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## Bilayer Formation by Aggregation of Polymeric Amphiphiles

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### ABSTRACT

Acrylic monomers possessing dialkyl(double-chain) and single-chain tails were prepared. The tail portions are made of characteristic structures common to the hydrophobic portion of bilayer-forming amphiphiles. They are dialkyl( $C_{12}$  to  $C_{18}$ ) chains connected by amide, glyceryl ether or glutamate moieties or single chains with rigid aromatic segments(azobenzene and biphenyl). These monomers were copolymerized by azobisisobutyronitrile with hydrophilic monomers such as acrylamide, acrylglycinamide and other charged monomers. Copolymers which contain as much hydrophobic units as possible were dispersed in water by sonication. They formed the bilayer aggregate spontaneously, as confirmed by electron microscopy, differential scanning calorimetry and small-angle laser light scattering. On average,  $10^2$  to  $10^3$  polymer molecules aggregate to form huge(molecular weight,  $10^6$ - $10^8$ ) bilayer aggregates. The bilayer assemblage of the polymeric amphiphiles appears to be inferior to that of the small-molecule counterparts, although introduction of the spacer between the polymer main chain and the side-chain portion improves the bilayer organization.

### INTRODUCTION

Bilayer membranes are formed by self-assembly of natural phospholipids as well as synthetic amphiphiles. We have shown that synthetic bilayer(and monolayer) membranes are obtainable from a large variety of amphiphiles including single-chain,<sup>(2)</sup> double-chain<sup>(3)</sup>, and triple-chain<sup>(4)</sup> compounds.

Stabilization of these molecular membranes has been investigated in connection with practical applications, beginning in 1980. Fujiki

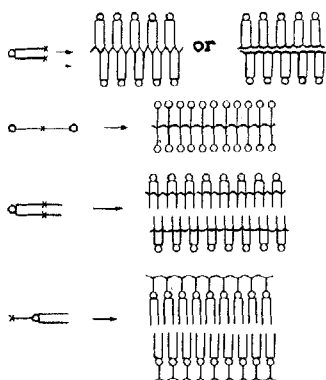


Fig. 1. Preparations of polymerized bilayer membranes.

in our group<sup>(5)</sup> and Regen et al.<sup>(6)</sup> described aggregation and polymerization of double-chain ammonium amphiphiles with a methacrylate moiety at the terminus of one hydrophobic chain. Shortly after, several amphiphilic diacetylenes were reported by three research groups of Chapman et al.<sup>(7)</sup>, Ringsdorf et al.<sup>(8)</sup>, and O'Brien et al.<sup>(9,10)</sup> More recently, butadiene amphiphiles<sup>(11,12)</sup> and additional methacryloyl<sup>(13,14)</sup> and olefinic<sup>(15)</sup> amphiphiles have been introduced. Polymerization is possible at various positions of the membrane, as summarized by Ringsdorf<sup>(13)</sup> in Figure 1.

Combination of polymeric and membranous properties is attainable by a method other than polymerization of pre-formed membranes. That is preparation of polymers capable of membrane formation. In this article, this approach in our group is described. A preliminary report has been published.<sup>(16)</sup>

## MATERIALS

### Monomers.

Monomer 1 was prepared from didodecylamine<sup>(18)</sup> and acryloyl chloride: 1( $n = 12$ ) mp  $\sim 0^\circ\text{C}$ ; 1( $n = 18$ )<sup>(19)</sup> mp  $30 - 40^\circ\text{C}$  (room temperature). In the preparation of monomer 2, dialkyl glyceryl ethers<sup>(20)</sup> were allowed to react with acryloyl chloride: 2( $n = 12$ ),<sup>(18)</sup> mp  $\sim 0^\circ\text{C}$ ;

$\underline{2}$  ( $n = 18$ )<sup>(19)</sup> mp 38 - 40°C (This is not sufficiently pure). Cationic monomer  $\underline{5}$  was obtained by reaction of acryloyl chloride and N,N-dimethylethanolamine and the subsequent quaternization with  $\text{CH}_3\text{Br}$ :  $\underline{5}$ , mp 148 - 149°C from ether.<sup>(18)</sup>

