ELSEVIER

Colloids and Surfaces A: Physicochem. Eng. Aspects 224 (2003) 213–220

COLLOIDS AND SURFACES A

www.elsevier.com/locate/colsurfa

# The research on the vesicle formation and transformation in novel Gemini surfactant systems

Zhangyi Liang, Chuanzhong Wang, Jianbin Huang *

*State Key Laboratory for Structural Chemistry of Unstable and Stable Species, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China*

Received 2 January 2003; accepted 8 April 2003

**Abstract**

Three new types of Gemini amphiphiles (*N*,*N*′-bis-(4-decyloxybenzylidene)-ethane-1,2-diamine, *N*,*N*′-bis-(4-decyloxy-2-hydroxybenzylidene)-ethane-1,2-diamine, and (*N*,*N*′-bis-(4-decyloxy-2-hydroxybenzylidene)-ethane-1,2-diamine)copper(II) were synthesized. Their aggregation behaviors were studied by TEM observation and monolayer experiments. It was found that the vesicle formation and transformation can be adjusted by molecular structure, pH value and the concentration of $Cu^{2+}$ in these surfactant systems.


*Keywords:* Gemini surfactant; Vesicle; Monolayer; pH effect; Metal ions

## 1. Introduction

Various kinds of organized assemblies, such as vesicles, micelles, multilayer composites etc. have been the subject of research for several decades [1–3]. Recently, researches about the control and adjustment of assembly structure and morphology have attracted broad attentions [4–7]. In the mean time, some new types of surfactants, such as Gemini [8] and Bola [9], were also developed. Since 1971, Bunton first synthesized a type of cationic Gemini surfactant [10], many kinds of Gemini surfactants have been prepared with the feature of cationic [11], anionic [12], or neutral [13] headgroups, and some particular physical–chemical properties, for examples, with very low critical micelle concentrations and low Krafft points [14,15], were also found. Considering the structural characteristics of Gemini amphiphile, which is constructed with two hydrophobic chains connected by one or two hydrophilic head groups, molecular design and tailoring in Gemini surfactants are of great importance to investigate the formation and properties of organized assemblies [16]. On the other hand, the aggregate formation is also influenced by environmental factors, such as temperature and additives [17–20]. Tanford and Nagarajan discussed the relation between the molecular packing parameter and the conformation of surfactant self-assembly [21,22]. Among the

* Corresponding author.
*E-mail address:* jbhuang@chem.pku.edu.cn (J. Huang).

0927-7757/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0927-7757(03)00167-5

numerous works, the effects of pH and metal ions on organized assemblies, especially on vesicles, are remarkable, since they are found to have profound influences in biochemical applications. The pH value around a damaged tissue is usually different from that around the normal one [23], and during the process of root hair growth, pH was found to influence the cell wall loosening, which can influence the vesicles enrichment [24].

In this work, we synthesized a Gemini amphiphile with two hydroxyl *N*,*N*′-bis-(4-decyloxy-2-hydroxybenzylidene)-ethane-1,2-diamine (BDHEM). For comparison, another Gemini amphiphile, *N*,*N*′-bis-(4-decyloxybenzylidene)-ethane-1,2-diamine (BDEM), and a ligand BDHEM with $Cu^{2+}$, (*N*,*N*′-bis-(4-decyloxy-2-hydroxybenzylidene)-ethane-1,2-diamine)copper(II) (BDHEM–Cu) were also synthesized. The effect of their structural difference on their capability of aggregate formation was compared. The influences of pH values and metal ion concentrations on the aggregate formation were also discussed.

