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Stability of Citral in Emulsions Coated with Cationic Biopolymer Layers

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ABSTRACT: Multilayer emulsions containing citral were prepared by the layer-by-layer deposition technique based on the electrostatic interaction between negatively charged emulsion droplets and two positively charged biopolymer coatings, chitosan (CS) and ε-polylysine (EPL). The optimum concentrations of both CS and EPL were determined through the ζ-potential and particle size measurements and were found to be 1.5 mg/mL for CS and 6 mg/mL for EPL. Quartz crystal microbalance with dissipation monitoring (QCM-D) was conducted to monitor the binding between emulsion droplets and cationic polymers, and our results proved the existence of strong interactions between emulsions and the cationic polymer coatings. The stability of citral and the production of the off-flavor compounds were analyzed by solid-phase microextraction gas chromatography (SPME−GC). The results suggested that the addition of the cationic CS interfacial layer was effective in improving the stability of citral during storage.

KEYWORDS: *Citral degradation, emulsions, layer-by-layer deposition, quartz crystal microbalance with dissipation monitoring (QCM-D)*

INTRODUCTION

Citrus flavors, which contribute to pleasant and fresh fruit aroma profiles, are widely used in the food and beverage industry. They are usually extracted from citrus fruits, such as oranges, grapefruits, lemons, and limes. Because of their limited water solubility, citrus oils are added into many food and beverage products in the form of emulsion.[1] 3,7-Dimethyl-2,6-octadienal (citral) is one of the most important flavor compounds in citrus oils. It is a mixture of two geometrical isomers neral and geranial, and both of the two isomers have sweet and strong lemon odors. Because of its monoterpene structure with an aldehyde group, citral can easily undergo a series of degradation reactions, especially under acidic aqueous conditions. These reactions not only reduce the fresh aroma intensity of citral but also generate a variety of off-odor compounds.[2] The detailed formation pathways of the off-odor compounds from citral degradation have been studied by Schiebrle et al.,[2] Kimura et al.,[3] Peacock and Kuneman,[4] and Ueno et al.[5] However, the degradation mechanism of citral is still not fully understood. It has been established[3,4] that the linear citral molecules undergo acid-catalyzed cyclization reaction first to form monoterpene alcohols, e.g., *p*-menthadien-8-ol and *p*-menthadien-4-ol. Further oxidation of these intermediate alcohols generate *p*-cymen-8-ol and its dehydration products α,*p*-dimethylstyrene, *p*-cymene, and *p*-cresol.

Numerous works have been reported to stabilize citral by encapsulating it in the form of emulsion or micelles, because encapsulation could isolate the active compound (i.e., citral) from the reactive molecules in the aqueous medium, such as protons and free radicals. For instance, Djordjevic et al.[6] used whey protein isolate (WPI)- and gum arabic (GA)-stabilized oil-in-water emulsions to stabilize citral, and the results showed that WPI was more effective than GA to inhibit the oxidation of citral. Choi et al.[7] examined the impact of Tween 80 micelles and polyglycerol polyicinoleate (PGPR) reverse micelles on the chemical stability of citral, and the results showed that both micelles and reverse micelles could be used to improve the stability of citral in beverage emulsions. Furthermore, oil-in-water (O/W) emulsions with improved protection capability for the encapsulated compound can be produced by engineering the interfacial membrane of the emulsion droplets using the layer-by-layer (LBL) technique.[8] The technique is based on the electrostatic interaction between oppositely charged molecules,[9,10] and it has been used in the colloid chemistry recently to modify the surface properties of emulsions, such as surface charges and thickness of the interfacial layer around the emulsion droplets.[11,12] For a conventional emulsion, the emulsifier determines the interfacial properties of the emulsion droplets, while in the multilayer emulsions, when an ionic emulsifier is used to prepare the primary emulsion, oppositely charged polymers, such as proteins and polysaccharides, could be adsorbed onto the droplet surface to create a secondary emulsion with modified surface properties. For the protection of citral, a cationic emulsion system is favorable because the positively charged interfacial layer can repel reactive species (e.g., protons and metal ions) away from the emulsion droplets; therefore, the degradation of citral, which is induced and accelerated by protons, metal ions, and free radicals, could be inhibited.

