

Effect of Hydrocarbon Parts of the Polar Headgroup on Surfactant Aggregates in Gemini and Bola Surfactant Solutions

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Cationic gemini surfactant homologues alkanediyl- α,ω -bis(dodecyldiethylammonium) bromide, $[\text{C}_{12}\text{H}_{25}(\text{CH}_3\text{CH}_2)_2\text{N}(\text{CH}_2)_S\text{N}(\text{CH}_2\text{CH}_3)_2\text{C}_{12}\text{H}_{25}]\text{Br}_2$, where $S = 4, 6, 8, 10, \text{ or } 12$, referred to as $\text{C}_{12}\text{C}_S\text{C}_{12}(\text{Et})$, and cationic bolaamphiphiles BPHEAB (biphenyl-4,4'-bis(oxyhexamethylenetriethylammonium) bromide), PHEAB (phenyl-4,4'-bis(oxyhexamethylenetriethylammonium) bromide) were synthesized, and their aggregation behaviors in aqueous solution were studied and compared by means of dynamic light scattering, fluorescence entrapment, and transmission electron microscopy. Spherical vesicles were found in the aqueous solutions of these gemini and bola surfactants, which can be attributed to the increase of the hydrocarbon parts of the polar headgroup of the surfactants. In combination with the result of the other gemini with headgroup of propyl group, the increase of the hydrophobic parts of the surfactant polar headgroup will be beneficial to enhance the aggregation capability of the gemini and bola surfactants. Both of the vesicles formed in the gemini and bola systems showed good stabilities with time and temperature, but different stability with salt due to the different membrane conformations of surfactant molecules in the vesicles.

Introduction

As two new types of surfactants, gemini surfactants and bolaform amphiphiles have attracted increasing attention over the last years, owing to their superior properties in comparison with those of conventional single-chain surfactants.^{1–5} The most pronounced differences for gemini surfactants are the extremely low critical micelle concentrations and low Krafft points.³ Considerable efforts have been made to design and synthesize new forms of gemini surfactants, and most of them has been focused on the effect of the spacer length or flexibility and hydrophobicity.⁶ The most widely studied gemini surfactants are dicationic quaternary ammonium compounds, which are referred to as $\text{C}_M\text{C}_S\text{C}_M(\text{Me})$, where M and S stand for the carbon atom number in the side alkyl chain and the methylene spacer, respectively.^{7,8} Moreover, the variation of headgroup of the gemini molecules usually affects aggregation behavior and then influences the surface and bulk properties.^{9,10} Although there are some works on the aggregation behaviors with the structural variation of the gemini surfactant's polar headgroup,^{11,12} research on the microstructure transformation with variation of the hydrocarbon part of the headgroup is still rare.

As to the conventional single-chain cationic quaternary ammonium surfactant, changing the trimethylammonium headgroup to a triethylammonium group or even a tripropylammonium group does not lead to any special variation of surface properties and aggregation behavior.^{13,14} However, when the headgroup was changed to tributylammonium, there was an obvious effect due to the large headgroup, such as on surface properties and aggregate microstructure in aqueous solution.¹⁴ Considering the fact that gemini and bolaform surfactants have two headgroups, the influence on the molecular properties by the variation of headgroups in the two surfactant systems may be greater than that in the conventional single-chain surfactant system. On the basis of this idea, in this paper, we designed and synthesized a series of gemini surfactants alkanediyl- α,ω -bis(dodecyldiethylammonium) bromide, $[\text{C}_{12}\text{H}_{25}(\text{CH}_3\text{CH}_2)_2\text{N}(\text{CH}_2)_S\text{N}(\text{CH}_2\text{CH}_3)_2\text{C}_{12}\text{H}_{25}]\text{Br}_2$, designated as $\text{C}_{12}\text{C}_S\text{C}_{12}(\text{Et})$ (where $S = 4, 6, 8, 10, 12$), and two kinds of cationic bolaamphiphiles BPHEAB (biphenyl-4,4'-bis(oxyhexamethylenetriethylammonium) bromide) and PHEAB (phenyl-4,4'-bis(oxyhexamethylenetriethylammonium) bromide). The molecules of alkanediyl- α,ω -bis(dodecyldimethylammonium) bromide are referred to as $\text{C}_{12}\text{C}_S\text{C}_{12}(\text{Me})$ in order to differentiate from their ethyl homologues. The effect of the above-mentioned gemini surfactant series and corresponding bolaamphiphiles on the aggregation properties were studied to investigate the effect of the hydrocarbon parts of the polar headgroup in unconventional surfactant systems with multipolar headgroups.

