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Controlling the Size of Vesicles Prepared from Egg Lecithin using a Hydrotrope

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Abstract—Phase diagrams were constructed using egg lecithin as a source of lipid, sodium xylenesulfonate as the hydrotrope, and various aqueous media. The resulted oil-in-water microemulsion (L_1) region was utilized to prepare unilamellar vesicles by diluting the microemulsion with equal amount of the aqueous media. When the ratio of lecithin/SXS was adjusted in the original micellar solution the average size of the corresponding vesicles that formed upon dilution of the L_1 phase changed accordingly. An increase in the ratio of lecithin/SXS resulted in a linear increase of average vesicle size, ranging from 75 to 500 nm. This correlation can be explained with the predictable change in the curvature of a vesicle when sufficient amount of hydrotrope was incorporated. The slope of this linear relationship is influenced by the polarity of the medium and the presence of molecules that can intercalate into the lipid bilayer.

Introduction

Liposomes are vesicles composed of surface-active amphiphiles, such as lipids, which contain a hydrophyllic (polar) head group and a hydrophobic (nonpolar) tail typically composed of long hydrocarbon chains. In water these molecules spontaneously form lipid bilayers, where the polar end stays in contact with the aqueous environment and the nonpolar tail points inward to form the inner portion of the bilayer. When lipid concentrations are high, the lipids form lamellar liquid crystals. However, if these solutions are diluted, the bilayers can dissociate and rearrange to form spherical vesicles.[1] The vesicles can vary in size, as well as in the number of lamellar layers that will form. Vesicles with a single lipid bilayer (4 nm thick membrane), referred to as liposomes, can range in size from 20 nm to 50 microns.[2] The study of liposomes is very important because of their similarity to biological membranes and their therapeutic value as delivery agents for enzymes, drugs, genetic manipulation, and diagnostic imaging.[3–5]

The behavior of a liposome as a biological agent is dependent upon the size, as well as other physical characteristics, including composition, lamellarity, and diffusion rates for small molecules.[6] For example, membrane fusion rates, cholesterol exchange rates, phospholipase activities, and influx and efflux rates are dependent upon curvature of the vesicle, internal volume and surface area.[6] Therefore, a method for controlling the size of vesicles upon formation would be very useful.

One successful preparation of vesicles comes from diluting low concentrations of an amphiphile with low surface activity by use of a hydrotrope.[7] Hydrotropes are short ionic molecules that help to increase the solubility of a surfactant and can disrupt the formation of lamellar liquid crystals.[8–10] At high concentrations hydrotropes will form aggregates to increase solubilization of the surfactant, but do not strongly interact with an interface.[9,11,12] Hydrotropes are, however, capable of affecting the curvature of the lipid bilayer, which will allow the control of the vesicle size upon formation.

The lipid chosen for this investigation was lecithin. This widely studied vesicle forming surfactant has gained much attention for its use in drug delivery systems, due to its natural abundance in cell membranes.[13–15] Sodium xylenesulfonate, a small 'block-like' molecule, was the model hydrotrope used in this investigation. The three different aqueous media used were water, 0.9% NaCl in water, and a mixture of 80% saline (0.9% NaCl), 10% ethylene glycol, and 10% glycerol. All three aqueous media are commonly found in many pharmaceutical formulations. The focus of this paper is to exam the effect on the size of the vesicles by quantitative addition of a hydrotrope to a vesicle-forming system.