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In this study, we examined the stability of citral encapsulated in palm kernel fat stabilized by lecithin (primary emulsion), chitosan (CS), and ε-polylysine (EPL) (secondary emulsions). Lecithin is widely used as an emulsifying, wetting, and dispersing agent. The presence of the functional head groups of various phospholipids makes lecithin an anionic emulsifier, which is suitable for the further LBL modification by cationic molecules, such as CS and EPL. CS is a linear polysaccharide comprising of co-polymers of D-glucosamine and *N*-acetyl-D-glucosamine. It has attracted increasing attention because of its potential wide range of industrial applications.[13] Because of the basic amine groups ($pK_a \approx 6.5$)[14] along the molecular chain, CS is soluble in water at low pH with positive charges. Another cationic biopolymer, EPL, is a homopolymer of L-lysine characterized by the isopeptide bond between ε-amino and α-carboxyl groups. In comparison to the chemically synthesized poly(L-lysine), EPL is naturally produced by the bacterium *Streptomyces albulus* and its characteristics depend upon the specific bacteria strains and culture conditions.[15] The isoelectric point of EPL is around 9.0.[16] Therefore, it is also positively charged at low pH. Three citral emulsion systems were prepared and analyzed in this study: anionic lecithin-stabilized emulsion and cationic lecithin−CS- or lecithin−EPL-stabilized multilayer emulsions. The objectives of this research were to (1) investigate the influence of different polymer concentrations on the emulsion physical properties (surface charges and particle sizes), (2) compare the stability of citral in three different formulations, and (3) evaluate the effects of different interfacial layers on the production of off-flavor compounds from citral under acidic conditions (pH 3.0).

MATERIALS AND METHODS

Materials. Palm kernel fat was a gift from Firmenich (Princeton, NJ). Alcolec PC 75 soy lecithin containing 75% phosphatidylcholine was a gift from American Lecithin Co. (Oxford, CT). EPL was purchased from Zhejiang Silver-Elephant Bioengineering Co. (Zhejiang, China). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Milli-Q water was used throughout the experiment.

Emulsion Preparation. The hot homogenization method[17] was used to prepare the primary emulsions. First, palm kernel fat was heated to 45 °C until completely melted. Citral and undecane (as the internal standard) were dissolved in the liquid lipid and homogenized with aqueous lecithin solution (in 10 mM citric acid/sodium hydroxide/sodium chloride at pH 3.0 buffer) by the ULTRA-TURRAX T-25 high-speed homogenizer (IKA Works, Inc., Willmington, DE) for 5 min at 45 °C. The coarse emulsions then went through a high-pressure homogenizer (EmulsiFlex-C3, Avestin, Inc., Ottawa, Canada) at the pressure of 150 MPa for 6 cycles to obtain primary lecithin-stabilized emulsions. The lecithin−CS- and lecithin−EPL-stabilized emulsions were prepared by adding the polymer solution into the primary emulsion and homogenized using the high-speed homogenizer at 1000 rpm for 1 min. The mixing processes were performed at room temperature. The same amount of pH 3.0 buffer solution was also added into the primary emulsions to make sure all of the samples (lecithin-, lecithin−CS-, and lecithin−EPL-stabilized emulsions) have the same contents of lipid (10 wt %), lecithin (5 wt %), citral (0.1 wt %), and undecane (0.01 wt %, as the internal standard). After sample preparation, citric acid was added when necessary to maintain the pH value (3.0) of all of the samples. A total of 10 mL of each emulsion dispersion was kept in a 20 mL amber glass vial with 10 mL of headspace. All of the samples were divided into two groups, with one group stored at 25 °C and the other group stored at 50 °C.

ζ-Potential Measurements. The surface charges of the lecithin-, lecithin−CS-, and lecithin−EPL-stabilized emulsions were measured using a Zetasizer Nano ZS-90 instrument (Malvern Instruments Ltd., Southboro, MA). All of the samples were diluted by pH 3.0 citric acid buffer before each measurement, and all of the measurements were performed at 25 °C.

Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) Measurements. The adsorption processes between CS or EPL and lecithin-stabilized primary emulsion were monitored using a commercial QCM-D apparatus (Q-Sense AB, Sweden) with a Q-Sense D300 electronic unit, which was controlled by the Q-Sense software (Q-Soft, Q-Sense). QCM-D is sensitive to small changes caused by deposited molecules on a gold crystal. A voltage is applied to the crystal to make it oscillate at a constant frequency; thus, any structural and/or mass changes on the crystal surface can induce a change in its resonant frequency. All of the experiments were performed at 25.00 ± 0.02 °C. Before each measurement, the gold-coated quartz crystal was soaked in 2 wt % sodium dodecyl sulfate solution for 30 min, then rinsed with Milli-Q water, and dried with nitrogen gas. The crystal was then cleaned in an ultraviolet (UV)/ozone chamber for 10 min, followed by soaking in a 1:1:5 (v/v/v) mixture of ammonia hydroxide (NH_4OH, 25%), hydrogen peroxide (H_2O_2, 30%), and Milli-Q water for 15 min at 75 °C. It was then rinsed with Milli-Q water, dried with nitrogen gas, and finally cleaned in a UV/ozone chamber for another 10 min. This cleaning procedure could remove the possible contaminants on the crystal surface. The crystal was then mounted in the QCM-D chamber and excited at their fundamental frequency (about 5 MHz), as well as the third, fifth, and seventh overtones (denoted by n = 3, 5, and 7, corresponding to frequencies of 15, 25, and 35 MHz, respectively). All of the samples were diluted 10 times before injecting into the QCM-D chamber to avoid clogging the instrument tubing, and samples were injected only when a stable Δf signal was obtained (Δf < 1 Hz in 5 min of the third overtone).

