Effect of Surface Charge on the Stability of Oil/Water Emulsions during Steam Sterilization

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Abstract □ Intravenous lipid emulsions are used for total parenteral nutrition and as carriers for lipophilic drugs. Exposure to the high temperature (121 °C) required for steam sterilization may cause coalescence and an increase in droplet size. The purpose of this study was to investigate whether an increase in the electrostatic repulsive force between oil droplets produced by formulation modification improves the thermal stability of lipid emulsions during autoclaving. The addition of a small amount, 0.66 or 1.32 mmol/kg (mm), of purified anionic phospholipid fractions (phosphatidic acid, phosphatidylglycerol, or phosphatidylinositol) to the standard formula increased the ζ potential from its normal value of –11 mV to –39 mV. Emulsions with the larger negative ζ potential did not exhibit any change in oil droplet size or distribution during steam sterilization at 121 °C for 15 min. The autoclaved emulsions having the larger negative ζ potential did not exhibit any evidence of coalescence when samples were stored for 1 month at 4 °C, room temperature, or 40 °C. Reduction of the negative surface charge of the oil droplets by the addition of stearylamine confirmed that the surface charge was an important factor, as emulsions having a reduced negative surface charge separated into two phases during autoclaving.

Introduction

Lipid emulsions have a long history of safe use as a source of intravenous nutrition. They have been used more recently for the intravenous administration of lipid-soluble drugs. Intravenous lipid emulsions must be sterile. The preferred sterilization process is steam sterilization. Exposure to the high temperature, 121 °C, required for steam sterilization may cause an increase in the mean droplet size.[1] The droplet size is important for the safe use of intravenous lipid emulsions, as the smallest capillaries have a diameter of 5 μm.[2−5] The desirable size range of oil droplets administered intravenously is approximately 0.4 to 1 μm,[2−5] which is approximately the same size as naturally occurring chylomicron in the blood.

Intravenous lipid emulsions are stabilized by a combination of forces such as electrostatic, hydration, and steric repulsive forces. Egg phospholipid, the emulsifier in most intravenous lipid emulsions, is a mixture of nonionic and anionic phospholipids. The anionic components have apparent pK_a values between 3 and 4[6] and contribute an electrostatic repulsive force within the pH range of intravenous lipid emulsions, i.e., pH 8.0−9.0. It has been proposed that phospholipids form a liquid crystal interfacial film structure at the oil−water interface by adsorption of water.[7] The phospholipid molecule can attach to itself up to 39 water molecules, resulting in a great increase in molecular volume.

The hydration and steric repulsive forces, which depend on adsorption, are inversely related to temperature. Thus, these stabilizing forces are less effective during exposure to 121 °C during steam sterilization. On the other hand, the electrostatic repulsive force is inversely related to the dielectric constant of the medium.[8] In the case of lipid emulsions, the medium is water and its dielectric constant decreases as temperature increases. Therefore, the electrostatic repulsive force is larger at elevated temperatures than at room temperature.

The electrostatic repulsive force has been increased in phospholipid-stabilized o/w emulsions by the addition of oleic acid, phosphatidylserine, phosphatidylglycerol, or phosphatidic acid.[9−13] Most of the studies focused on reducing the rate of coalescence when the lipid emulsion was mixed with electrolytes in an admixture for total parenteral nutrition. In general, the stability of emulsions to the addition of electrolytes was directly related to the magnitude of the negative surface charge. Yamaguchi et al.[13] reported that when the ζ potential of a lipid emulsion was increased from −8 to −18 mV by the addition of 17.5 mM oleic acid, the emulsion exhibited no change in mean particle size after autoclaving at 121 ° C for 20 min. The mean particle size of the control emulsion (without the added oleic acid) increased from 0.20 to 0.32 μm during autoclaving. No information was presented regarding the particle size distribution. Thus, although the mean droplet size (as measured by photon correlation spectroscopy) did not change during autoclaving, coalescence may have occurred. A second technique capable of monitoring droplets larger than 1 μm, such as single-particle optical sensing, was needed to determine if coalescence occurred during autoclaving.

