

# Morphology Observation of Novel 3, 4-Bis-Alkyloxycarbonyl-Hexanedioic Acid Monolayer on Water Surface by Dropping Method

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## Abstract

We have investigated the morphology of novel 3, 4-bis-alkyloxycarbonyl-hexanedioic acid monolayer (alkyl chain number  $n = 12, 14,$  and  $16$ ) formed on the water surface by dropping method using Surface Tension measurement (STm) and Brewster Angle Microscopy (BAM). Surfactant of 3, 4-bis-alkyloxycarbonyl-hexanedioic acid has a gemini type structure.  $\pi$ -A isotherm curves (STm) of two gemini surfactants of  $n = 12$  and  $14$  showed gradual increase in surface pressure, while that of  $n = 16$  showed gradual increase, a stagnant range and finally steep increase. BAM observation showed each image corresponding to the result of STm measurement. From STm and BAM results of surfactants, it is found that surfactant of  $n = 12$  and  $14$  form Liquid-Expand (LE) type monolayer, while that of  $n = 16$  formed Liquid-Condensed (LC) type monolayer including LE-LC phase transition.

## Keywords

3, 4-Bis-Alkyloxycarbonyl-Hexanedioic Acid, Gemini Surfactants, Gemsurfs, Monolayer, Morphology

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## 1. Introduction

Gemini surfactant essentially has a dimer structure at which two monosurfactants are connected by a spacer

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group near those hydrophilic head groups [1]-[3]. Compared to the corresponding monosurfactant, gemini surfactant has various characteristics such as low Krafft temperature, low critical micelle concentration, and a low surface tension. Since Zana and Talmon [1] synthesized gemini surfactant and studied their solution properties, these surfactant are being used in various biological and industrial applications such as vehicles for gene delivery into cells [4], understanding cell fusion and fission processes [5], effective gelation of water [6], and soft template for anisotropic gold particles [7]. Studies related to air-water interface such as aggregation behavior of cationic gemini surfactants and interaction with nucleoside bases [8], formation of gemini surfactant-DNA complex monolayer for surface patterning and nanofabrication [9], miscibility between gemini surfactant-stearic acid for elucidation of biological membrane and self-assembly [10] have been also reported.

In year 2004, Chukyo Yushi Co., Japan [11] synthesized a novel gemini surfactant 3, 4-bis-alkyloxycarbonylhexanedioic acid commonly known as “Gemsurf” whose structure is shown in **Figure 1**. Gemsurf is different from previously reported gemini surfactant in following characteristics: two hydrophilic head groups are connected directly by one carbon-carbon single bond. Gemsurf is also similar to the structure of amphiphilic molecule of biomembranes. Gemsurf commercial synthesis has been established and surfactant currently is being used in cosmetics and emulsion preparations, as dispersants, bubble stabilizers, and cleaners. Solution properties of Gemsurf-inorganic and -organic substances [11] and complex of Gemsurf-monosurfactants [12] have been investigated. However, there is no report of surface properties of Gemsurf behavior at air-water interface.

In this context we have investigated for the first time the morphology of the novel Gemsurf monolayer formed on the water surface by the dropping method [13] using Surface Tension measurement (STm) and Brewster Angle Microscopy (BAM).

## 2. Experimental

### 2.1. Materials

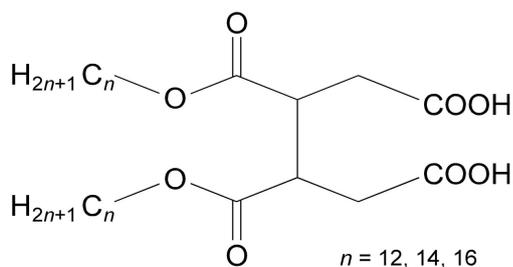
Gemsurf of alkyl chain numbers  $n = 12, 14,$  and  $16$  in **Figure 1** was gifted from Chukyo Yushi Co., Japan and used without further purification. Spreading solvent chloroform (99.0%) was procured from Wako Pure Chemical Industries Ltd., Osaka, Japan. Purified water with conductance  $< 0.07 \mu\text{S}/\text{cm}$  for subphase was obtained using a Super Water Purifying System (WL-21P; Yamato Scientific Corp. Ltd., Tokyo, Japan).