Experimental Section

Surface Tension. The surface tension measurement was conducted using the drop volume method at 25.00 ± 0.01 °C.¹⁵ Critical micelle concentration (cmc) is determined by the cross point of the two lines before and after cmc on the γ -log c curve.

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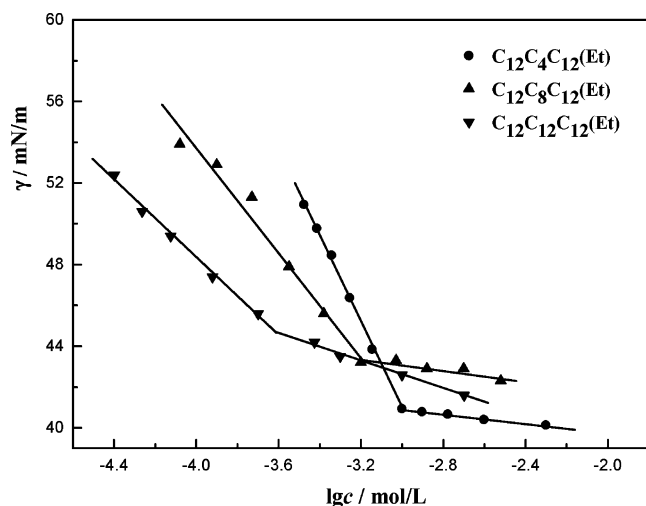


Figure 1. γ – $\log c$ curves of $C_{12}C_5C_{12}(Et)$ series ($S = 4, 8, 12$) at 25.0 °C.

Dynamic Light Scattering (DLS). DLS measurements are performed with a spectrometer (ALV-5000/E/WIN Multiple Tau Digital Correlator) and a Spectra-Physics 2017 200 mW Ar laser (514.5 nm wavelength). The scattering angle is 90°, and the intensity autocorrelation functions are analyzed by using the methods of Contin and Cumulant. All measurements were made at 25.0 °C.

Transmission Electron Microscopy (TEM). Micrographs were obtained with a JEM-100CX II transmission electron microscope by the negative-staining method and freeze–fracture technique.

Negative-Staining Method. Uranyl acetate solution (1%) was used as the staining agent. One drop of the solution was placed onto a carbon Formvar-coated copper grid (230 mesh). Filter paper was used to absorb the excess liquid.

Freeze–Fracture Technique. Fracturing and replication were carried out in an EE-FED.B freeze–fracture device equipped with a JEE-4X vacuum evaporator.

Entrapment of Riboflavin and Gel Filtration. Vesicles were prepared in 1×10^{-4} mol/L riboflavin solution. 0.5 mL of this solution is loaded onto a pre-equilibrated Sephadex G-50 column (18 cm \times 1 cm) and eluted with an identical ionic strength NaBr solution to separate vesicle-entrapped riboflavin from the free riboflavin. The fluorescence emission due to riboflavin is measured at 514 nm upon excitation at 374 nm using a Hitachi F-4500 spectrofluorometer.

Differential Scanning Calorimetry (DSC). The measurement was carried on the micro-DSC (Setaram-France) instrument, and the measurements were made at 1 °C/min in the temperature range 5–95 °C.