This study was undertaken to determine if the formulation of o/w emulsions can be modified by the addition of purified anionic phospholipid fractions to increase the naturally occurring negative surface charge and if emulsions having a larger negative surface charge will undergo less coalescence during steam sterilization.

Experimental Section

Miglyol 812 is a neutral oil composed of caprylic and capric triglyceride (Huls, Hillside, NJ). Olive oil was obtained from ICN Biomedical, Aurora, OH. Egg phospholipid was a gift of Pharmacia & Upjohn, Clayton, NC. Phosphatidic acid, phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol were obtained from Avanti Polar Lipid, Alabaster, AL. Stearylamine was obtained from Sigma Chemical Co., St. Louis, MO.

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Table 1—Effect of Autoclaving on the Properties of the Standard Emulsion

	before autoclaving	after autoclaving
acid value, mg KOH/g emulsion	0.247 ± 0.003	0.348 ± 0.009
pH	8.0 ± 0.0	7.2 ± 0.0
ζ potential, mV	−10.5 ± 2.4	−23.6 ± 0.8
Mean diameter, μm	0.28 ± 0.01	0.40 ± 0.00
Volume of oil present as droplets larger than 1 μm, % v/v	0.060 ± 0.005	0.473 ± 0.046

The following formula was the standard emulsion used in this study: Miglyol 812 10 g olive oil 10.0 g; butylated hydroxytoluene 0.2 g; egg phospholipid 1.2 g; glycerol 2.4 g; doubly distilled water, qs 100.0 g.

The emulsion was produced by preparing the aqueous phase by dispersing the egg phospholipid in a solution of glycerol and water using a constant speed stirrer at 1500 rpm. Nitrogen was bubbled into the glycerol and water solution for 15 min before the egg phospholipid was added. The oil phase was prepared by combining Miglyol 812 and olive oil and bubbling nitrogen into the solution for 15 min. Butylated hydroxytoluene was dissolved in the oil phase. A primary emulsion was prepared by mixing the oil and aqueous phases for 10 min using a high speed mixer with a propeller blade (Multimix 4642, Braun). The primary emulsion was passed through a microfluidizer (Model 110 Y, Microfluidics Corp., Newton, MA) five times at 20000 psi. The cooling coil and interaction chamber were packed with ice. The final pH of the emulsion was adjusted to 8.0 by the addition of 0.5 to 1.0 mL of 0.1 N NaOH per 300 g of emulsion.

The temperature of emulsions produced by the microfluidizer has been reported to increase by 5 °C for each pass when no cooling is used.[14,15] The use of ice on the cooling coil and interaction chamber in this study controlled the temperature of the discharged emulsion in the range of 16 to 22 °C.

The emulsion was packaged in 10 mL vials (Type 1 glass) and sealed with a pulp and plastic screw cap. The emulsion was purged with nitrogen before sealing. Samples of the emulsion in the 10 mL vials were autoclaved for 15 min at 121 °C, 15 psi.

The pH of the emulsion and the results of all of the other tests were reported as the mean of four measurements from two sample vials. The acid value was determined by a standard procedure and is expressed as the milligrams of potassium hydroxide required to neutralize the free acids in 1 g of emulsion.[16] The mean droplet diameter was determined by dynamic light scattering (Nicomp 370, Particle Sizing Systems, Santa Barbara, CA) and was based upon volume weighting. The emulsion was diluted 1:100 immediately before measurement with doubly distilled water that had been passed through a 0.22 μm membrane filter. This dilution procedure was selected after a preliminary experiment indicated that the mean droplet diameter remained the same when measured immediately after dilution or 10, 15, or 30 min after dilution. The percentage of oil present as droplets larger than 1 μm was determined using a single-particle optical sensing system (Accusizer 770, Particle Sizing Systems, Santa Barbara, CA). The emulsion was diluted immediately before measurement with doubly distilled water that had been passed through a 0.22 μm membrane filter. The dilution ratio was adjusted to obtain a droplet count between 3000 and 8000 droplets/mL. The ζ potential was determined by Doppler electrophoretic light scattering analysis (DELSA 440, Coulter, Hialeh, FL). The emulsion was diluted with 0.01 M HEPES buffer at pH 7.5 (Fisher, Fairlawn, NJ) which contained 5 mM NaCl.

