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Formation and Stabilization of Antimicrobial Delivery Systems Based on Electrostatic Complexes of Cationic−Non-ionic Mixed Micelles and Anionic Polysaccharides

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Lauric arginate (LAE) is a cationic surfactant that is of great interest to the food industry because of its strong antimicrobial activity. However, its application within foods and beverages is currently restricted because of its limited solubility in aqueous solutions and its bitter taste, which have been associated with its cationic nature. This study examines whether electrostatic complexes between cationic LAE mixed micelles and anionic polysaccharides could be used to improve LAE functionality. Two types of pectin (high and low methoxyl) were titrated into buffer solutions containing either LAE micelles or LAE/Tween 20 mixed micelles (pH 3.5, 50 mM citrate buffer). The electrical characteristics of the micelles or micelle/pectin complexes were assessed by microelectrophoresis measurements, while their stability to aggregation was evaluated by light scattering measurements. LAE micelle/pectin complexes formed large aggregates that rapidly sedimented. On the other hand, mixed micelle/pectin complexes (1:1 LAE/Tween 20, w/w) were stable to aggregation and formed clear solutions. The electrical charge of mixed micelles changed from +8 to −15 mV when the pectin concentration was increased (0.00−0.05 wt %), indicating an electrostatic interaction between anionic pectin molecules and cationic micelles. Lower concentrations of low methoxyl pectin were required (0.01 wt %) to change the net charge of mixed micelles from positive to negative than high methoxyl pectin (0.025 wt %). Our results suggest that the addition of pectin to mixed LAE/Tween 20 micelles leads to the formation of electrostatic complexes that may be useful as functional ingredients in food and other products.

KEYWORDS: Cationic surfactant; lauric arginate; antimicrobial; Tween 20; pectin; electrostatic complex

INTRODUCTION

Amino-acid-based surfactants consist of an amino acid residue as the hydrophilic head group and a nonpolar long-chain compound as the hydrophobic tail group (*1*). They are obtained by combining natural saturated fatty acids, alcohols, and amines with different amino acid head groups through ester and amide linkages. The combination of polar amino acid (hydrophilic moiety) and nonpolar long-chain compound (hydrophobic moiety) produces molecules with high surface activity (*2*). Because they are broken down into natural food-grade constituents within the gastrointestinal tract after ingestion, they have low toxicity and are suitable for food, pharmaceutical, and cosmetic applications (*3*).

Lauric arginate (N^{α}-lauroyl-L-arginine ethyl ester monohydrochloride, LAE) (**Figure 1a**) is an amino-acid-based surfactant that is a derivative of lauric acid, L-arginine, and ethanol (*1*, *3*, *4*). LAE is a cationic surfactant that has been approved as generally recognized as safe (GRAS) within the United States for certain food applications (*5*). LAE has been shown to be a highly potent antimicrobial against a wide range of food pathogens and spoilage organisms (*3*, *5*). The high antimicrobial activity of LAE has been attributed to its action on the cytoplasmic membranes of the microorganisms in such a manner that their metabolic processes are altered and their normal cycle is inhibited but without cellular lysis (*3*, *6*). In addition, it has a low oil−water equilibrium partition coefficient ($K_{OW} < 0.1$), which means that it tends to concentrate in the water phase of products, where most bacterial activity occurs (*5*). LAE is hydrolyzed in the human body by chemical and metabolic pathways, which quickly break the molecule into its natural components: lauric acid and L-arginine (*4*). The morphology of the surfactant micelles formed by LAE depends upon the temperature, composition, and electrolyte content of the system (*1*, *7*).

Its low toxicity and high antimicrobial activity make it a valuable tool for controlling or preventing microbial growth in food products. On the other hand, its application within the food industry may be limited for a number of reasons: (i) its potency as

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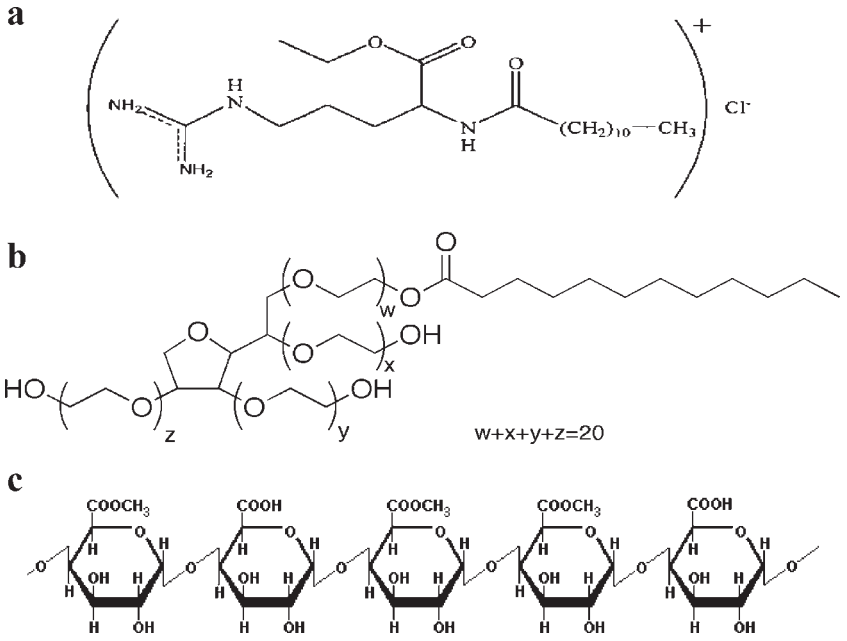

Figure 1. Molecular structure of (**a**) LAE, (**b**) Tween 20 [polyoxyethylene (20) sorbitan monolaurate; molecular formula, $C_{58}H_{114}O_{26}$], and (**c**) pectin.

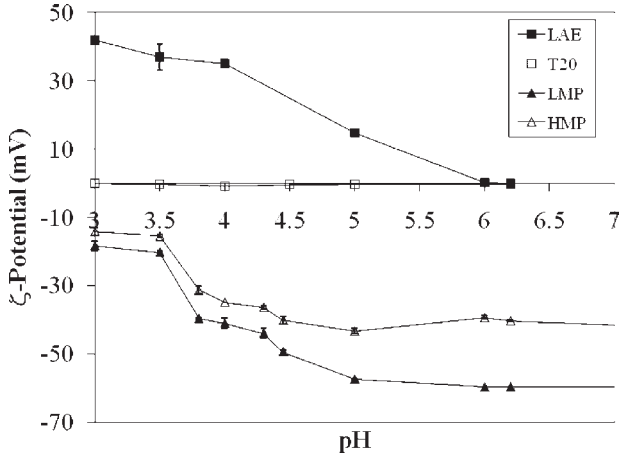

Figure 2. pH dependence of the electrical charge (ζ potential) of each of the individual components of LAE (0.25 wt %/citrate buffer), T20 (0.25 wt %/citrate buffer), and pectins (0.05 wt % HMP and LMP/citrate buffer). All solutions were prepared in 50 mM Na−citrate buffer (pH 3.0−7.0).