In the preparation of monomer  $\underline{6}$ , N,N-dialkylsuccinamides (mp 35°C for  $n = 12$ , and mp 63 - 64°C for  $n = 18$ ) were condensed with distilled tetra(ethylene glycol) (10 times excess) in the presence of dicyclohexylcarbodiimide (DCC) (and dimethylaminopyridine in the latter case) in dry tetrahydrofuran (THF) by stirring at room temperature for one day. After the usual work-up, the products were isolated by column chromatography. The pale yellow oils were allowed to react with excesses of acryloyl chloride in dry ether in the presence of triethylamine by stirring at room temperature for 2 h. Extraction, washing and silica-gel column chromatography (ether) gave colorless oils:  $\underline{6}$  ( $n = 12$ ) Anal. Found: C, 68.20%; H, 10.74%; N, 2.01%. Calcd for  $\text{C}_{39}\text{H}_{73}\text{O}_8\text{N}$ : C, 68.52%; H, 10.67%; N, 2.05%.  $\underline{6}$  ( $n = 18$ ) Anal. Found: C, 71.35%; H, 11.38%; N, 1.63%. Calcd for  $\text{C}_{51}\text{H}_{97}\text{O}_8\text{N}$ : C, 71.87%; H, 11.47%; N, 1.64%.

Monomer  $\underline{7}$  was prepared as follows. After 1,3-dioctadecyl-2-(2-hydroxyethyl)glyceryl triether and 1.5 times NaH were stirred in dry THF for 30 min at 70°C, a large excess of succinic anhydride was added and the mixture stirred for 10 h. After decomposition of NaH with methanol, the product was extracted with  $\text{CHCl}_3$ , washed and recrystallized from ethanol/methanol. The pale brown solid obtained (mp ca. 30°C) was condensed in dry THF with 10 times excess of distilled tetra(ethylene glycol) in the presence of DCC and dimethylaminopyridine. After stirring for 1 h in an ice bath and for four days at room temperature, the product was separated as usual and purified by column chromatography with ether as eluent. The colorless waxy product obtained in 25% yield was allowed to react with acryloyl chloride by procedures similar to those described above: colorless wax, mp < 30°C. Anal. Found: C, 68.60%; H, 10.90%. Calcd for  $\text{C}_{56}\text{H}_{106}\text{O}_{12} \cdot 1/2\text{H}_2\text{O}$ : C, 68.60%; H, 11.00%.

Acrylglycinamide  $\underline{8}$  was obtained from acryloyl chloride and glycinamide: mp 126 - 127°C, lit<sup>(21)</sup> mp 128°C.

p-( $\omega$ -Carboxydecyloxy)-p'-dodecyloxyazobenzene, mp 136 - 138°C, was prepared as described elsewhere.<sup>(22)</sup> This compound was converted to the acid chloride by refluxing  $\text{SOCl}_2$ , which was subsequently allowed

to react with excess tetra(ethylene glycol) in the presence of triethylamine and  $\text{Na}_2\text{CO}_3$  overnight: yellow powder, mp 96 - 98°C after recrystallization from acetone. The product was then allowed to react with acryloyl chloride in dry THF (see above) and the product (9) recrystallized from acetone: yellow wax, mp 82 → 85°C (the arrow indicates the liquid-crystalline range). Anal. Found: C, 69.60%; H, 9.07%; N, 3.59%. Calcd for  $\text{C}_{46}\text{H}_{72}\text{O}_9\text{N}_2$ : C, 69.32%; H, 9.11%; N, 3.51%.