Particle Size Measurements. The physical stability of all of the emulsion samples was evaluated by measuring their particle size changes at different storage temperatures (25 and 50 °C) with time. The particle sizes were measured by a photon correlation spectroscopy (PCS)-based BIC 90 plus particle size analyzer equipped with a Brookhaven BI-9000AT digital correlator (Brookhaven Instrument Corp., New York). The light source of the particle size analyzer is a solid-state laser operating at 658 nm with 30 mW power, and the signals were detected by a high-sensitivity avalanche photodiode detector. All of the samples were diluted 100 times to avoid a multiple scattering effect, and all of the measurements were made at a fixed scattering angle of 90° and a temperature of 25.0 ± 0.1 °C.

Measurement of Citral. Analyses of citral and its degradation products were conducted on Agilent 6850 gas chromatography (GC) equipped with a J&W DB-5MS capillary column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness) and a flame ionization detector (FID). The oven temperature was increased from 60 to 150 °C at 4 °C/min, then increased to 230 °C at 20 °C/min, and held at 230 °C for 5 min. The gas flow was controlled as follows: hydrogen flow at 40.0 mL/min, air flow at 45.0 mL/min, and helium as the carrier gas flow at 45.0 mL/min. The FID temperature was 250 °C. A 0.75 mm inner diameter solid-phase microextraction (SPME) injection sleeve was employed to minimize the broadening effect compared to a 2.0 mm injection glass linear. For SPME analysis, 10 mL of each solid lipid nanoparticle (SLN) dispersion was stored in a 20 mL amber glass vial containing a magnetic stir bar under stirring. The glass vial was sealed with a polytetrafluoroethylene (PTFE)/silicone septa and a screw cap. The 65 μm polydimethylsiloxane−carboxen (PDMS−DVB) SPME fiber was exposed to the sample headspace manually for 30 min at 25 °C (for the 25 °C storage samples) and 50 °C (for the 50 °C storage samples). After the absorption process, the fiber was inserted immediately into the injection port of GC and held for 5 min to ensure a complete thermal desorption. The quantification of citral and the degradation products were analyzed by computing their peak areas versus the internal standard (undecane) peak area.

GC−Mass Analysis of the Degradation Products of Citral. An Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass detector and a J&W DB-5MS capillary column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness) was used. The gas flow was

controlled as follows: hydrogen flow at 40.0 mL/min, air flow at 45.0 mL/min, and nitrogen flow at 45.0 mL/min. The injection port was kept at 230 °C. The oven temperature was increased from 60 to 150 °C at 4 °C/min, then increased to 230 °C at 20 °C/min, and held at 230 °C for 5 min. The ionization voltage was held at 70 eV, and the ion temperature was 280 °C.

Statistical Analysis. All experiments were conducted twice in duplicate, and all data were expressed as means ± standard deviations. When appropriate, data were analyzed using the *t* test by SPSS software (SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Influence of CS and EPL Concentrations on the Surface Charges of Emulsion Droplets. The surface charges of lecithin−CS- and lecithin−EPL-stabilized emulsions with different CS and EPL concentrations (at pH 3.0) were measured by ζ potential. There are two purposes for the ζ-potential measurements: first, changes of the surface charge could prove the adsorption between the oppositely charged emulsion droplets and the polymers (i.e., CS and EPL, respectively); second, the optimum concentrations for both CS and EPL could be identified to prepare stable lecithin−CS- and lecithin−EPL-coated secondary emulsion dispersions in the following experiments to encapsulate citral. A series of secondary emulsion systems were prepared with various concentrations of CS (0−1.75 mg/mL) and EPL (0−8 mg/mL), and the dependence of the surface charges upon different CS and EPL concentrations at pH 3.0 was shown in Figure 1.

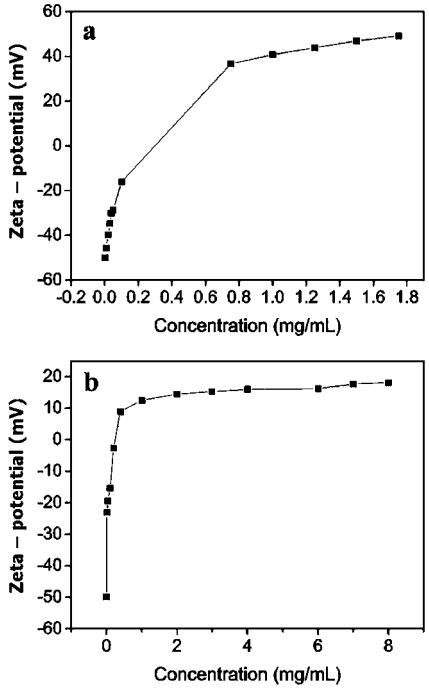

Figure 1. Plots of emulsion ζ potential versus the concentrations of (a) CS and (b) EPL at pH 3.0. Some error bars lie within data points.