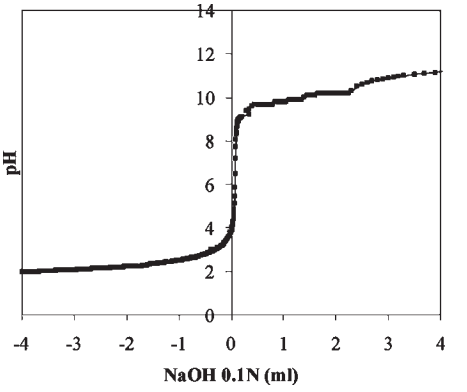

Figure 3. Titration of LAE (0.25 wt %/water) with 0.1 N NaOH or 0.1 N HCl. pH versus the amount of hydroxide ions added to the first sample, with negative values indicating the amount of chloride ions added to the second sample (the two samples are described in the text).

an antimicrobial may be affected if it interacts with anionic components within the food matrix; (ii) it may bind to anionic biopolymers (mucin) within the mouth, leading to perceived bitterness; and (iii) it tends to precipitate from solution at pH > 4.5 and high ionic strength.

A recent study has shown that the tendency for LAE to precipitate in aqueous solutions can be reduced by mixing it with a non-ionic surfactant (Tween 20) (**Figure 1b**) to form mixed micelles (*8*). In the present study, we examine the possibility of further improving the stability and performance of LAE by forming electrostatic complexes between cationic mixed micelles (LAE/Tween 20) and an anionic biopolymer (pectin). Pectin is a heteropolysaccharide with a backbone consisting of partially esterified α-1,4-linked D-galacturonides (**Figure 1c**), containing varying amounts of covalently attached rhamnose and branches of L-arabinose, D-galactose, D-xylose, and L-rhamnose (*9*, *10*). The functional properties of a pectin ingredient mainly depend upon its degree of esterification (DE): high ester or high methoxyl pectins (HMPs) have a DE above 50%, and low ester or low methoxyl pectins (LMPs) have a DE below 50% (*11*).

Pectin functions as a gelling, thickening, and stabilizing agent in foods. This functionality is related to its molecular weight (MW), DE, distribution of ester groups along the backbone, presence of nonuronide components, and other structural characteristics (*12*−*14*). Pectin has carboxylic acid side groups that are negatively charged across a wide range of pH ($pK_a \approx 3.5$). HM pectin has been widely used as a stabilizer in acidified milk drinks (pH 3.6−4.3), because it aids in preventing flocculation of milk proteins (*15*, *16*). Interactions of charged pectins with the cationic groups of proteins play a major role in the stabilizing effect of pectins in acidified dairy drinks and emulsions (*17*−*19*).

Mixed micelle/pectin complexes would be expected to have a lower positive charge (or even a net negative charge) when compared to mixed micelle systems alone. Previous researchers have shown that decreasing the electric charge on cationic compounds decreases their bitterness (*20*), and hence, the formation of mixed micelle/pectin complexes may help improve the perceived mouthfeel of LAE-containing products. In addition, mixed micelle/pectin complexes may have a reduced tendency to

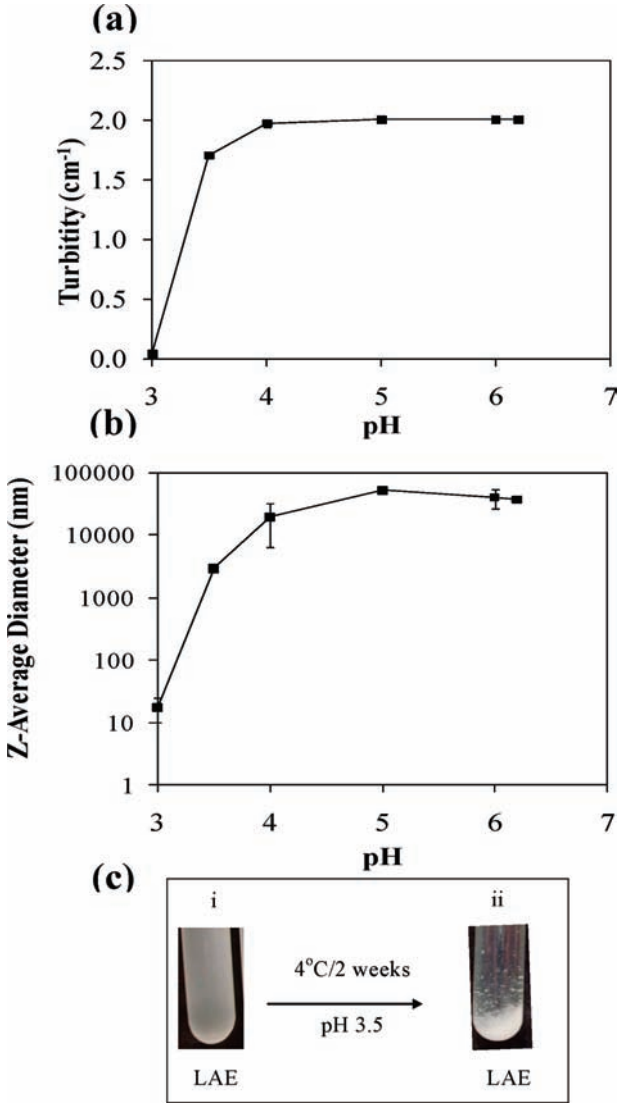

Figure 4. pH dependence of (**a**) turbidity and (**b**) Z average diameter (in nanometers) of LAE micelles (0.25 wt %/citrate buffer). All solutions were prepared in 50 mM Na−citrate buffer (pH 3.0−7.0). (**c**) Photographs of LAE micelles (0.25 wt % in 50 mM Na−citrate buffer (pH 3.5): (i) at room temperature and (ii) at 4 °C for 2 weeks.

interact with other anionic species in food products, thereby increasing the compatibility of LAE with many food matrices.

