oil, 0.23 wt % SDS, 100 mM acetic acid, 2.00 wt % fish gelatin, pH 3.0). The stabilities of primary and secondary emulsions with the same oil concentration to thermal processing, ionic strength, and pH were assessed by measuring particle size distribution, ζ potential, microstructure, destabilized oil, and creaming stability. The droplets in secondary emulsions had good stability to droplet aggregation at holding temperatures from 30 to 90 °C for 30 min, [NaCl] ≤ 100 mM, and pH values from 3 to 8. This study shows that the ability to generate emulsions containing droplets stabilized by multilayer interfacial membranes comprised of two or more types of emulsifiers, rather than a single interfacial layer comprised of one type of emulsifier, may lead to the development of food products with improved stability to environmental stresses.

KEYWORDS: Emulsion; gelatin; SDS; stability; ζ potential

INTRODUCTION

Emulsions are thermodynamically unstable systems because of the unfavorable contact between oil and water phases, and so they will always break down over time (*1*−*4*). Conventionally, oil-in-water (O/W) emulsions are created by homogenizing an oil phase and an aqueous phase together in the presence of one or more emulsifiers, because emulsifiers increase the short- and long-term kinetic stability of food emulsion systems (*4*, *5*). Emulsifiers are surface-active ingredients that adsorb at interfaces and facilitate the production of small droplets by lowering the interfacial tension during homogenization (*6*, *7*). Emulsifiers also improve the stability of emulsions to droplet aggregation by generating repulsive forces between the droplets and/or by forming interfacial membranes around the droplets that are resistant to rupture (*4*−*6*, *8*).

A wide variety of synthetic and natural emulsifiers, including small-molecule surfactants, phospholipids, proteins, and polysaccharides, is legally available for utilization in food emulsions (*9*−*11*). However, these emulsifiers have considerably different abilities to form and stabilize emulsions. In other words, each type of emulsifier varies in its effectiveness at producing small oil droplets during homogenization and in its ability to prevent droplet aggregation under various environmental stresses such as pH, ionic strength, heating, freezing, and drying. For example, some emulsifiers are highly effective at generating small emulsion droplets during homogenization but are less effective at providing long-term stability against droplet aggregation and vice versa (*8*). Consequently, there is no single emulsifier ideal for every type of food product.

Recently, our laboratory has utilized a technology that enabled us to combine the beneficial attributes of different kinds of emulsifiers to create emulsions with improved stability to environmental stresses (*12*−*18*). This technique is based on layer-by-layer deposition of polyelectrolytes onto oppositely charged surfaces or colloidal particles due to electrostatic attraction. This technology provides food scientists with an extremely powerful new method of improving the resistance of food emulsions to environmental stresses. Nevertheless, it is essential to select an appropriate combination of emulsifier and biopolymer to create a stable emulsion with the desired physicochemical characteristics.

In this study, we intend to prepare emulsions containing cationic droplets surrounded by thick interfacial membranes

* To whom correspondence should be addressed. Tel.: +1-413-545-1019. Fax: +1-413-545-1262. E-mail: mcclements@foodsci.umass.edu.

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using the electrostatic deposition technique described above. Emulsions containing cationic droplets coated with relatively thick interfacial layers may have important applications in the food industry, because they could provide better stability to particle aggregation (*19*−*23*) and lipid oxidation (*24*−*26*) than those coated with thinner layers or those containing anionic droplets. For this reason, we selected sodium dodecyl sulfate (SDS) as an anionic surfactant and fish gelatin (FG) as a cationic biopolymer for preparation of two-layered interfacial membranes. SDS was chosen as a model anionic surfactant, because its physicochemical properties have been widely studied and reported in the literature. It should be noted that SDS is not a food-grade emulsifier, but it does represent a number of small-molecule anionic surfactants that are commonly used in foods, such as fatty acid salts, lecithin, SLS, DATEM, and CITREM (*11*). Previously, we have shown that SDS rapidly adsorbs to the surfaces of lipid droplets formed during homogenization to produce primary emulsions containing small anionic droplets. On the basis of our previous work with other biopolymers, we expected that addition of cationic FG to this primary emulsion would produce secondary emulsions containing cationic droplets coated with two-layered interfacial membranes. FG is regarded as a potential stabilizer for food emulsions because of its unique functional attributes, natural abundance, and underutilization (*27*−*30*).

Another possible application of the layer-by-layer deposition method mentioned above is to improve the stability of emulsified oils to lipid oxidation. In a previous study, our laboratory developed a technology that used proteins (whey, casein, soy) to produce relatively thick cationic emulsion droplets (*31*). This thick and positively charged (at pH < pI) membrane can be used to produce physically and oxidatively stable emulsions. However, most proteins have pI values in the range of 4.5−5.5 and, thus, cationic emulsion droplets can only be produced at pH values below 4.0. On the other hand, FG has a relatively high isoelectric point (pI ≥ 7.0) (*32*), and so it was selected as the outer layer for preparation of secondary emulsions which would potentially have cationic droplets over a wider pH range.

The primary objective of the current investigation was to improve the physical stability of conventional monolayered emulsions to environmental stresses by incorporating FG as a second layer. We prepared and characterized O/W emulsions stabilized by SDS−FG membranes created by electrostatic deposition. In particular, we compared the stabilities of the primary and secondary emulsions to thermal processing, ionic strength, and pH.