Keywords: hydrotrope; vesicles; liposomes; lecithin; sodium xylenesulfonate; micelles; lamellar liquid crystals; phase diagrams; lipids; surfactant.
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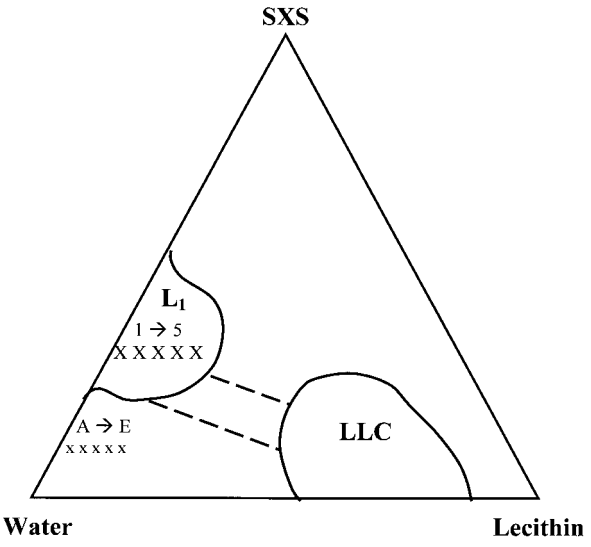

Figure 1. Phase diagram prepared using lecithin, SXS and water.

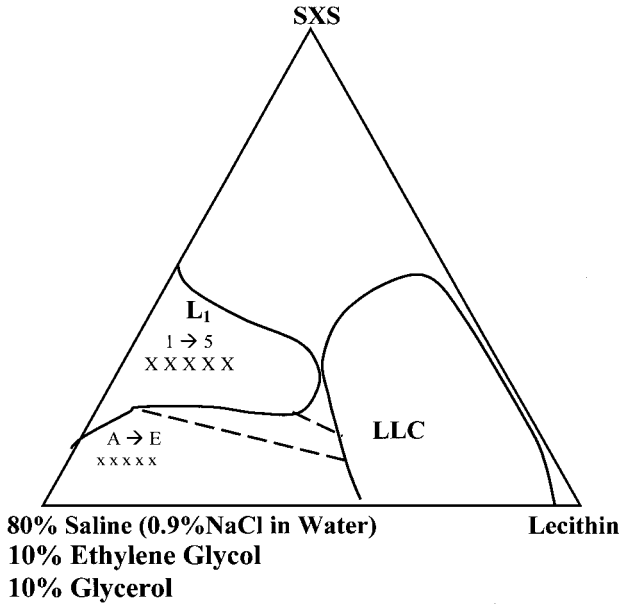

Figure 3. Phase Diagram prepared using lecithin, SXS, and the control medium (80% saline solution that is 0.9% NaCl by weight, 10% ethylene glycol, and 10% glycerol).

Results and Discussion

Phase diagrams were used to determine the micellar (L_1) and lamellar liquid crystal (LLC) regions. Although only the micellar region was used to obtain the vesicles, it was necessary to determine the boundaries between these two distinct regions. To create vesicles, dilutions were made from the micellar region of the L_1 phase. Each dilution of the micellar phase brought the composition of each sample to a region between the micellar and LLC phases. The tie lines in Figs.1–3 that connect both phase boundaries illustrate this composition. Fig. 1 shows that SXS was soluble in water up to 49% by weight of the hydrotrope (L_1), for which the solutions were isotropic. The solubility of the lecithin in water was low (less than 0.1%), but when water was added to lecithin the lamellar liquid crystal phase (LLC) was formed between 14% and 50% water by weight. The SXS showed a limited solubility of 25% in the LLC phase, and lecithin showed a limited solubility of 22% in the micellar (L_1) phase. The phase diagram represented in Fig. 2 shows that SXS was soluble in water containing 0.9% NaCl up to 49% by weight of the hydrotrope, with a limited solubility of 39% for the surfactant in this region. The solubility of SXS in water containing NaCl was very similar to the solubility of SXS in water only, but the solubility of lecithin in the L_1 phase significantly increased from 22% in water to 39% in water containing NaCl. The increase in solubility of lecithin in the L_1 phase was the result of sodium chloride ions breaking up the liquid lamellar crystals. Again, the solubility of lecithin in water was low, but the addition of water to the surfactant led to the formation of the LLC phase between 2 and 37% water by weight. The LLC phase appeared earlier when sodium chloride was added to water. This shift is clearly the result of increased ion strengths. The addition of the salt decreased the water penetration into the lipid bilayer and increased the rigidity of the liquid crystals.[16] The solubility of the hydrotrope in this region increased to 36% SXS by weight as the result of increased ionic strengths. Fig. 3 represents the phase diagram for lecithin and SXS in an aqueous medium of 80% saline (0.9% NaCl), 10% ethylene glycol, and 10% glycerol. From this diagram the solubility of hydrotrope was determined to be ca. 51% SXS by weight. The solubility for the surfactant in this region was found to be 52% lecithin by weight. The addition of ethylene glycol and glycerol did not significantly change the shape of the L_1 region, only that the L_1 region became narrower. The addition of water to the surfactant resulted in the formation of the LLC phase from 3 to 42% water by weight, with a limited solubility of 47% SXS by weight. The further expansion of the liquid crystal region might be due to the decrease in polarity of the medium caused by the addition of ethylene glycol and glycerol. The alcohols acted as cosolvents that penetrated into the lipid bilayer and reduced the effects of the hydrotrope.[17–19]