## 2. Experimental

### 2.1. Materials and methods

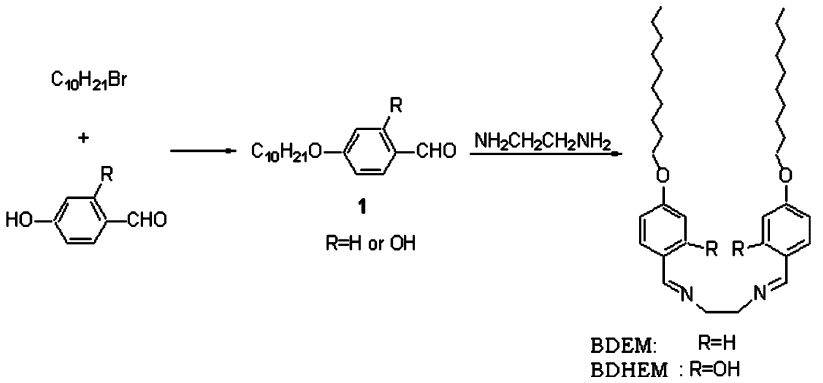
#### 2.1.1. Synthesis

*2.1.1.1. N,N′-Bis-(4-decyloxy-2-hydroxybenzylidene)-ethane-1,2-diamine.* 1.0 g (3.6 mmol) of 4-decyloxy-2-hydroxybenzaldehyde and 0.108 g (1.8 mmol) of ethylenediamine were dissolved in 20 ml of ethanol. The solution was gently stirred for 6 h at room temperature, and yellow precipitate was observed in the solution. The precipitate was filtered off and thoroughly washed with acetone to give 0.84 g of BDHEM (80%). FT-IR (KBr pellet): 2921, 2852, 1625, 1574 $cm^{-1}$. 0.88 (t, $CH_3$, 6H), 1.27 (m, $CH_2$, 28H), 1.76 (m, $CH_2$, 4H), 3.84 (s, $CH_2N$, 4H), 3.94 (t, $CH_2O$, 4H), 6.35 (d, phenyl ring, 2H), 6.39 (s, phenyl ring, 2H), 7.05 (d, phenyl ring, 2H), and 8.19 (s, CHN, 2H). UV–vis (nm, ethanol): 283, 313, 410.

*2.1.1.2. N,N′-Bis-(4-decyloxybenzylidene)-ethane-1,2-diamine.* 1.0 g (3.8 mmol) of 4-decyloxybenzaldehyde and 0.114 g (1.9 mmol) of ethylenediamine were dissolved in 20 ml of ethanol. The solution was gently stirred for 6 h at room temperature, and white precipitate was observed in the solution. The precipitate was filtered off and thoroughly washed with acetone to give 0.78 g of BDEM (75%). FT-IR (KBr pellet): 2924, 2848, 1640, 1607 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, ppm): 0.88 (t, $CH_3$, 6H), 1.27 (m, $CH_2$, 28H), 1.78 (m, $CH_2$, 4H), 3.91 (s, $CH_2N$, 4H), 3.97 (t, $CH_2O$, 4H), 6.90 (d, phenyl ring, 4H), 7.64 (d, phenyl ring, 4H), and 8.20 (s, CHN, 2H).

The synthesis route about BDHEM and BDEM are shown in Scheme 1.

*2.1.1.3. (N,N′-Bis-(4-decyloxy-2-hydroxybenzylidene)-ethane-1,2-diamine)copper(II).* $Cu(Ac)_2$ 0.0401 g (0.201 mmol) were




Scheme 1. The synthesis route about BDHEM and BDEM.

dissolved in 13 ml of ethanol (a). BDHEM 0.1166 g (0.201 mmol) were dissolved in 5:1 ethanol–chloroform (b). a was added to b slowly drop by drop, while stirring at room temperature for 15 min. The solution was refluxed for 4 h then cooling in room temperature. The precipitate was recrystalized in the solution of methanol:chloroform = 7:1 (v/v) (Scheme 2).

### 2.1.2. Vesicle preparation

The ligand–lipid vesicles of BDEM, BDHEM and BDHEM–Cu, were prepared by reverse-phase evaporation, adding samples in chloroform solution and then removing the solvent thoroughly under vacuum.