MATERIALS AND METHODS

Materials. Sodium dodecyl sulfate (SDS), analytical grade sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH), Oil Red O, and sodium azide (NaN_3) were purchased from the Sigma Chemical Co. (St. Louis, MO). Acetic acid was purchased from Fisher Science (Chicago, IL). Food/pharmaceutical grade dry fish gelatin (FG) was obtained from Norland Products Inc. (Lot # 1253KD, Cranbury, NJ). As stated by the manufacturer, the fish gelatin (solids, 85% minimum; weight loss on drying, 15% maximum; total ash, 2.0% maximum; heavy metals, <10 ppm; arsenic, <0.8 ppm; chromium, <10 ppm; lead, <1.5 ppm; sulfur dioxide, <50 ppm), type A gelatin, was prepared by acid hydrolysis of collagen from cod skins. The average molecular weight was 60 kDa, and the isoelectric point (IEP) was around 7. Corn oil was purchased from a local supermarket and used without further purification. 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 into water and then adjusting the pH to 3.0 using 1 M HCl. An emulsifier solution was prepared by dispersing 20 mM (0.58 wt %) SDS into the stock buffer solution and stirring overnight to ensure complete dissolution. An FG solution was prepared by dispersing 4.0 wt % FG into the same buffer (pH 3.0) and stirring overnight to ensure complete hydration. The pH of these two solutions was then adjusted back to pH 3.0 using 1 M HCl.

Emulsion Preparation. An oil-in-water emulsion was prepared by blending 20 wt % corn oil and 80 wt % aqueous emulsifier solution (0.58 wt % SDS, pH 3.0) together using a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland) for 2 min. This coarse emulsion was then passed through a two-stage high-pressure valve homogenizer (LAB 1000, APV-Gaulin, Wilmington, MA) three times: 4500 psi at the first stage and 500 psi at the second stage. A 0.04 wt % portion of sodium azide (NaN_3) was added to the emulsion as an antimicrobial agent. This primary emulsion (20 wt % corn oil, 0.46 wt % SDS, pH 3.0) was diluted with an aqueous FG stock solution (4.0 wt %, pH 3.0) and 100 mM acetate buffer solution to yield secondary emulsions with the following compositions: 10 wt % corn oil, 0.23 wt % SDS, 0−2.0 wt % FG, and 100 mM acetic acid (pH 3.0). The secondary emulsions were stirred for 10 min with or without subsequent ultrasound treatment for 60 s at a frequency of 20 kHz, an amplitude of 40%, and a duty cycle of 0.5 s (Model 500 sonic dismembrator, Fisher Scientific, Pittsburgh, PA). Emulsions were then stored at ambient temperature for 24 h before being analyzed.

Emulsion Environmental Stresses. We compared the influence of environmental stresses (temperature, ionic strength, and pH) on the mean particle diameter, ζ potential, microstructure, oil destabilization, and creaming stability of primary and secondary emulsions with same oil concentration.

Thermal Processing. The primary emulsions were diluted with 100 mM acetic acid buffer (pH 3.0) to adjust the oil concentration to the same value as in the secondary emulsion (10 wt %). Emulsion samples (10 g) were transferred into glass test tubes (internal diameter 15 mm, height 125 mm), which were then incubated in a water bath for 30 min at different temperatures ranging from 30 to 90 °C. After incubation the emulsion samples were immediately placed at room temperature, where they were stored for 24 h prior to analysis.

NaCl Stability. Primary and secondary emulsion samples were diluted with 100 mM acetic acid buffer (pH 3.0) to obtain the same final oil concentration (2.0 wt %) but different NaCl concentrations (0−500 mM). Emulsion samples (10 g) were then transferred into glass test tubes (internal diameter 15 mm, height 125 mm) and stored at room temperature for 24 h before being analyzed.

pH Stability. Primary and secondary emulsion samples were diluted with 100 mM acetic acid buffer (pH 3.0) to obtain the same final oil concentration (2.0 wt %). The pH of the diluted primary and secondary emulsions was then adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 using NaOH solutions. The emulsions were then stored at room temperature for 24 h before being analyzed.

Particle Size Measurements. To avoid multiple scattering effects, emulsions were diluted to a droplet concentration of approximately ~0.005 wt % using buffer solution at the pH and NaCl concentration of the sample and stirred continuously throughout the measurements to ensure the samples were homogeneous. The particle size distribution of the emulsions was then measured using a laser light scattering instrument (Mastersizer, Malvern Instruments, Worcestershire, U.K.). This instrument measures the angular dependence of the intensity of laser light (λ = 632.8 nm) scattered by a dilute emulsion and then finds the particle size distribution that gives the best fit between experimental measurements and predictions based on light scattering theory. The particle size was reported as volume-surface mean diameter, d_{32} ($=\sum n_i d_i^3/\sum n_i d_i^2$, where n_i is the number of particles with diameter d_i), or volume-weighted mean diameter, d_{43} ($=\sum n_i d_i^4/\sum n_i d_i^3$). All measurements were made on at least two freshly prepared samples, and results are reported as averages and standard deviations. It should be noted that the theory used to calculate the particle size distribution assumes that the particles are spherical and homogeneous, but the particles in emulsions containing flocs are nonspherical and non-homogeneous. In addition, dilution and stirring are likely to disrupt weakly flocculated droplets, and stirring may form large oil droplets in emulsions that exhibit extensive oiling off. Therefore, the particle

size data on flocculated and highly coalesced emulsions should be interpreted with caution.

ζ Potential Measurements. The prepared emulsions were diluted to a droplet concentration of approximately ~0.005 wt % using buffer solution at the pH and NaCl concentration of the sample. The ζ potential of the emulsion was determined using a particle electrophoresis instrument (ZEM5003, Zetamaster, Malvern Instruments, Worcestershire, U.K.), measuring the direction and velocity of droplet movement in the applied electric field. The ζ potential was reported as the average and standard deviation of measurements made on two freshly prepared samples, with five readings taken per sample.