MATERIALS AND METHODS

Materials. The cationic surfactant LAE ($C_{20}H_{41}N_4O_3Cl$; MW, 421.0 g mol^{-1}), available commercially under the trade name Mirenat-N [10.5% (w/v) LAE in 89.5% (w/v) propylene glycol solvent] was provided by Vedeqsa Grupo LAMIRSA (Terrassa, Spain). HMP with a DE of ≈70% (pectin 1400) and LMP with a DE of ≈30% (pectin LM 35) were provided by TIC Gums (Belcamp, MD). Polyoxyethylene 20 sorbitan monolaureate (Tween 20) (T20), citric acid, and sodium monocitrate were purchased from Sigma Chemical Co. (St. Louis, MO). Distilled and deionized water was used for the preparation of all solutions.

Solution Preparation. A stock buffer solution was prepared and then used to make all other solutions (50 mM citrate at pH 3.5). LAE solutions were prepared by dispersing Mirenat-N in stock buffer. T20 solutions were prepared by dispersing T20 in stock buffer. Pectin solutions were prepared by dispersing powdered pectin in stock buffer, sealing them in a bottle, heating them in an autoclave at 120 °C under pressure for 15 min, and then continuously stirring them for 10 min. Mixed surfactant solutions (LAE/T20) were prepared by mixing 5 mL of T20 solution (0−0.6 wt %) with 5 mL of LAE solution (0.25 wt %) in glass vials. Micelle/pectin solutions or mixed micelle/pectin solutions were prepared by dispersing 5 mL of pectin solutions (0−0.025 wt %) into glass vials containing either 5 mL of LAE solution (0.25 wt %) or 5 mL of mixed surfactant solution (0.25 wt % LAE and 0.25 wt % T20). The resulting solutions were then mixed thoroughly and stored overnight prior to analysis.

pH Stability. The pH stability of the various surfactant systems prepared was determined by measuring changes in the particle size and solution turbidity when acid or alkali was added. LAE, T20, and/or pectin solutions were dissolved in 50 mM Na−citrate buffer (pH 3.0−7.0) to obtain samples with different pH values but the same final surfactant/pectin composition: 0.25 wt % LAE, 0.25 wt % T20, and 0.05 wt % pectin. The samples were then stored at room temperature for 24 h prior to analysis.

LAE Buffering Capacity. The buffering capacity of LAE in aqueous solution was determined by measuring the pH versus acid or alkali concentration. Two 0.25 wt % LAE solutions were prepared in distilled water, and acid (0.1 N HCl) was added to one, while alkali (0.1 N NaOH) was added to the other. The change in pH of the solutions was then measured as a function of the acid or alkali concentration added.

Turbidity, Electrical Charge, and Size Titrations. The optical turbidity (at 600 nm) of the surfactant and pectin/surfactant solutions was measured using an ultraviolet−visible (UV−vis) spectrophotometer (Spectronic 21D, Milton Roy, Rochester, NY) at room temperature. The samples were contained within 1 cm path-length optical cells, and distilled water was used as a control. Turbidity measurements were carried out on duplicate samples. The electrical charge (ζ potential) and mean diameter (Z average) of the particles in the solutions were measured using an instrument capable of electrophoresis and dynamic light scattering measurements (Zetasizer Nano-ZS, model ZEN3600, Malvern Instruments, Worchester, U.K.). The ζ potential was calculated from the measurement of the electrophoretic mobility of particles in an applied oscillating electric field using laser Doppler velocimetry. The mean diameter of the particles was calculated from their Brownian motion via the Stokes−Einstein equation. In some samples, precipitates were formed that were too large to analyze using this instrument. Measurements were conducted on duplicate samples and repeated 3 times per sample.

Optical Microscopy. The microstructures of selected surfactant systems were observed using an optical microscope (Nikon Eclipse E400, Nikon Corp., Tokyo, Japan). A drop of surfactant solution was placed on a microscope slide and then covered by a coverslip. An image of the sample was acquired using digital image processing software (Micro Video Instruments, Inc., Avon, MA) and stored on a personal computer.

RESULTS AND DISCUSSION

Solution Properties of Individual Components: LAE, T20, and Pectin. Initially, we examined the pH dependence of the solution properties of each of the individual components used in our experiments: LAE, T20, and pectin. The ζ potential of the particles present within the LAE solutions was strongly positive at acidic pH (e.g., +42 mV at pH 3) but became much less positive at higher pH values (**Figure 2**). The pH dependence of the ζ potential of the LAE particles can be attributed to the electrical characteristics of their head groups. LAE has an amino head group that partly loses its positive charge in this pH range, as demonstrated by the loss of buffering capacity in the pH range from 4 to 9 (**Figure 3**). T20 had a slightly negative charge (≈ −0.3 mV) that was close to zero across the entire pH range (**Figure 2**), which is to be expected for a non-ionic surfactant with a polyoxyethylene head group. The pectin molecules had a relatively high and constant negative charge from pH 7 to 5 but became progressively less negative as the solution was adjusted from pH 5 to 3 (**Figure 2**). This decrease in charge magnitude at low pH can be attributed to the fact that the anionic groups on the pectin chains are carboxylic acids with a $pK_a \approx 3.5$ (*21*). At the same pH, the magnitude of the negative charge on the pectin molecules was greater for LMP than for HMP, which can be attributed to the fact that LMP has a higher linear charge density, i.e., more carboxylic acid groups per unit chain length (*22*).

