

# Self-supporting Molecular Bilayer Lipid Films in Protic and Aprotic Organic Solvents. Existence of Organized Bilayer Structure and Phase Transition

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Self-supported polyion-complexed lipid films could retain organized bilayer structures and a fundamental bilayer characteristic (phase transition) even in protic and aprotic organic solvents.

Synthetic bilayer membranes possess fundamental physico-chemical properties similar to those of biomembranes in aqueous solution.<sup>1</sup> Immobilization of the synthetic bilayers is of considerable current interest, because it facilitates their use as novel organic functional thin films with biomembrane-mimic properties both in the aqueous<sup>2</sup> phase and under an air atmosphere.<sup>3</sup> If bilayer characteristics are maintained in organic media, novel possibilities in fundamental studies and applications in membrane science should arise. Recently, Kunitake *et al.*<sup>4</sup> reported dispersed bilayer formation of phosphate amphiphiles by complexation with Ca<sup>2+</sup> ion in chloroform.

In the present study, we have examined the thermal behaviour and X-ray analyses of self-supported films of the polyion-complexed lipid  $2C_{18}N^+PSS^-$  in organic solvents in order to shed light on the existence of organized structures in nonaqueous media. The preparation of  $2C_{18}N^+PSS^-$  lipid films has been described before.<sup>2</sup> The films were soluble in chloroform, tetrahydrofuran, and benzene, but insoluble in other organic media used here.† Differential scanning calorimetry (DSC) was conducted with Seiko-Denshi, SSC-560U, and Shimadzu, DSC-50 instruments. The lipid films were fabricated in DSC sample vessels by casting from chloroform (amount of lipid film applied 1.0 mg). The vessels were sealed after addition of solvent, and heated at 1 °C/min.

As shown before, the phase transition temperature ( $T_c$ ) of the  $2C_{18}N^+PSS^-$  lipid films in H<sub>2</sub>O is 45 °C,<sup>2a</sup> a value which is similar to that for the corresponding aqueous bilayer dispersions of dioctadecyldimethylammonium bromide ( $T_c$  45 °C).<sup>5</sup> We investigated the thermal behaviour of the films in eleven organic solvents and surprisingly found that the lipid films possess an endothermic peak in some of these media. Figure 1(A) shows results for alcoholic solvents and water. DSC peaks were clearly observed in methanol, ethanol, n-propanol, and n-butanol at 22.3, 17.0, 12.5, and 6.0 °C, respectively. In the series of protic media from H<sub>2</sub>O to n-butanol, a drop in  $T_c$  values and decrease in  $\Delta H$  (transition enthalpy) values accompanied an increase in the polarity of the media. We also examined seven aprotic media and found that there is a phase transition even in nitromethane, acetonitrile, *N,N*-dimethylformamide (DMF), and acetone at 34.2, 34.0, 27.0, and 25.0 °C, respectively (Figure 1B). However, a phase transition could not be detected in ethyl acetate, dioxane, or n-pentane.

Transition entropies ( $\Delta S$ ) show the degree of randomness in the hydrocarbon chain.<sup>6</sup> In this study,  $\Delta S$  values per methy-

lene unit of the lipid films were 4.93 J K<sup>-1</sup> mol<sup>-1</sup> in H<sub>2</sub>O and 1.3–4.6 J K<sup>-1</sup> mol<sup>-1</sup> in organic media, almost the same as those for liposomal lipid membranes<sup>6</sup> and synthetic aqueous bilayers.<sup>5</sup> All these results suggest that the endothermic peaks observed in the organic media arise from a transition between the crystalline state and liquid crystalline state of the lipid films similar to those observed for aqueous bilayer membranes.