Optical Microscopy. Emulsions were gently agitated in a glass test tube before analysis to ensure that they were homogeneous. A drop of emulsion was placed on a microscope slide and then covered with a cover slip. The microstructure of the emulsion was then observed using conventional optical microscopy (Nikon microscope Eclipse E400, Nikon Corp., Japan). The images were acquired using a CCD camera (CCD-300-RC, DAGE-MTI, Michigan City, IN) connected to Digital Image Processing Software (Micro Video Instruments Inc., Avon, MA) installed on a computer.

Destabilized Oil Measurements. The amounts of destabilized oil present in the emulsions were determined using the dye dilution technique described in detail by Palanuwech et al. (*33*). Emulsions were agitated in a glass test tube before analysis to ensure that they were homogeneous. A 3 g portion of corn oil containing a known amount of oil-soluble red dye (0.0015 wt % Oil Red O) was added to the top of 5 g of the emulsion to be tested. Samples were mixed by vortexing for 30 s and then centrifuged at 20 000 rpm for 20 min at 25 °C (Sorvall Centrifuges T-1270, DuPont Co., Wilmington, DE). The dyed free oil on top of the emulsion was removed with a pipet and placed into a 1.5 mL cuvette, where its absorbance was measured at 517 nm using UV−visible spectrophotometer (UV-2101 PC, Shimadzu Corp., Japan). The percentage of destabilized oil (ϕ_d) was calculated using the equation ϕ_d (%) $= 100m_o(a - 1)/m_e\phi$, where m_o is the mass of added dye solution, m_e is the mass of the emulsion, ϕ is the mass fraction of oil in the emulsion, and a is the ratio of the absorbance of the standard dye solution to that of the dye solution extracted from the top of the emulsion sample.

Creaming Stability Measurement. A 10 g amount of the emulsion was transferred into a test tube (internal diameter 15 mm, height 125 mm), which was tightly sealed with a plastic cap and then stored for 1 day and 7 days at room temperature. After storage, emulsions were separated into an optically opaque "cream" layer at the top and a transparent (or turbid) "serum" layer at the bottom. We defined the serum layer as the sum of the turbid and transparent layers. The total height of the emulsions (H_E) and the height of the serum layer (H_S) were measured. The extent of creaming was characterized by serum (%) $= 100(H_S/H_E)$. The percent of serum provided indirect information about the extent of droplet aggregation in an emulsion. All measurements were made on at least two freshly prepared samples.

Statistical Analysis. Experiments were performed twice using freshly prepared samples. Averages and standard deviations were calculated from these duplicate measurements.

RESULTS AND DISCUSSION

Influence of Fish Gelatin (FG) Concentration on Droplet Characteristics and Stability of Secondary Emulsions. The purpose of these experiments was to establish the optimum FG concentration required to prepare stable secondary emulsions. The electrical charge, mean particle diameter, microstructure, and oil destabilization of secondary emulsions (10 wt % corn oil, 0.23 wt % SDS, 100 mM acetic acid buffer, pH 3.0) containing different FG concentrations (0−2.0 wt %) were measured (Figures 1−4).

In the absence of FG, the net charge on the emulsion droplets was ~−40 mV, because the SDS used to stabilize the droplets in the primary emulsion has a negative charge at pH 3. On the other hand, the FG used to prepare the secondary emulsions has a positive charge at pH 3 because its isoelectric point (pI) is ~7.0. The net charge on the droplets therefore switched from negative to positive as the FG concentration in the emulsions was increased (Figure 1). The droplet charge was neutralized at ~0.04 wt % FG concentration for nonsonicated emulsions and ~0.08 wt % for sonicated ones, which corresponded to FG-to-SDS mass ratios (R) of ~0.17 and ~0.35 g/g, respectively. The charge on the droplets eventually reached a relatively constant positive value (~+30 mV) when the FG concentration exceeded about ~0.20 wt % ($R \approx 0.87$ g/g). The ability of charged polyelectrolytes to adsorb to the surface of oppositely charged colloidal particles and cause charge reversal is well established in the literature (*34*−*38*). We should mention that the observed change in interfacial composition of the emulsion

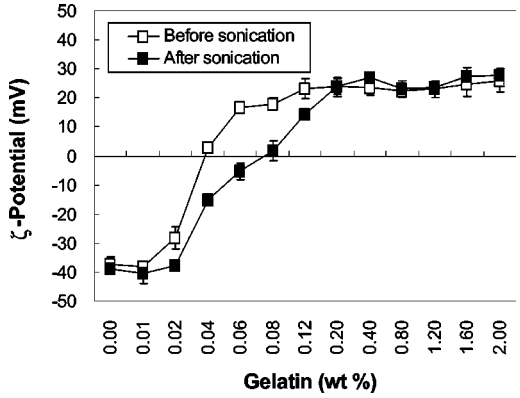

Figure 1. Dependence of electrical charge of emulsion droplets (ζ potential) on fish gelatin (FG) concentration for secondary emulsions (10 wt % corn oil, 0.23 wt % SDS, 100 mM acetic acid, pH 3.0) after dilution with buffer solution.