Results and Discussion

The variations of the surface tension γ with the surfactant concentration c are shown in Figure 1. The plots of γ vs $\log c$ show a break at a concentration corresponding to the critical micelle concentrations (cmc) of $C_{12}C_5C_{12}(Et)$ series ($S = 4, 8, 12$), and they are 1.00, 0.65, and 0.24 mmol/L at 25 °C, respectively. The cmc values of $C_{12}C_5C_{12}Br_2(Et)$ ($S = 4, 8, 12$) are almost equal to those of their methyl homologues, while the cmc values are much lower than those of the conventional cationic quaternary ammonium surfactant dodecyl triethylammonium bromide, which is regarded as the monomer of the gemini (DTEAB, cmc = 1.3×10^{-2} mol/L).

At room temperature (~ 22 °C), the influence on the formation of molecular organized assemblies by the variation of headgroups in gemini and bola systems was studied. The aqueous solutions of the $C_{12}C_5C_{12}(Et)$ series ($S = 4, 8, 12$) were prepared at several concentrations; clear and transparent solutions could be obtained. The organized assemblies formed in the systems were investigated

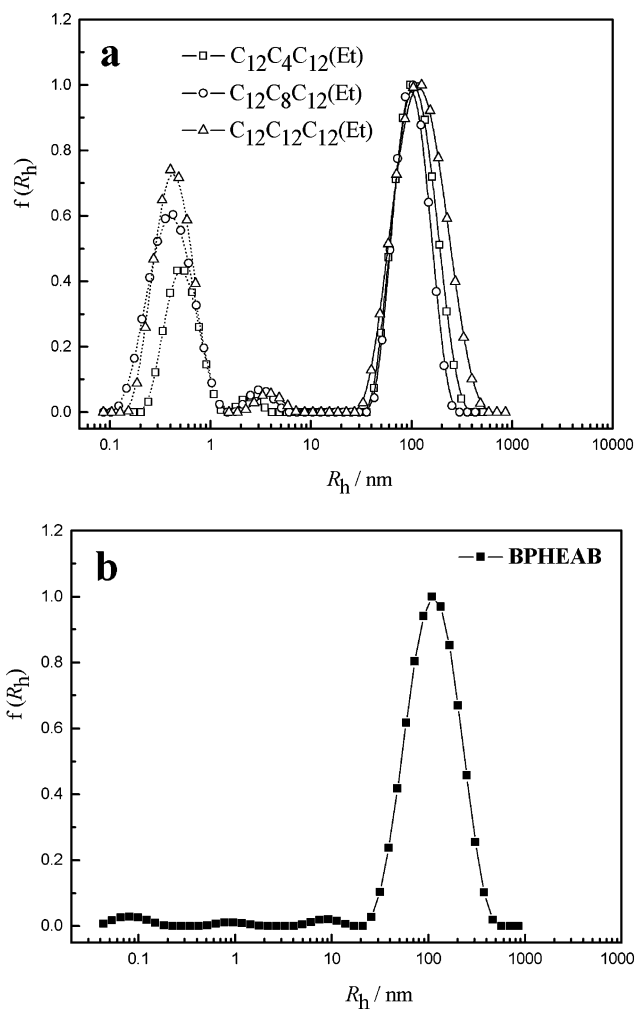


Figure 2. Hydrodynamic radius (R_h) distributions of the aggregates formed in the aqueous solution at the concentration of 10 mmol/L at 25.0 °C: (a) $C_{12}C_5C_{12}(Et)$ series ($S = 4, 8, 12$); (b) BPHEAB.

