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SHORT COMMUNICATION

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Spontaneous formation of monodisperse vesicles near the cloud point of an aqueous amphiphilic system

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Abstract Monodisperse vesicular structures have been produced spontaneously from an almost structure-less aqueous amphiphilic system: water/di(ethylene glycol) mono-hexyl ether (C_6E_2)/sodium cholate. Individually, neither C_6E_2 nor the bile acid produces noticeable aggregate structures in an aqueous solution. However, the presence of small amounts of bile acid in the C_6E_2/water binary system is found to produce large microstructures, besides pushing the miscibility gap to higher temperatures. Dynamic light scattering studies indicate the presence of very monodisperse structures of sizes ranging from 15 to 50 nm in radii. The radii of these structures show strong dependence on the concentrations of the components and on the distance in temperature from the cloud point. For a given set of conditions the sizes are very stable and reproducible. Electron microscopy and conductivity measurements confirm these structures as vesicles. These may be formed due to the association of bile acid with C_6E_2 producing the geometrical parameters necessary for the formation of vesicles.

Key words Monodisperse vesicles – spontaneous formation – hydrotropes – bile acid – amphiphiles – cloud point – critical phenomenon – light scattering – electron microscopy

Introduction

An amphiphilic molecule consists of one part, which is hydrophilic, and one part that is hydrophobic. These molecules, depending on the individual structures, show very rich polymorphism when dissolved in water. The molecules may self assemble into spherical aggregates, rod-like aggregates, lamellar sheets, and vesicles in aqueous solutions [1]. Of these structures, the vesicular structure was thought to be a meta-stable structure and only amphiphiles with two hydrophobic tails were observed to form vesicles [2]. Even with double-tailed amphiphiles, it has been hard to produce vesicles by spontaneous self-assembly [2]. In 1989, Murthy et al. [3, 4] first observed the spontaneous formation of stable vesicles in aqueous solutions of two ionic surfactants with oppositely charged head groups. This work initiated theoretical interest among the physicists and Safran et al. [5, 6] proposed a theoretical interpretation for this formation of vesicles in a cationic surfactant/anionic surfactant/water system. They showed theoretically that this stabilization is driven by interactions between the two surfactants and the compositional asymmetry of the inner and outer layers of the vesicle. The ionic attractions between oppositely charged head groups simulated the double-tail behavior in forming the vesicles observed in Refs. [3, 4]. In the present work, this double-tail behavior is possibly simulated by complexation or hydrogen bonding between the acidic groups of the bile acid and the alcohol group of C_6E_2 amphiphile. The striking features of the vesicles formed in this system are (a) their spontaneous production in this short-tailed amphiphilic system and (b) their monodispersity combined with the high sensitivity of their sizes to temperature.

The C_6E_2/water binary system has an upper miscibility gap [7] with its lower critical temperature being 0 °C. At room temperature, this binary system exhibits two phases with C_6E_2 mostly residing in the upper phase. This clouding phenomenon is very common with nonionic amphiphiles. The addition of hydrotropic salts and/or other hydrophilic amphiphiles is known to push the cloud point of a binary system to higher temperatures [8]. These salts produce the shift in the cloud point either by altering the structure of water or by increasing the hydrophilicity of the amphiphile molecule through incorporation in the water/amphiphile interfacial layer.

Simple bile acid such as sodium cholate is known to reduce the rigidity of biological membranes and at sufficient concentrations a vesicle-to-micelle transition has been observed [9–12]. This bile salt contains a bulky aromatic hydrophobic side, a hydrophilic side and short hydrophilic tail. It is very soluble in water and aggregates to form very small micelles. Bile acids act as co-surfactants in that they enhance the solubilization power of phosphatidylcholine vesicles. Because of its high solubility in water, we selected the simple hydrotrope, sodium cholate, to push the upper miscibility gap of the C_6E_2/water binary system to temperatures well above room temperature. Sodium cholate did push the upper miscibility gap to higher temperatures. Beyond this expected effect, it also introduced highly monodisperse aggregate structures that are not typical of these amphiphilic molecules.

There is a range of concentrations of the C_6E_2/water/bile acid system that is in one-phase at temperatures around room temperature. We first noticed opalescence, indicating the presence of large aggregates, in some of the samples in the one-phase region of this ternary system. We investigated these samples further and found the aggregate structures to be monodisperse vesicles. This interesting phenomenon may be due to the complexation or the hydrogen bonding between the C_6E_2 molecules and sodium cholate molecules. This may provide the required compositional asymmetry and the necessary geometrical parameters for the formation of vesicles by spontaneous self-assembly. Supporting evidence from dynamic light scattering, conductivity data and electron microscopy are presented.