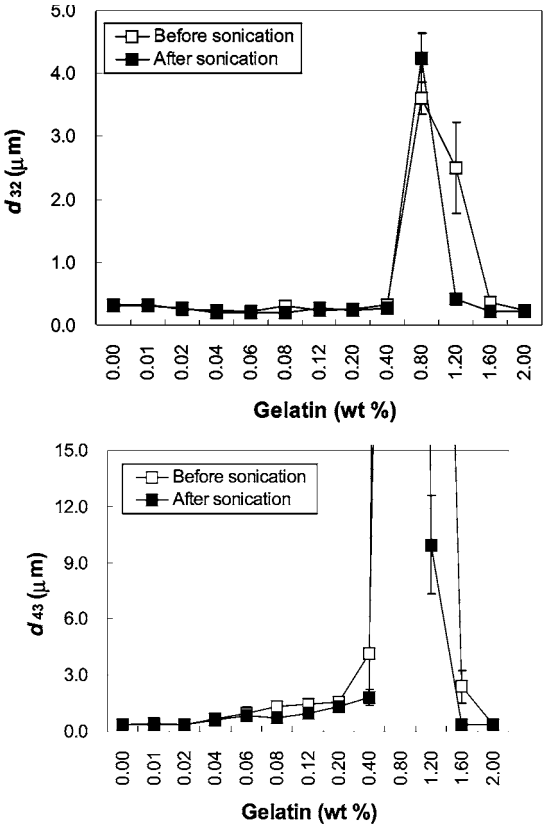

Figure 2. Dependence of mean particle diameters on FG concentration for secondary emulsions (10 wt % corn oil, 0.23 wt % SDS, 100 mM acetic acid, pH 3.0) after dilution with buffer solution: (a, top) d_{32}; (b, bottom) d_{43}.

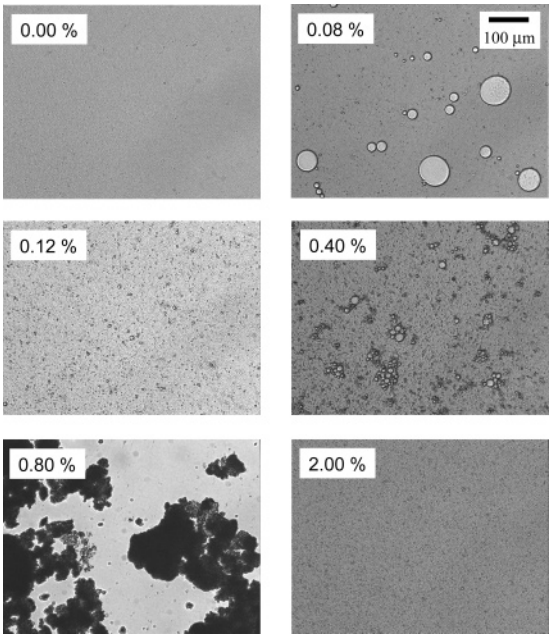

Figure 3. Photomicrographs of SDS-coated emulsions stabilized with FG (10 wt % corn oil, 0.23 wt % SDS, 0–2.00 wt % FG, 100 mM acetic acid, pH 3).

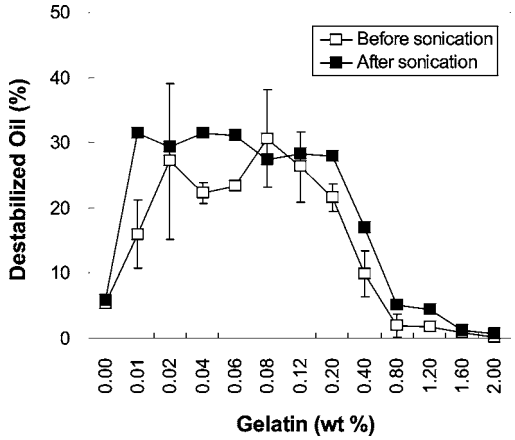

Figure 4. Destabilized oil in secondary emulsions (10 wt % corn oil, 0.23 wt % SDS, 100 mM acetic acid, pH 3.0) as a function of different FG concentrations.

droplets (as demonstrated by the ζ potential measurements) could also have been attributed to competitive adsorption effects: i.e., displacement of SDS from the droplet surfaces by gelatin at sufficiently high gelatin concentrations (*4*). Nevertheless, we believe this mechanism is less likely because of the strong electrostatic attraction between the anionic SDS and cationic gelatin.

In the absence of FG, the mean particle diameter of the emulsion droplets was small (~0.3 μm for both d_{32} and d_{43}) and no creaming (serum = 0%) was observed during storage over 7 days, which indicated that SDS was effective at generating small emulsion droplets that were stable to aggregation. At FG concentrations from 0.01 to 0.40 wt %, there was no appreciable change in d_{32} (~0.2−0.3 μm) and the emulsions appeared to have good creaming stability (serum = 0%), which suggested that the majority of the droplets in the emulsions were not aggregated. Nevertheless, d_{43} increased appreciably with increasing FG concentration, reaching 4.1 μm at 0.40 wt % FG (Figure 2b), which suggested that a fraction of the droplets in the emulsions were aggregated. Optical microscopy also indicated that there was evidence of droplet coalescence and/or flocculation in many of the emulsions containing between 0.01 and 0.40 wt % FG (Figure 3). In addition, the amount of destabilized oil in the emulsions containing between 0.01 and 0.40 wt % FG was relatively high (Figure 4), which suggested that the droplets were prone to coalescence. These measurements highlight the importance of using a range of different analytical techniques to characterize the stability of emulsions prone to droplet aggregation. We postulate that the instability of the emulsions to droplet flocculation and coalescence over this range of FG concentrations was due to the fact that the surfaces of the emulsion droplets were not saturated with gelatin. Instead, there were negatively charged regions of SDS and positively charged regions of SDS−gelatin on the droplet surfaces, which would have promoted destabilization through charge neutralization and bridging flocculation effects (*5*, *34*, *39*). Coalescence was probably enhanced in this gelatin concentration range, because flocculation caused the droplets to be brought into close proximity for extended periods (*4*).