p-Dodecyloxy-p'-hydroxybiphenyl<sup>(23)</sup> was condensed with bromoundecanoic acid in ethanolic KOH by refluxing for one day. The precipitate was removed and colorless crystals obtained upon cooling was recrystallized from ethyl acetate: mp 102 → 112°C. The product was converted by  $\text{SOCl}_2$  to the corresponding acid chloride, which was then allowed to react with tetra(ethylene glycol) at 60°C overnight. Colorless powders (mp 104 - 106°C) obtained by extraction by  $\text{CHCl}_3$  and recrystallization from acetone were converted to monomer 10 by acryloyl chloride: colorless powder from ethyl acetate, mp 84 - 88°C. Anal. Found: C, 70.83%; H, 9.45%. Calcd for  $\text{C}_{46}\text{H}_{72}\text{O}_9 \cdot 1/2\text{H}_2\text{O}$ : C, 71.01%; H, 9.45%.

Monomers 11 were prepared from dialkyl glutamates<sup>(24)</sup> and acryloyl chloride in the presence of triethylamine: 11 (n = 12), colorless needles, mp 58 - 59°C. Anal. Found: C, 71.31%; H, 11.02%; N, 2.51%. Calcd for  $\text{C}_{32}\text{H}_{59}\text{NO}_5$ : C, 71.46%; H, 11.06%; N, 2.60%. 11 (n = 16), colorless powder, mp 68 - 69°C. Anal. Found: C, 73.86%; H, 11.67%; N, 2.16%. Calcd for  $\text{C}_{40}\text{H}_{95}\text{NO}_5$ : C, 73.91%; H, 11.63%; N, 2.15%.

Monomers 12 were synthesized by introducing the hydrophilic spacer to monomers 11 by the procedure similar to that used for 6. Thus, dialkyl glutamates were first acylated with succinic anhydride in dry THF in the presence of triethylamine, and the acid products were allowed to react with ethylene glycol, di(ethylene glycol), or tetra(ethylene glycol) in the presence of DCC and dimethylamino-pyridine. After the usual work-up, the ester products were purified by silica-gel chromatography or by recrystallization, and allowed to react with acryloyl chloride. Monomer 12 (n = 12, m = 1), colorless powder, mp 35 - 36.5°C, Anal. Found: C, 67.02%; H, 10.01%; N, 2.01%. Calcd for  $\text{C}_{38}\text{H}_{67}\text{NO}_9$ : C, 66.93%; H, 9.90%; N, 2.05%. Monomer 12 (n = 12, m = 2), colorless wax, Anal. Found: C, 65.81%; H, 9.98%; N, 1.87%.

Calcd for  $C_{40}H_{71}NO_{10}$ : C, 66.18%; H, 9.86%; N, 1.93%. Monomer  $\underline{12}$  ( $n = 12, m = 4$ ), colorless oil, Anal. Found: C, 64.97%; H, 9.88%; N, 1.90%.  
 Calcd for  $C_{44}H_{79}NO_{12}$ : C, 64.92%; H, 9.78%; N, 1.72%. Monomer  $\underline{12}$  ( $n = 16, m = 1$ ), colorless powder, mp 48 - 49°C. Anal. Found: C, 69.66%; H, 10.71%; N, 1.82%.  
 Calcd for  $C_{46}H_{83}NO_9$ : C, 69.57%; H, 10.53%; N, 1.76%. Monomer  $\underline{12}$  ( $n = 16, m = 4$ ), colorless powder, mp 35 - 36.5°C, Anal. Found: C, 67.69%; H, 10.47%; N, 1.69%.  
 Calcd for  $C_{52}H_{95}NO_{12}$ : C, 67.42%; H, 10.34%; N, 1.51%.