Results and Discussion

The effect of autoclaving at 121 °C for 15 min on the properties of the standard emulsion is presented in Table 1. The acid value increased following autoclaving, indicating the formation of free fatty acids. It is likely that the elevated temperature accelerated the hydrolysis of the oil phase and the egg phospholipid used as the emulsifier. The rate of hydrolysis of phospholipids has been found to follow the Arrhenius equation in the temperature range of 5 to 90 °C.[17] The fatty acids formed by the hydrolysis of egg phospholipid are probably also responsible for the decrease in pH observed after autoclaving. The ζ potential became more negative following autoclaving. This may be the result of the increased fatty acid concentration and/or the redistribution of the egg phospholipid from the aqueous phase where it is present as liposomes to the interface during autoclaving.[18]

Table 2—Effect of Adding Egg Phospholipid Fractions (0.66 or 1.32 mm) on the ζ Potential

egg phospholipid fraction added	ζ potential ± SD, mV	
	0.66 mm	1.32 mm
none	−10.5 ± 2.4	−10.5 ± 2.4
phosphatidylcholine	−12.2 ± 3.4	
phosphatidic acid	−35.1 ± 1.7	−38.4 ± 1.4
phosphatidylglycerol	−39.4 ± 1.2	−40.0 ± 1.8
phosphatidylinositol	−32.7 ± 0.3	−37.3 ± 0.4

Autoclaving caused an increase in the mean droplet size from 0.28 to 0.40 μm. It is likely that coalescence occurred during exposure to 121 °C which resulted in an increased mean droplet size. Further evidence that coalescence occurred is the increase in the percentage of oil present as droplets larger than 1 μm. This parameter is more critical for safety than the mean droplet size, as the smallest capillary has a diameter of 5 μm.[2−5] Some oil droplets which formed during autoclaving became large enough to rise to the surface of the emulsion and were seen without magnification as oil droplets on the surface.

Egg phospholipid is a mixture of many components.[19] The major components are phosphatidylcholine (PC) and phosphatidylethanolamine which exhibit no net charge at physiological pH. The minor components, which comprise 2−5% of the total lipid, are phosphatidylserine, phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylinositol (PI), sphingomyelin, cholesterol, and lysophosphatidylcholine. Phosphatidylserine, PA, PG, and PI are negatively charged at pH 7.0. Along with free fatty acids, these anionic fractions are probably responsible for the negative ζ potential of the standard emulsion (Table 1).

The effect of increased negative ζ potential on the physical stability of the standard emulsion during autoclaving was tested by adding approximately 0.05% w/w (0.66 millimolal (mm)) of purified egg phospholipid fractions which are anionic (PA, PG, or PI). The nonionic fraction, PC, was included at the same concentration as a control. As seen in Table 2, the addition of 0.66 mm of the anionic fractions caused the ζ potential to increase from −11 mV to approximately −39 mV. The nonionic egg phospholipid fraction, PC, did not cause a statistically significant change in the ζ potential. The formula modification did not affect the production of the emulsion as the mean droplet diameter of the emulsions containing an additional 0.66 mm of nonionic or anionic egg phospholipid fractions was the same as the standard formulation (Figure 1).

The effect of increased electrostatic repulsive force was seen when the emulsions containing 0.66 mm egg phospholipid fractions were autoclaved. The mean droplet size of the emulsions having a larger negative ζ potential (PA, PG, PI) were unchanged during autoclaving (Figure 1). In contrast, the mean droplet size increased during autoclaving in the standard emulsion and the emulsion containing additional nonionic fraction, PC. The change in mean droplet diameter during autoclaving for the standard emulsion and the emulsion containing additional PC was statistically significant at *p*-values of 0.001 and 0.003, respectively.