At a FG concentration of 0.80 wt % ($R \approx 3.48$ g/g), there were large increases in mean particle diameters ($d_{32} \approx 3.6$ μm, $d_{43} \approx 93.1$ μm) and evidence of creaming instability in the emulsions (42.5% serum after 1 day and 45.1% after 7 days). Optical microscopy indicated that the large increase in droplet diameter was due to extensive droplet flocculation in the emulsions at this gelatin concentration (Figure 3). The ζ potential of the droplets in these emulsions was relatively high ($|\zeta| > 20$ mV), and therefore one might have expected the droplets to be stable to aggregation because of a strong electrostatic repulsion between them. The fact that they aggregated may have been because of a bridging flocculation mechanism; i.e., a gelatin molecule from one droplet could have been simultaneously adsorbed to the surface of another droplet. Presumably, there was insufficient FG in the aqueous phase when the primary emulsion was mixed with the gelatin to completely cover the droplet surfaces before the droplets collided with each other. Appreciable droplet flocculation was also observed in the emulsions containing 1.20 wt % FG, which can be attributed to the same mechanisms as discussed for the emulsions containing 0.80 wt % FG.

At FG concentrations of ≥1.60 wt % there was no evidence of droplet aggregation, creaming, or oiling off in the emulsions (Figures 2−4). We postulate that at these relatively high biopolymer concentrations the droplet surfaces were completely saturated with FG and therefore protected against droplet aggregation by a highly charged and relatively thick SDS−FG membrane. The fact that little bridging flocculation was observed in these emulsions suggests that the time required for the droplets to become saturated with FG was considerably faster than the time between droplet collisions.

In previous studies, it was found that ultrasound could be used to disrupt flocs formed during the preparation of secondary emulsions, because it could generate forces sufficiently large to physically cleave or detach biopolymer molecules from all but one of the flocculated droplets (*13*, *15*, *17*, *40*). For this reason, we examined the possibility of using sonication to break up the flocs in the secondary emulsions produced in this study (Figures 1, 2, and 4). Sonication caused a slight increase in the magnitude of the negative charge on the emulsion droplets at relatively low FG concentrations (<0.20 wt %) but had little effect at higher FG concentrations. In addition, sonication had little influence on the mean particle diameter (Figure 2) or amount of oil destabilization (Figure 4) observed in the secondary emulsions. Hence, the effect of sonication on the

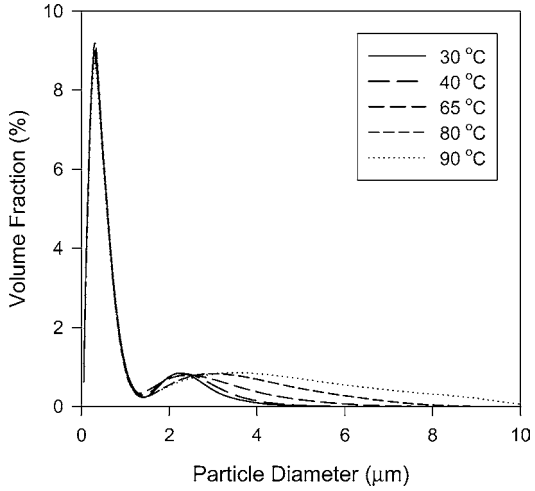

Figure 5. Particle size distributions of secondary emulsions (10 wt % corn oil, 10 mM SDS, 2.00 wt % FG, 100 mM acetic acid, pH 3.0) after storage at different temperatures for 1 day.

stability of the emulsions stabilized by SDS−gelatin membranes was much less than that observed previously for emulsions stabilized by SDS−chitosan membranes (*17*).

Taking the above results together, we used secondary emulsions containing 2.0 wt % FG without ultrasound treatment for the remainder of this study, because these emulsions contained small highly charged droplets that were stable to droplet aggregation, creaming, and oil destabilization.

Influence of Thermal Processing on Properties of Primary and Secondary Emulsions. The purpose of these experiments was to examine the influence of thermal processing on the stability of emulsions containing droplets coated by SDS membranes (primary emulsions) and those coated by SDS−FG membranes (secondary emulsions). Primary and secondary emulsions were held at temperatures ranging from 30 to 90 °C for 30 min, cooled to room temperature, and then stored for 1 day prior to analysis. There was no significant effect of heating on the ζ potential of the negatively charged droplets in the primary emulsion (-40 ± 0 mV) or on the positively charged droplets in the secondary emulsion ($+32 \pm 1$ mV). In addition, there were no significant changes in the mean diameters of the particles in the primary ($d_{32} = 0.32 \pm 0.00$ μm, $d_{43} = 0.34 \pm 0.00$ μm) and secondary emulsion ($d_{32} = 0.22 \pm 0.01$ μm, $d_{43} = 0.43 \pm 0.04$ μm) with holding temperature. On the other hand, thermal processing did have some impact on the particle size distribution in the secondary emulsions (Figure 5). The emulsions stabilized by SDS−FG membranes were bimodal, with a major peak corresponding to a large fraction of relatively small droplets and a minor peak corresponding to a small fraction of relatively large particles. The major peak was centered at a diameter of 0.29 μm regardless of holding temperature, whereas the minor peak was centered at a diameter of 2.1 μm at 30 °C but shifted upward with increasing temperature, to a diameter of 3.3 μm at 90 °C. This suggested that the majority of the droplets in the emulsions were unaffected by heating but that there was a small fraction of larger particles that grew upon heating. The origin of this effect is unknown but may have been due to some partial desorption of gelatin molecules from the droplet surfaces at elevated temperatures that led to bridging flocculation. Even so, the change in particle size distribution observed in these emulsions was much less than that observed in emulsions stabilized by globular proteins (*41*). In addition, there were no evidence of creaming or oiling off in the primary and secondary emulsions, which indicated that they were all stable to thermal processing at the holding temperatures used in this study.