### 2.1.3. Microscopic studies

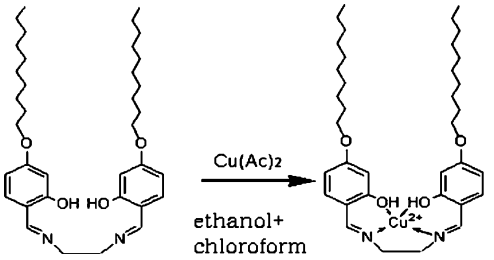
Micrographs were obtained with an electron microscope (JEM-100CX) using freeze-fracture techniques. For freeze-fracture sample preparation: fracturing and replication were carried out in a high vacuum freeze-etching system (Balzers BAF-400D).

### 2.1.4. Monolayer experiments

The monolayer experiments were carried out on a Krüss Filmbalance FB-1. The isotherms were recorded at 30.0 °C. The monolayer compression speed of sample BDEM was 1.4 $Å^2$ $min^{-1}$ per molecule, and the speed of sample BDEHM was 1.1 $Å^2$ $min^{-1}$ per molecule.

## 3. Results and discussion

The aqueous systems of BDHEM and BDEM ($1 \times 10^{-3}$ mol $dm^{-3}$) were prepared by sonication for 1 h at 60 °C. The resulting ligand–lipid vesicle solution was translucent. TEM observation showed that no vesicles formed in BDEM or BDHEM aqueous solutions. Increasing the pH in the BDEM system seemed to have no effect to the vesicle formation. However, for the BDHEM system, the situation was different. With the pH variation, vesicles were found at pH 10–13 and no vesicle existed at pH 14. This result is quite different from the systems of conventional amphiphiles. Usually the aggregation ability of conventional neutral surfactants is not sensitive with the change of pH. The results and some TEM images are shown in Table 1 and Fig. 1.




Scheme 2. The synthesis route about BDHEM–$Cu^{2+}$.

It is well known that the type of aggregate forming in a system will depend on the value of molecular packing parameter [25]. $P = V_c/A_0 l_c$, where $V_c$ and $l_c$ are the volume and chain length of the hydrophobic group, respectively, and $A_0$ is the optimum area of per polar group. For vesicle formation, the proper value of $P$ lies in the range of 1/2–1 and for bilayer or reverse micelle, more than 1, whereas, for globular micelles, $P$ lies in 0–1/2. Usually, ($V_c/L_c$) is nearly constant, hence the variation of the optimum area of per polar group $A_0$ will influence the parameter $P$ and have the chance to adjust the type of the surfactant aggregates.

Generally, in the same surfactant system, the molecular packing of the organized assemblies (vesicle, micelle, etc.) is similar to the molecular packing of the surface layer (adsorption layer or insoluble film). Thus it is useful to study the

Table 1
Vesicle formation of BDHEM and BDEM systems at different pH

| System | PH | TEM observation |
|---|---|---|
| BDEM | 6–7 ($H_2O$) | – |
| BDEM | 10 | – |
| BDEM | 12 | – |
| BDHEM | 6–7 ($H_2O$) | – |
| BDHEM | 10 | + |
| BDHEM | 12 | + |
| BDHEM | 13 | + |
| BDHEM | 14 | – |

"+", Vesicle; "–", no vesicle.