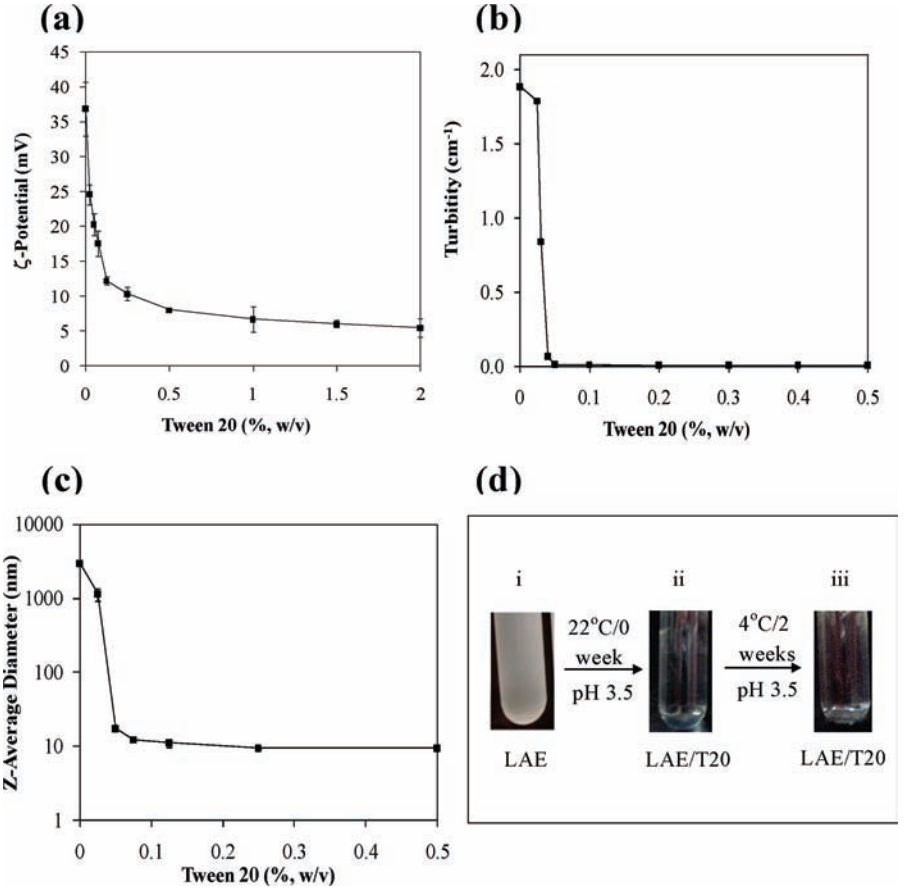

Figure 5. Change in (**a**) ζ potential, (**b**) turbidity, and (**c**) Z average diameter (in nanometers) of LAE micelles (0.25 wt %) upon the addition of T20 (0−0.6 wt %) and (**d**) photographs of (i) LAE micelles (0.25 wt %), (ii) LAE/T20 mixed micelles (1:1, v/v), and (iii) LAE/T20 mixed micelles (1:1, v/v) stored at 4 °C for 2 weeks. All solutions were prepared in 50 mM Na−citrate buffer (pH 3.5).

The pH dependence of aggregate formation was also measured for aqueous solutions of LAE, T20, and pectin (panels **a** and **b** of **Figure 4**). The turbidity of T20, HMP, and LMP solutions remained relatively low (< 0.05 cm^{-1}) across the entire pH range studied (pH 3−7), indicating that aggregates large enough to scatter light strongly were not formed (data not shown). The turbidity of LAE solutions (≈ 0.03 cm^{-1}) and the mean diameter of the particles present within these solutions ($d \approx 18$ nm) were both relatively low at pH 3 but increased appreciably at higher pH values (panels **a** and **b** of **Figure 4**), indicating that extensive aggregation occurred. Indeed, visual observation of these samples showed that large white precipitates were formed after mixing, which rapidly sedimented to the bottom of the containers (**Figure 4c**).

A similar phenomenon has been observed with other ionic surfactants (*7*, *23*). The stability of ionic micelles to aggregation is largely due to the relatively strong electrostatic repulsive interactions acting between them. At low pH, the LAE micelles have a strong positive charge, and therefore, the micelles are relatively stable toward aggregation. However, as the pH increases, some of the cationic head groups become deprotonated ($NH_3^+ \rightarrow NH_2$), thereby losing their net charge and promoting aggregation above a particular pH. There is a wide diversity of pH values in different kinds of foods, ranging from less than pH 3 to greater than pH 7, and hence, the addition of LAE to some foods may be problematic because of its tendency to form large aggregates that sediment.

Practically, food ingredients are often used in foods that are stored under refrigerated conditions, and therefore, it was important to examine the impact of cold storage on the stability of LAE dispersions. LAE dispersions were stored under quiescent conditions at 4 °C for 2 weeks and then observed visually. We found that a thick white sediment formed at the bottom of the tubes after cold storage at all pH values studied (pH 3−7), which indicated that the LAE solutions were highly unstable to chilling (**Figure 4c**). This would be a major limitation to the application of LAE in many types of food products, e.g., chilled beverages, dressings, sauces, or desserts. Aqueous solutions of T20, LMP, and HMP remained transparent after storage at 4 °C for 2 weeks, indicating that they were stable to cold storage (data not shown). These initial studies highlighted the need to identify effective strategies to overcome the poor pH and cold stability of LAE in aqueous solutions.

Characterization of LAE/T20 Interactions. Initially, we examined the possibility of forming mixed LAE/T20 micelles to improve the functional performance of LAE. Previous studies have shown that mixing LAE with a non-ionic surfactant can be used to control the electrical charge and solution properties of micelle dispersions (*8*). The change in turbidity, mean particle diameter, and ζ potential of mixed surfactant systems were measured when different amounts of T20 (0−2 wt %) were added to a LAE dispersion (0.25 wt % LAE and 50 mM citrate buffer at pH 3.5).

In the absence of T20, the ζ potential of the LAE aggregates was highly positive ($\zeta = +37$ mV), which can be attributed to the positively charged surfactant head groups. With an increasing T20 concentration, the ζ potential of the particles decreased from +37 to +6 mV (**Figure 5a**), which suggested that LAE molecules were incorporated into mixed LAE/T20 micelles. The electrical charge on T20 micelles alone at this pH was close to zero (−0.3 mV). As more T20 was added to the system, the positively charged LAE

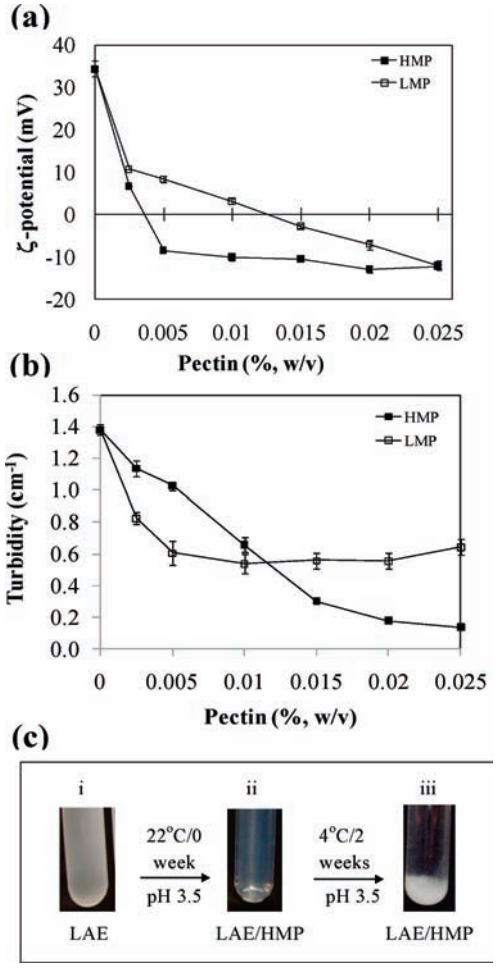

Figure 6. Influence of the addition of HMP and LMP (0−0.025 wt %) on LAE micelles (0.25 wt %) on (**a**) ζ potential and (**b**) turbidity and (**c**) photographs of (i) LAE micelles (0.25 wt %), (ii) LAE/HMP micelles (0.25 wt % LAE and 0.01 wt % HMP), and (iii) LAE/HMP micelles stored at 4 °C for 2 weeks. All solutions were prepared in 50 mM Na−citrate buffer (pH 3.5).

