

## Letters

### Special Surface and Aggregation Behavior of a Novel Amino Acid Amphiphile

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An amino acid amphiphile, *N*-(4-decyloxy-2-hydroxybenzylidene)glycine, was synthesized. Surface tension measurement, dynamic light scattering, and transmission electron microscopy were used to investigate its surface activity and aggregation in solution. All behavior was sensitive to the pH value of the solution. It was found that these properties occur at pH = 12, which is different from the usual situation for carboxylate amphiphiles. The abnormal behavior can be attributed to the formation of a dimeric structure at pH = 12.0. Vesicles were found in this kind of system, which are stable even at the concentration of transition metal ions up to 1 mM. This phenomenon was attributed to the formation of complexes.

#### Introduction

Self-organized molecular assemblies, such as vesicles, have been attracting great attention for application of biomembrane model systems or carriers in drug delivery systems (DDSs). Many works have been performed on the pharmacological and pharmaceutical applications of these assemblies.<sup>1</sup> A substantial subject in this area is the releasing control of the entrapped materials under the various desired conditions. Many attempts have been made to design vesicles having the characteristics of perceiving the variation of environmental conditions, such as pH,<sup>2</sup> temperature,<sup>3</sup> and UV light.<sup>4</sup> The pH-sensitive vesicles are especially noteworthy as DDSs since that the pH value around damaged tissue is always different from that around the normal one.<sup>2</sup> However most pH-sensitive vesicular systems are not stable with the existence of transition metal ions in solution.<sup>5</sup> The vesicular aggregates

will be destroyed if even a trace of the metal ions is added. It was thought that the trouble may be solved by introducing coordinating points in the structures of amphiphiles to make them form so-called organometallic amphiphiles. This kind of amphiphile will form complexes with metal ions, and the metal ions will in turn be a part of aggregates. In fact, organized molecular assemblies formed from organometallic amphiphiles have been one of the most attractive subjects, for its wide application in both academic and practical fields,<sup>6–9</sup> such as constructing a supramolecular catalytic system,<sup>10,11</sup> producing size-quantized semiconductors,<sup>12</sup> and other functions.<sup>13,14</sup>

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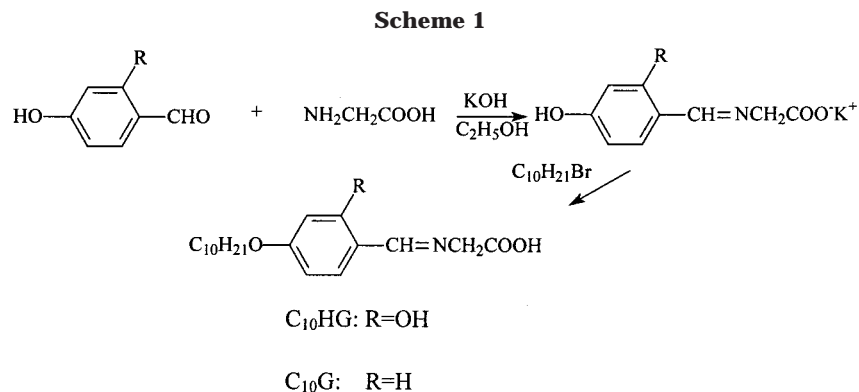
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In this study, an organometallic amphiphile with an amino acid moiety *N*-(4-decyloxy-2-hydroxybenzylidene)-glycine (abbreviated as  $\text{C}_{10}\text{HG}$ ) was synthesized, and the properties of surface adsorption, micellization, and vesicle formation were studied.