### 2.2. Monolayer Formation

Details of the monolayer formation using the dropping method have been reported in a previous article [13]. 1 mM solution of Gemsurf-chloroform was spread on the purified water surface using a 100  $\mu\text{l}$  microsyringe (Ge-0583-04; Hamilton Co., Nevada, USA). Spreading was conducted at 1  $\mu\text{l}/\text{min}$  intervals. Gemsurf-chloroform droplet was dropped gently and the next drop was added after the previous droplet expanded on the water surface. The completion of monolayer formation was confirmed by the fact that the formation of lenses of droplet was observed on the water surface, and value of surface tension as described in *Sections 2.3* did not change even if droplet continued being dropped.

### 2.3. Surface Tension Measurement (STm)

STm for each Gemsurf monolayer was carried out on a Surface Tensiometer (CBVP-A3; Kyowa Interface Science Corp. Ltd.). As mentioned in *Section 2.2*, Gemsurf-chloroform solution was spread on the purified water surface by dropping method with a platinum Wilhelmy plate. The surface pressure of each monolayer was



**Figure 1.** Molecular structure of Gemsurf ( $n = 12, 14,$  and  $16$ ).

recorded as a function of spreading volume at  $26.0^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$  and measurements were conducted more than three times and experimental errors were  $\pm 0.1$  mN/m.

## 2.4. Brewster Angle Microscopy (BAM)

Brewster angle microscopy (BAM) for visualizing the morphology of each Gemsurf monolayer was carried out on a Brewster angle microscope (EMM633K; Filgen Inc., Nagoya, Japan) and USB-CAP type (SD-USB2CUP3; AREA Co. Ltd, Tokyo, Japan) imaging analysis software. A *p*-polarized light of 632.8 nm wavelength was irradiated at the Brewster angle of  $53.1^{\circ}$  on each monolayer surface. The reflected light was detected by a CCD camera (C5948-70; Hamamatsu Photonics, Hamamatsu, Japan). The lateral resolution of BAM was about  $1\ \mu\text{m}$ . The formation process of each monolayer was observed at the arbitrary surface pressure as described in Section 3.2. The BAM measurement was conducted at  $26^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and measurements were conducted more than three times.

## 3. Results

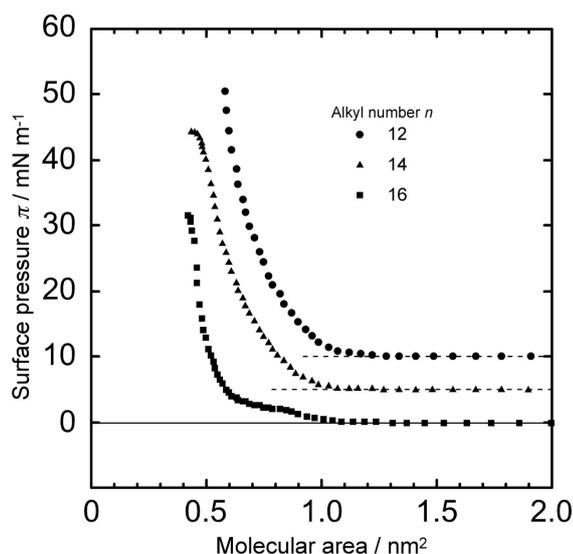
### 3.1. $\pi$ -A Isotherm Curves

Figure 2 shows the surface pressure versus molecular area ( $\pi$ -A) isotherm curves of three Gemsurf surfactants ( $n = 12, 14,$  and  $16$ ) monolayers obtained at  $26^{\circ}\text{C}$  using the dropping method. Horizontal axis represents molecular area calculated from molecular numbers in the dropping volume. Vertical axis represents surface pressure measured when enough time passed after dropping of Gemsurf-chloroform droplet and the pressure value became constant. At Gemsurf of  $n = 12$  and  $14$ , surface pressure values increased 10 and 5 units each on surface pressure scale, respectively.