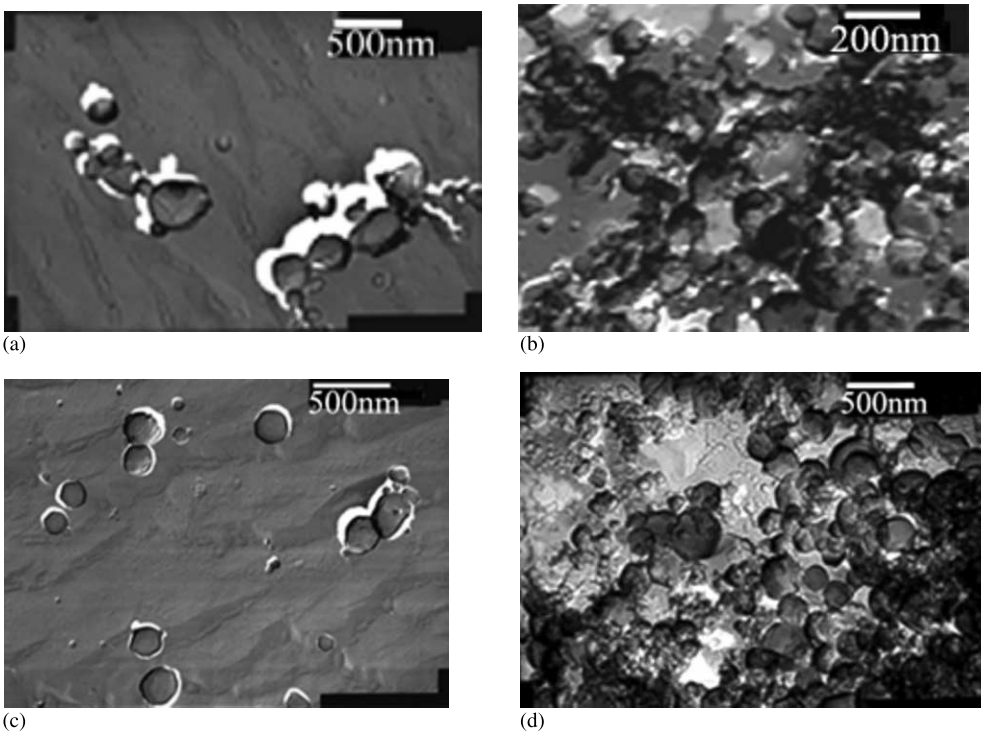



Fig. 1. TEM for vesicle formation of BDHEM system at (a) pH 10; (b) pH 12; (c) pH 13; (d) BDHEM–$Cu^{2+}$ in pure water.

limiting molecular area $a_0$ in the surface film to have better understanding to the formation of organized assemblies. Since the solubility of BDHEM and BDEM is poor, the surface pressure–area isotherms of monolayers were investigated to understand the molecular packing in the monolayer. Some results are shown in Fig. 2 and Table 2.

Fig. 2 and Table 2 show that the molecular packing of BDHEM monolayer on the water subphase is obviously influenced by the pH value and there exist a minimum for limiting area at pH 12. This phenomenon may be attributed to the formation of the inner-molecular hydrogen bonds in BDHEM. In our previous work, it was shown that the hydroxyl group just ionizes 1/2 when pH 12 and the inner-molecular hydrogen bonds formed [26]. The formation pulls the aromatic rings together and causes the molecular area reduction (Fig. 3). Consequently, the optimum area of per polar group $A_0$ of BDHEM aggregate will also decrease, and P will increase (maybe fall in the range of 0.5–1), when the pH comes to 12 in BDHEM systems. Hence, this is understandable why it is beneficial to vesicle formation in the range of pH 10–13. On the other hand, since there are no inner-molecular hydrogen bonds in BDEM systems due to the absence of hydroxyl groups, the pH value in subphase has less effect on the molecular area of BDEM molecules. In fact the packing parameter P may be calculated by the Tanford formula [21]:

Table 2
The limiting areas ($a_0$) of BDHEM and BDEM monolayers at 30.0 °C on a water surface with various pH values

| pH | $a_0$ of BDHEM (Å$^2$) | $a_0$ of BDEM (Å$^2$) |
|---|---|---|
| 6–7 ($H_2O$) | 108 | 119 |
| 10 | 96 | 116 |
| 12 | 76 | 119 |
| 13 | 94 | |
| 14 | 162 | 118 |

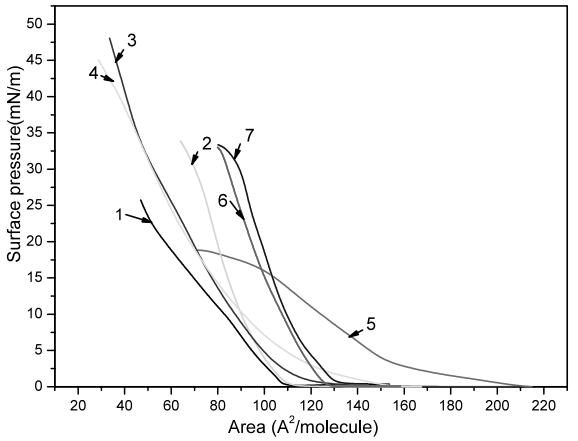



Fig. 2. Surface pressure–area isotherms of BDHEM and BDEM monolayers at 30.0 °C on the subphase: (1) BDHEM, pH 6–7 (water); (2) BDHEM, pH 10; (3) BDHEM, pH 12; (4) BDHEM, pH 13; (5) BDHEM, pH 14; (6) BDEM, pH 6–7 (water); (7) BDEM, pH 12.