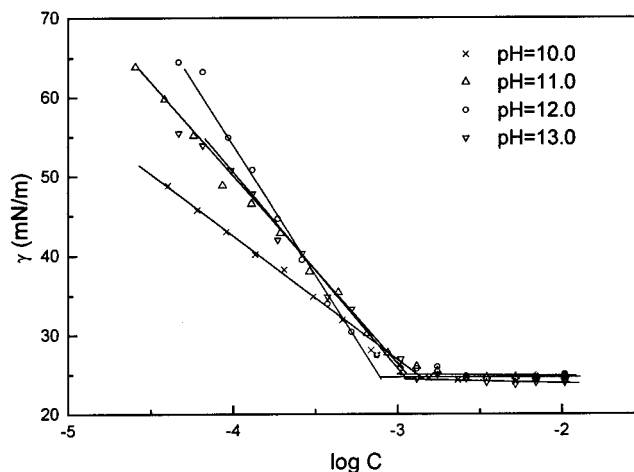
### Experimental Section

**Method.**  $^1\text{H}$  NMR spectra were recorded on a Varian 200M spectrometer, with tetramethylsilane as internal reference. IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer with KBr pellets. UV-vis spectra were recorded on a Shimadzu UV-250 spectrophotometer. Surface tension measurements were carried out using the drop volume method<sup>15</sup> at  $30.0 \pm 0.1$  °C. Light scattering measurements were made using a spectrometer of standard design (ALV-5000/E/WIN Multiple Tau Digital Correlator) and a Spectra-Physics 2017 200mW Ar laser (514.5 nm wavelength). The scattering angle was set at 90°, and the intensity autocorrelation functions were analyzed using the methods of Cumulant and Contin. Transmission electron microscopy (TEM) was carried out on a JEM-100CX II electron microscope, using a negative-staining method (uranyl acetate). Potentiometric pH titration was performed on a PHS-2 acidity meter.

**Materials.** The purities of the surfactants were examined based on a surface curve, no minimum point was found on the curves of the surface tension versus the logarithm of the surfactant concentration. The pH value of aqueous solution was controlled by Borax and potassium hydroxide. The ion strength was kept at  $0.10 \text{ mol}\cdot\text{kg}^{-1}$  by KCl. Water was distilled twice from aqueous solution of  $\text{KMnO}_4$  which was prepared at more than 24 h before use. Potassium *N*-2,4-dihydroxybenzylidene-glycine (potassium *N*-4-hydroxybenzylidene-glycine) was prepared by strongly stirring a mixture of 2,4-dihydroxybenzaldehyde (4-hydroxybenzaldehyde) and glycine at room temperature.<sup>16</sup>

***N*-(4-Decyloxy-2-hydroxybenzylidene)glycine.** Potassium *N*-2,4-dihydroxybenzylidene-glycine (233 mg, 1 mmol) was added to a stirred suspension of potassium hydroxide (56 mg, 1 mmol) in 10 mL of anhydrous methanol. The mixture was refluxed for 1 h. Then 1-bromodecane (221 mg, 1 mmol) was added. After the mixture was refluxed for 6 h, the solvent was removed and the residue was dissolved in 10 mL of water and then acidified by diluted HCl. The precipitate was filtered off and thoroughly washed with water. The product was recrystallized from alcohol to give 150 mg of  $\text{C}_{10}\text{HG}$  (40%) as a brown solid. The potassium salt was prepared from an alcohol solution of potassium hydroxide.  $^1\text{H}$  NMR (200 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  0.853 (s, 2H,  $\text{CH}_2$ ), 1.241 (m, 21H,  $\text{C}_{10}\text{H}_{21}$ ), 1.970 (s, 1H, CH), 6.961 (m, 3H, phenyl H). FT-IR(KBr,  $\text{cm}^{-1}$ ): 3423(OH), 2956, 2926, 2855 ( $\text{CH}_3(\text{CH}_2)_9$ ), 1725 (C=N), 1626 (C=O). UV-vis (nm, pH = 12.0): 365, 260.

***N*-(4-Decyloxybenzylidene)glycine.** The synthesis is similar to *N*-(4-decyloxy-2-hydroxybenzylidene)glycine except that potassium *N*-2,4-dihydroxybenzylidene-glycine was replaced by potassium *N*-4-hydroxybenzylidene-glycine. A yellow solid was



**Figure 1.** Surface tension curves of  $\text{C}_{10}\text{HG}$  at different pH values ( $I = 0.10 \text{ mol}\cdot\text{kg}^{-1}$  (KCl),  $T = 30.0$  °C).

