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LETTERS

Vesicles with Superior Stability at High Temperature

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The formation of vesicles in the synthetic bolaamphiphiles SEDA/DEAB mixed systems in the temperature range of (25−80 °C) was found by FF-TEM. No phase transition temperature was detected in this temperature range by both DSC and fluorescent probe measurements. In addition, the results of the IR spectra showed that the hydrocarbon chains in the mixed systems are in highly ordered arranging state as the temperature increased to higher temperature. All these results showed the superior thermal stability of the vesicles in the SEDA/DEAB mixed systems.

Introduction

Vesicles are widely used as model cell membranes, drug delivery systems and microreactors.[1−3] In contrast to metastable phospholipid vesicles,[4] catanionic surfactant vesicles[5,6] are usually thought to form spontaneously and have many advantages. To facilitate the practical implementation of this kind of spontaneous formed vesicles as the microreactors and templates,[7] it is desirable to prepare the vesicle of superior stability at the extreme environment such as high temperature or low pH value. Unfortunately, phase transition usually occurs when the vesicle suspensions are heated to 20−70 °C,[8−11] except that of archaebacteria, which can exist at extreme environment of 85 °C and low pH.[12] Recently great attention has been paid to the research of vesicle of bolaamphiphiles[13−16] (molecules with two headgroups connected by one or two hydrocarbon chains) since their structures are similar to that of the membrane molecules of archaebacteria. However, as far as we know few synthetic bolaamphiphile systems or their mixtures with other amphiphiles have met this expectation.[17] In this report, vesicles were prepared from the mixture of simple structured bolaamphiphile, sodium eicosanedioate ($NaOOC(CH_2)_{18}COONa$, or SEDA), and conventional cationic surfactant dodecyltriethylammonium bromide (DEAB). By means of freeze-fracture TEM, variable temperature IR spectra (VT-IR), differential scanning calorimetry (DSC), and fluorescence probe technique, it is interesting to note that unusual vesicle stability at high temperature was found in this kind of systems.

Experimental Section

Materials. SEDA was synthesized according to the following procedure: eicosanedioic acid was dissolved in anhydrous ethanol under heating. Sodium hydroxide was then added into the solution. After stirring for 5 h, the mixture was cooled and filtered to obtain white solids. The crude products was then recrystalized three times in ethanol/water solution to get the final products. DEAB are recrystalized five times from an ethanol/acetone mixture. Pyrene was obtained from Sigma Co. and used as received. Water was distilled from the $KMnO_4$

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containing deionized water**.** Other reagents were from Beijing Chemical Co. of A. R. grade.

Preparation of Samples. Catanionic surfactant systems were prepared by dissolving SEDA in an aqueous DEAB solution, or by the dissolution in water of both the solid amphiphiles. In any case, gentle heating is needed, and the pH was controlled to 9.2 by 0.01 mol/dm^3 $Na_2B_4O_7 \cdot 10H_2O$. All concentrations are given in a molar basis, and the solutions were kept in airtight containers to equilibrate a few days at room temperature before they were further analyzed.

Characterization Techniques. *FF-TEM.* Freeze-fracture transmission electron microscopy (FF-TEM) observations on the replication of samples were performed by using a JEOL-TEM 200CX electron microscope. For the preparation of replica, a small amount of sample was placed in a gold cup. The gold cup was warmed by high thermal capacity instruments before preparing the sample replica of high temperature. Then the gold cup was swiftly plunged into liquid Freon which was cooled with liquid nitrogen. The frozen samples were fractured and replicated in a freeze-fracture apparatus BAF 400 (Bal-Tec, Balzer, Liechtenstein) at −140 °C. Pt/C was deposited at an angle of 45°.

DSC. A differential scanning calorimetry (DSC) measurement was carried on the micro DSC (Setaram, France) instrument. A 0.8 mL aliquot of sample solution was transferred into the copper cup and then sealed with a copper cap. The measurements were made at 0.5 °C or 1 °C/min in the temperature range of 10−90 °C.

VT-IR. Variable temperature infrared spectra (VT-IR) were measured by a Nicolet Magna-IR 750 Fourier transform spectrometer with 4 cm^{-1} resolution. Sample solutions in D_2O were kept on the CaF_2 plates, and then the spectra were recorded at variable temperatures.

Fluorescence Probe Research. Ethanol solution of Pyrene was introduced into a solution of SEDA/DEAB. The total surfactant and Pyrene concentrations were 2×10^{-2} and 7×10^{-7}mol/dm^3 respectively, and the ethanol volume is less than 1% of the total solution volume (usually the effect of ethanol at this concentration on the surfactant aggregates is insignificant[18,19]). A Hitachi 850 spectrafluorometer was used to determine the micro polarity at various temperatures with the excitation wavelength of 338 nm, the emission wavelength of 350−470 nm, and bandwidths of 5 nm. I_3 and I_1 represent the intensities of the third and the first vibronic peaks in the fluorescence emission spectrum of Pyrene.