Table 3—Effect of Adding 0.66 mm Egg Phospholipid Fractions on the Free Fatty Acid Content, pH, and ζ Potential Following Autoclaving at 121 °C for 15 min

egg phospholipid fraction added	acid value, mg KOH/g emulsion		pH[a]		ζ potential mV	
	before autoclaving	after autoclaving	before autoclaving	after autoclaving	before autoclaving	after autoclaving
none	0.247 ± 0.003	0.348 ± 0.009	8.0	7.2	–10.5 ± 2.4	–23.6 ± 0.8
phosphatidylcholine	0.236 ± 0.002	0.359 ± 0.005	8.0	7.2	–12.2 ± 3.4	–22.4 ± 0.5
phosphatidic acid	0.275 ± 0.003	0.319 ± 0.004	8.0	7.4	–35.1 ± 1.7	–37.7 ± 1.5
phosphatidylglycerol	0.270 ± 0.004	0.303 ± 0.002	8.0	7.3	–39.4 ± 1.2	–40.3 ± 0.8
phosphatidylinositol	0.269 ± 0.007	0.303 ± 0.005	8.0	7.1	–32.7 ± 0.3	–35.2 ± 3.0

[a] The standard deviation was ± 0.0.

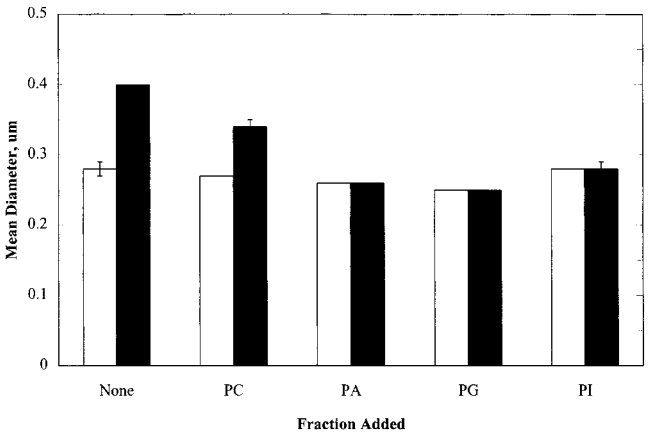

Figure 1—Effect of adding 0.66 mm egg phospholipid fractions on the mean droplet size following autoclaving at 121 °C for 15 min. The standard deviation is indicated on each bar when its value was greater than 0.00. Key: open bar, before autoclaving; hatched bar, after autoclaving; PC, phosphatidylcholine; PA, phosphatidic acid; PG, phosphatidylglycerol; PI, phosphatidylinositol.

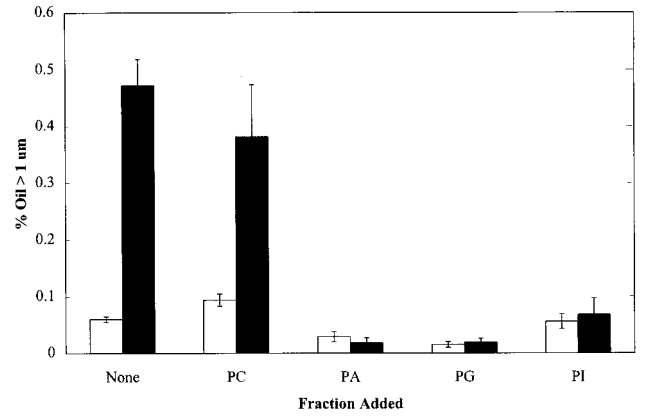

Figure 2—Effect of adding 0.66 mm egg phospholipid fractions on the percentage of oil present as droplets larger than 1 μm following autoclaving at 121 °C for 15 min. The standard deviation is indicated on each bar. Key: open bar, before autoclaving; hatched bar, after autoclaving; PC, phosphatidylcholine; PA, phosphatidic acid; PG, phosphatidylglycerol; PI, phosphatidylinositol.