by dynamic light scattering (DLS) first. The plot of the apparent hydrodynamic radius distribution $f(R_{h,app})$ was calculated by the CONTIN method. From the DLS plot (Figure 2a), there was one obvious peak with an average apparent hydrodynamic radius ($\langle R_{h,app} \rangle$) of 100 nm, reflecting the typical size of a big aggregate. The peaks centered at $\langle R_{h,app} \rangle$ smaller than 1 nm did not correspond to micelles. DLS results (not shown in this paper) indicated that the small peaks may be induced by the characteristic molecular structure of gemini and reflected some interaction in the unconventional surfactant systems with multipolar headgroups. By TEM observation, both the negative-staining method and the freeze–fracture method were used, and spherical vesicles were observed in the aqueous solution of $C_{12}C_5C_{12}(Et)$ series ($S = 4, 8, 12$) (Figure 3a–c), indicating that the big aggregates in the DLS plot should be assigned to vesicles combined with the fact that the apparent viscosity (η_a) of the solution is quite close to the viscosity of water. The riboflavin entrapment measurement (Figure 2 in Supporting Information) also proved the formation of vesicles in the aqueous solution for the $C_{12}C_5C_{12}(Et)$ series ($S = 4, 8, 12$). Vesicle peaks were clearly showed in the fluorescence plots, which indicated that the vesicles were separated successfully in the eluting process. Similar results were also observed for other $C_{12}C_5C_{12}(Et)$ series of $S = 6, 10$, indicating that the vesicle formation is a general phenomena in the systems of $C_{12}C_5C_{12}(Et)$ series ($S = 4, 6, 8, 10, 12$). Further experiments were performed in the systems of alkanediyl- α,ω -bis(dodecyl-dipropylammonium) bromide, abbreviated to $C_{12}C_5C_{12}(Pro)$, of

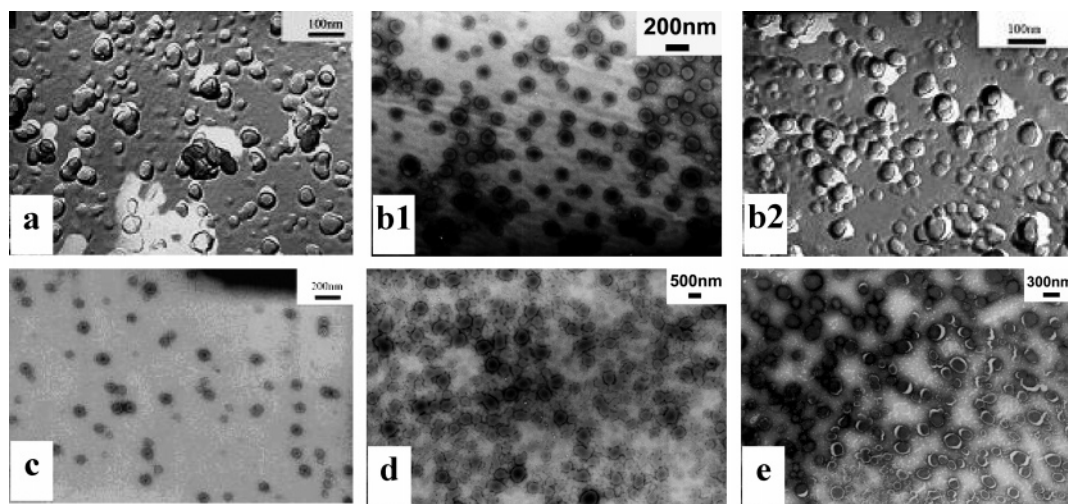


Figure 3. TEM micrographs for the gemini and bola surfactants of 10 mmol/L aqueous solution: (a) $C_{12}C_4C_{12}(Et)$; (b) $C_{12}C_8C_{12}(Et)$; (c) $C_{12}C_{12}C_{12}(Et)$; (d) $C_{12}C_4C_{12}(Pro)$; (e) BPHEAB. (a and b2 by the freeze-fracture method, b1 and c–e by the negative-staining method).