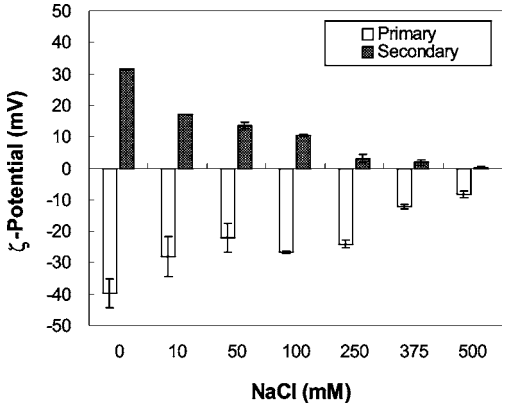

Figure 6. Dependence of particle electrical charge (ζ potential) on NaCl concentration for primary (20 wt % corn oil, 0.46 wt % SDS, 100 mM acetic acid, pH 3.0) and secondary emulsions (10 wt % corn oil, 0.23 wt % SDS, 2.00 wt % FG, 100 mM acetic acid, pH 3.0).

Influence of Ionic Strength on Properties of Primary and Secondary Emulsions. A number of studies have shown that secondary emulsions are more stable to droplet aggregation than primary emulsions in the presence of salt (*14*, *15*, *17*). The purpose of these experiments was therefore to compare the influence of NaCl (0−500 mM) on the stabilities of SDS-coated primary and SDS−FG-coated secondary emulsions. After preparation, the emulsions were stored for 24 h at room temperature and then their electrical charge, mean particle diameter, and microstructure were measured (Figures 6−8). In addition, the creaming stability (after 1 week) and percentage of oil destabilization of the emulsions was measured (data not shown).

The ξ potential of the SDS stabilized droplets in the primary emulsions remained negative at all NaCl concentrations (Figure 6). However, the magnitude of the ξ potential decreased as the NaCl concentration increased, which can be attributed to electrostatic screening effects (*42*, *43*). The ξ potential of the SDS−FG-stabilized droplets in the secondary emulsions remained positive at all ionic strengths, but the magnitude of the ζ potential decreased appreciably as the NaCl increased, which could be attributed to electrostatic screening effects. However, some of the reduction in positive charge on the emulsion droplets in the secondary emulsions may also have been due to desorption of FG from the droplet surfaces as the ionic strength increased. In other words, the attraction between the negatively charged SDS molecules and the positively charged FG molecules could have been weakened at high ionic strength, thus allowing partial desorption of some of the cationic biopolymer. The fact that the ζ potential was still positive in the droplets at high ionic strengths suggests that at least some of the gelatin molecules remained attached to the droplet surfaces.

In the primary emulsion, the mean particle diameters remained fairly constant as the salt concentration was increased from 0 to 50 mM NaCl but then increased appreciably at higher levels (Figure 7a,b). The optical microscopy observations indicated that droplet coalescence occurred in the emulsions at $\geq$50 mM NaCl (Figure 8), and the oil destabilization measurements indicated that there was extensive "oiling off" at $\geq$50 mM NaCl, with up to 70% of the emulsified oil being destabilized at 500 mM NaCl (data not shown). The droplet aggregation observed in the primary emulsions at these high salt concentrations was presumably due to screening of the electrostatic repulsion between the droplets and/or changes in the optimum curvature