Experimental

C_6E_2 and sodium cholate (BA) were purchased from Aldrich. We made 22 samples ranging in concentrations from mostly water with a small amount of C_6E_2 to mostly C_6E_2 with a small amount of water. We mapped the phase diagram of these samples and then started adding the bile acid incrementally from a stock solution of 10% bile acid in water. The phase behavior of these samples mixed with bile acid was then recorded. Figure 1 shows the phase boundary of the binary system and the upward shift of the upper miscibility gap of C_6E_2/water with increasing concentrations of bile acid. The samples that were in the two-phase region at room temperature showed clear visual transition to the one-phase region with the addition of BA. Some of the one-phase samples that have the shifted cloud point slightly above room temperature appeared blue, indicating the presence of microstructures. We present the quantitative data on the size of the structures for four samples, labeled c8–c11, identified in Fig. 1. All these samples have the same (10% BA)/water volume ratio of 0.05.

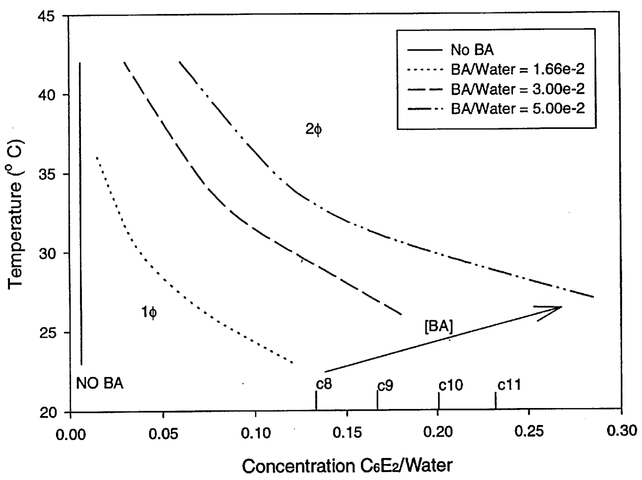

Fig. 1 Phase diagrams illustrating the upward shift of the upper miscibility gap of C_6E_2/water with increasing concentrations of bile acid. Samples c8–c11 were selected for quantitative measurements

Sizes of the microstructures in the selected samples were measured by dynamic light scattering (DLS). The scattering system was built at Smith College using a 100 mW air-cooled Ar laser with a wavelength of 514 mm and a beam diameter of 0.82 mm. ALV-5000 correlator was used to collect the data [13]. All measurements were done at a 90° scattering angle and analyzed using second cumulant analysis. This analysis gives the diffusion coefficient from which the sizes were calculated using the Stoke–Einstein relationship [14]. For each of the compositions, c8–c11 (see Fig. 1), the vesicle sizes and polydispersity were measured for a series of temperatures below the cloud point and the data was plotted as radii versus reduced temperature. Here the reduced temperature is taken as the difference between the cloud point and the sample temperature. Figure 2 shows the dependence of vesicle size on the reduced temperature for increasing concentrations of C_6E_2 at a given BA to water ratio. All

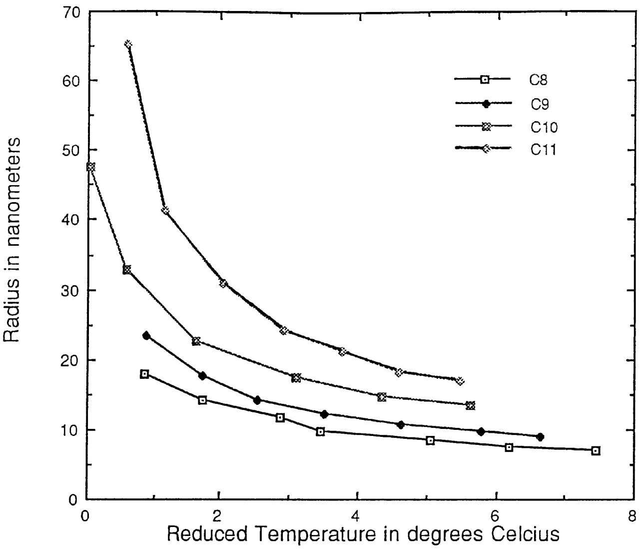

Fig. 2 Vesicle radius plotted as a function of reduced temperature for each of the samples c8–c11. Reduced temperature is defined as the difference between the cloud point and the sample temperature

measurements showed high monodispersity in the sizes. The polydispersity values obtained for these structures from cumulant analysis are comparable to those obtained for polystyrene microsphere standards with a standard deviation of less than 1.5% in their sizes.

One of the samples that showed large structures at room temperature was used to obtain electron micrographs. Micrographs were made using freeze fracture technique [3, 15]. Replicas were observed on a Philips CM-10 electron microscope at the University of Massachusetts. The sizes of the spherical structures seen in the micrograph (Fig. 3A and B) agree with the results from light scattering. It is important to note here the very fact that the structures survive the freeze fracture process confirms the vesicular nature of these structures.