In the preparation of  $\underline{13}$ , didodecyl glutamate hydrochloride in ether was stirred for 2 h at room temperature together with three equivalents of terephthaloyl chloride and two equivalents of triethylamine. The acid chloride product (colorless powder, mp 61 - 62°C) obtained upon separation of triethylamine hydrochloride and solvent removal was allowed to react with ethylene glycol or tetra(ethylene glycol) in dry THF in the presence of triethylamine at room temperature. After washing, extraction of the organic products into ether and solvent removal, the hydrolyzed starting material was separated in methanol and the condensation product was recrystallized from hexane: mp 70 - 73°C for  $m = 1$  and mp 34 - 35°C for  $m = 4$ . These products were allowed to react with acryloyl chloride as usual, and recrystallized from methanol or hexane. Monomer  $\underline{13}$  ( $m = 1$ ), colorless powder, mp 46 - 50°C, Anal. Found: C, 69.06%; H, 9.37%; N, 1.98%.  
 Calcd for  $C_{42}H_{67}NO_9$ : C, 69.10%; H, 9.25%; N, 1.92%.  
 Monomer  $\underline{13}$  ( $m = 4$ ), pale yellow powder, Anal. Found: C, 67.45%; H, 9.25%; N, 1.76%.  
 Calcd for  $C_{48}H_{79}NO_{12}$ : C, 66.87%; H, 9.24%; N, 1.62%.

### Polymerization.

Copolymerizations of hydrophobic and hydrophilic monomers were conducted in mixtures of ethanol and benzene with AIBN initiator in degassed ampoules. Small amounts of water were added when monomers are insoluble in organic solvents. Copolymers were recovered by precipitation, purified by reprecipitation and dried. In some cases, solvent was removed from the polymerization mixture and the solid residues were reprecipitated. The copolymer composition was determined by elemental analysis.

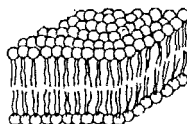
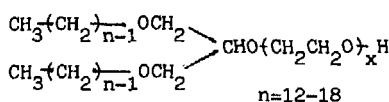
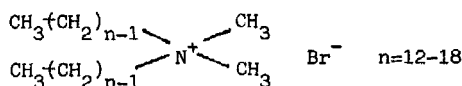
### Measurements.

Aqueous solutions of copolymers were obtained by sonication with a Branson Sonifier 185(microtip). Electron micrographs were obtained with a Hitachi H-500 instrument.<sup>(25)</sup> Differential scanning calorimetry(DSC) was conducted for 20-mm samples with a Daini-Seikosha SSC/560 instrument.<sup>(26)</sup> The molecular weight was determined by a small-angle light scattering apparatus with a He-Ne laser source (Toyo Soda, LS-8).<sup>(2)</sup>

## RESULTS AND DISCUSSION

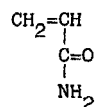
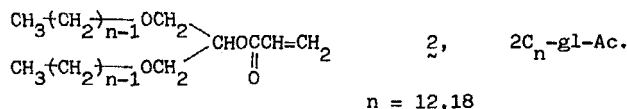
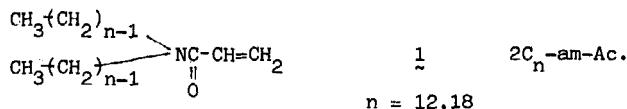
### Copolymers with Directly-Bonded Dialkyl Chains. (18,19)

Simple dialkylammonium salts<sup>(3)</sup> and 1,3-dialkylglyceryl amphiphiles<sup>(20)</sup> form stable bilayer membranes. Therefore, copolymers of dialkyl monomers(1 and 2) and hydrophilic monomers(3, 4 and 5) may provide similarly stable bilayer aggregates.

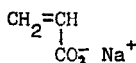


Typical dialkyl amphiphiles and their bilayer membrane.

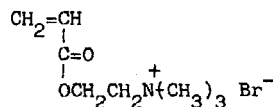
Bilayer membrane of double-chain amphiphiles



3 AcAm



4



5

In the case of aqueous dispersions of  $2C_{12}amAc-AcAm$  copolymer (dialkyl monomer unit, 5 - 7 mol%) large aggregates were not detected by electron microscopy. By elongation of the alkyl chain, definite aggregates became observable. Formation of spherical aggregates (diameter, 100 - 200 Å) was found for  $2C_{18}amAc-AcAm$  copolymers (dialkyl monomer unit, 2 - 5 mol%), and vesicles with diffuse layer structures (width, ca. 100 Å) were observed in an electron micrograph of  $2C_{18}amAc-AcAm$  (8.2 mol%).