Gemsurf of  $n = 12$  has presented a  $\pi$ -A isotherm curve similar to a Liquid-Expand (LE) type monolayer [14]. Therefore, the surface pressure increases gradually from about  $1.2\ \text{nm}^2$  molecular area and the pressure increased monotonously up to  $40\ \text{mN/m}$ . The shape of the curve is smooth and different from the steep curve observed in a liquid-condensed (LC) type monolayer. The limiting molecular area ( $A_0$ ) is calculated to be *ca.*  $0.74 \pm 0.02\ \text{nm}^2/\text{molecule}$ .

Gemsurf of  $n = 14$  has also presented an isotherm curve similar to LE type monolayer, the surface pressure increases gradually from about  $1.1\ \text{nm}^2$  molecular area and the pressure increased monotonously up to  $39\ \text{mN/m}$ . The shape of curve is smooth and  $A_0$  is calculated to be *ca.*  $0.69 \pm 0.02\ \text{nm}^2/\text{molecule}$ .

Gemsurf of  $n = 16$ , on the other hand, has presented a different isotherm curve compared to that of  $n = 12$  and  $14$ . Therefore, the surface pressure increased gradually at  $1.1\ \text{nm}^2$  molecular area and after a stagnation of



**Figure 2.** Surface pressure versus molecular area ( $\pi$ -A) isotherm curve of Gemsurfs ( $n = 12, 14,$  and  $16$ ) at  $26^{\circ}\text{C}$ . ●: Gemsurf of  $n = 12$ , ▲:  $n = 14$ , ■:  $n = 16$ .

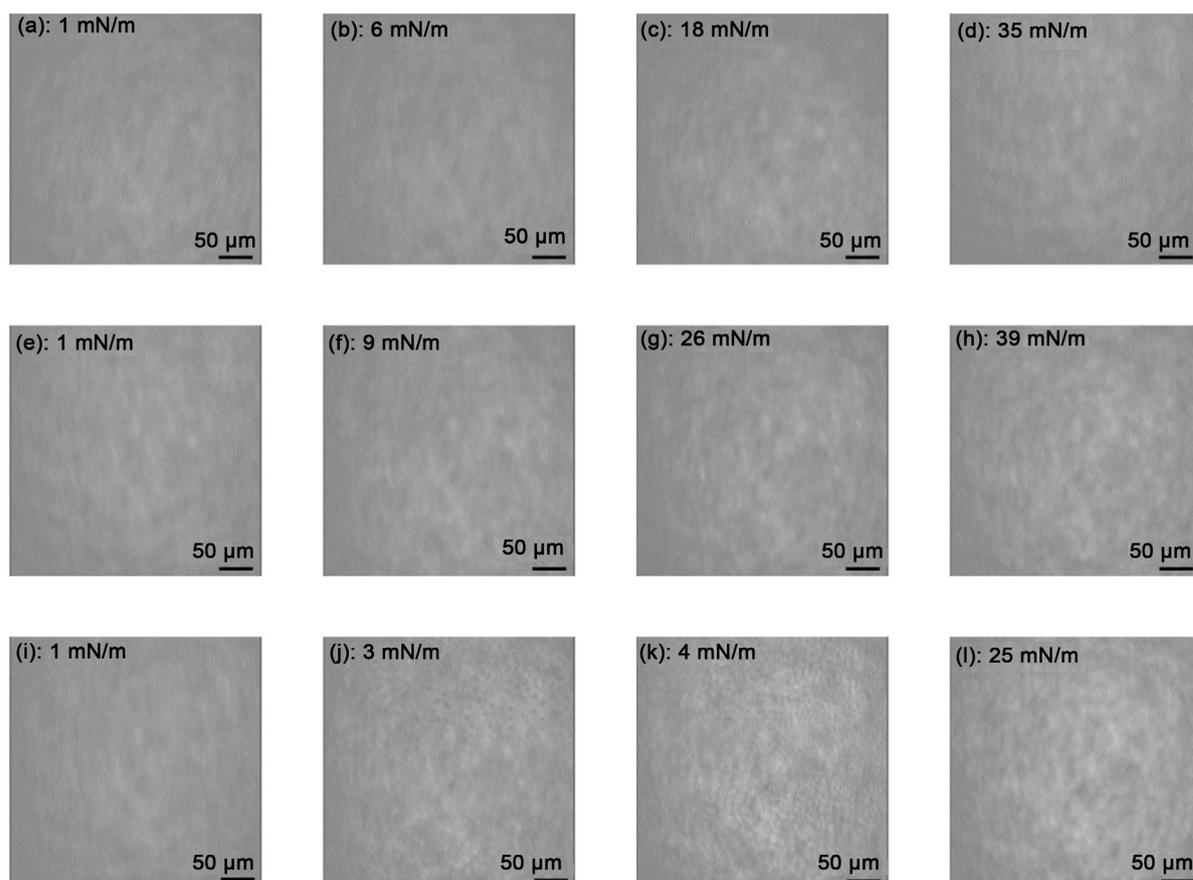
surface pressure at 2 mN/m in molecular area range of 0.70 to 0.85 nm<sup>2</sup>, it increased steeply up to 32 mN/m.  $A_0$  is calculated to be *ca.* 0.53 ± 0.02 nm<sup>2</sup>/molecule. Such curve is similar to that of dipalmitoyl phosphatidyl choline (DPPC) monolayer [13]-[15] at which the surface pressure increases gradually at low surface pressures as a LE state and it also shows a steep increase at high surface pressures as a LC state. The LE-LC phase transition occurs at a surface pressure 10 mN/m.

