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Direct Observation of Ordered Bilayers in Cast Films of Double-Chain Ammonium Amphiphiles by Transmission Electron Microscopy

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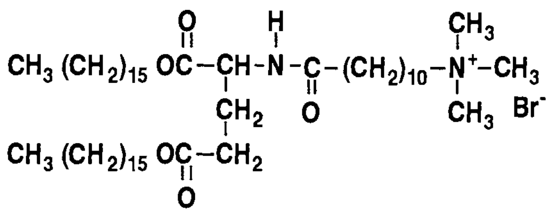
Individual molecular bilayers in cast films of double-chain amphiphiles were observed directly by transmission electron microscopy upon appropriate staining. Ordering of bilayers was affected by a minor structure change of the component amphiphile.

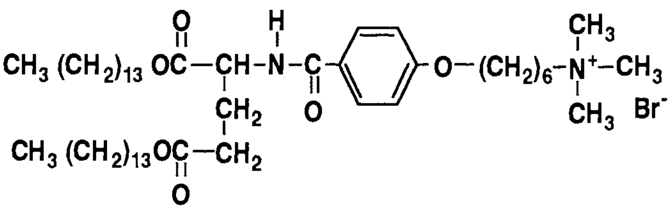
Organic ultra thin films attract much attention as potential functional materials. They are usually formed by spin coating, the Langmuir-Blodgett technique, and vapor deposition. The self-organizing property of component molecules plays a decisive role in producing ordered molecular architectures in these cases.

Casting of aqueous bilayer membranes is a convenient method for obtaining regular multilayer films of molecular bilayers.$^{1)}$ The rich variety of the bilayer component and self assembling of the unit bilayer render the cast film system very appealing as matrices for anisotropic ordering$^{2,3)}$ of functional molecules and for preparing two-dimensional organic and inorganic materials.$^{4,5)}$ The molecular organization in these cast films can be examined by many physicochemical techniques including X-ray diffraction,$^{4)}$ anisotropic ESR spectroscopy,$^{2)}$ and scanning electron microscopy.$^{6)}$ These results, however, remain indirect or of insufficient resolution. In the present study, we applied transmission electron microscopy (TEM) to observation of individual bilayers by improving the negative staining procedure. We selected two ammonium amphiphiles **1** and **2** as typical examples. They commonly possess two alkyl chains as hydrophobic tail and the glutamate residue as connector. Apart from the alkyl chain length, their structures differ only in the spacer portion: the latter contains the phenylene unit.



$CH_3(CH_2)_{15}OC(=O)-CH(-CH_2-CH_2-C(=O)O(CH_2)_{15}CH_3)-NH-C(=O)-(CH_2)_{10}-N^+(CH_3)_3\ Br^-$

amphiphile **1**



amphiphile **2**

Amphiphiles **1** and **2** were dispersed in deionized water by ultrasonication (Branson Ultrasonics Co., Sonifier II model 250). The resulting transparent dispersions (30 mM, 2 ml) were cast on porous Teflon sheets

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for 3 days at 25 ℃ and 60% relative humidity to give self-supporting films with ca. 50 μm thickness and 2.5 cm diameter.

These cast films were stained for TEM observation. In the initial trial, cast films were exposed to OsO_4 vapor directly in a sealed bottle, according to the conventional procedure. This procedure did not yield satisfactory staining, as clear TEM images could not be found. In the subsequent experiments, the tartrate anion that is known to be stained readily by OsO_4 was introduced into the cast films by ion exchange. Upon soaking of the cast films in aqueous Rochelle salt (potassium sodium tartrate, 1 M) for 24 h, the bromide anion was completely replaced with tartrate anion, as confirmed by appearance of carboxylate IR peaks and by loss of Br^- in X-ray photoelectron spectroscopy. These films were then treated with OsO_4 vapor in a sealed bottle for 24 h, sliced by a microtome (Reichert-Jung, Ultracut N), and subjected to high-resolution TEM observation (Hitachi, H-9000, acceleration voltage 300 kV).