Multi-walled vesicles were observed for the transparent supernatant of a milky dispersion of  $2C_{12}-gl-Ac-AAm$  (dialkyl monomer unit, 10.1%). Spherical particles (ca. 100 Å) and fibers (diameter, ca. 100 Å) were found for  $2C_{18}-gl-Ac-AcAm$  (dialkyl monomer unit, 0.8 mol%), and typical multi-walled vesicles (diameter, 1000 - 3000 Å; layer width, ca. 50 Å) were seen by increasing the dialkyl monomer unit to 1.9 mol%. A further increase in the dialkyl unit made the copolymer water-insoluble.

In any of these comonomer combinations, the phase transition behavior which is characteristic of the bilayer assemblage<sup>(26)</sup> was not observed in the DSC experiment. Therefore, it is concluded that these copolymers are not suitable for formation of definite bilayer structures.

Copolymers obtained from dialkyl monomer 1 and charged hydrophilic monomers 4 and 5 similarly could not be shown to produce bilayer aggregates.

#### Copolymers with Spacer-Mediated Dialkyl Side Chains.

The results described in the preceding section point to the following conclusion:

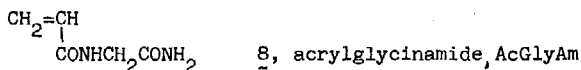
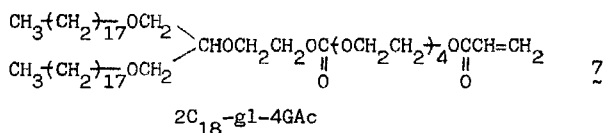
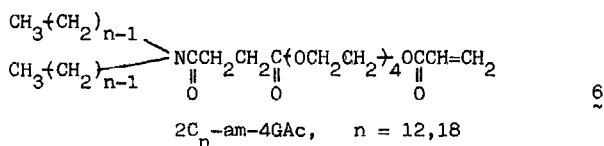
(1) It is necessary to increase the content of the dialkyl side chain in order to induce its ready assemblage. However, the polymer must remain dispersed in water. This factor sets an upper limit in the dialkyl content, although appropriate choices of hydrophilic comonomers may alleviate the solubility problem.

(2) The polymer main chain must not interfere with the side chain packing.

In order to avoid these two difficulties, we decided to place hydrophilic spacers between the polymerizable group and the



bilayer-forming side chain. Two types of the hydrophobic monomer (6 and 7) were prepared for this purpose, and a strongly hydrophilic acrylglycinamide 8 was additionally used as comonomer.



The aggregation behavior of aqueous copolymers of dialkyl monomers 6 and 7 with acrylamide(AcAm) or acrylglycinamide(AcGlyAm) is summarized in Table I. Hydrophilicity of copolymers is enhanced by the use of acrylglycinamide as comonomer and the tetra(ethylene glycol) spacer. For example,  $2\text{C}_{12}\text{-am-4GAc-AcGlyAm}(x = 12.4)$  gives a slightly turbid dispersion, while  $2\text{C}_{12}\text{-am-4GAc-AcAm}(x = 6.9)$  cannot be dispersed. Acrylglycinamide is quite effective.  $2\text{C}_{18}\text{-am-4GAc-AcGlyAm}(x = 16.0)$  gives an almost transparent dispersion, while  $2\text{C}_{18}\text{-gl-Ac-AcGlyAm}(x = 12.1)$  cannot be dispersed. This exemplifies the usefulness of the spacer.

Molecular weights of these aqueous dispersions are huge. They ranges from 5 to 100 millions, and are typical of the bilayer aggregate. The copolymers are supposedly molecularly dispersed in  $\text{CHCl}_3$ , and, therefore, the value of molecular weight( $10^4$ - $10^6$ ) should correspond to those of single polymer molecules. Comparisons of molecular weight determined in  $\text{H}_2\text{O}$  and  $\text{CHCl}_3$  show that 500 to 1000 polymer molecules assemble in water to produce huge aggregates.