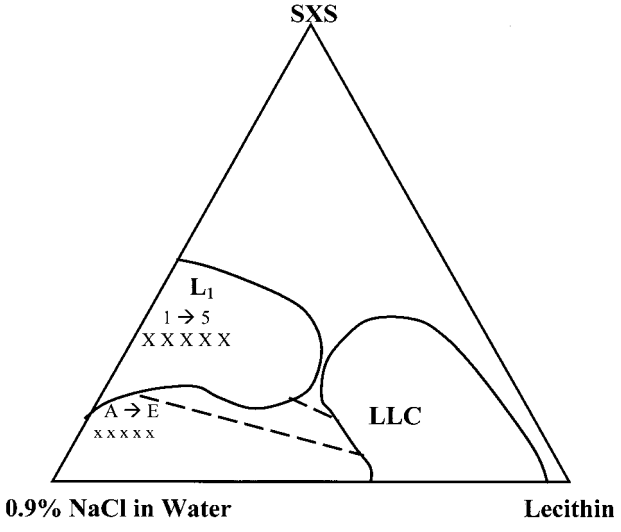

Figure 2. Phase Diagram prepared using lecithin, SXS, and water containing 0.9% NaCl.

Table 1. The interlayer spacing of the LLC region for the phase diagram of lecithin/SXS/water

Lecithin (%)	SXS (%)	Water (%)	Interlayer spacing (Å)
76.6	8.4	15	44.207
72.0	8.0	20	44.726
62.8	7.2	30	51.175
58.2	6.8	35	53.827
53.6	6.4	40	55.834

It has been demonstrated by Friberg et al. that vesicles can be prepared through the dilution of a micellar solution with water.[20–23] In this study, five samples in the L_1 region were chosen (Points 1→5 in Figs. 1–3) and diluted with equal amounts of aqueous media to give vesicle solutions (Points $A \rightarrow E$ in Figs. 1–3). The final vesicle solutions were formed in the phase regions between the L_1 and the LLC phases. The tie lines indicate where the L_1 phase is in equilibrium with the LLC phase. The formation of lamellar liquid crystals can be reduced when micellar solutions are diluted into the two-phase region using a shear mixing technique.[23,24]

To confirm the intercalation of SXS into the lipid bilayer, the impact of SXS on the interlayer spacing of the LLC was investigated. Small X-ray scattering was used to measure the interlayer spacing of five points chosen from the LLC region of Fig. 1. Two important parameters of LLC phase can be obtained through linear fit of the small angle X-ray data: the interlayer spacing at zero water content, d_0, and the fraction of water penetrating from the aqueous layer to the hydrophobic region, α, according to Eq. (1), where d is the interlayer spacing and R is equal to the water volume ratio.

$$d = d_0[1 + (1 - \alpha)R] \quad (1)$$

The composition of the liquid crystal and the interlayer spacing values are listed below in Table 1. A graphical representation of Table 1 is shown in Fig. 4. From this graph, one can see that the interlayer spacing increased as the volume of water in the system increased. By extending the line formed by the five points to the zero point for the volume ratio of water, the thickness of the hydrophobic layer of the system is found to be approximately 37 Å, an expected value for lecithin. The water penetration factor, α, can be calculated from Eq. (2), where S is the slope found in Fig. 4.