X-Ray diffraction analyses were performed in order to get direct proof of the existence of oriented structures. The self-supporting lipid film of  $2C_{18}N^+PSS^-$ , prepared by casting from CHCl<sub>3</sub> on a Teflon plate, was set in the sample holder of a Rigaku Denki, RAD-RC X-ray diffractometer equipped with a small-angle scattering goniometer. As shown in Figure 2 (trace a), the lipid film shows a sharp diffraction peak

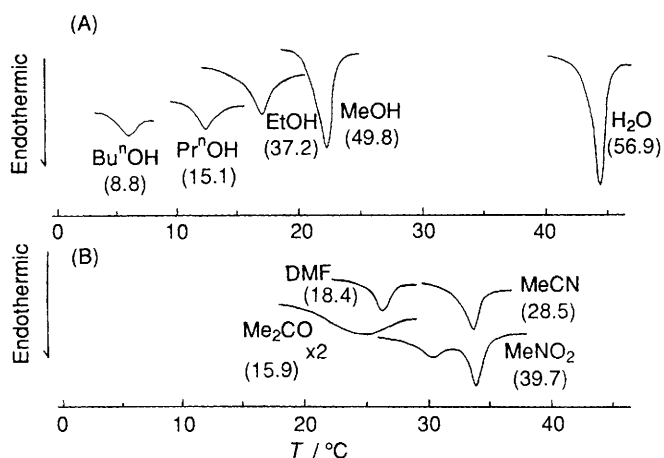


Figure 1. DSC thermograms of the  $2C_{18}N^+PSS^-$  lipid film in protic (A) and aprotic (B) solvents, at a heating rate of 1.0 °C/min. The figures in parentheses are the enthalpy values for the transition (kJ/mol).

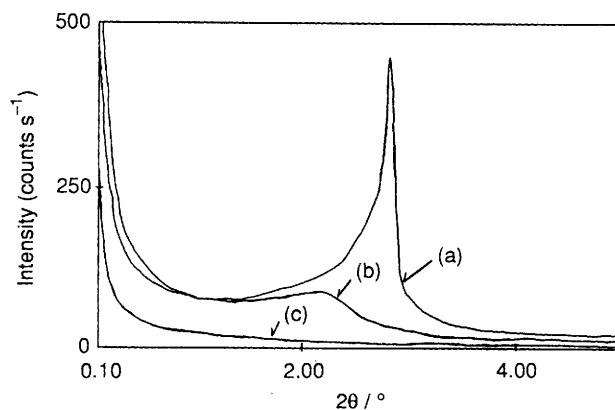
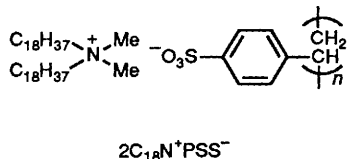


Figure 2. X-Ray diffraction diagrams of the  $2C_{18}N^+PSS^-$  lipid film in (a) ethanol and (b) air. Background is shown in trace (c).



† Spectrophotometric grade methanol, ethanol, and *N,N*-dimethylformamide were used; other solvents were purified by distillation.

(maximum scattering angle,  $2\theta$ ,  $2.82^\circ$ ) in ethanol and the  $d$  spacing is calculated to be 31 Å from the Bragg equation; this value corresponds to the thickness of a double layer of the lipid. A broad diffraction peak was observed near  $2.2^\circ$  ( $d$ , 41 Å) under an air atmosphere (trace b). The results provide convincing evidence for the existence of bilayer structures in ethanol.

The tightness of ion-pairing interactions of the polyion-complexed lipid head groups and the extent of penetration of organic solvents into the dialkyl chain parts of the lipid are expected to be important factors in determining the existence of organized structures in nonaqueous solvents. The former factor would contribute to the prevention of solvent penetration into the hydrophobic dialkyl regions and the retention of ordered structures in nonaqueous media.

We conclude that the polyion-complexed lipid films could retain organized bilayer structures and fundamental bilayer characteristics (phase transition) even in protic and polar aprotic media. The findings open new possibilities for studies on supported bilayer membrane films in organic solvents.

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