The phase transition peak(DSC) is found for these aqueous dispersions when the alkyl chain length is  $\text{C}_{18}$ . The phase transition temperature( $T_c$ ) is  $40^\circ\text{C}$  for the dialkylamide type side chain(6) and 48 to  $50^\circ\text{C}$  for the 1,3-dialkylglycerol type side chain. These values are close to those obtained for the bilayer aggregate of simple double-chain( $\text{C}_{18}$ ) ammonium salts.<sup>(26)</sup>

TABLE I Aggregate Characteristics of Dialkyl-Substituted Copolymers

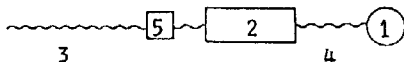
Copolymer	0.01 unit M		mol. wt.		T(°C)	DSC peak AH( $\frac{\text{kcal}}{\text{unit mol}}$ )	electron microscopy
	aq. dispersion	in H <sub>2</sub> O	in CHCl <sub>3</sub>				
<b>2C<sub>n</sub>-am-4GAC-AcGlyAm</b>							
n = 12	x = 5.8	clear	5.0 x 10 <sup>6</sup>	—	}	no peak	no structure
	6.1	clear slightly turbid	—	8.1 x 10 <sup>5</sup>			
	12.4						
n = 18	x = 7.7	clear	7.2 x 10 <sup>7</sup>	5.4 x 10 <sup>4</sup>	40.4	9.4	lamella & vesicic
	16.0	slightly turbid	9.4 x 10 <sup>7</sup>	2.1 x 10 <sup>5</sup>	40.4	6.2	—
<b>2C<sub>18</sub>-gl-4GAC-AcGlyAm</b>							
	x = 1.9	clear	3.3 x 10 <sup>7</sup>	3.2 x 10 <sup>4</sup>	47.7	8.1	prelamella
	5.6	clear	7.2 x 10 <sup>6</sup>	4.7 x 10 <sup>4</sup>	51.3	10.1	no structure
<b>2C<sub>12</sub>-am-4GAC-AcAm</b>							
	x = 6.9	turbid					
<b>2C<sub>n</sub>-gl-Ac-AcGlyAm</b>							
n = 12	x = 8.8	clear	9.7 x 10 <sup>6</sup>	—	}	no peak	prelamella
	16.7	turbid	—	—			
n = 18	x = 4.3	clear	1.1 x 10 <sup>7</sup>	—	49.5	4.2	prevesicle
	6.4	clear	—	—	48.0	1.8	—
	12.1	turbid	—	—	—	—	—

x: dialkyl monomer unit in %  
 unit M: molar concentration of the dialkyl monomer unit.

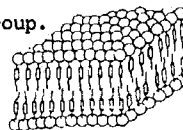
Electron microscopy indicates formation of the bilayer structure, as noted in Table I. However, definite bilayer structures (vesicles and lamellae) such as observed for small-molecule aggregates cannot be seen. Apparently, oriented side-chain aggregation is not sufficiently developed in these polymers.

#### Copolymers with Spacer-Mediated Single-Chain Side Groups.

We have established that stable bilayer assemblies are formed spontaneously in water from single-chain amphiphiles with rigid segments.<sup>(2)</sup> These amphiphiles are typically composed of five structural units: 1, flexible tail, 2, rigid segment, 3, hydrophilic head, 4, spacer, and 5, additional interacting group.

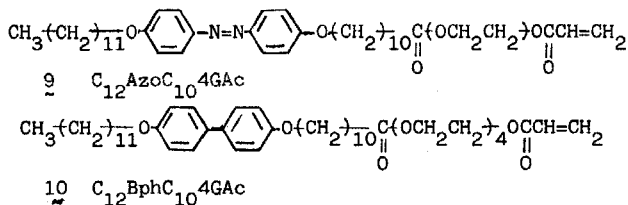


General structure of bilayer-forming single-chain amphiphile.