$$\alpha = 1 - S/d_0 \quad (2)$$

For samples obtained using water as the medium α was determined to be 0.987, which indicates a significant amount of water penetration into the hydrocarbon layer due to the intercalation of SXS.[25]

The sizes of the vesicles were then determined using dynamic light scattering (DLS) method. As shown in Figs. 5–7, there is a linear relationship between the particle size and the ratio of surfactant (lecithin) to hydrotrope (SXS) in all three media (water, 0.9% NaCl in water, and a mixture of 80% saline (0.9% NaCl), 10% ethylene glycol, and 10%

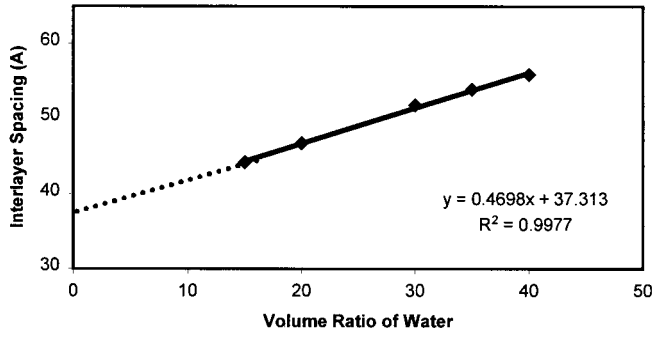

Figure 4. An increase in the interlayer spacing of the LLC region for the phase diagram of lecithin/SXS/water as the amount of water increases.

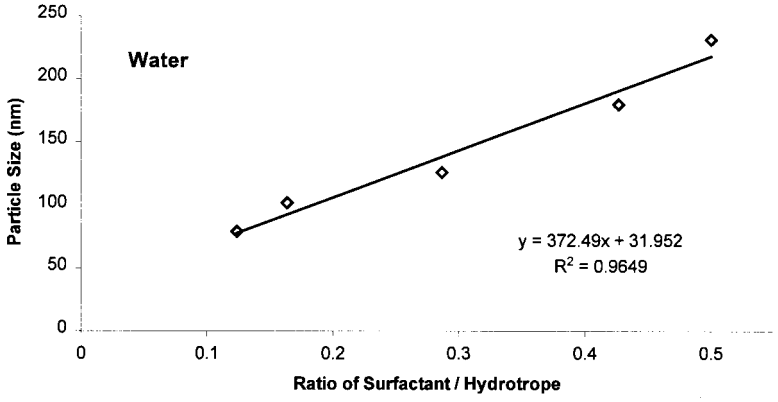

Figure 5. The average particle size of the vesicles formed from lecithin, SXS, and water, versus the ratio of the surfactant (lecithin) to hydrotrope (SXS).

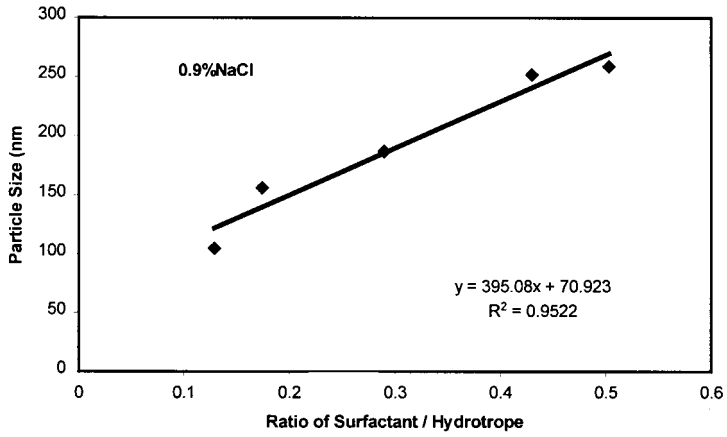

Figure 6. The average particle size of the vesicles formed from lecithin, SXS, and 0.9% NaCl in water, versus the ratio of the surfactant (lecithin) to hydrotrope (SXS).