In the absence of either CS or EPL, the surface charge of lecithin-stabilized emulsion was −49.9 mV. For the emulsion droplets coated with CS, the surface charge became less negative with the increase of the CS concentration, which suggested that the positively charged CS adsorbed onto the negatively charged emulsion droplet surfaces by forming an extra CS layer around the droplets. There is a CS concentration gap between 0.1 and 0.75 mg/mL, as shown in Figure 1a, mainly because it would be very difficult to measure the ζ potential for samples coated with CS in that concentration range. During the sample preparation process, when the CS aqueous solution (with a concentration between 0.1 and 0.75 mg/mL) was mixed with the lecithin-stabilized emulsion, a thick gel formed within hours; therefore, no ζ potential can be measured. The most likely explanation for this phenomenon is that particle aggregation substantially dominated within that CS concentration range, and consequently, gel formed because of the bridging flocculation between the negatively charged emulsion droplets and positively charged CS molecular chains. When the CS concentration was high enough, the surface charge values reached a plateau at ∼45 mV, indicating that the initially negatively charged emulsion droplets were saturated with CS.

For the EPL-coated emulsion samples, their surface charges also switched from negative to positive with the increase of the EPL concentration and then reached a plateau at around 15−18 mV when the EPL concentration was high enough (Figure 1b). The surface charges of the EPL-coated emulsions were much smaller than the CS-coated emulsions. For example, the EPL-coated emulsions showed lower surface charges (∼18 mV) than that of the CS-coated emulsions (∼45 mV). This was due to the different polymer characteristics, such as molecular chain structures and charge densities along the molecular chains. Besides the surface charges, another marked difference between CS- and EPL-coated emulsions is that no concentration gap was observed for the EPL-coated emulsions. For the CS-coated emulsions, there existed a CS concentration range where bridging flocculation dominated to cause the emulsion droplets to aggregate and form gel, as discussed above; however, there was no such concentration range for EPL, as shown in Figure 1b. During the preparation of EPL-coated samples, particle flocculation happened very fast when the EPL concentration was between 0.2 and 4 mg/mL. Therefore, within this concentration range, the EPL-coated samples were vortexed for 30 s prior to the ζ-potential measurement. Different polymer coatings resulted in different phase behaviors, such as gelation for CS-coated samples and particle flocculation for EPL-coated samples, which could be related to different polymer characteristics. It was suggested that various polymers could exert different influences on the formation process of the multilayer emulsions, and the influencing factors related to the polymers are their concentrations, molecular weights, structures, and charge densities, among which the concentrations and charge densities are the most important factors.[8] The high surface charge for CS-coated emulsion indicated that CS is a strongly charged polymer; when the emulsion droplets cannot be fully covered, the CS molecular chains were likely adsorb onto two of the droplet surfaces to induce bridging flocculation, which is an irreversible phenomenon to result in the subsequent gel formation because of the strong interaction between the CS molecules and the emulsion droplets. While EPL is a weakly charged molecule, the interaction between EPL and the emulsion droplets is also weak to make adsorption an unfavorable process to induce the polymer depletion flocculation, which is a reversible phenomenon. Different surface charge intensities caused by different molecules lead to different emulsion flocculation behaviors, which has led to the different performances of the two cationic polymer-coated systems.