Bilayer membrane of single-chain amphiphile

Typical single-chain amphiphiles contain the biphenyl and azobenzene units as rigid segment. Monomer 9 and 10 were newly synthesized on the basis of this generalization.

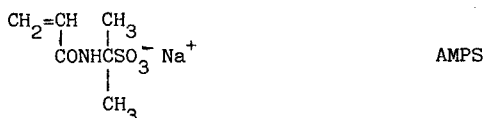


Copolymerization of 9 and 10 with acrylamide did not give water-soluble polymers when the mole fraction of 9 and 10 in copolymer was 10 - 20 %. Solubility was improved considerably when acrylglycinamide was used as comonomer. As shown in Table II, clear dispersions are obtainable when the content of the single-chain monomer is 15 - 20 %. Sharp phase transition peaks were noted with these aqueous solutions; however, the aggregate morphology was not sufficiently developed. Thus, these copolymers appear to give only limited bilayer assemblage.



TABLE III Aggregate Characteristics of Copolymers with Dialkyl Glutamate Side Chains

Copolymer	0.01 unit M aq. dispersion	mol. wt.		DSC peak T(°C), $\Delta H$ ( $\frac{\text{kcal}}{\text{unit mol}}$ )	electron micrograph
		in H <sub>2</sub> O	in CHCl <sub>3</sub>		
2C <sub>12</sub> GluAc-AcAm	x = 7 clear	—	—	not detected	diffuse lamella
2C <sub>12</sub> GluIGAc-AcAm	x = 9 colloidal	—	1.9 x 10 <sup>4</sup>	29	vesicle & lamella
2C <sub>12</sub> Glu2GAc-AcAm	x = 8 colloidal	—	—	11	—
2C <sub>12</sub> Glu4GAc-AcAm	x = 6 colloidal	1 x 10 <sup>6</sup>	—	—	fragmentary bilayer
	11	—	3.9 x 10 <sup>4</sup>	13	vesicle & lamella
2C <sub>12</sub> Glu-ph-4GAc-AcAm	x = 7 clear	—	—	32	fragmentary bilayer
2C <sub>16</sub> GluAc-AcAm	x = 14 colloidal	3 x 10 <sup>6</sup>	—	43	—
2C <sub>16</sub> Glu4GAc-AcAm	x = 7 colloidal	2 x 10 <sup>6</sup>	—	43	globule
2C <sub>16</sub> Glu4GAc-AcGlyAm	x = 78 colloidal	9 x 10 <sup>6</sup>	1.9 x 10 <sup>4</sup>	40, 47	leaflet
2C <sub>16</sub> Glu4GAc-AMPS	x = 24 clear	—	3.8 x 10 <sup>4</sup>	46	lamella
2C <sub>16</sub> Glu4GAc-TMEAc	x = 75 colloidal	—	—	46	lamella



The aggregation behavior of some of these copolymers is summarized in Table III. In general, dialkyl glutamate-derived polymers show improved solubilities in water. For example,  $2\text{C}_{12}\text{-am-4GAc-AcAm}$  ( $x = 6.9$ ) (see Table I) gave a turbid solution, whereas an aqueous dispersion of  $2\text{C}_{12}\text{Glu4GAc-AcAm}$  ( $x = 11$ ) was only slightly turbid (or colloidal). When more hydrophilic acrylglycinamide, AMPS of TMEAc(5) were used as comonomer, the dialkyl content could be greatly increased and copolymers containing 70 - 80 % dialkyl monomer units could be dispersed in water.

Molecular weights measured in  $\text{CHCl}_3$  are  $2 \sim 4 \times 10^4$ , and are enhanced to  $1 \sim 10$  millions in water. Copolymers were dispersed by sonication for 1 h and aged before measurement at room temperature overnight. This indicates as in the preceding section that the aqueous aggregates are composed of 100 - 500 polymer molecules.