The enhanced physical stability of the emulsions having an increased negative ζ potential was also seen when the percentage of oil present as droplets larger than 1 μm was determined (Figure 2). The three emulsions containing 0.66 mm anionic egg phospholipid fractions and having an increased negative ζ potential exhibited no change in the percentage of oil present as droplets larger than 1 μm as a result of autoclaving. This behavior is in sharp contrast to the standard formulation and the formulation containing an additional 0.66 mm PC. The percentage of oil present as droplets larger than 1 μm in these emulsions increased from less than 0.1 to 0.4 during autoclaving. Oil droplets were also visible on the surface of the standard emulsion and the emulsion containing additional PC. The three emulsions having a larger negative ζ potential did not have any visible oil droplets on the surface after autoclaving. Thus, the hypothesis that additional electrostatic repulsive force would inhibit coalescence during autoclaving was supported.

Table 4—Mean Droplet Size[a] of Autoclaved Emulsions Containing 0.66 mm Egg Phospholipid Fractions during Storage at 4 °C, Room Temperature (rt), and 40 °C

egg phospholipid fraction added	mean diameter, μm			
	initial	1 mo, 4 °C	1 mo, RT	1 mo, 40 °C
none	0.40	0.41	0.40	0.41
phosphatidylcholine	0.34	0.33	0.33	0.34
phosphatidic acid	0.26	0.26	0.26	0.26
phosphatidylglycerol	0.25	0.25	0.25	0.25
phosphatidylinositol	0.28	0.28	0.28	0.28

[a] All standard deviations were ± 0.01 or less.

Exposure to the elevated temperature of autoclaving caused hydrolysis of the egg phospholipid in the standard emulsion which was seen as an increase in the acid value, a decrease in the pH, and an increase in the negative ζ potential (Table 1). These parameters changed in a similar way during autoclaving of the emulsions containing additional egg phospholipid fractions (Table 3).

The effect of a higher concentration of added anionic egg phospholipid fractions was studied by repeating the experiments using 1.32 mm (approximately 0.10%) egg phospholipid fractions. The ζ potentials of the emulsions containing 1.32 mm PA, PG, and PI (Table 2) were −38.4, −40.0, and −37.3 mV, respectively. These values are only slightly increased in comparison to the values obtained when half that amount was added (Table 2). The mean droplet size of emulsions containing 1.32 mm PA, PG, or PI did not change during autoclaving. The percentage of oil present as droplets greater than 1 μm also did not change in these emulsions during autoclaving. Thus, increasing the concentration of added anionic egg phospholipid fractions above 0.66 mm (approximately 0.05%) did not significantly increase the surface charge or improve the physical or chemical stability of the emulsion to autoclaving.

The autoclaved standard emulsion and emulsions containing an additional 0.66 mm egg phospholipid fractions were stored at 4 °C, room temperature, and 40 °C for 1 month. As seen in Table 4, the mean droplet size of all of the emulsions was stable at all three temperatures. Likewise, the percentage of oil present as droplets larger than 1 μm did not significantly change during the storage period (Table 5). Thus, the addition of small amounts of anionic egg phospholipid fractions to reduce the rate of coalescence during autoclaving did not adversely affect the rate of coalescence during storage.

The preceding experiments demonstrate that the thermal stability of o/w emulsions can be improved by increasing the electrostatic repulsive forces between oil droplets.

Table 5—Percentage of Oil Present as Droplets Larger than 1 μm in Autoclaved Emulsions Containing 0.66 mm Egg Phospholipid Fractions During Storage at 4 °C, Room Temperature (rt), and 40 °C

egg phospholipid fraction added	% oil present as droplets larger than 1 μm, % v/v ± SD			
	initial	1 mo, 4 °C	1 mo, rt	1 mo, 40 °C
none	0.473 ± 0.046	0.452 ± 0.037	0.235 ± 0.028	0.352 ± 0.027
phosphatidylcholine	0.382 ± 0.092	0.452 ± 0.067	0.395 ± 0.073	0.399 ± 0.062
phosphatidic acid	0.018 ± 0.009	0.015 ± 0.002	0.025 ± 0.002	0.009 ± 0.002
phosphatidylglycerol	0.019 ± 0.007	0.016 ± 0.009	0.010 ± 0.003	0.010 ± 0.001
phosphatidylinositol	0.068 ± 0.029	0.022 ± 0.002	0.032 ± 0.008	0.019 ± 0.001