Influence of CS and EPL Concentrations on the Particle Size. The mean particle diameters of lecithin−CS- and lecithin−EPL-stabilized particles with different CS and EPL concentrations (at pH 3.0) were measured at pH 3.0, and the results are shown in Figure 2. In the absence of either CS or EPL, the mean particle diameter of the lecithin-stabilized emulsion droplets was ∼100 nm. For the CS-coated emulsions, three phases could be observed for the particle size changing trend, as shown in Figure 2a: (1) the particle size slightly increased when the CS concentration increased from 0 to 0.1 mg/mL; (2) the particle size greatly increased when the CS concentration increased from 0.1 to 1 mg/mL; and (3) the particle size reached a constant value of ∼450 nm when the CS concentration exceeded 1 mg/mL. A similar trend could also be observed for the EPL-coated emulsions, as shown in Figure 2b, where the mean particle diameter first slightly and then dramatically increased with the increase of the EPL concentration, finally decreased, and reached a plateau of ∼600 nm when the EPL concentration was high enough (≥6 mg/mL). Similar particle size changing trends were also reported in several previous studies.[12,18] Before the coating processes of the cationic biopolymers CS and EPL, lecithin-stabilized emulsions had a relatively smaller particle size and good physical stability during storage (which will be discussed in the next section). During the initial stage of adding polymers (both CS and EPL) into the lecithin-stabilized emulsion, polymer chains began to adsorb onto the emulsion droplet surfaces because of the electrostatic interaction between the negative charges on the emulsion droplets and the positive charges on the polymer molecular chains, which caused the increase of the particle size. For example, at the concentration of 0.1 mg/mL for both CS and EPL, the mean diameter for CS-coated emulsion was 289.4 nm, while for EPL-coated emulsion, the mean diameter was 474.5 nm. In the second stage, with a further increase of the polymer concentrations, most of the emulsion droplets were partially coated with polymers; therefore, bridging flocculation happened. At this stage, the polymer chains could act as "bridges" between the opposite charges on different emulsion droplets, which means one CS or EPL molecular chain could adsorb onto more than one droplet surface simultaneously. The bridging effect resulted in the sharp increase of the particle size. For example, the CS-coated emulsion droplets became larger than 600 nm when the CS concentration exceeded 0.1 mg/mL, and the mean diameter for the EPL-coated emulsion showed a ∼5 times increase when the EPL concentration increased from 0.1 to 0.2 mg/mL. At the final stage when the polymer concentrations were high enough, the polymer-coated emulsion droplets gradually became saturated; consequently, bridging disappeared, and the electrostatic repulsion was strong enough to avoid emulsion aggregation.

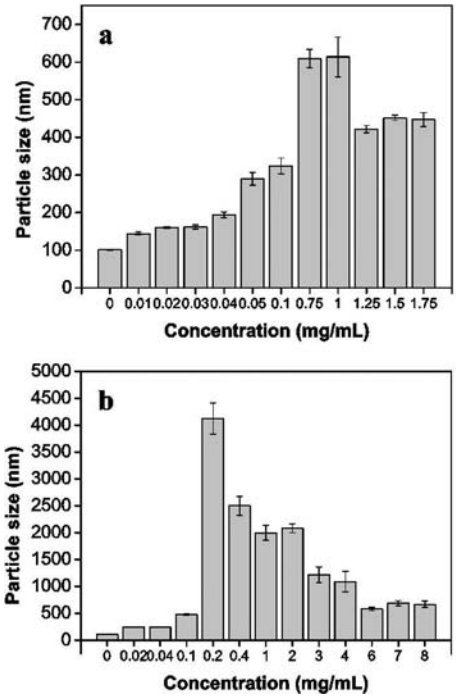

Figure 2. Dependence of the mean particle diameters of emulsions upon the concentrations of (a) CS and (b) EPL.

Adsorption between CS and the Lecithin-Stabilized Emulsion Studied by QCM-D. Both the ζ-potential and the particle size results proved the adsorption between the emulsion droplets and the polymers to a certain extent; however, direct evidence is lacking in most of the similar studies. In this research, QCM-D was used to monitor the adsorption process between the emulsion and polymers directly. Figure 3a showed the frequency shift for the adsorption between CS and the lecithin-stabilized emulsion starting with the deposition of CS on the quartz crystal sensor surface. With the injection of the emulsion into the QCM-D chamber, the frequency decreased immediately, which was associated with the strong interaction between CS and the emulsion. Furthermore, the additional buffer wash did not cause a significant frequency change, which indicated that the interaction between CS and emulsion was robust enough to

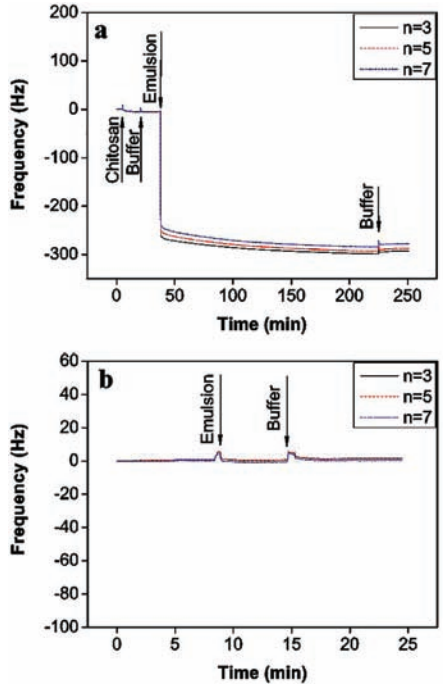

Figure 3. Representative QCM-D frequency shifts for (a) the adsorption between CS and lecithin-stabilized emulsion and (b) the adsorption of lecithin-stabilized emulsion onto the crystal sensor surface without CS at three frequency overtones (n = 3, 5, and 7).