$$V_c = 27.4 + 26.9n_c; \qquad L_c = 1.5 + 1.265n_c;$$
$$P = V_c/(A_0 \times L_c),$$

Where $n_c$ is the number of carbon atoms embedded in the hydrocarbon chain; $V_c$, the volume occupied by an alkyl chain of $n_c$; $L_c$, the maximum length for a chain with $n_c$; and $A_0$ is the area of headgroup. Here we used two $V_c$ to stand for $V_c$ in the original formula, since BDHEM are with double nonpolar tails. In pure water, the $P$ value of BDHEM system is near 0.40, but in the solution of pH 12 and 14, P is around 0.55 and 0.26, respectively. Considering the fact that pH 12 is the best condition for the formation of inner hydrogen bonds, it is logical to conclude that the system of BDHEM aggregates possesses a minimum optimum area of per polar group $A_0$. It is also understandable that there existed a maximum density of vesicles at the solution of pH 12 (Fig. 1).

Table 3
Vesicle formation in copper solution with different concentration

| System | $C_{Cu^{2+}}$ (mol dm$^{-3}$) | TEM observation |
|---|---|---|
| BDEM | $10^{-3}$ | – |
| BDEM | $10^{-4}$ | + |
| BDEM | $10^{-5}$ | – |
| BDHEM | $10^{-3}$ | – |
| BDHEM | $5 \times 10^{-4}$ | – |
| BDHEM | $10^{-4}$ | + |
| BDHEM | $10^{-5}$ | + |

"+", Vesicle; "–", no vesicle. $C_{Cu^{2+}}$: the concentration of copper ion.

The effect of copper ion concentration on aggregates formation about BDHEM and BDEM was also studied. The samples of BDHEM and BDEM were prepared by $10^{-3}$ mol dm$^{-3}$. After sonication for 1 h, there still remained some precipitates. Vesicles were observed in the BDHEM systems as well as BDEM systems if the $Cu^{2+}$ concentration is low. However, vesicles disappeared after the $Cu^{2+}$ concentration is equal to or bigger than $10^{-3}$ mol dm$^{-3}$ (Table 3). Some TEM images are shown in Fig. 4.

From Table 3 and Fig. 4, we can find that addition of small amount of $Cu^{2+}$ will be helpful to the vesicle formation in these two systems, for example, in the systems of BDHEM and BDEM vesicles were observed at the $Cu^{2+}$ concentration

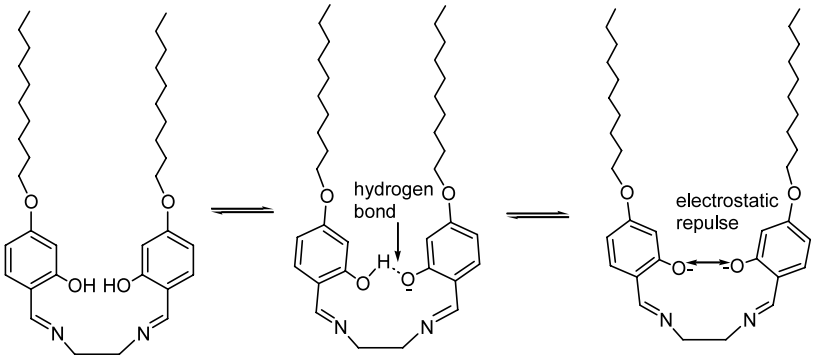



Fig. 3. The conformation change with increasing pH value of BDHEM.