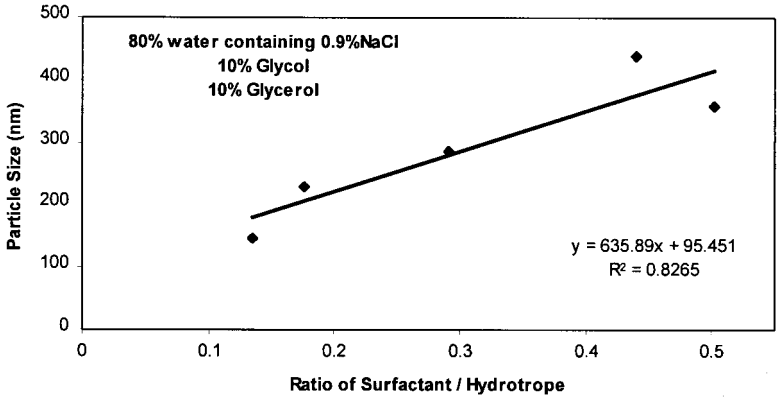

Figure 7. The average particle size of the vesicles formed from lecithin, SXS, and the control medium (80% by volume of 0.9% NaCl in water, 10% propylene glycol, and 10% glycerol), versus the ratio of the surfactant (lecithin) to hydrotrope (SXS).

glycerol). The linear plot shown in Fig. 6 has a larger *y*-intercept than that of Fig. 5, which means that the initial particle size is slightly larger. However, the slopes in Figs. 5 and 6 are quite similar, which means that the influence of the hydrotrope on the particle size is relatively independent from the ionic strengths or the polarity of the media.

In Fig. 7, the *y*-intercept is very similar to that of Fig. 6, but

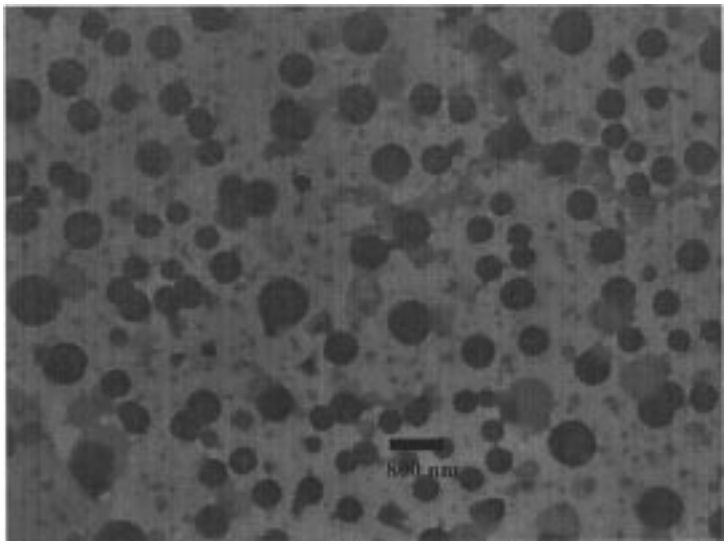

Figure 8. The TEM photographs that were taken of the vesicles produced from the lecithin/SXS/water, approximate diameter of average vesicle is 800 nm.

the slope is significantly larger than the other two graphs. The greater the slope the more sensitive the particle size change is to the amount of hydrotrope present in the system. In the presence of a hydrotrope, the penetration of the water into the bilayer is greater. In other words, the alcohols assist the penetration of the water into the lipid bilayer. Because the alcohol medium can cause a greater change in the size of the vesicle, larger vesicles up to 0.5 microns can be produced. The TEM photograph shown in Fig. 8 depicts the average particle size (400 nm) of vesicles produced from a representative lecithin/SXS/water sample.