Results and Discussions

At room temperature (∼25 °C), the SEDA/DEAB mixed solutions of different mixing ratio are all clear and homogeneous even after being kept for 6 months. The aggregates formation in these systems was investigated by FF-TEM. Global vesicles with a diameter of about 20−60 nm in the SEDA/DEAB systems were observed. It seems that the mixing ratio has no obvious effect on the size of vesicles (Figure 1). The results of vesicle formation in the SEDA/DEAB systems were listed in Table 1.

Furthermore, the stability of vesicles in SEDA/DEAB systems against the temperature was also studied by FF-TEM. The electron micrographs revealed the presence of vesicles in SEDA/DEAB systems in the temperature range of 25−80 °C (Figure 1a and Figure 2). To make a comparison, we also examined the vesicle formation of the sodium laureate (SL)/DEAB conventional catanionic system at 60 and 80 °C. However, no vesicles were found in these systems of different mixing ratio.

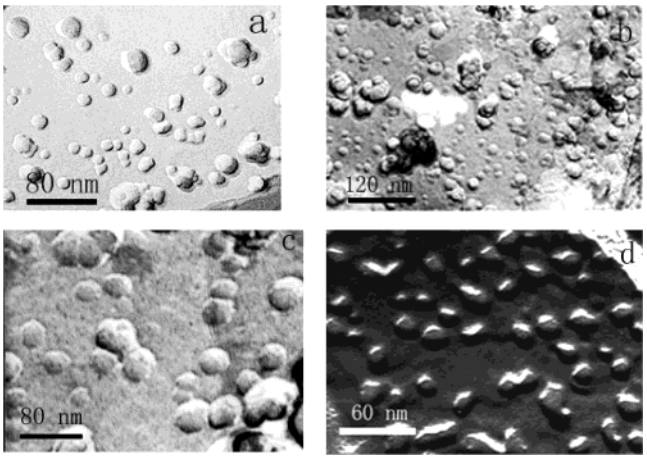


Figure 1. Freeze fracture electron micrograph of vesicles in SEDA/DEABsystems (The total concentrations C are all 0.01 mol/dm^3, pH = 9.2). The mixing ratios are (a) 1:2, (b) 1:3, (c) 1:7, (d) 1:12.

TABLE 1: Vesicle Formation in SEDA/DEAB Systems at Different Mixing Ratios

molar mixing ratio (SEDA/DEAB)	concentration (mol/dm^3)	appearance of solution	FF-TE observation
1:2	0.001∼0.02	clear	+
1:3	0.001∼0.04	clear	+
1:7	0.005∼0.08	clear	+
1:12	0.01∼0.1	clear	+

[a] +: vesicle. −: no vesicle.

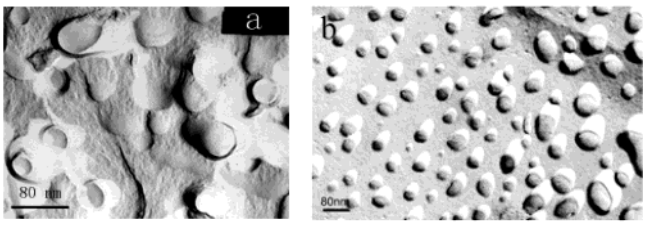


Figure 2. FF-TEM images of vesicles formed in 1:2 SEDA/DEAB mixed system(C=0.01 mol/dm^3, pH=9.2) at (a) 50 °C and (b) 80 °C.

In addition, the vesicles in SL/dodecyltrimethylammonium bromide(DTAB) were found disrupted at temperature higher than 50 °C.[20]

It is surprising that there is still vesicle existence at the temperature of 80 °C since usually the vesicles composed of synthetic surfactants or natural phospholipids are disrupted. J. Guilbot et al.[21] reported the vesicles prepared from asymmetric bolaamphiphiles being disrupted when the temperature was higher than 60 °C. Moreover, a lot of work reported the phase transition behaviors of surfactant vesicles and phospholipid liposome,[9−11] indicating vesicle membranes began to melt when the temperature reaches 23−75 °C.[9−11] That is to say, the vesicles formed in SEDA/DEAB systems possess superior thermal stability, which is a situation different from that of conventional vesicle systems but similar to that of archaebacteria.[12,22,23]

DSC measurements were also conducted to confirm the superior thermal stability of vesicles in SEDA/DEAB systems (Figure3). In contrast to an obvious phase transition temperature (65 °C) occurring in the conventional SL/DEAB = 1:1, 1:2, and 1:3 (in mole) catanionic surfactant systems, no phase transitions were detected in the SEDA/DEAB solutions under the same conditions, which means that the membrane of vesicles formed in the SEDA/DEAB systems is not destroyed even at temperatures higher than 80 °C.