**Table 1. Surface Chemical Properties of Compound  $\text{C}_{10}\text{HG}$  at  $30.0$  °C with  $I = 0.10 \text{ mol}\cdot\text{kg}^{-1}$  (KCl)**

pH	$10^6 \Gamma_{\text{max}}$ (mol/m)	cmc ( $10^{-3}\text{M}$ )	$A_{\text{min}}$ ( $\text{nm}^2$ )
10.0	2.9	1.49	0.58
11.0	3.6	1.48	0.46
12.0	6.1	0.65	0.27
13.0	4.2	0.91	0.40

obtained (180 mg, 50%). FT-IR(KBr,  $\text{cm}^{-1}$ ): 2921, 2849 ( $\text{CH}_3(\text{CH}_2)_9$ ), 1746 (C=N), 1688 (C=O). UV-vis (nm, pH = 12.0): 328, 240.

### Results and Discussion

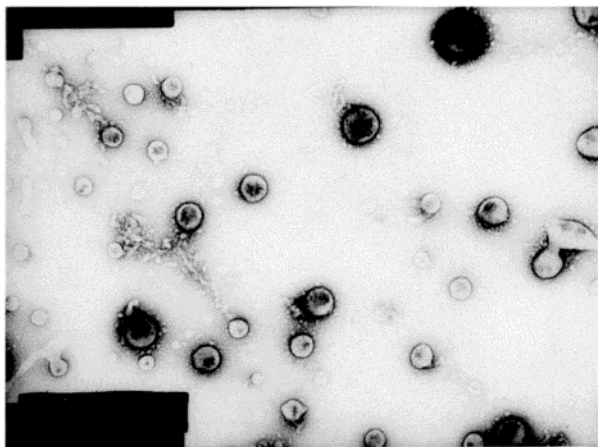
The synthesis of  $\text{C}_{10}\text{HG}$  is shown in Scheme 1.

The surface tension curves of  $\text{C}_{10}\text{HG}$  at various pH values are shown in Figure 1. The values of critical micelle concentration (cmc) can be determined from the inflection points in the  $\gamma$ - $\log c$  curves. The adsorption of amphiphile ( $\Gamma$ ) was calculated according to the Gibbs adsorption equation  $\Gamma = -d\gamma/RTd \ln c$ . Then the area per amphiphile molecule ( $A$ ) was obtained from the saturation adsorption by  $A = 1/(N_A \Gamma)$ , where  $N_A$  is Avogadro's constant.<sup>15</sup> The saturation adsorptions ( $\Gamma_{\text{max}}$ ) and the minimum area per amphiphilic molecule ( $A_{\text{min}}$ ) were calculated by using the slopes of the straight parts of  $\gamma$ - $\log c$  curves for adsorption equation. The values of  $\Gamma_{\text{max}}$ , cmc, and  $A_{\text{min}}$  are listed in Table 1. It was found that they were obviously affected by the pH value of solution.

It is well-known that the surface activity of conventional carboxylate increases with the decrease of pH value in solution, since that the concentration of its hydrolysis product (carboxylic acid), which is of high surface activity,

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**Figure 2.** Transmission electron micrograph (negative staining method) of vesicles formed from  $C_{10}HG$  (0.05 M) at pH 12.0.

would increase with the decrease of pH. Therefore, the electrostatic repulsion in surface adsorption layer and aggregates of surfactant in solution would be weakened, leading to the increase in surface adsorption, decrease in cmc, and more tightening in molecular packing. However, a novel result was found in the aqueous system of  $C_{10}HG$ ; i.e., with the increase of pH in the range of 10–12, the saturated adsorption increases and then decreases at the higher pH. Thus, there exists a maximum in the curve of  $\Gamma_{\max}$  varying with pH. Correspondingly, there is a minimum cmc at the same pH value, showing the supercapability of aggregation at this pH.