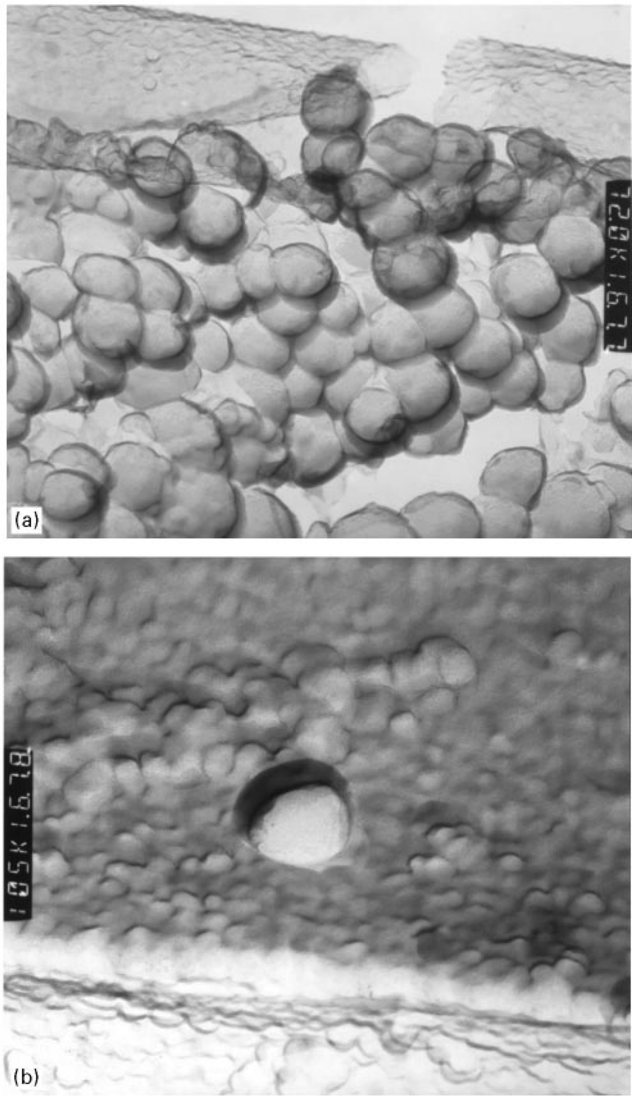

Fig. 3 Electron micrographs, using the freeze fracture technique, of vesicles in the sample c11 with BA/water = 5.0×10^{-2}. The sizes agree with the light scattering data at room temperature (A) magnification = 72.0 K; (B) magnification = 105 K

In addition to electron microscopy, conductivity measurements confirmed the presence of an entrapped volume. We used a 0.2% NaCl solution instead of water to prepare the samples. NaCl is a simple lyotrope which pushes the cloud point of the C_6E_2/water system to lower temperature. However, the addition of a small amount of BA restores the cloud point back to the original temperature and structures form as before. The NaCl concentration is the same inside and outside of the structures, but the salt trapped in the interior does not contribute to the conductivity. Thus, the conductivity of the sample decreases as the structures are formed. This method has been used successfully for observing the vesicle to micelle transition [16]. Figure 4 shows the conductivity data. The conductivity of the sample with structures is lower than that of the control sample and decreases with the increasing size of the structures as the temperature approaches the cloud point, as seen in the figure. At the cloud point, when the vesicles break, the conductivity value jumps up to that of the control sample as all the NaCl ions are released to contribute to the conductivity. These data further confirm the presence of an entrapped volume in the structures.

Discussions

Highly monodisperse vesicles form spontaneously by adding sodium cholate to the C_6E_2/water binary system.