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resist washing. To prove that the frequency shift was not caused by the physical deposition of emulsion because of gravity, a control measurement was taken, as shown in Figure 3b. During the control measurement, the frequency remained at 0, which suggested that the frequency shift resulted from the interaction between CS and emulsion and not from the physical deposition of emulsion. The same adsorption measurements were also taken for EPL-coated emulsions; however, the frequency shift soon exceeded the instrument limitation even at very low EPL concentrations. Therefore, only data from CS-coated emulsions were shown here. The most likely reason for the abnormal frequency shifts caused by EPL might be attributed to the depletion flocculation induced by the weakly charged EPL molecules. The ζ-potential data already suggested that the EPL-coated emulsions had lower surface charge intensities than the CS-coated emulsions; therefore, the interaction between EPL and the emulsion droplets might not be sufficient to maintain a stable adsorption between EPL and the emulsions. Consequently, the free, non-adsorbing polymer molecules induced the emulsion depletion flocculation[26] in the QCM-D measurement chamber to cause the abnormal frequency behaviors.

In this study, QCM-D was used as supplementary means to determine the optimal concentration of CS. In combination of the ζ-potential and particle size measurement results, five different CS concentrations were chosen for the QCM-D measurements, and the results are shown in Figure 4. When the

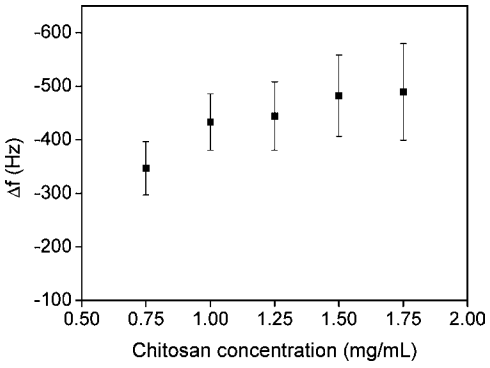

Figure 4. Dependence of the QCM-D frequency shifts upon CS concentrations.

CS concentration increased from 0.75 to 1.75 mg/mL, the frequency shifts also increased at first and then reached a plateau. Consequently, the ultimate CS concentration of 1.5 mg/mL was used to prepare citral-loaded emulsion, and the final EPL concentration of 6 mg/mL was chosen based on the ζ-potential and particle size results.

Physical Stability of Citral-Loaded Emulsions with Different Polymer Coatings. Particle sizes of citral-loaded emulsions stabilized by lecithin, lecithin−CS, and lecithin−EPL were measured at days 0, 30, and 60 during storage under 25 and 50 °C. The mean particle diameter data are listed in Table 1. Lecithin-stabilized emulsion had the smallest particle size of 109.2 nm, while both CS and EPL coatings increased the particle size to 451.6 and 580.5 nm, respectively. EPL-coated emulsion was very unstable, and phase separation happened during storage, even at 25 °C. Therefore, no particle size was measured at days 30 and 60. The most likely reasons are the following: (1) EPL-coated emulsion droplets had the largest size among all of the formulations, and (2) this group of samples also had the smallest surface charge (~18 mV). A large

Table 1. Mean Particle Diameter (nm) Changes during Storage for Citral-Loaded Emulsions Stabilized by Lecithin, Lecithin−CS, and Lecithin−EPL Stored under 25 and 50 °C

T (°C)	samples	day 0	day 30	day 60
25	lecithin	109.2 ± 3.0	123.3 ± 4.7	132.7 ± 9.8
	lecithin−CS	451.6 ± 7.4	494.7 ± 12.5	512.3 ± 15.7
	lecithin−EPL	580.5 ± 28.1		
50	lecithin	109.2 ± 3.0	148.9 ± 3.3	186.1 ± 7.9
	lecithin−CS	451.6 ± 7.4	601.8 ± 38.3	646.7 ± 41.2
	lecithin−EPL	580.5 ± 28.1		

particle size plus not enough electrostatic repulsion between the emulsion droplets contributed to the instability problem of the EPL-coated emulsion. For the other two formulations (lecithin- and lecithin−CS-stabilized emulsions), the particle size increased during storage with time, especially at a higher temperature (50 °C). However, both of them still showed good physical stability because the particle size increments were not significant enough to cause obvious creaming or phase separation, which might be associated with the high surface charges and the consequent large repulsive force between the emulsion droplets.