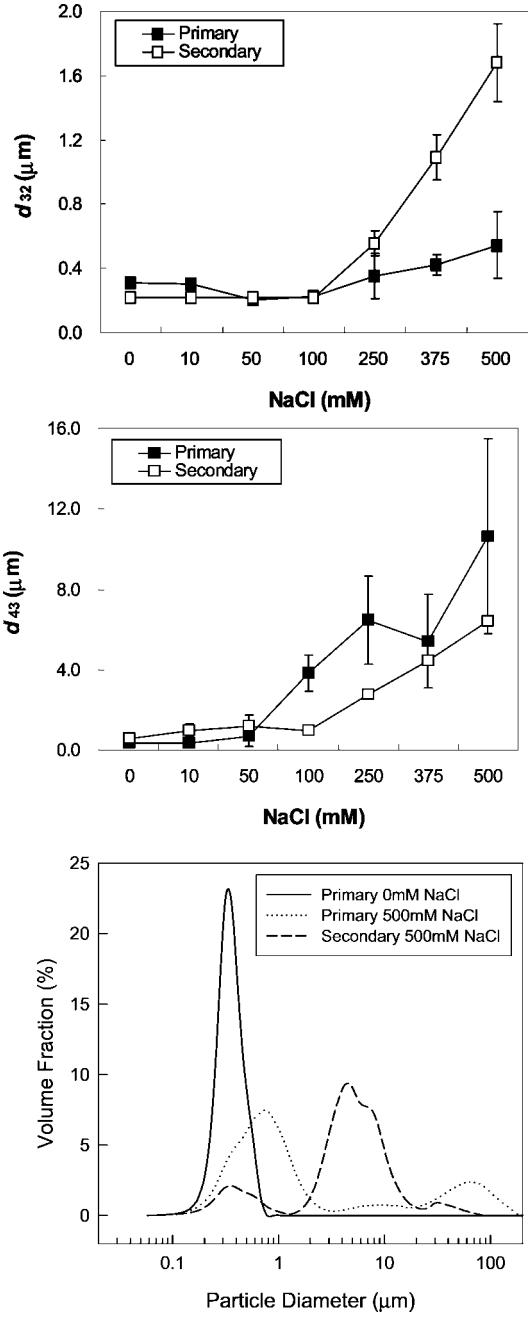

Figure 7. Dependence of mean particle diameter on NaCl concentration for primary (20 wt % corn oil, 0.46 wt % SDS, 100 mM acetic acid, pH 3.0) and secondary emulsions (10 wt % corn oil, 0.23 wt % SDS, 2.00 wt % FG, 100 mM acetic acid, pH 3.0): (a, top) d_{32}; (b, middle) d_{43}. (c, bottom) Particle size distributions of primary and secondary emulsions at 500 mM NaCl.

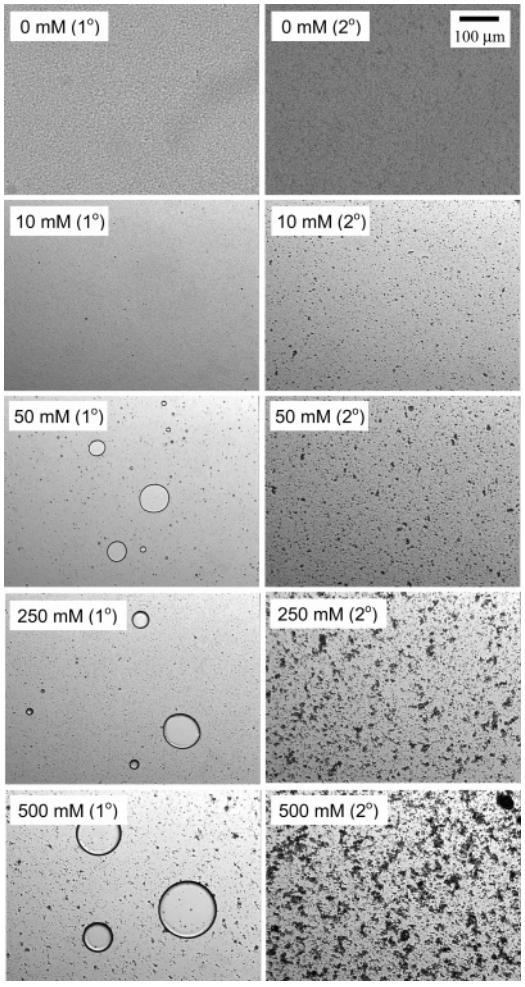

Figure 8. Photomicrographs of SDS-coated emulsions stabilized with FG (10 wt % corn oil, 0.23 wt % SDS, 2.00 wt % FG, 100 mM acetic acid, pH 3) at different NaCl concentrations.

of the SDS membranes surrounding the droplets (*42*, *43*). It seems that the relatively thin and mobile interfacial membranes formed by SDS in the presence of salt were not capable of preventing droplet coalescence (*44*, *45*). On the other hand, no creaming was observed in these emulsions after 1 week of storage, which suggested that many of the droplets were not aggregated.

In the secondary emulsions, the mean particle diameters increased slightly as the salt concentration was increased from 0 to 100 mM NaCl but then increased steeply at higher salt concentrations (Figure 7a,b). There was no evidence of oil destabilization in any of the secondary emulsions, which suggests that they were resistant to coalescence (*44*, *45*). Optical microscopy showed that there was some droplet flocculation in the secondary emulsions, even at 10 mM NaCl, and that this increased with increasing salt concentration (Figure 8). This highlights the fact that particle size measurements carried out by laser light scattering on diluted emulsions do not always accurately reflect the level of droplet flocculation in nondiluted emulsions. Our results suggests that the flocs formed in the secondary emulsions at intermediate salt concentrations were only held together by fairly weak attractive interactions that could easily be disrupted by dilution and stirring during the light scattering experiments. The secondary emulsions had higher d_{32} and lower d_{43} values as compared to those of the primary emulsions at ≥100 mM NaCl, which can be attributed to the higher volume fraction of relatively large particles in the primary emulsions compared to the secondary emulsions (Figure 7c). It is interesting to note that the droplets in the secondary emulsions were unstable to flocculation but not coalescence or oil destabilization, as was the case in the primary emulsions. This is probably because the relatively thick SDS−FG membranes prevent the droplets from coming close enough together to coalesce, due to a strong short-range steric repulsion generated by the biopolymer (*44*, *45*). The droplets in the secondary emulsions containing high salt concentrations (≥100 mM NaCl) were also prone to extensive creaming (serum ≈ 92%), which

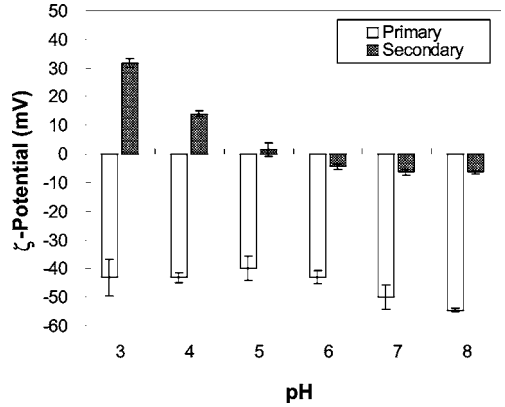

Figure 9. Influence of storage pH on particle electrical charge (ζ potential) of primary (20 wt % corn oil, 0.46 wt % SDS, 100 mM acetic acid, pH 3.0) and secondary emulsions (10 wt % corn oil, 0.23 wt % SDS, 2.00 wt % FG, 100 mM acetic acid, pH 3.0).