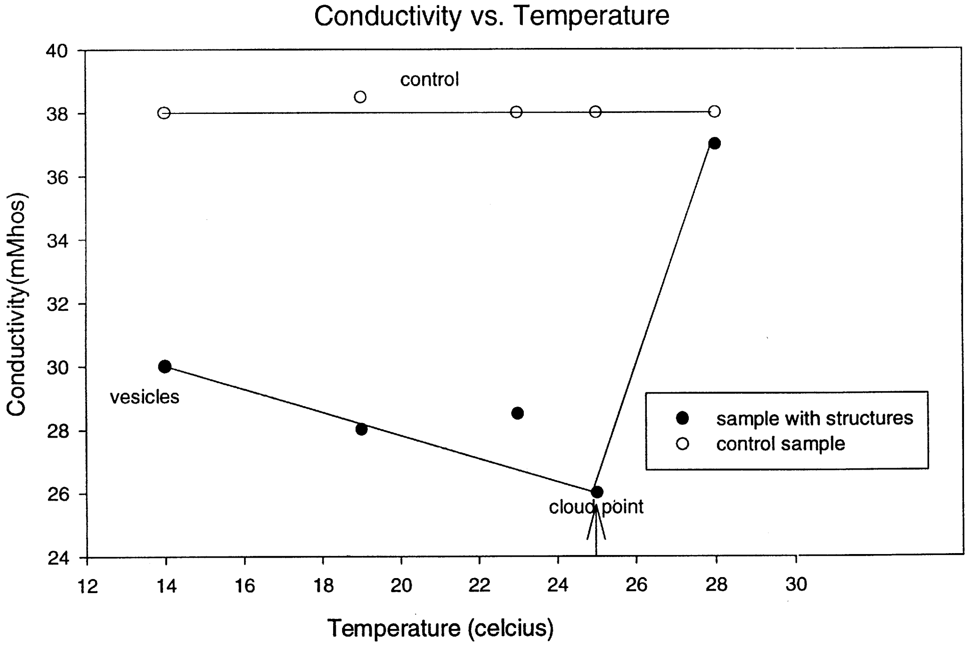

Fig. 4 Conductivity plotted as a function of temperature in a sample with structures (solid circles) and in a control sample (open circles). The samples were prepared in a 0.2% NaCl solution instead of water. The graph shows the decreasing conductivity of the sample with increasing vesicle size as the temperature approaches the cloud point. At the cloud point, the conductivity recovers to that of the control sample

The vesicle sizes range from 15 to 50 nm in radii. The sizes decrease with increasing concentrations of sodium cholate and increase with increasing concentrations of C_6E_2. The decrease in rigidity of the bilayer due to the increase in BA concentrations allows for more curvature, and hence smaller structures. Vesicle sizes also vary with the distance in temperature from the cloud point. When the system is driven towards the cloud point, there is a steep rise in the vesicle sizes, and finally the system phase separates. Upon cooling immediately to room temperature, the original vesicle sizes are reproduced with the same temperature dependence.

In conclusion, these results indicate that the bile salt is not acting as a simple hydrotrope or a phase mixing agent. Instead, it is forming a pseudo complex with C_6E_2 molecule and is inducing a self-aggregation hitherto not seen in either water/C_6E_2 or water/bile salt binary systems. A possible explanation for this unusual behavior is complexation or hydrogen bonding between the acidic groups of the bile acid and the alcohol group of C_6E_2. Because of critical phenomenon, aggregation is possible only when the system is below and close to the cloud point. Above the cloud point, the length scales diverge due to the critical phenomenon, and hence the observed steep rise in the vesicle sizes. The high level of monodispersity among the vesicles at a specific temperature, and the ability to control the sizes of the structures through temperature may prove useful in a variety of applications. More detailed studies of conductivity in order to characterize entrapment volume and entrapment efficiency are underway. Also, the effect of amphiphile hydrophobicity, and hydrophilicity on this spontaneous vesicle formation will be discussed in a forthcoming publication.

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References

1. Israelachvili JN (1985) Intermolecular and Surface Forces. Academic Press, Orlando, FL
2. Israelachvili JN, Mitchell DJ, Ninham, BW (1976) J Chem Soc Faraday Trans II 72:1525
3. Murthy K, Rodriguez B, Zasadzinski J, Kaler EW (1989) Paper presented in 63rd Colloid and Surface Science Symposium Seattle, WA, USA, June 18–21; Science 245:1371
4. Murthy K, (1991) Invited Paper at the American Physical Society Annual Meeting, Cincinnati, OH, USA, March 18–22
5. Safran SA, Pincus PA, Andelman, D (1990) Science 248:534

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6. Safran SA, Pincus PA, Andelman D, MacKintosh FC (1991) Phys Rev 43A:1071
7. van Os NM, Haak JR, Rupert LAM (1993) Physico-Chemical Properties of Selected Anionic, Cationic and Nonionic Surfactants, Elsevier, NY, p 207 & 265
8. Khalweit M, Strey R (1985) Angew Chem Int Ed Engl 24:654
9. Carey MC, Small DM (1970) Amer J Med 49:590
10. Mazer NA, Carey MC, Kwasnik RF, Benedek GB (1979) Biochemistry 18:3064
11. Thurmond RL, Lindblom G, Brown MF (1991) Biophys J 60:728
12. Maza A, Parra JL (1997) Colloids Surf A127:125
13. Ohlmann, Kristin (1995) Honors Thesis, Smith College, Northampton, MA01063
14. Pecora R (1985) Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy. Plenum Press, NewYork
15. Bailey SM, Chiruvolu S, Israelachvili JN, Zasadzinski JAN (1990) Langmuir 6:1326
16. Schonfelder E, Hoffman H, Ber. Bunsenges (1994) Phys Chem 98:842