monomers would be distributed among a larger number of mixed micelles, and hence, the positive charge on each individual micelle would decrease. In addition, each positively charged LAE head group would be further away from its neighbors, which would be expected to reduce the electrostatic repulsion between head groups in the same micelle, thereby reducing the tendency for ion condensation to occur.

In the absence of T20, the turbidity (>1 cm^{-1}) and mean particle diameter ($>10\,000$ nm) of the LAE dispersions were relatively high (panels **b** and **c** of **Figure 5**), which can be attributed to the presence of LAE aggregates that were large enough to scatter light (**Figure 5d**). From 0 to 0.05 wt % T20, there was a steep decrease in solution turbidity and mean particle diameter, which can be attributed to the incorporation of LAE monomers into mixed LAE/T20 micelles, thereby disrupting the relatively large LAE aggregates initially present in the dispersion. From 0.05 to 2 wt % T20, the turbidity of the dispersion was low (<0.01 cm^{-1}), the mean particle diameter was small and relatively constant (≈ 9 nm), and the solutions appeared transparent (**Figure 5d**), which indicated that all of the LAE had been incorporated into mixed LAE/T20 micelles. The mean particle diameter of the T20 micelles alone at this pH was measured to be about 8.3 ± 0.09 nm, indicating that the incorporation of LAE only caused a slight change in their overall size.

The impact of T20 on the cold stability of the mixed micelle systems was determined by storing them at 4 °C under quiescent conditions for 2 weeks. The amount of precipitate formed was then determined visually (results not shown). In the absence of T20, large aggregates were formed in the samples that rapidly sedimented to the bottom of the test tubes, suggesting complete micelle instability (**Figure 4c**). In the presence of ≥ 0.05 wt % T20, the dispersions containing mixed micelles remained stable to aggregation during cold storage for 2 weeks, with the solutions remaining transparent and no sediment being formed (**Figure 5d**).

Overall, these experiments indicate that the addition of a nonionic surfactant (T20) to LAE dispersions at a mass ratio of T20/LAE > 1:5 (w/w) can be used to improve their aggregation stability by forming mixed micelles. Nevertheless, the mixed micelles formed still have a net positive charge, which may adversely impact their interactions with anionic components within foods (e.g., biopolymers and minerals) or surfaces that the foods come into contact (e.g., packaging materials or the human tongue).

Characterization of LAE/Pectin Interactions. We therefore examined the possibility of creating LAE-containing complexes that were anionic and had improved aggregation stability based on interactions between the cationic LAE and an anionic biopolymer (pectin). We hypothesized that LAE may be able to form complexes with pectin that had better stability to pH and cold storage than LAE micelles alone. The impact of the electrical charge of the polysaccharide was examined using pectin ingredients with different degrees of methoxylation: HMP (DE ≈ 70%) and LMP (DE ≈ 32.6%). Varying amounts of pectin were added to a solution of LAE micelles (0.25 wt % in 50 mM citrate buffer at pH 3.5), and the changes in ζ potential and turbidity were measured (panels **a** and **b** of **Figure 6**).

In the absence of pectin, the ζ potential of the LAE micelles was positive ($\zeta = +37$ mV). As the pectin concentration was increased, the ζ potential became less positive and then eventually became negative, with the point of zero charge being around 0.005 wt % pectin for HMP and around 0.01 wt % pectin for LMP. This result indicates that the anionic pectins bound to the cationic surfactants and formed molecular complexes, with charge neutralization depending upon the linear charge density of the pectin.

The addition of pectin to LAE solutions caused appreciable changes in solution appearance and turbidity. In the absence of pectin, the turbidity of the solutions was relatively high, indicating the presence of LAE particles large enough to scatter light (**Figure 6b**). When LMP was added to the LAE solutions, large aggregates could be observed visually that rapidly sedimented to the bottom of the test tube, which meant that we could not accurately measure their turbidity. When the HMP concentration was increased, there was a progressive decrease in solution turbidity (panels **b** and **c** of **Figure 6**), which indicated that there was a decrease in the size and/or concentration of the complexes formed. Nevertheless, the solutions were still relatively turbid (>0.2 cm^{-1}) even at high pectin concentrations, indicating that relatively large colloidal particles were still present.

The impact of complex formation with pectin on the cold stability of LAE systems was also examined. LAE/pectin mixtures were stored under quiescent conditions at 4 °C for 2 weeks, and the amount of precipitate formed was determined (**Figure 6c**). Large sediments were formed at the bottom of all samples containing pectin after storage, indicating that pectin alone was unable to improve their cold storage stability. Indeed, in many samples, both LMP and HMP promoted additional precipitation and sedimentation after cold storage. Precipitation of surfactant micelles is typically associated with a loss of their functionality (such as detergency, emulsification, and antimicrobial activity)