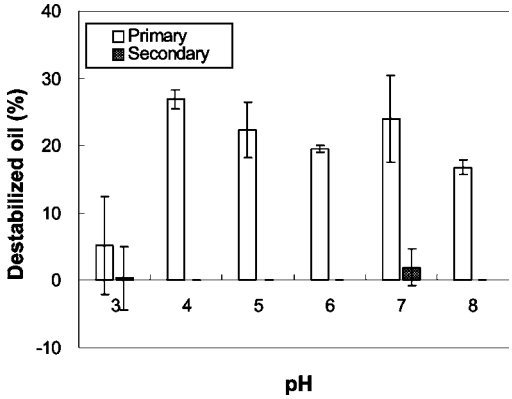

Figure 10. Destabilized oil in primary (20 wt % corn oil, 0.46 wt % SDS, 100 mM acetic acid, pH 3.0) and secondary emulsions (10 wt % corn oil, 0.23 wt % SDS, 2.00 wt % FG, 100 mM acetic acid, pH 3.0) as a function of different pH from 3 to 8.

can be attributed to the fact that the majority of particles within them had aggregated.

Overall, these results show that the instability of the primary emulsions to ionic strength could be improved below a critical salt concentration (≤100 mM NaCl) by coating the SDS-stabilized droplets with FG.

Influence of Storage pH on Stability of Primary and Secondary Emulsions. The purpose of these experiments was to identify the influence of pH on the stabilities of primary and secondary emulsions. The pH dependence on the ζ potential and mean particle diameter of diluted primary and secondary emulsions was measured after they had been stored at room temperature for 24 h (Figure 9).

The ζ potential of the SDS-stabilized droplets in the primary emulsions was negative at all pH values without a significant change (−46 ± 6 mV). The ζ potential of the droplets in the secondary emulsions went from positive (+31.9 mV) to negative (−6.3 mV) as the storage pH increased from below to above the FG's pI (∼7.0), and had a value close to zero somewhere between pH 5 and 6. The reversal of charge on the emulsion droplets suggests that either the FG lost some of its positive charge when the pH was increased or that it was (partially) desorbed from the droplet surfaces. The ζ potential of droplets in SDS−FG-stabilized emulsions was slightly negative even at pH values below the pI ($\zeta \approx -5$ mV at pH 6−7), due to the negative charge on the SDS molecules outweighing the positive charge on the FG molecules. FG would be expected to lose its net charge and be detached from the droplet surfaces around its pI value (pH 7.0). Interestingly, the ζ potential of the particles was much less negative in the secondary emulsions than in the primary emulsions at pH 7−8, which suggests that at least some of the FG molecules may have had a residual positive charge and remained adsorbed to the droplet surfaces. It has been reported that there is a clearly defined patch of positive charge on the protein molecule surface at pH > pI, although the protein carries a net negative charge at this pH, which allows the protein to adsorb to the negatively charged molecules (*39*).

In the primary emulsions, the mean particle diameter was relatively small from pH 3 to 8 ($d_{32} = 0.28 \pm 0.06$ μm, $d_{43} = 1.46 \pm 1.18$ μm), presumably because the electrostatic repulsion between the droplets was sufficiently strong to prevent extensive droplet aggregation. Nevertheless, the primary emulsions were not stable to coalescence at pH ≥4 (Figure 10). A possible explanation for this observation is that the sodium ion (Na^+) concentration increased appreciably when the pH was adjusted from 3 to 8 (due to addition of NaOH), which would have reduced the electrostatic repulsion between the droplets and may have altered the optimum curvature of the SDS membranes, thus making the droplets more susceptible to coalescence (*42*, *46*). In the secondary emulsions, the electrostatic attraction between the droplet surfaces and the FG molecules would have decreased as the pH was increased because of the loss of positive charge on the FG and the increase in solution ionic strength. Consequently, one would expect that some of the FG molecules may have either partially or fully desorbed from the droplet surfaces (*4*, *39*, *47*). However, no creaming (% serum = 0), no significant increase in mean particle diameter ($d_{32} \approx 0.22 \pm 0.00$ μm, $d_{43} \approx 0.43 \pm 0.01$ μm), and no changes in overall microstructure (data not shown) were observed in the secondary emulsions from pH 3 to 8. In addition, there was little evidence of oil destabilization over this pH range in the secondary emulsions (Figure 10). These results suggest that the droplets in the secondary emulsion were highly stable to flocculation, coalescence, and creaming, which may be due to the fact that the SDS−FG membrane is highly resistant to rupture and generates strong repulsive interactions between the droplets.

In summary, this study has shown that the stability of SDS-coated emulsion droplets to environmental stresses can be improved by coating the droplets with fish gelatin (FG). The driving force for FG adsorption was presumably the electrostatic attraction between the negatively charged droplets and positively charged FG. The droplets in the secondary emulsions stabilized by SDS−FG membranes had relatively good stability to droplet aggregation at holding temperatures of 30−90 °C for 30 min, ≤100 mM NaCl, and pH values from 3 to 8, regardless of the magnitude of electrical charge on the droplets. We postulate that the SDS−FG membranes formed around the droplets were relatively thick, which generated a steric repulsion between the droplets that was strong enough to inhibit droplet flocculation and coalescence. The unique attributes of the fish gelatin used in this study (i.e. high hydrophilicity, low thermal denaturation temperature, and relatively high IEP), may lead to the generation of emulsions that are stable over a wide range of pH values.

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