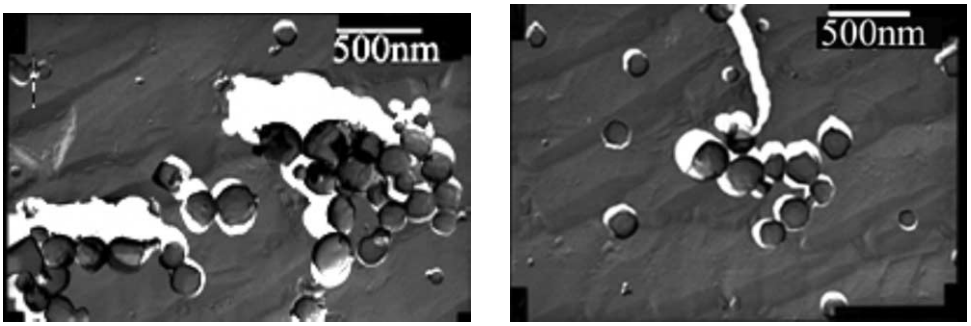



Fig. 4. Vesicle formation in the system of (a) BDEM, $C_{Cu^{2+}} = 10^{-4}$ mol dm$^{-3}$, by freeze fracture method; (b) BDHEM, $C_{Cu^{2+}} = 10^{-4}$ mol dm$^{-3}$, by freeze fracture method.

of $10^{-4}$ mol dm$^{-3}$. The results of the isotherms of monolayers in the systems of BDHEM and BDEM titrated with $Cu^{2+}$ in the subphase also showed that the limiting molecular area $a_0$ of BDHEM and BDEM both decrease with the addition of $Cu^{2+}$ (Fig. 5 and Table 4). When the $Cu^{2+}$ concentration increased from 0 to $10^{-4}$ mol dm$^{-3}$, for the systems of BDHEM and BDEM, $a_0$ decreased from 108 to 86 and 119 to 98 Å$^2$, respectively. This phenomenon may be attributed to the coordination interaction between the $Cu^{2+}$ and BDHEM or BDEM. As pointed above, the closer molecular packing is helpful to the vesicles formation according to the geometry rule. However, when the $Cu^{2+}$ concentration is equal to or bigger than $10^{-3}$ mol dm$^{-3}$, no vesicles were observed for the system of BDHEM and BDEM, which indicating that high metal concentration can destroy vesicles formation [27]. The excessive $Cu^{2+}$ may induce the formation of precipitates BDHEM–$Cu^{2+}$ or BDEM–$Cu^{2+}$ and greatly reduce the actual BDHEM or BDEM concentration in the solution. Thus vesicles disappear in these systems.

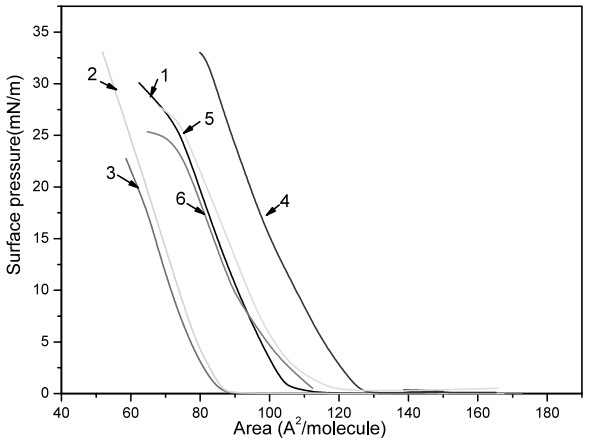



Fig. 5. Surface pressure–area isotherms of BDHEM and BDEM monolayers at 30.0 °C on the subphase: (1) BDHEM, 0 (water); (2) BDHEM, $[Cu^{2+}] = 10^{-5}$ mol l$^{-1}$; (3) BDHEM, $[Cu^{2+}] = 10^{-4}$ mol dm$^{-3}$; (4) BDEM, 0 (water); (5) BDEM, $[Cu^{2+}] = 10^{-5}$ mol dm$^{-3}$; (6) BDEM, $[Cu^{2+}] = 10^{-4}$ mol dm$^{-3}$.