Stability of Citral During Storage. The stability of citral was determined in various formulations (lecithin-, lecithin−CS-, and lecithin−EPL-stabilized emulsions) by calculating the loss of the isomers of citral, neral and geranial, during storage. At 25 °C, both neral and geranial showed similar degradation trends, as shown in Figure 5. Lecithin−EPL-stabilized emulsion was

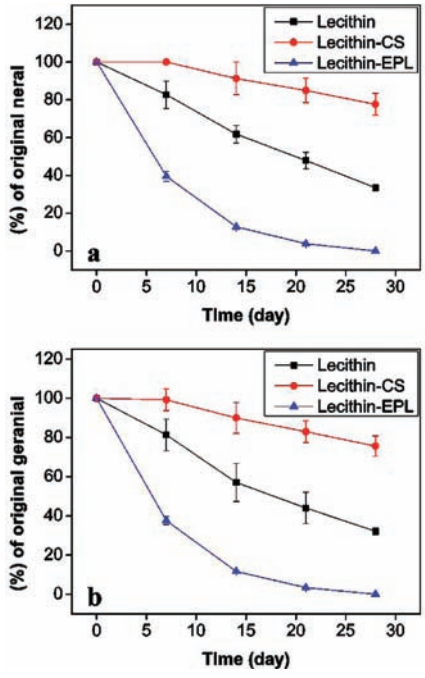

Figure 5. Degradation of (a) neral and (b) geranial in lecithin-, lecithin−CS-, and lecithin−EPL-stabilized emulsions stored at 25 °C.

the worst formulation because both neral and geranial degraded very fast compared to the other two groups of samples. Only ~3% citral was left after 3 weeks, and no citral could be detected after 4 weeks. The fast degradation of citral in the lecithin−EPL-stabilized formulation was caused by phase separation of the emulsion. The phase-separated emulsion sample was basically divided into two layers, which are the top

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lipid layer and the bottom aqueous layer. Because citral is a partially polar molecule with certain water solubility, it may exist in both layers. Furthermore, phase separation is a phenomenon that often involves creaming and coalescence; the break of the emulsion structure will eventually happen. Therefore, the protection of the emulsion for citral no longer existed. Lecithin-stabilized emulsion showed certain protection for citral when 82.7% neral and 81.3% geranial were left after the first week, and 33.5% neral and 32.1% geranial still remained in the sample after 4 weeks. In comparison to the lecithin-stabilized emulsion, more than 2 times the amount of citral was left in the lecithin−CS-stabilized formulation. For example, almost no neral and geranial degraded after the first week, and 77.6% neral and 75.6% geranial remained in the sample at the end of the measurement (4 weeks). The extra CS coating had a positive effect to inhibit citral from degradation. Similar to our previous study,[19] all of the samples were also stored at a relatively higher temperature (50 °C) to accelerate citral degradation (Figure 6). As expected, both neral and

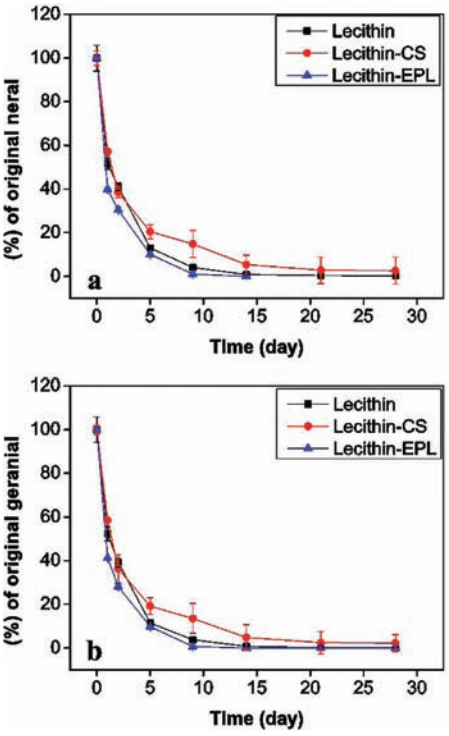

Figure 6. Degradation of (a) neral and (b) geranial in lecithin-, lecithin−CS-, and lecithin−EPL-stabilized emulsions stored at 50 °C.

geranial degraded much faster than the samples stored at 25 °C, so that the difference between different formulations could not be differentiated. For instance, ~48% citral was lost for lecithin-stabilized emulsion, ~43% citral was lost for CS-coated emulsion, and ~60% citral was lost for the EPL-coated emulsion only after 1 day of storage at 50 °C. Several off-flavor compounds were also detected, and the analysis results will be discussed in the following section.

Evaluation of Off-Flavor Compounds for Citral-Loaded Emulsions. Citral was completely degraded and almost totally converted to its degradation products during storage at 50 °C for 4 weeks. As shown in our previous study,[19] similar GC spectra were obtained in this study, and the major off-flavor compounds were listed in Table 2. Both citral and

Table 2. Major Degradation Products Formed from Citral-Loaded Emulsions Stored at 50 °C for 4 Weeks[19]

compound	ID method[a]
2-heptanone	A
1-octen-3-ol	A
delta-2-carene	A
p-cresol	B
α,p-dimethylstyrene	B
butanoic acid	A
p-metha-1,5-dien-8-ol	B
p-methylacetophenone	B

[a]Compounds were identified on the basis of the following criteria: A, mass spectrum agrees with that of the Wiley mass spectral database, and the compounds can only be considered as "tentatively identified"; B, mass spectrum and retention index agree with those of authentic compounds purchased from Sigma-Aldrich (St. Louis, MO).