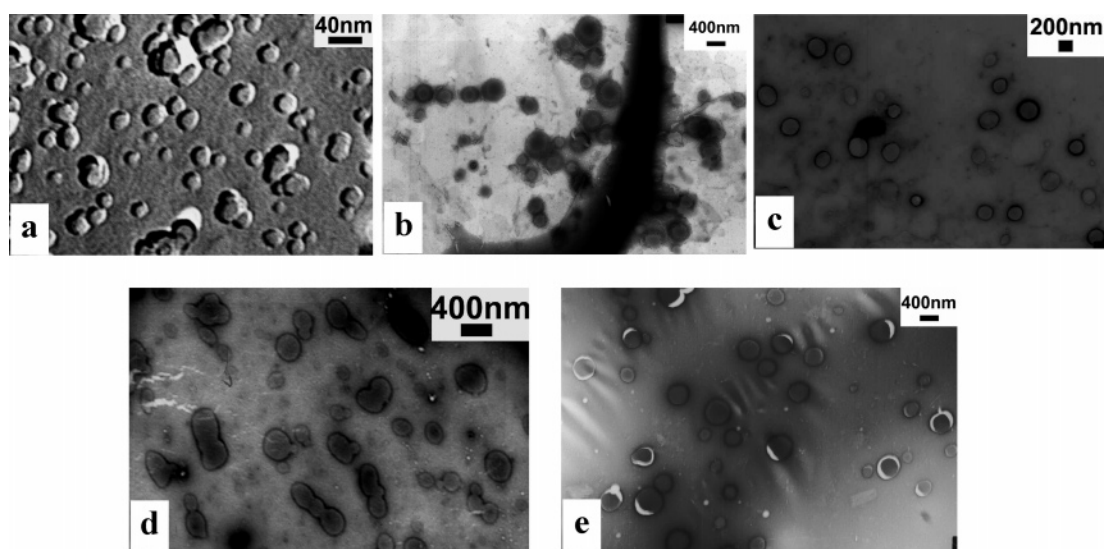


Figure 4. TEM micrographs for gemini and bola surfactant at the concentration of 10 mmol/L: (a) $C_{12}C_{12}C_{12}(Et)$, fresh solution at 80 °C by the freeze–fracture method; (b) $C_{12}C_{12}C_{12}(Et)$, 6 months at room temperature by the negative-staining method; (c) BPHEAB, fresh solution at 60 °C by the negative-staining method; (d) BPHEAB; and (e) PHEAB with the addition of 0.1 mol/L NaBr.

which the ethyl groups in the headgroups were changed to propyl groups. Vesicles were also observed in the aqueous solution of $C_{12}C_4C_{12}(Pro)$ (Figure 3d). However, for the $C_{12}C_S C_{12}(Me)$ series ($S = 4, 8, 12$), the micrographs showed only densely packed spheroidal micelles, and vesicles can be observed only in the solution of two homologues with a very long spacer, namely, $C_{12}C_{16}C_{12}(Me)$ and $C_{12}C_{20}C_{12}(Me)$.⁸

As for bolaform amphiphiles, the influence on the formation of molecular organized assemblies by the variation of headgroups was also studied, and similar results were obtained. Two kinds of cationic bolaamphiphiles BPHTAB (biphenyl-4,4'-bis(oxyhexamethylenetriethylammonium) bromide) and BPHEAB (biphenyl-4,4'-bis(oxyhexamethylenetriethylammonium) bromide) were also synthesized and studied. Only micelles were found to be formed in the aqueous solution of BPHTAB with methyl headgroup.¹⁶ However, when the number of headgroups increased from methyl groups to ethyl ones, i.e., BPHEAB, besides micelles, vesicles were also observed by TEM (Figure 3e). DLS measurement also proved the peaks assigned to vesicles with the average apparent hydrodynamics radius ($R_{h,app}$) of 100 nm (Figure 2b), of which the size is consistent with the TEM observed result.