It is noteworthy that the molecules pack very tightly in the surface adsorption layer at pH = 12.0 ( $A_{\min} = 0.27 \text{ nm}^2$ ) in the system of  $C_{10}HG$ . According to the geometrical rule,<sup>17</sup> the form of surfactant aggregates will depend on the value of molecular packing parameter  $P = V_c/l_c A_0$ , where  $V_c$  and  $l_c$  are the volume and chain length of the hydrophobic group, respectively, and  $A_0$  is the appropriate area for the hydrophilic group. The increase of  $P$  value with the decrease of  $A_0$  will be beneficial to vesicle formation. Dynamic light scattering results showed two kinds of aggregates existing in the aqueous solution of  $9 \times 10^{-4} \text{ M } C_{10}HG$  at pH 12.0 and 25.0 °C. The two kinds of aggregates are of different size. The larger one has an average of 170 nm and the smaller 20 nm. Combining with the TEM observation, the larger aggregates were assigned to be vesicles, while the smaller aggregates were rodlike micelles. To our knowledge, few carboxylate derivatives can form vesicles at such a low concentration and such a high pH value.<sup>18</sup> The morphology of the aggregates obtained with transition electron microscope is shown in Figure 2. At pH 12.0, a great number of vesicles

**Table 2.** Surface Chemical Properties of Compound  $C_{10}G$  at 30.0 °C with  $I = 0.10 \text{ mol}\cdot\text{kg}^{-1}$  (KCl)

pH	$10^6 \Gamma_{\max}$ (mol/m)	cmc ( $10^{-3}\text{M}$ )	$A_{\min}$ ( $\text{nm}^2$ )
10.0	3.7	2.49	0.45
12.0	1.7	3.97	0.98

were clearly observed in the  $C_{10}HG$  system; while at pH 11.0 and 13.0, there were only a few. The average size of vesicles at pH = 12.0 provided by electron microscopy is about 150 nm in diameter (Figure 2), which is well consistent with the result of DLS.

It is also interesting to note that the vesicles in  $C_{10}HG$  solution remained stable when the concentrations of  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Cd}^{2+}$  were up to 1 mM, while the vesicles in the conventional carboxylate system were destroyed by addition of transitional metal ions. Since  $C_{10}HG$  is also a ligand in the viewpoint of coordination chemistry, it is understandable that the above metal ions will form complexes rather than precipitate with the anionic surfactants. This result introduces a new way for preparing pH-sensitive and metal-ion-resisted organized assemblies.

As a comparison, another amphiphile, *N*-(4-decyloxybenzylidene)glycine (abbreviated as  $C_{10}G$ ), was synthesized.  $C_{10}G$  is similar to  $C_{10}HG$ , but a hydrogen atom takes the place of a hydroxyl group on phenyl ring. The surface tensions of  $C_{10}G$  at different pH values were measured under the same conditions as those for the  $C_{10}HG$  system. The experimental results are listed in Table 2.

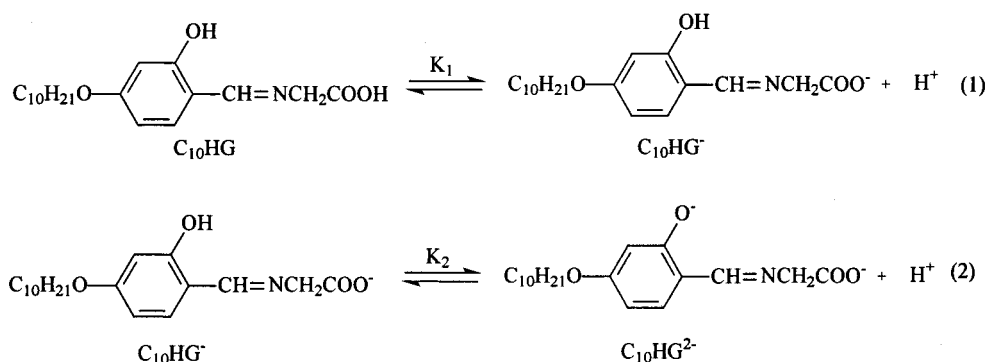
It is seen that the  $\Gamma_{\max}$  of  $C_{10}G$  at pH = 12.0 is much smaller than that at pH = 10.0. Meanwhile the cmc of the former is larger than that of the latter one. So is  $A_{\min}$ . TEM also showed that the  $C_{10}G$  system vesicle could not be observed at pH 12. These facts indicated that the properties of  $C_{10}G$  are different from those of  $C_{10}HG$  but similar to those of the conventional carboxylate amphiphiles. Therefore, the abnormal properties in surface activity and aggregation behavior of the  $C_{10}HG$  system should be attributed to the effect of hydroxyl group in the  $C_{10}HG$  molecule.