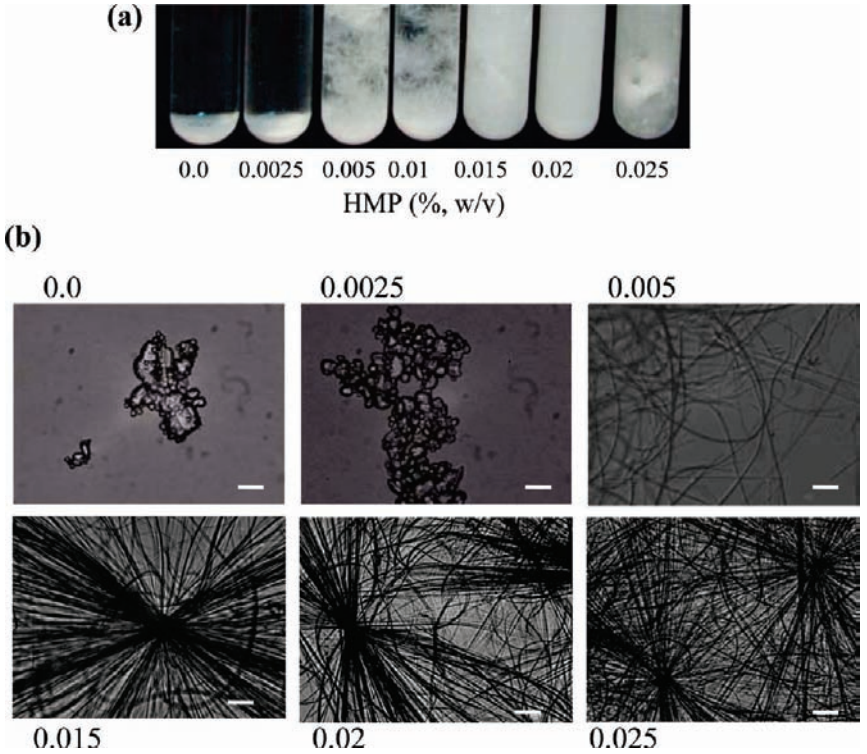

Figure 7. (**a**) Effect of the concentration of HMP (0−0.025 wt %) on the formation of aggregates of LAE micelles (0.25 wt %), after storage at room temperature for 2 weeks under quiescent conditions. (**b**) Light microscopy imaging of LAE micelles to which various amounts of HMP were added, as indicated in panel **a**. Scale bars = 100 μm. All solutions were prepared in 50 mM Na−citrate buffer (pH 3.5).

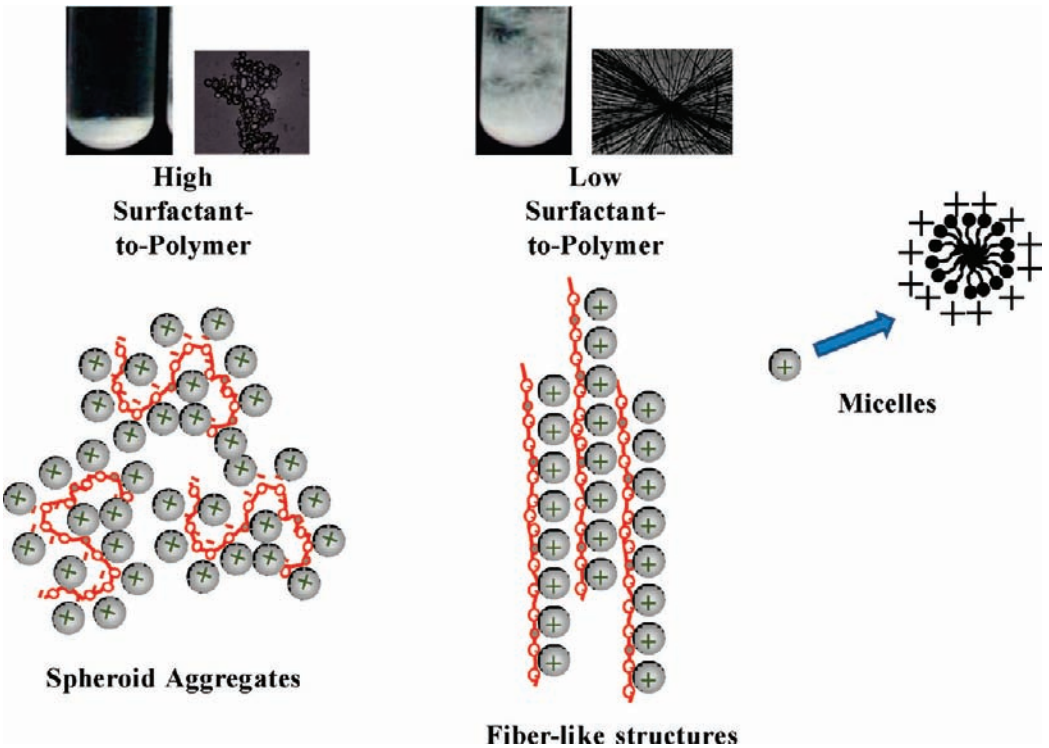

Figure 8. Schematic representation of a molecular interpretation for the difference in structural organization of the LAE/HMP complexes at different surfactant/polymer ratios: high and low.

and, hence, is undesirable in formulations where this particular functionality is required.

These results show that pectin alone is unable to improve the stability of LAE to aggregation in solution at ambient or cold storage temperatures. Nevertheless, it could be used to form LAE/pectin complexes that had a net negative charge, which may be advantageous for certain applications, e.g., to avoid adverse interactions with anionic components or to prevent a bitter mouthfeel.