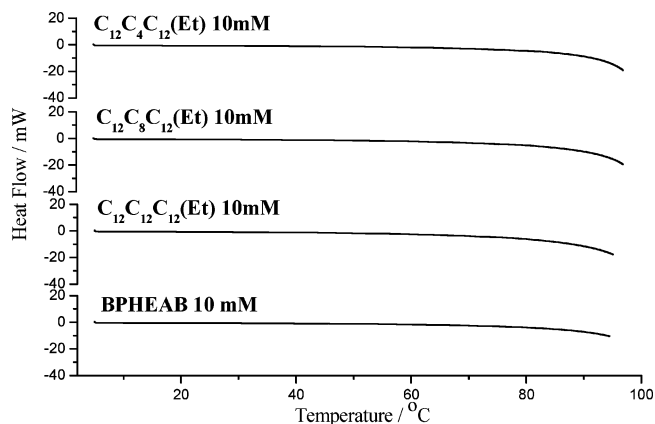


Figure 5. DSC curves of gemini $C_{12}C_S C_{12}(Et)$ series ($S = 4, 8, 12$) and bolaamphiphile BPHEAB at the concentration of 10 mmol/L.

All the results in the methyl, ethyl, and propyl headgroup series of gemini or bola surfactants indicated that the increase of the hydrocarbon parts of the polar headgroup will be beneficial

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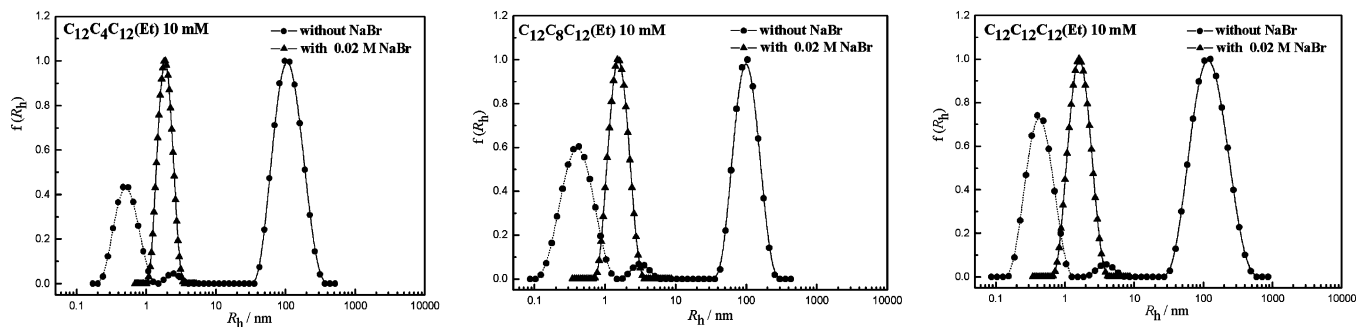


Figure 6. Hydrodynamic radius (R_h) distributions of the aggregates in the homologues of the $C_{12}C_5C_{12}(Et)$ series ($S = 4, 8, 12$) with and without the addition of 0.02 mol/L NaBr at 25.0 °C.

to enhance the aggregation capability of the surfactants. With the increase of the hydrocarbon parts on the surfactant headgroup, the hydrophobic effect of the whole amphiphile was also correspondingly increased. These structure effects will be more obvious for the surfactant having multipolar headgroups, such as gemini and bola surfactants, since the stronger hydrophobic interaction and the resultant strong aggregation tendency allow the molecules to form larger aggregates than micelles.

The characteristics of the vesicles formed in the gemini and bola surfactant systems were studied and compared. Due to the desirability of practical implementation of vesicles as model cell membranes, drug delivery systems, and microreactors,^{17,18} vesicle stability is an important topic to be investigated, and many works have been focused on phospholipid vesicles^{19,20} and cationic surfactant vesicles,^{21–23} which are usually thought to form spontaneously and have many advantages. However, so far the reports on the stability of the vesicles formed in the gemini surfactant systems on time and temperature are still rare. In contrast to the vesicles composed of synthetic surfactants or natural phospholipids, which are disrupted and present phase transition behaviors when the temperature reaches 23–75 °C,^{24–26} it is surprising that there are still vesicles at 80 °C in $C_{12}C_5C_{12}(Et)$ ($S = 4, 8, 12$) (Figure 4a). DSC measurements were also conducted to confirm the superior thermal stability of vesicles in $C_{12}C_5C_{12}(Et)$ ($S = 4, 8, 12$) (Figure 5). In contrast to an obvious phase transition temperature occurring in most phospholipid vesicle systems, no phase transitions were detected in $C_{12}C_5C_{12}(Et)$ ($S = 4, 8, 12$) solutions, which means that the membranes of vesicles formed in $C_{12}C_5C_{12}(Et)$ ($S = 4, 8, 12$) systems are not destroyed even at temperatures higher than 80 °C. Moreover, the micrographs (Figure 4b) of the $C_{12}C_{12}C_{12}(Et)$ solution showed that spherical vesicles still existed after 6 months at room temperature, although they became bigger than those in the fresh solution. That is to say, the spontaneously formed vesicles in the $C_{12}C_5C_{12}(Et)$ series are stable for at least 6 months without the use of sta-