### 3.2. BAM Observation

**Figure 3** shows a series of BAM images on three Gemsurf ( $n = 12, 14,$  and  $16$ ) monolayers obtained at 26°C using the dropping method. BAM observations were performed on each monolayer at the dropping volume corresponding to characteristic of four surface pressures of each monolayer.

For Gemsurf of  $n = 12$  (**Figures 3(a)-(d)**), we obtained similar images like that observed in the case of LE type monolayer. Therefore, at a low surface pressure of 1 mN/m ((a)), the image of monolayer was a little brighter compared to that of water surface. According to an increase in surface pressure (6 and 18 mN/m, (b) and (c)), those images became brighter and the formation of monolayer continued homogeneously. At 35 mN/m ((d)), the image became further brighter and finally the homogeneous monolayer was formed and completed. Such a gradual increase in brightness of monolayer over the pressure range corresponded to the gradual increase in the surface pressure on  $\pi$ - $A$  isotherm curve (**Figure 2**).

For Gemsurf of  $n = 14$  (**Figures 3(e)-(h)**), images were similar to LE type monolayer as observed in  $n = 12$ . According to an increase in surface pressure (1, 9, and 26 mN/m, (e), (f), and (g)), those images became brighter over the whole and the formation of monolayer continued homogeneously. At 39 mN/m ((h)), image became further brighter and the homogeneous monolayer was completed. Brightness of image of (h) was somewhat lighter than that of 35 mN/m of Gemsurf of  $n = 12$  ((d)). The difference is related to monolayer thickness and its



**Figure 3.** BAM images of Gemsurfs ( $n = 12, 14,$  and  $16$ ) on various surface pressures at 26°C. (a)-(d) Gemsurf of  $n = 12$ , (e)-(h)  $n = 14$ , and (i)-(l)  $n = 16$ .

refractive index of monolayer [16] [17].

For Gemsurf of  $n = 16$  (Figures 3(i)-(l)), monolayer images were different from that of Gemsurf of  $n = 12$  and 14. At a low surface pressure of 1 mN/m ((i)), image was a little bright. After the LE-LC phase transition started at about 2 mN/m, image ((j)) showed bright dark contrast and hole or island type domain appeared in the image. At a surface pressure of about 4 mN/m (image (k)), the contrast became clearer and with assembled domains. At a surface pressure of 25 mN/m (image (l)) the image became further brighter and homogeneous monolayer was formed on the water surface.

## 4. Discussion

From the results of STm and BAM, it is found that Gemsurf of  $n = 12$  and 14 is LE type monolayer, while Gemsurf of  $n = 16$  is LC type monolayer with LE-LC phase transition. In a handbook [14], it has been shown that in LE type monolayer, alkyl chains in monolayer molecules have gauche structure and have random orientation. Whereas, in LC type monolayer, those have trans structure and oriented uniformly on the water surface.