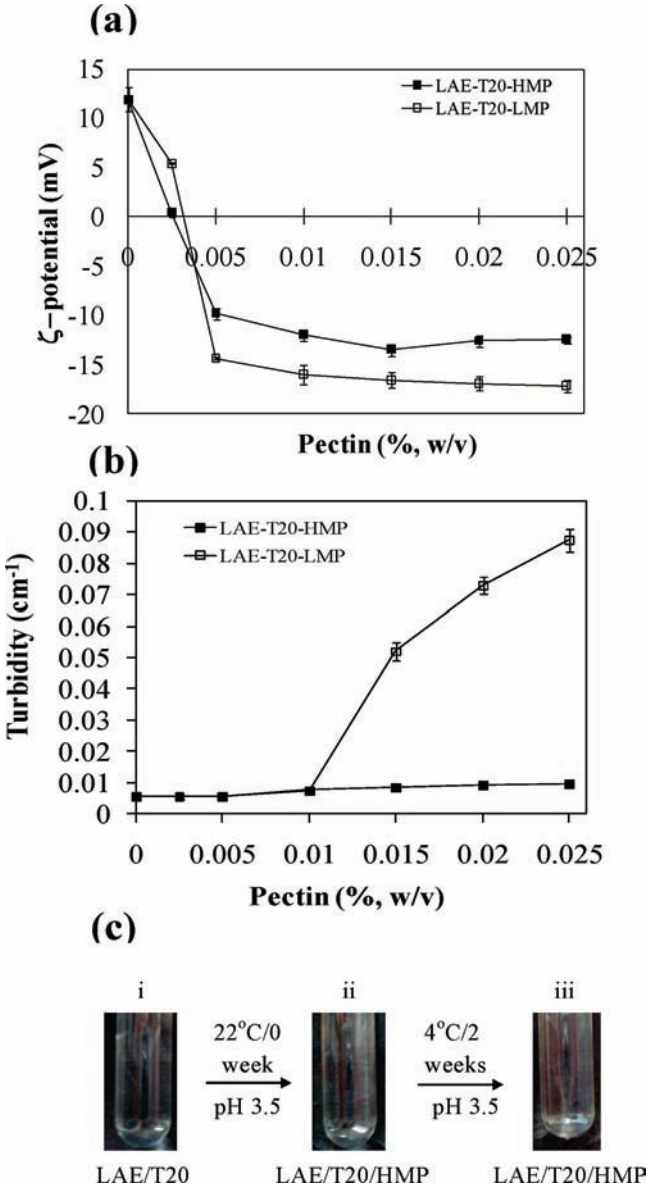

Figure 9. Change in (**a**) turbidity and (**b**) ζ potential of mixed micelles of LAE/T20 (0.25 wt % LAE and 0.25 wt % T20) upon the addition of HMP and LMP (0−0.025 wt %) and (**c**) photographs of (i) LAE/T20 mixed micelles (1:1, v/v), (ii) LAE/T20/HMP micelles (0.25 wt % LAE/0.25 wt % T20/0.01 wt % HMP), and (iii) LAE/T20/HMP micelles stored at 4 °C for 2 weeks. All solutions were prepared in 50 mM Na−citrate buffer (pH 3.5).

When LAE/HMP complexes were stored at room temperature for 2 weeks, large white aggregates were observed (**Figure 7a**). At low HMP concentrations (0−0.0025 wt %), the samples did not appear highly viscous and the white aggregates formed a sediment at the bottom of the test tubes after storage. Increasing the concentration of HMP to 0.005−0.01 wt % increased the viscosity, and white fibrous aggregates were observed that remained suspended in the test tubes (**Figure 7a**). When the HMP concentration exceeded 0.01 wt %, the solution became opaque with a marked increase in viscosity evidenced by the formation of gel-like solutions.

Optical microscopy examination of these samples showed that compact spheroid aggregates were formed at low HMP concentration (0−0.0025 wt %) but fiber-like structures were formed at higher HMP concentrations (**Figure 7b**). For example, at 0.005−0.01 wt % HMP, these fibers were long and thin and were arranged into bundles (about 30−50 mm) that were centrally connected as radial arrays. The bundles decreased in size (10−20 mm) and increased in number when the HMP concentration was increased above 0.01 wt % (**Figure 7b**).

On the basis of the above observations, we proposed a molecular interpretation for the difference in structural organization of the LAE/HMP complexes at different surfactant/polymer ratios (**Figure 8**). At high surfactant/polymer ratios (i.e., low HMP concentrations), each pectin molecule binds many LAE micelles, which leads to charge neutralization. These primary LAE/pectin complexes can then associate with each other through van der Waals and electrostatic interactions to form compact spheroid structures. At low surfactant/polymer ratios (i.e., high HMP concentrations), there are fewer LAE micelles available per pectin molecule, which means that there may be extensive positive and negative regions available on the surfaces of each primary LAE/pectin complex. Consequently, these primary complexes may be able to assemble into long thin fibers by creating sandwich-type structures of LAE and pectin (**Figure 8**). At present, the reason that radial structures of fibers are formed at higher pectin concentrations is unknown. Clearly, further work is needed to identify the molecular and physicochemical phenomena involved. A number of previous studies have also reported the formation of fibrous structures, e.g., when cationic surfactants are mixed with anionic surfactants (*24*), or when cationic surfactants are dispersed in water under certain conditions (*25*). Aggregation/gelation has also been reported in various other types of polymer−surfactant mixtures (*26*, *27*).

Characterization of LAE/T20/Pectin Interactions. In this set of experiments, we examined the possibility of forming LAE/T20/pectin complexes with improved functional performance. Our hypothesis was that the addition of T20 would improve the aggregation stability of the LAE, while the addition of pectin would enable the formation of anionic complexes. The LAE/T20/pectin complexes were formed by mixing anionic pectin with cationic LAE/T20 micelles.

In the absence of pectin, the ζ potential of the LAE/T20 mixed micelles was positive (ζ = +12 mV). As the pectin concentration was increased, the ζ potential became less positive and then eventually became negative, with the point of zero charge being around 0.025 wt % for both HMP and LMP (**Figure 9a**). This result indicates that the anionic pectins bound to the cationic mixed micelles to form LAE/T20/pectin complexes. The magnitude of the negative charge on these complexes was higher for LMP than for HMP, which can be attributed to the fact that it has the higher linear charge density.

The addition of pectin to mixed micelle dispersions caused appreciable changes in solution turbidity (**Figure 9b**). In the absence of pectin, the turbidity of the mixed micelle dispersions was relatively low because of the ability of the non-ionic surfactant to suppress LAE aggregation (**Figure 9b**). When the HMP concentration was increased, there was a slight increase in solution turbidity, which indicated that there was a slight increase in either the size and/or the concentration of the complexes formed. Nevertheless, the dispersions still had a low turbidity ($< 0.02\ cm^{-1}$) even at high pectin concentrations, indicating that the complexes formed were relatively small.

At LMP concentrations ≤0.01 wt %, the turbidity of the solutions remained fairly low ($< 0.01\ cm^{-1}$), indicating that any complexes formed were relatively small and did not scatter light strongly. Nevertheless, when the LMP concentration exceeded 0.01 wt %, there was an appreciable increase in solution turbidity (**Figure 9b**), which suggested that large aggregates had formed. The physicochemical origin of the differences in the aggregation behavior of the LMP and HMP is currently unknown. At 0.01 wt %