bilizers. All these results strongly suggest that the vesicles formed in the $C_{12}C_5C_{12}(Et)$ series are relatively stable with time and possess superior thermal stability, which is a different situation from that of conventional vesicle systems, while the vesicles formed in the bolaamphiphile BPHEAB also showed superior thermal stability. Both the DSC and TEM results (Figure 5 and Figure 4c) were used to prove it. The superior thermal stability of vesicles in bolaamphiphile system also be exhibited in the mixed system of bolaamphiphile and its oppositely charged conventional surfactant.²⁷

The effect of salt on the formation of vesicles was also investigated. The TEM results showed that the vesicles no longer existed in the $C_{12}C_5C_{12}(Et)$ ($S = 4, 8, 12$) systems with the addition of 0.02 mol/L NaBr. DLS results also showed that the peak assigned to vesicles with the average hydrodynamic radius (R_h) of 100 nm completely disappeared, leaving solely the “micelle peak” with the (R_h) of 2 nm (Figure 6). In combination with TEM results, it can be concluded that vesicles were destroyed and completely transformed into micelles in the aqueous solution of $C_{12}C_5C_{12}(Et)$ series ($S = 4, 8, 12$) with 0.02 mol/L NaBr addition. However, the situation was different when NaBr was added into the bolaamphiphile of 10 mmol/L BPHEAB solution. The vesicles still existed in the BPHEAB system even as the addition concentration of NaBr reach 0.1 mol/L, although some vesicles fused (Figure 4d). In fact, the addition of NaBr was beneficial to the formation of vesicles instead of destroying the vesicles in the bola system. With the cationic bolaamphiphile PHEAB (phenyl-4,4'-bis(oxyhexamethylenetriethylammonium) bromide) as an example, although the headgroup is ethyl, micelles only and no vesicles were found to be formed in the aqueous solution of PHTAB due to its good solubility and large cmc value. However, with 0.1 mol/L NaBr added, vesicles were formed in the aqueous solution of PHTAB (Figure 4e). The observed different salt addition effects on the stability in the two types of vesicles may be attributed to the membrane-spanning conformation of surfactant molecules in the vesicles. In comparison with bilayer membranes formed by conventional surfactants or gemini surfactant as those with shorter spacers, monolayer membranes are usually formed by bolaamphiphiles based on their special structure, namely, BPHEAB molecules adopt a membrane-spanning conformation in the vesicles. The monolayer membrane systems may provide great convenience for the membrane mimetic investigation due to their stability and fluidity.^{28–30}

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Conclusion

In conclusion, the headgroup plays an important role in regulating the aggregate microstructure in the surfactant systems, especially for the surfactant having multipolar headgroups, such as gemini and bola surfactants. The increase of the hydrophobic effect induced by the hydrocarbon parts of the polar headgroup will be beneficial to the aggregation of the molecules. Moreover, the vesicles formed in the gemini and bola surfactant systems were compared with each other, and their similarities and differences were obtained. Studies concerning the surface properties and the variation of the headgroup of gemini and bola surfactant systems are ongoing in our laboratory.

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Supporting Information Available: Experimental details for the synthesis of gemini surfactants and bolaamphiphiles and the results of riboflavin entrapment measurement. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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