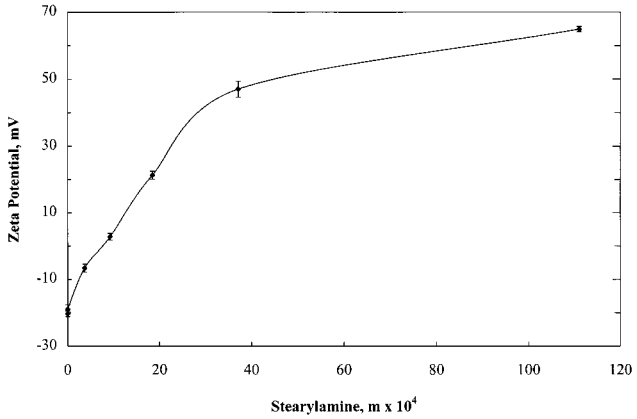

Figure 3—ζ potential of emulsions containing various concentrations of stearylamine. The standard deviation is indicated for every emulsion.

Table 6—Effect of Autoclaving at 121 °C for 15 min on the Mean Droplet Size of Emulsions Containing Stearylamine

experiment	concentration of stearylamine, mm	mean droplet diameter ± SD, mm	
		before autoclaving	after autoclaving
1	0	0.27 ± 0.01	0.37 ± 0.00
1	0.371	0.75 ± 0.02	cracked[a]
1	0.925	0.62 ± 0.00	cracked[a]
1	1.85	0.40 ± 0.01	cracked[a]
2	0	0.27 ± 0.00	0.42 ± 0.01
2	3.71	0.29 ± 0.00	0.28 ± 0.00
2	11.1	0.28 ± 0.00	0.27 ± 0.00

[a] The emulsion had separated into two phases.

To verify that the surface charge plays an important role in the stability of o/w emulsions, emulsions were prepared which had reduced negative ζ potentials as well as positive ζ potentials. The ζ potential of the standard emulsion containing 1.2% egg phospholipid was modified by incorporating concentrations of stearylamine ranging from 0.371 to 11.1 mm. The emulsions were prepared and autoclaved as two experiments. The first experiment consisted of the standard emulsion and emulsions containing 0.371, 0.925, and 1.85 mm stearylamine. The second experiment consisted of the standard emulsion and emulsions containing 3.71 and 11.1 mm stearylamine.

The ζ potential of the standard emulsion in the first experiment was −20.3 ± 0.8 mV and −19.0 ± 1.4 mV in the second experiment (Figure 3). Addition of 0.371 mm (approximately 0.01%) stearylamine reduced the negative ζ potential to −6.6 mV. This result indicates that the interface contains both anionic egg phospholipid fractions and stearylamine. The mean droplet diameter of this emulsion (Table 6) prior to autoclaving was larger (0.75 μm) than the standard emulsion. The mean droplet diameter prior to autoclaving was also larger than the standard emulsion when 0.925 and 1.85 mm stearylamine was included in the formulation. However, emulsions containing 3.71 or 11.1 mm of stearylamine exhibited initial mean droplet diameters which were virtually identical to the standard emulsion. This behavior suggests that coalescence occurred prior to autoclaving in the emulsions containing 0.371, 0.925, and 1.85 mm stearylamine.

Table 7—Effect of Autoclaving at 121 °C for 15 min on the Percent of Oil Present as Droplets Larger than 1 μm in Emulsions Containing Stearylamine

experiment	concentration of stearylamine, mm	% oil present as droplets larger than 1 mm ± SD	
		before autoclaving	after autoclaving
1	0	0.005 ± 0.000	0.115 ± 0.077
1	0.371	0.257 ± 0.119	cracked[a]
1	0.925	0.237 ± 0.079	cracked[a]
1	1.85	0.201 ± 0.030	cracked[a]
2	0	0.051 ± 0.008	0.486 ± 0.067
2	3.71	0.099 ± 0.024	0.103 ± 0.038
2	11.1	0.019 ± 0.001	0.029 ± 0.10

[a] The emulsion had separated into two phases.