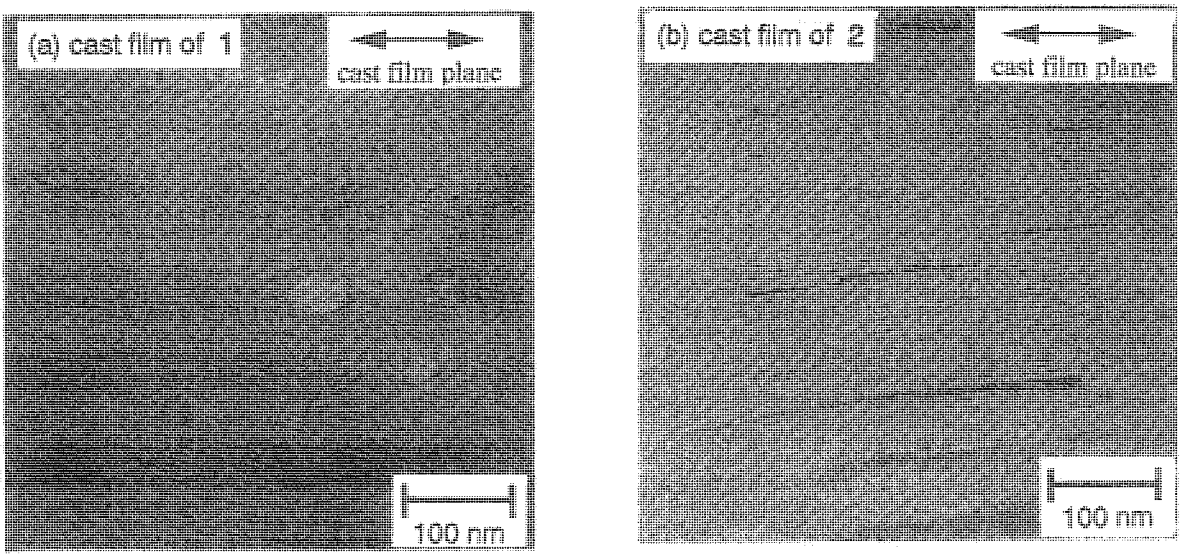


Fig. 1. Cross section TEM images of cast films of (a) amphiphile **1** and (b) amphiphile **2.**

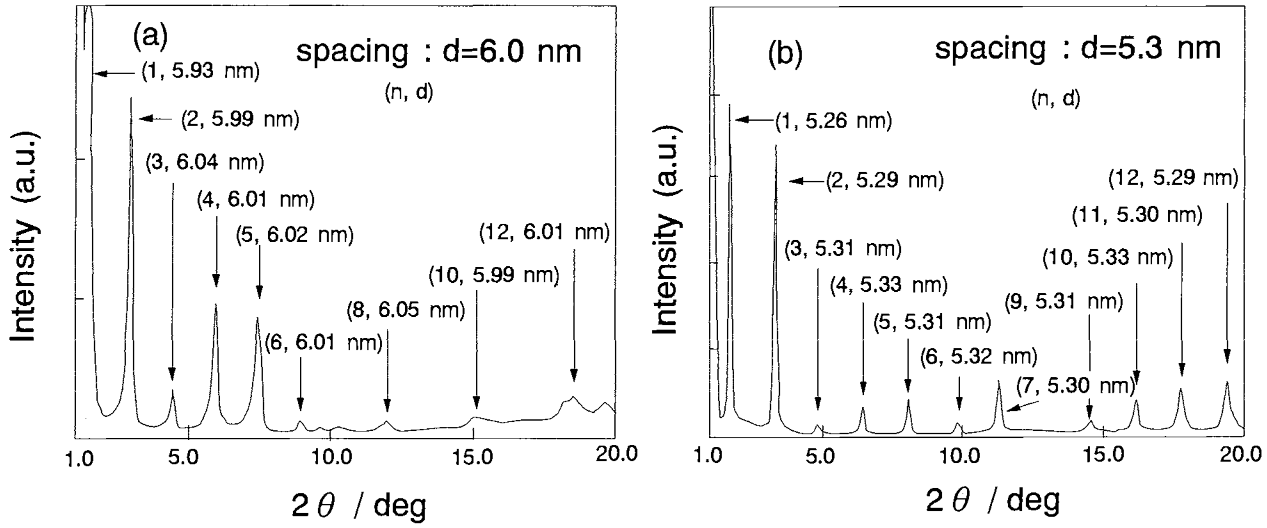
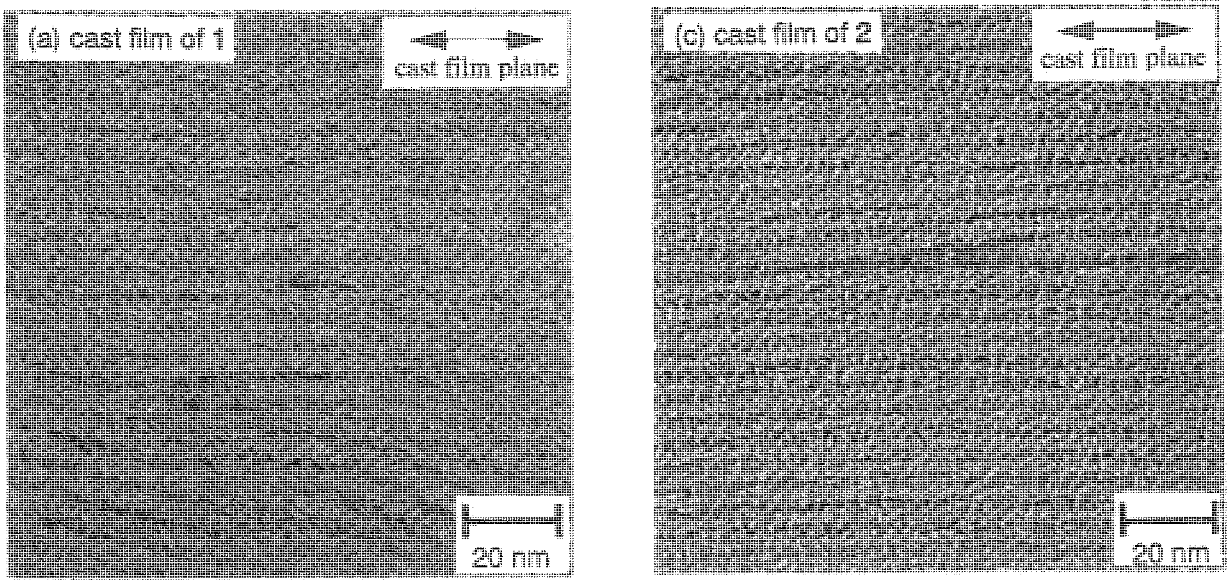