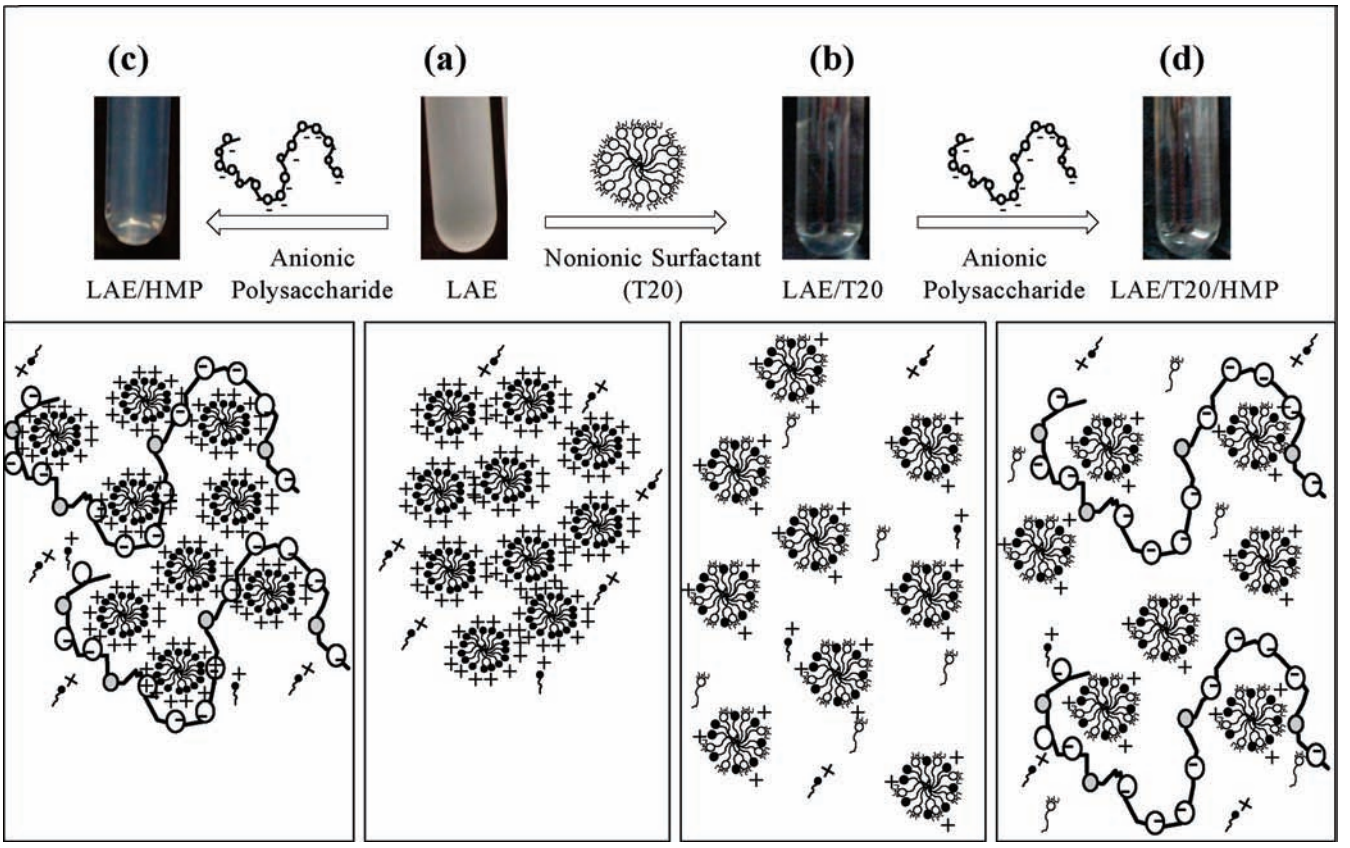

Figure 10. Schematic representation of the formation of mixed micelles and double-layered (masked) micelles with mixed micelles and oppositely charged food biopolymers (pectin): (**a**) LAE cationic micelles (0.25 wt %), (**b**) formation of mixed micelles of cationic LAE/non-ionic T20 micelles, (**c**) interaction between cationic LAE micelles and anionic pectin, and (**d**) interaction between mixed micelles of cationic LAE/non-ionic T20 micelles and anionic pectin. All solutions were prepared in 50 mM Na−citrate buffer (pH 3.5).

pectin, the electrical charge on the mixed micelle/LMP complexes was higher than that on the mixed micelle/HMP complexes (**Figure 9a**). Therefore, one might have expected the electrostatic repulsion to have been greater for the LMP system than for the HMP system. Presumably, some form of bridging flocculation occurred in the LMP system, because of sharing of cationic mixed micelles between numerous anionic pectin molecules.

To determine the cold stability of mixed micelle/pectin systems, samples were stored under quiescent conditions at 4 °C for 2 weeks and then the amount of precipitate formed was determined visually (**Figure 9c**). In the absence of T20, a white sediment was formed in the bottom of the tubes, indicating that they had poor cold stability (**Figure 6c**). In the presence of T20, mixed micelle/pectin systems were stable to aggregation over a range of HMP concentrations (0.005−0.025 wt %) (**Figure 9c**) and LMP concentrations (0.005−0.01 wt %).

The visual appearance and proposed structural organization of the surfactants and polymers within the various systems is shown schematically in **Figure 10**. We propose that soluble complexes of mixed micelles and pectin are formed under certain conditions that lead to transparent solutions that are stable to precipitation and sediment formation. The pure LAE micelles are prone to aggregation at higher pH values because of a reduction in their head group charge reduces the electrostatic repulsion between them (**Figure 10a**). LAE/T20 mixed micelles are more stable to aggregation because the hydrophilic polymeric head group of the non-ionic surfactant increases the steric repulsion between the micelles (**Figure 10b**). LAE/pectin complexes are unstable to aggregation because of charge neutralization and bridging flocculation effects (**Figure 10c**). LAE/T20/pectin complexes are relatively stable to aggregation because of the reduced ability of pectin to promote charge neutralization and bridging flocculation effects, because there are less cationic sites on the surface of the mixed micelles for pectin molecules to bind.

This study has shown that the solution properties of a cationic surfactant (LAE) can be appreciably altered by forming molecular complexes with a non-ionic surfactant (T20) and/or an anionic biopolymer (pectin). In the absence of these additives, LAE tends to aggregate and sediment at relatively high pH values (pH > 3) and under cold-storage conditions (4 °C). The pH and cold stability of LAE can be greatly improved by mixing it with T20, although the mixed micelles formed are cationic and may, therefore, have adverse interactions with anionic components and surfaces (such as food ingredients, packaging, or the human tongue). Mixtures of pectin and LAE micelles formed aggregates that sedimented, and therefore, pectin alone could not be used to improve the solution properties of the cationic surfactant. However, the addition of pectin to LAE/T20 mixed micelles led to the formation of transparent solutions that were more stable to pH changes and cold storage than LAE alone. In addition, the pectin/mixed micelle complexes formed were anionic, which should limit adverse interactions with anionic components and surfaces. This work has shown that the solution properties of a functional cationic surfactant (LAE) can be greatly improved by blending it with food-grade ingredients (non-ionic surfactant and anionic biopolymer), which may extend its applications in a variety of foods and other products. In future studies, we will examine the impact of complex formation on the sensory perception and antimicrobial activity of LAE.

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