The standard emulsions without added stearylamine (Table 6) exhibited the same increase in mean droplet size during autoclaving as was reported in Table 1. The emulsion with a reduced negative ζ potential (0.371 mm stearylamine) coalesced to such a degree during autoclaving that it completely separated into two phases. The surface charge of the emulsions became positive (+2.8 mV) when the concentration of stearylamine was 0.925 mm or higher (Figure 3). The ζ potential was +64.9 mV at the highest stearylamine concentration (11.1 mm). Only the two emulsions having the highest positive ζ potentials failed to exhibit an increase in mean droplet size during autoclaving (Table 6). These two emulsions were also the only ones which did not exhibit an increase in the percent oil present as droplets larger than 1 μm (Table 7). Although there was no significant change following autoclaving in the mean droplet size or percentage of oil present as droplets greater than 1 μm in the emulsions containing 3.71 or 11.1 mm stearylamine, examination of the surface of the emulsions after autoclaving revealed that the emulsion containing 11.1 mm stearylamine was more stable. No oil droplets were observed on the surface of the emulsion containing 11.1 mm stearylamine after autoclaving. In contrast, oil droplets were visible on the surface of the emulsion containing 3.71 mm stearylamine after autoclaving.

The thermal instability of the emulsions containing 0.371 or 0.925 mm stearylamine may be attributed to the small surface charge, −6.6 or + 2.8 mV, respectively. The electrostatic repulsive forces present in these emulsions may be insufficient to prevent coalescence during autoclaving. The emulsion containing 1.85 mm stearylamine had a ζ potential of +21.3 mV. The absolute value of the ζ potential was similar to the standard emulsions (−20.3 and −19.0 mV). Since the standard emulsion never separated into two phases during autoclaving (Tables 1 and 6), it was

surprising that the emulsion containing 1.85 mm stearylamine cracked during autoclaving. Previous experiments showed that autoclaving the standard emulsion caused the formation of free fatty acids and increased the negative ζ potential (Table 1). Thus, it is likely that the positive ζ potential of the emulsion containing 1.85 mm stearylamine became less positive during autoclaving. It is believed that the reduced positive ζ potential did not provide enough electrostatic repulsive force to prevent coalescence. Unfortunately, the ζ potential of this emulsion after autoclaving could not be measured because the emulsion separated into two phases during autoclaving.

The emulsions containing 3.71 and 11.1 mm stearylamine did not separate during autoclaving. Measurement of the ζ potential after autoclaving yielded reduced positive ζ potentials of $+7.5 \pm 0.9$ and $+31.5 \pm 1.5$ mV, respectively. The formation of free fatty acids may explain why the emulsion containing 11.1 mm stearylamine exhibited better thermal stability than the emulsion containing 3.71 mm stearylamine. The ζ potential of the emulsion containing 11.1 mm stearylamine was always greater than +31.5 mV during autoclaving while the ζ potential of the emulsion containing 3.71 mm stearylamine decreased to a minimum ζ potential of +7.5 mV during autoclaving.

Conclusions

This study demonstrates the important role of the electrostatic repulsive force in the thermal stability of o/w emulsions. We have shown that positively charged egg phospholipid emulsions are not desirable, as the positive ζ potential is neutralized during autoclaving. In commerce, o/w emulsions stabilized by egg phospholipid are autoclaved at an alkaline pH (pH 8−9) in order to maintain an adequate negative ζ potential for thermal stability.

Formulation modification by the addition of small amounts of anionic phospholipid fractions is a convenient means of increasing the ζ potential. This approach may be an attractive alternative to alterations of the autoclaving process when coalescence occurs during steam sterilization. The results of this study may also be relevant to observed batch-to-batch differences in the stability of egg phospholipid to autoclaving. As a natural product, the composition of egg phospholipid may vary in terms of nonionic and anionic components. All other factors being equal, one lot of egg phospholipid having a larger fraction of anionic components may produce a more stable emulsion at elevated temperatures than another lot having a smaller fraction.

The mechanical properties of the interfacial film may also be an important factor in the thermal stability of o/w emulsions. This factor will be investigated in a subsequent study.

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