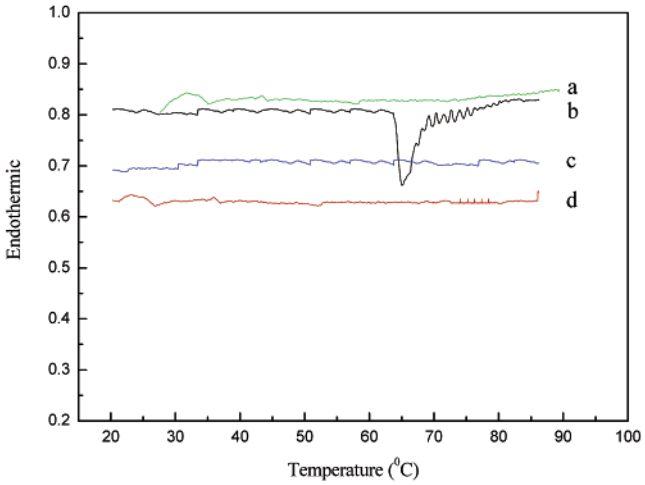


Figure 3. DSC curves of SEDA/DEAB systems (C = 0.02 mol/dm^3, pH = 9.2) (a) 1:2, (c) 1:3, (d) 1:7, and (b) 1:1 SL/DEAB (C = 0.02 mol/dm^3, pH = 9.2.).

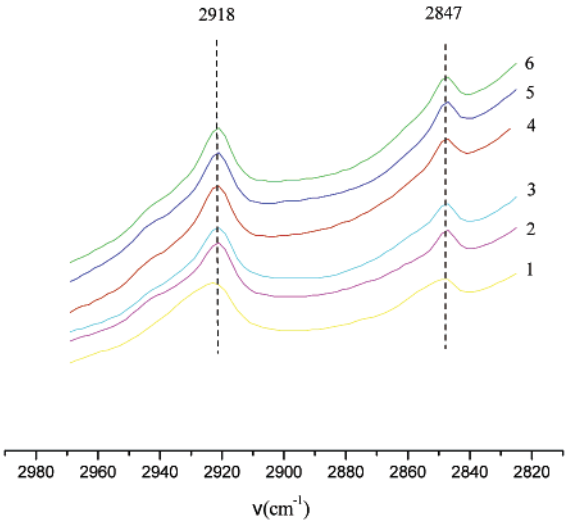


Figure 4. VT-IR spectra of CH_2 υ_{as} and υ_s band region for the self-assembly in the 1:2 SEDA/DEAB mixed solutions (C = 0.02 mol/dm^3, pH = 9.2). Curves 1−6 are corresponding to 20, 30, 40, 50, 80, and 90 °C, respectively.

Variable temperature FT-IR spectroscopy (VT-IR) was also used to investigate the vesicle stability in the SEDA/DEAB-systems. It is known that the CH_2 vibration is sensitive to the order of molecular packing.[24−26] The CH_2 asymmetric υ_{as} and symmetric stretching υ_s vibration bands usually shift from 2929 and 2956 cm^{-1} respectively, to lower frequency (lower than 2920 and 2850 cm^{-1}) when molecular packing turn from disordered state to highly ordered one. Part of the VT-IR spectra of 1:2 SEDA/DEAB systems is shown in Figure 4. It is seen that the υ_{as} and υ_s of CH_2 in this system appear at 2918 and 2847 cm^{-1}, respectively, which indicates that the methylene chains stretch in the membrane and are in the highly ordered gel state with an all-trans conformation.[24−26] Moreover, the wavenumber of CH_2 vibration bands does not increase with the increase of temperature, demonstrating that the observed microstructures did not change at high temperatures.[27]

Further confirmation was made by the measurement of micropolarity by the use of pyrene as the fluorescence probe.[28] The intensity ratio of the first and third vibronic peaks of pyrene I_1/I_3 reflects the polarity of the microenvironment in which the pyrene molecules are located. Thus, the plot of I_1/I_3 (or I_3/I_1) values against temperatures is usually used to determine the phase transition temperature of bilayers.[29] As shown in Figure 5, the values of I_1/I_3 in SEDA/DEAB mixed solutions kept around 0.88, which indicates the microenvironment of pyrene is similar to that of 2-propanol.[30] The I_1/I_3 values versus T gave almost horizontal plots, suggesting no phase transition occurs in the whole experimental temperature range (20−90 °C). This result agrees well with that of DSC and VT-IR measurements.