The phase transition was observed invariably except for  $2\text{C}_{12}\text{GluAc-AcAm}$ . The identical results were obtained regardless of the starting temperature ( $0^\circ\text{C}$  or  $-50^\circ\text{C}$ ). A typical DSC curve is shown in Figure 2. In the case of the didodecyl glutamate series with different spacers ( $2\text{C}_{12}\text{Glu-mG-Ac-AcAm}$ ), the highest  $T_c$  was found at  $29^\circ\text{C}$  for the copolymer with the mono(ethylene glycol) spacer. The introduction of the benzene ring in the spacer portion enhanced  $T_c$ . In the dihexadecyl series, the phase transition remained in a narrow

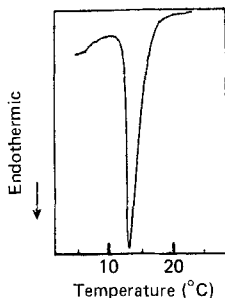


Fig. 2 DSC thermogram of  $2\text{C}_{12}\text{-Glu4GAc-AcAm}$  ( $x = 11$ ) copolymer.

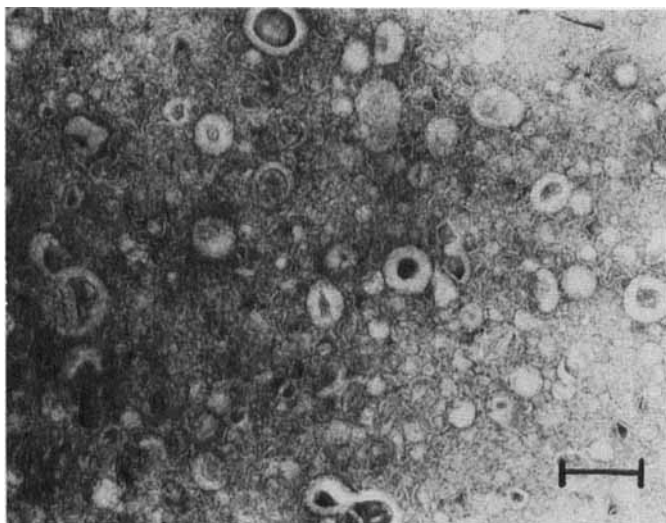


Fig. 3. Electron micrograph of  $2C_{16}Glu4GAc-AcAm(x = 11.6)$  copolymer stained by uranyl acetate, Scalebar, 5000 Å.

temperature region(43 - 46 °C), regardless of the spacer length and comonomer. The influence of the polymer main chain on the side chain assemblage appears to be small in these cases.

Electron microscopy showed that the bilayer structure is more or less produced in all cases examined. However, the morphologies are not as clear as those of small-molecule counterparts. Figure 3 is a representative electron micrograph of the aqueous copolymer. Multiwalled vesicles are seen abundantly. The layer width is larger than two times the side chain length. The polymer main chain may be included in the layer.

#### CONCLUSION

It is established that stable bilayer assemblies are spontaneously formed in water from vinyl copolymers of hydrophobic and hydrophilic monomers. The bilayer structure is formed by side chain aggregation, as schematically shown in Figure 4. Their tendencies to form bilayers are related to the ease of assemblage of the corresponding small-molecule amphiphile, although the assemblage of the polymers appears

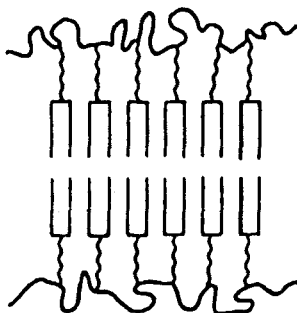


Fig. 4 Schematic illustration of bilayer membranes of polymeric amphiphiles.

to be inferior from electron microscopy. Further improvements should be possible by appropriate monomer design. Since the bilayer of polymeric amphiphiles possesses head group structures which are covalently bound, it may display unique bilayer characteristics. This is currently under investigation in our group.

The present study complements polymerized vesicles currently being investigated by other groups, in terms of combination of the polymeric nature and the bilayer organization.

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