It is known that alkyl chain structure in monolayer molecules maintains the original bulk structure and properties of molecules. Differential Scanning Calorimetry (DSC) has been conducted of Gemsurf of  $n = 12$ , 14, and 16. Gemsurf of  $n = 12$  showed the peak of phase transition at 0.4 and 92.3 °C. In  $n = 14$ , phase transitions were at 24.8°C and 95.0°C, and  $n = 16$ , at 40.8°C and 99.3°C. It seems that the peak appeared at low temperature for each Gemsurf corresponds to the melting of alkyl chain and reorientation of molecules from crystal to liquid crystal phase, and at high temperature it corresponds to the bulk melting point. The phase of the above 3-dimension bulk state does not always reflect the state of 2-dimension monolayer, but DSC results indicate that the Gemsurf of  $n = 12$  and 14 is a monolayer of gauche structure of alkyl chain and  $n = 16$  is a monolayer of trans of alkyl chain at 26°C.

The apparent molecular area of hydrophilic group (dicarboxylic acid part) of Gemsurf ( $A_{h-g}$ ) was estimated to be *ca.* 0.4 nm<sup>2</sup> from roughly calculation by molecular model (molecular model kit (MOL-TALOU Models, basic-A set, Koriyama, Japan)). From the value of above molecular area, two alkyl chains of gauche structure in Gemsurf of  $n = 12$  and 14 are spread larger than area of  $A_{h-g}$  on the water surface, thereby  $A_0$  would become a larger value.  $A_0$  of  $n = 12$  was larger than that of  $n = 14$ . Because the alkyl chain length of  $n = 14$  is longer than that of  $n = 12$ , the stronger interaction between alkyl chains of  $n = 14$  would make  $A_0$  of  $n = 14$  small compared to that of  $n = 12$ . In Gemsurf of  $n = 16$ , two alkyl chains have trans structure that would promote the interactions between alkyl chains. As a result, the spread of alkyl chain on the water surface is restrained compared to that of  $n = 12$  and 14, and also  $A_0$  of  $n = 16$  would become smaller than that of  $n = 12$  and 14. Molecular area of trans saturated acyl chains ( $A_{a-c}$ ) is *ca.* 0.2 nm<sup>2</sup> [18],  $A_0$  of  $n = 16$  is slightly larger than  $A_{h-g}$  and sum of two  $A_{a-c}$ . This indicates that two alkyl chains open between each other or tilt a little from water surface normal.

The above dependence and behavior of alkyl chain length on the measured Gemsurfs monolayer is quite similar to that of dilauroyl phosphatidyl choline (DLPC,  $n = 12$ ), dimyristoyl phosphatidyl choline (DMPC,  $n = 14$ ), and dipalmitoyl phosphatidyl choline (DPPC,  $n = 16$ ) which are biomembrane model substances [14]. Therefore, DLPC and DMPC form LE type monolayer, whereas DPPC forms LC type monolayer including LE-LC phase transition. The alkyl chain of DLPC and DMPC has gauche structure and DPPC has trans. The order of  $A_0$  is DLPC > DMPC > DPPC. Measured Gemsurfs are quite different from composition and structures of hydrophilic groups and hydrophilic lipophilic balance (HLB) compared to phospholipids, however, Gemsurfs showed monolayer morphology similar to phospholipids formed on the water surface. This implies that Gemsurfs have a possibility with the surfactant ability similar to that of phospholipids. Nakahara and co-workers [19] [20] have reported the importance of the function and the role as lung surfactants by DPPC and palmitic acid molecules. On the pattern of applications of lipid surfactants, we expect that Gemsurfs would not only be used in cosmetic and industrial products but also have applications in *in-vivo* components to substitute biomolecules.

## 5. Conclusion

We have investigated the morphology of the novel Gemsurfs ( $n = 12$ , 14, and 16) monolayer formed on the water surface at 26°C using STm and BAM methods. It was found that  $n = 12$  and 14 formed LE type monolayer and that of  $n = 16$  LC type monolayer where LE to LC phase transition existed. Morphology of Gemsurf monolayers was similar to those of phospholipids series of biomolecules. Gemsurfs have capable of surface activity as well as phospholipids and also a possibility of application as biosurfactant.

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