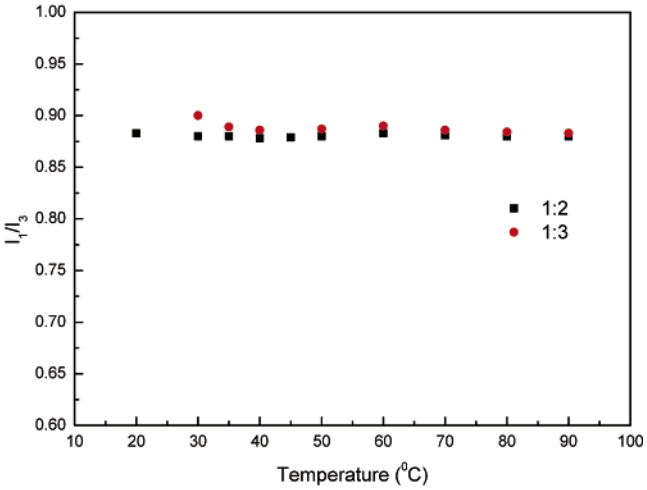


Figure 5. I_1/I_3 of SEDA/DEAB systems at different temperatures. The concentration of total surfactant and pyrene are 0.02 and 7.0 × 10^{-7} mol/dm^3 in pH = 9.2 solutions.

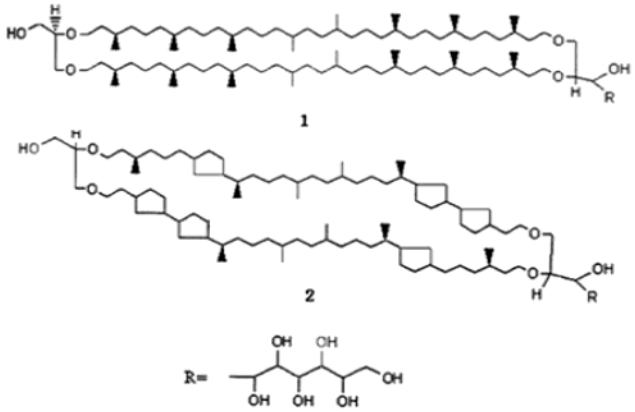
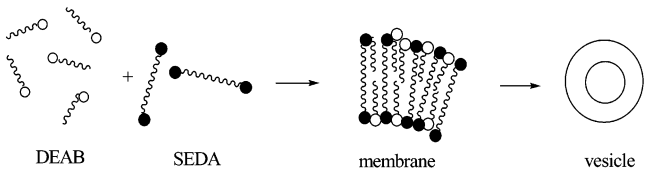


Figure 6. Example of natural bolaamphiphiles in archaebacteria membrane.

SCHEME 1: Possible Process of Vesicle Formation in SEDA/DEAB Mixed Solutions



The superior thermal stability of vesicles in SEDA/DEAB system may be attributed to the membrane spanning conformation of SEDA molecules in the vesicles and the strong electrostatic interaction between the opositely charged headgroups of the two molecules. As is known, vesicles in single bolaamphiphiles aqueous solutions or coventional catanionic surfactant systems are usually destroyed at higher temperatures. It is well-known that the membrane of archaebacteria which is composed of typical natural bolaaphiphiles (Figure 6) can maintain a superior stability at the high temperature (>80 °C).[21,22] Thus it is resonable to attribute the superior thermal

stability of vesicles in SEDA/DEAB systems to the combined effect of the bola-form molecular structure of SEDA and the strong intereaction of cationic and anionic surfactants. The IR results that the SEDA molecules adopt a membrane spanning conformation in the vesicles, giving strong support to our conclusion that the membrane spanning sturcture of SEDA are responsible for the unusual thermal stability of SEDA/DEAB vesicles. On the basis of the above conclusion, a possible vesicle formation mechanism was proposed as Scheme 1. The studies concerning the thermal stable vesicles of bolaamphiphile/oppositely charged conventional surfactant mixed systems are going on in our laboratory.

Conclusions

In conclusion, we found vesicles with superior thermalstability in the mixed systems of SEDA/DEAB at high temperatures (>80 °C). As far as we know, this is the first report of synthetic mixed vesicles which can exist at the temperature over 80 °C. Consequently, it will open a great vista of the practical application of membrane mimetic chemistry and will especially shed a light on facilitating the use of catanionic vesicle as microreactors and templates.

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