It is also interesting to note that BDHEM seemed have stronger interaction with $Cu^{2+}$ than that of BDEM with $Cu^{2+}$. When the $Cu^{2+}$ concentration is $10^{-5}$ mol dm$^{-3}$, a great deal of BDHEM vesicles were observed, in the contrast to the fact that little vesicles of BDEM existed at the same $Cu^{2+}$ concentration. The results of the isotherms of monolayers in the systems of BDHEM and BDEM showed that for BDHEM, $a_0$ decreased from 108 Å$^2$ on pure water subphase to 83 Å$^2$. Nevertheless, for BDEM, $a_0$ is from 119 to 104 Å$^2$ (Table 4 and Fig. 5). This may be accredited to the different ligation binding sites

Table 4
The limiting areas ($a_0$) of BDHEM and BDEM monolayers at 30.0 °C on different $Cu^{2+}$ concentration subphase

| Concentration of $Cu^{2+}$ (mol dm$^{-3}$) | $a_0$ of BDHEM (Å$^2$) | $a_0$ of BDEM (Å$^2$) |
|---|---|---|
| Water | 108 | 119 |
| $10^{-5}$ | 83 | 104 |
| $10^{-4}$ | 86 | 98 |

of BDHEM–$Cu^{2+}$ and BDEM–$Cu^{2+}$. For BDHEM, there are four binding sites for a copper ion: two oxygen atoms and two nitrogen atoms. On the other hand, for BDEM, there are only two nitrogen atoms that can act as binding site. Hence the interaction of BDEM–$Cu^{2+}$ will be much weaker than BDHEM–$Cu^{2+}$.

To confirm our conclusion, another compound BDHEM–Cu was also synthesized. TEM observation demonstrated that vesicles formed in aqueous BDHEM–Cu solution (Fig. 1(d)). However, adding $10^{-3}$ mol $dm^{-3}$ $Na_2S_2O_3$, $Na_2S_2O_4$, or EDTA to the BDHEM–Cu (concentration $10^{-4}$ mol $dm^{-3}$) solutions, respectively, no vesicles were observed in any case. This may be accredited to the facts that Red-Ox reaction or ligand exchange reaction occurred in such systems. For the addition of $Na_2S_2O_3$ and $Na_2S_2O_4$, the Red-Ox reaction is [28].

$$Cu^{2+}(BDHEM)^0 + e \rightarrow [Cu^{2+}(BDHEM)^-]^+ + e \rightarrow [Cu^{2+}(BDHEM)^{2-}]^0$$

One or two electrons can be delocalized across the ligand. The reduction of the two-electron decreases the density of intramolecular ion pair, which diminishes monomer amphiphilicity and ultimately deaggregates the assemblies [28]. Another possibility is that when $Na_2S_2O_3$ or $Na_2S_2O_4$ was added in the BDHEM–Cu solution, the double bond of nitrogen (C=N) may disappear accompanied by breaking the planar structure of the ring.

For the case of EDTA addition, EDTA combines $Cu^{2+}$ more steadily than that with BDHEM. Therefore, after sonication, a more stable compound of Cu(EDTA) is produced:

$$BDHEM\text{–}Cu + EDTA \rightarrow Cu(EDTA) + BDHEM.$$

Thus, adding $Na_2S_2O_3$, $Na_2S_2O_4$, or EDTA can reduce the actual concentration of copper ions in the system and finally destroy vesicles. These results support our conclusion that copper ions may influence the vesicle formation of BDHEM and BDEM systems.

## 4. Conclusion

Three new types of Gemini amphiphiles were synthesized. The effect of molecular structure and environmental factors (pH value and metal ions) on vesicle formation was investigated. In contrast to the case of the BDEM system, the pH value has strong influence on the vesicle formation of BDHEM system. Vesicles were observed in the pH range of 10–13, and pH 12 was found to be the best condition for vesicle formation. The measurement of limiting area by surface pressure–area isotherms provided the useful molecular packing information to understand the nature of the vesicle formation.