lipid degradation products were detected from all of the formulations. It is well-known that lipid degradation will generate many off-flavor compounds, such as 2-heptanone, 1-octen-3-ol, and butanoic acid, in this study, especially at high temperatures. A cationic interfacial membrane is supposed to increase the lipid oxidative stability in an oil-in-water emulsion system based on several previous studies.[20−22] However, both polymer coatings did not show significant effects to inhibit lipid oxidation in this study. For the citral degradation, it has been accepted that citral degradation started from acid-catalyzed reactions first to form several compounds, such as *p*-cymene, *p*-menthadien-8-ols, and *p*-menth-2-ene-1,8-diols.[5,23,24] In this study, only *p*-metha-1,5-dien-8-ol was produced as the acid-catalyzed reaction product from citral after encapsulation. While the EPL coating had no effect to reduce the production of *p*-metha-1,5-dien-8-ol, the CS coating worked to inhibit its production, as shown in Figure 7. The other citral degradation products, such as delta-2-carene, *p*-cresol, *α,p*-dimethylstyrene, and *p*-methylacetophenone, were produced from the oxidation reactions of citral. In comparison to the lecithin-stabilized emulsion, the CS coating reduced the production of delta-2-carene and *p*-cresol and had the similar concentration level of *p*-methylacetophenone, but unfortunately, it also promoted the production of *α,p*-dimethylstyrene. While the EPL coating increased the production of delta-2-carene, *α,p*-dimethylstyrene, and *p*-methylacetophenone, interestingly, it also decreased the production of *p*-cresol in the CS-coated sample. Most of the citral oxidation products were not detected in all of the formulations, such as *p*-cymen-8-ol and 8-hydroperoxy-*p*-cymene.[3,5] There might be two reasons to explain this phenomenon: First, previous studies proposed that most of the oxidation products of citral were produced from a series of dehydration and/or oxidation reactions of many intermediate compounds, such as *p*-menthadien-8-ols and *p*-mentha-1,4-(8),5-triene.[5,25] These intermediate compounds resulted from the acid-catalyzed reactions of citral induced by protons in the acidic aqueous medium. It had been shown that most of the acid-catalyzed products were inhibited after citral was encapsulated in this study, because encapsulation was an effective way to isolate citral from the reactive species, such as protons and metal ions, in the acidic aqueous medium. Consequently, the oxidation reaction pathways were interrupted to produce the commonly detected oxidation products of citral. The second reason might be due to the presence of lipid and phospholipids (palm kernel fat and lecithin) in all of

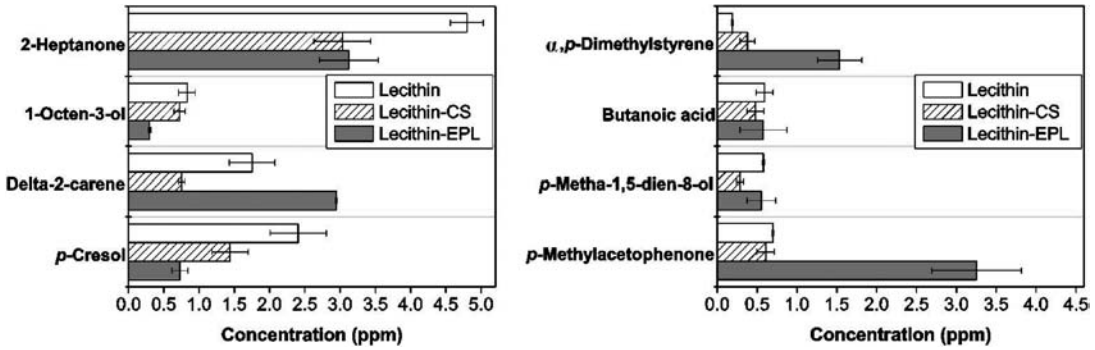

Figure 7. Concentrations for the major degradation compounds produced from citral-loaded emulsions stored at 50 °C for 4 weeks.

the formulations. However, the mechanisms are still unknown, and more research is needed to study how the other ingredients, such as lipid and phospholipids, influence the reaction pathways of the degradation of citral.

In summary, citral was encapsulated in anionic lecithin-stabilized emulsion and two cationic lecithin−CS- and lecithin−EPL-stabilized emulsions. ζ-Potential, particle size, and QCM-D measurements have confirmed the feasibility to prepare different emulsion systems with different polymer coatings based on the electrostatic interaction between oppositely charged emulsion droplets and cationic polymers. EPL-coated particles were not stable enough to protect citral because of the small surface charge and the formation of very large particles. The lecithin−CS-stabilized emulsion dispersion not only possessed good physical stability during storage but also worked better than the solely lecithin-stabilized particles to inhibit citral from degradation.

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