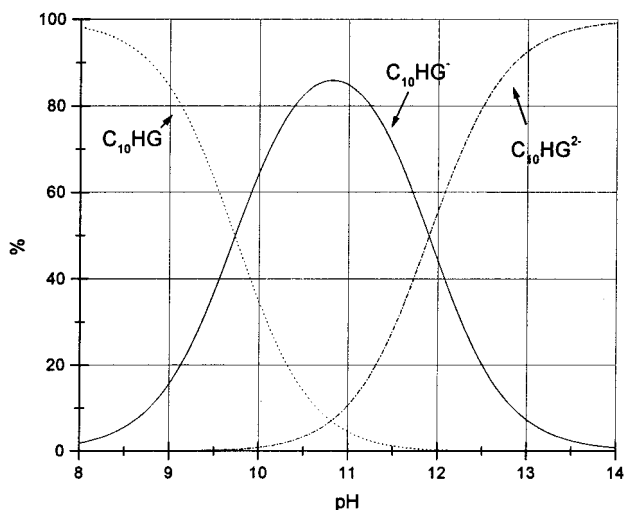
Another question is why the series of abnormal behavior appear at pH 12 in the  $C_{10}HG$  system. Potentiometric pH titration was carried out in an aqueous solution of 0.01 M  $C_{10}HG$  at 30.0 °C. The titration data were analyzed for equilibria 1 and 2 (Scheme 2).

Values of 9.73 and 11.89 were obtained for the deprotonation constants  $\log K_1$  and  $\log K_2$ , respectively. The compositions of the  $[C_{10}HG]_{\text{total}} = 0.01 \text{ M}$  system at various pH values and 30.0 °C are shown in Figure 3. It was found that the molar ratio of  $C_{10}HG^-$  and  $C_{10}HG^{2-}$  is nearly 1:1 at pH = 12.0.

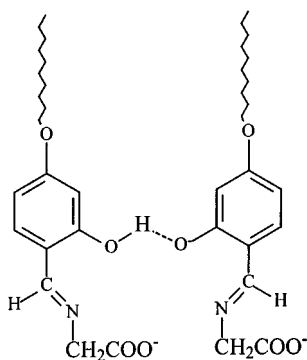
It is seen that at pH 12 half of the hydroxyl groups of  $C_{10}HG^-$  are deprotonated. The negatively charged oxygen

**Scheme 2**





**Figure 3.** Species distribution resulting from an aqueous solution of 0.01 M  $C_{10}HG$  at 30.0 °C.



**Figure 4.** The dimeric structure of  $C_{10}HG$ .

in  $C_{10}HG^{2-}$  would attack the hydrogen atom in the hydroxyl group of a  $C_{10}HG^-$  anion. Thus a dimeric structure would be formed (as shown in Figure 4). A similar phenomenon was reported by Maeda for an alkyl dimeth-

ylamine oxide system.<sup>19,20</sup> The dimer formation results in more tightly packing in the adsorption layer and aggregates in the  $C_{10}HG$  system, consequently inducing the abnormal surface and aggregation behaviors mentioned above.

Furthermore, this dimer has two hydrophobic chains and two hydrophilic carboxylic groups, which is similar to the gemini surfactants, bringing it some properties similar to most of gemini surfactants. For example, it can easily form a vesicle at a very low concentration. On the other hand, vesicles formed in the solution are easily destroyed by increasing or decreasing the pH value of the system, since its spacer is formed by a hydrogen bond instead of a covalent bond. Thus we call it "pseudogemini", for it is similar to gemini surfactant to some extent. Such a "pseudogemini" has a novel character, i.e., its structure, properties, and, hence, functions can be controlled by pH adjustment.

In summary, a novel amphiphile  $C_{10}HG$  was synthesized and a kind of dimeric structure was formed through a hydrogen bond at pH 12, which leads to abnormal surface and aggregation behavior. Vesicles in this system can coexist with transition metal ions, rather than being destroyed by them. In our opinion similar phenomena should exist in many surfactant systems, and the research may open a new vista of surface science. Further work is going on in our laboratory.

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