Addition of $Cu^{2+}$ is also a good method to adjust the vesicle formation and transformation in this kind of systems. It was found that suitable concentration of $Cu^{2+}$ promotes the vesicle formation but higher $Cu^{2+}$ concentration will destroy the vesicles. BDHEM–Cu was also synthesized and it was found to form vesicle easily. Furthermore, adding $Na_2S_2O_3$, $Na_2S_2O_4$ or EDTA, decreases the actual $Cu^{2+}$ concentration and destroys the vesicles in all these systems. All these results provided a simple way to control vesicle formation in this kind of systems and shed a light to the desired organized microstructure research on controlling aggregate behavior by the adjustment of molecular design and the effect of pH value and metal ions concentration.

## Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 20233010, 20073002).

## References

[1] E. Marques, A. Khan, M.G. Miguel, B. Lindman, J. Phys. Chem. 97 (1993) 4729.
[2] J.B. Huang, G.X. Zhao, Colloid Polym. Sci. 273 (1995) 156.
[3] B. Yang, H. Matsumura, K. Katoh, H. Kise, K. Furusawa, Langmuir 17 (2001) 2283.

[4] J.B. Huang, Y. Zhu, B.Y. Zhu, R.K. Li, H.I. Fu, J. Colloid Interf. Sci. 15 (2001) 201.
[5] M. Bergsma, M.L. Fielden, J.B.F.N. Engberts, J. Colloid Interf. Sci. 243 (2001) 491.
[6] M.L. Fielden, C. Perrin, A. Kremer, et al., Eur. J. Biochem. 268 (2001) 1269.
[7] A. Bernheim-Groswasser, R. Zana, Y. Talmon, J. Phys. Chem. B 104 (2000) 12192.
[8] R. Zana, Adv. Colloid Interf. 97 (2002) 205.
[9] F. Hentrich, C. Tschierske, S. Diele, C. Sauer, J. Mater. Chem. 4 (1994) 1547.
[10] C.A. Bunton, L. Robinson, J. Schaak, M.F. Stern, J. Org. Chem. 36 (1971) 2346.
[11] S. Bhattacharya, S.K. De Geroge, Chem. Commun. (1997) 2287.
[12] Y.P. Zhu, A. Masuyama, M. Okahara, J. Am. Oil Chem. Soc. 67 (1990) 459.
[13] J.M. Pestman, K.R. Terpstra, M.C.A. Stuart, H.A. van Doren, A. Brisson, R.M. Kellog, J.B.F.N. Engberts, Langmuir 13 (1997) 6857.
[14] F.M. Menger, C.A. Littau, J. Am. Chem. Soc. 115 (1993) 10083.
[15] R. Zana, Y. Talmon, Nature 362 (1993) 228.
[16] S.K. Hait, S.P. Moulik, Curr. Sci. India 82 (2002) 1101.
[17] M. Gradzielski, H. Hoffmann, J.C. Panitz, A. Wokaun, Colloid Interf. Sci. 169 (1995) 103.
[18] A.S. Luk, E.W. Kaler, S.P. Lee, Biochemistry-US 36 (1997) 5633.
[19] M. Mao, J.B. Huang, B.Y. Zhu, H.Q. Yin, H.L. Fu, Langmuir 18 (2002) 3380.
[20] M. Mao, J.B. Huang, B.Y. Zhu, J.P. Ye, J. Phys. Chem. B 106 (2002) 219.
[21] C. Tanford, J. Phys. Chem. 176 (1972) 21.
[22] R. Nagarajan, Langmuir 18 (2002) 31.
[23] D. Collins, D.C. Litzinger, L. Huang, Biochim. Biophys. Acta 234 (1990) 1025.
[24] E. Ryan, M. Steer, L. Dolan, Protoplasma 215 (2001) 140.
[25] J.N. Israelachvili, Intermolecular and Surface Forces, Academic Press, New York, 1992.
[26] C.Z. Wang, J.B. Huang, S.H. Tang, B.Y. Zhu, Langmuir 17 (2001) 6389.
[27] J.H. Fendler, Membrane Mimetic Chemistry, Wiley, 1982.
[28] S. Munoz, G.W. Gokel, J. Am. Chem. Soc. 115 (1993) 4899.