All three types of media reveal a linear relationship between the particle size and the ratio of surfactant/hydrotrope. As the ratio of surfactant/hydrotrope increased the size of the vesicle increased. These three figures show that for the first time one can prepare vesicle with different particle sizes at will. With this method, one can prepare vesicle with an average particle size anywhere from 75 to 500 nm by simply adjusting the molar ratio of lipid to hydrotrope.

Conclusion

Phase diagrams constructed using lecithin and sodium xylenesulfonate in various aqueous media can be used as a roadmap for the preparation of vesicles with predictable sizes. It is clear that the incorporation of a hydrotrope into the lipid bilayer perturbs its preferred curvature, which translates to a change in average vesicle sizes. Therefore, vesicles with predictable sizes can be prepared by adjusting the amount of hydrotrope. After the formation of vesicles during the initial dilutions, samples can be further diluted without disrupting the integrity of the system.

Experimental

Materials

Sodium xylenesulfonate was purchased from Aldrich Chemical Company, Inc, Milwaukee, WI. L-α-Phosphatidyl-lcholine (L-α-lecithin) produced from dried egg yolk was purchased from Sigma, St. Louis, MO. The so-called control medium was prepared by combining 80% by volume sodium chloride solution that was 0.9% NaCl by weight, with 10% by volume ethylene glycol and 10% by volume glycerin. Both ethylene glycol and glycerin were purchased from Fisher Chemical, Fair Lawn, NJ.

Phase diagrams

Three phase diagrams were created using egg lecithin as the vesicle-forming surfactant and sodium xylenesulfonate as the hydrotrope. Lecithin is a mixture of double-chained phosphatidylcholine occurring naturally in the cell membranes of both plants and animals. The phase diagrams differed in the type of aqueous media that were used, which was either water, water containing 0.9% NaCl, or water containing 80% by volume of sodium chloride solution (0.9% NaCl), 10% ethylene glycol, and 10% glycerol by volume. Boundaries for the micellar (L_1) and lamellar liquid crystalline (LLC) phases were determined visually and with the aid of a microscope equipped with crossed polarizers. The LLC region was confirmed by measuring the interlayer spacing with small angle X-ray diffraction.

Interlayer spacing measurements

To measure the interlayer spacing of the lamellar liquid crystal, or thickness of the lecithin layer, small angle X-ray diffraction was used. A small amount of sample was drawn into a glass capillary of 0.5 mm in diameter, sealed at both ends, and placed in a brass sample holder with a 2 mm hole-diameter. The X-ray radiation was CuKα filtered by nickel foil to yield a wavelength of 0.1524 nm at 40 kV and 18 mA. The system is a Siemens crystalloflex 4 using a Kiessig low-angle camera (Richard Seifer) and an ORDELA detection system, which allows the angle 2θ to range between 0.7 and 5.7°.

Dynamic light scattering (DLS)

The DLS measurements were performed on a Brookhaven Instruments System, with a water-cooled argon ion laser light sources (Model 85, Lexel Laser) that operated at a wavelength of 514.5 nm. The sampling setup consisted of a BI-DS photomultiplier tube, a stepper-motor-controlled goniometer (BI-2000SM) and a BI-2030AT digital correlator. The DLS was performed at a 90° angle with the sample maintained at a constant temperature of 23.0±0.5°C. The sizes of the vesicles were determined using the cumulants analysis of the experimental measured autocorrelation functions: $ln[g^1(\tau)] = -K_1\tau K_2(\tau/2!)$, where τ equals the mean decay constant, and the cumulants K_1 and K_2 represent the average diameter of the particle and the relative width of the particle distribution respectively.

Transmission electron microscopy (TEM)

The samples were prepared by mixing 6 drops of the lecithin/SXS/water solution with 1 ml of 0.3% phosphotungsted acid and a droplet of the mixed solution was then applied to a small 3 mm, 200 mesh carbon coated copper grid plate. Excess solution was blotted off with a piece of filter paper and the grid was dried for approximately 15 min before examination by TEM. Observations were made on the JOEL 1210 TEM operating at 120 kV, and the images were recorded on Kodak® TEM plate film.

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