Fig. 2. XRD pattern of cast film of (a) amphiphile **1** and (b) amphiphile **2.**
n, the order of diffraction ; d, bilayer thickness.

Figure 1 displays TEM images of the cross section of cast films of **1** and **2**. The films were resistant against electron beam during observation. Dark striations represent OsO_4 particles deposited in the polar region of the interbilayer space. It is immediately apparent that cast film **1** gives well-developed, flexible layers. In contrast, cast film **2** possesses a much more regular, rigid-looking multilayer. The layers run parallel to the film plane in **2**, but the layer direction is not very regular in **1**. Earlier, we showed that the presence of the benzene ring in the spacer portion improved the regularity of bilayer organization.[7,8] The improved molecular orientation appears to be related to a difference in the multibilayer regularity of Fig. 1.



(b) cast film of **1**

(d) cast film of **2**

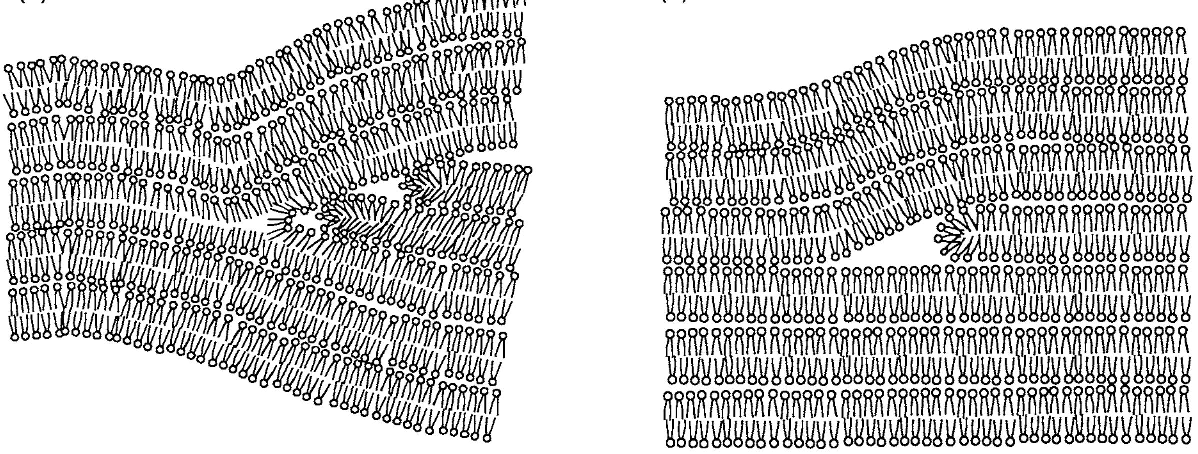


Fig. 3. Enlarged cross section TEM images of cast films of (a) amphiphile **1** and (c) amphiphile **2** and schematic illustrations of their microscopic morphologies.

The layer thickness estimated from TEM images is 5.7 nm for **1** and 5.0 nm for **2**. These data are consistent with long spacings determined by X-ray diffraction (XRD) (without staining). Figure 2 compares reflection (XRD) patterns of the two cast films. The long spacings are 6.0 nm and 5.3 nm for **1** and **2**, respectively. Sharp diffraction peaks up to 12th order are seen for **2**. Peak sharpness is lessened in **1** . This difference reflects that in multilayer regularity by TEM.

As can been seen from Fig. 1 the multilayer structure contains defects and dislocations. Figure 3 gives enlarged images of typical dislocations and their schematic illustrations. The layers in cast film **1** (Fig. 3a) are undulating in the whole area. This surface curvature will be produced by partially disordered molecular alignment or by coexistence of multiple lamellar phases. Bifurcation would be caused by smooth conversion of the planar bilayer structure into micellar and reversed micellar structures (concave and convex) and must be related to the flexible bilayer structure. On the other hand, the individual layer of cast film **2** (Fig. 3c) is much more regular, and the dislocations appear to be produced by discontinuous layers. Therefore, the dislocation will be highly localized (Fig. 3d), unlike gradual translocation of molecules in bilayer **1**.

In conclusion, we provided direct information on the supramolecular structure of cast multibilayer films. The two amphiphiles we used in this study are very similar in their molecular structures. Minor structural difference (the benzene unit in the spacer portion) exerts a profound influence on the supramolecular structure of a cast film. The present finding provides an important guideline for development of cast films as regular, two dimensional matrices.

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