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# IMMOBILIZATION OF AMMONIUM BILAYER MEMBRANES BY COMPLEXATION WITH ANIONIC POLYMERS[1]

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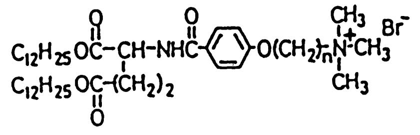
Water-insoluble polyion complexes were prepared by mixing aqueous solutions of ammonium bilayer membranes and anionic polymers. The complexes retained typical bilayer characteristics and gave transparent films by casting.

Synthetic bilayer membranes are formed spontaneously in water by self-assembly of a large variety of amphiphiles.[2] Immobilization of these aqueous bilayers facilitates their uses as novel functional materials. Immobilization techniques described in the past few years include: blending with poly(vinyl chloride),[3] coating of nylon capsules,[4] casting on glass,[5] composite films with poly(vinyl alcohol),[6] films sandwitched with cellulose acetate layers,[7] and Langmuir-Blodgett films.[8,9]

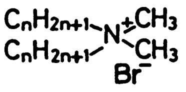
Polyion complexes have been prepared from polyelectrolytes of opposite charges, and are reported to display unique structural and functional properties.[10,11] Component polymer ions are usually water-soluble and the product complexes are water-insoluble. Since aqueous bilayers are stable aggregates and behave like polymers in many respects, neutralization of charged bilayers with polymers of opposite charges would give water-insoluble complexes. Based on this presumption, we developed a new immobilization technique of bilayers.

A typical preparative procedure is as follows: cationic bilayer components (3 x $10^{-5}$ mol) were dispersed in 2 ml of water by sonication. The solutions were mixed with aqueous polymers(3 x $10^{-5}$ mol of the negative charge) in 2 ml of water. The precipitates were collected by centrifuge, washed and dried. The recoveries were 40 to 70%, and the compositions(ratio of the negative and positive charges) as determined by elemental analysis varied between 0.2 and 5, depending on the conditions. Transparant films were obtained by casting chloroform solutions (0.5 mg in 100 µl) on quartz plates.

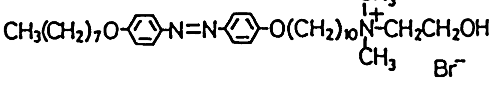
Bilayer components and polymers used in this study are as follows.



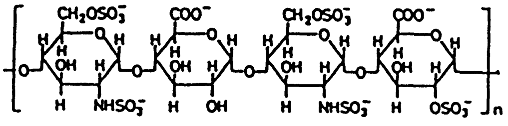
2C$_{12}$-Glu-ph-C$_n$N$^+$



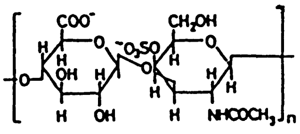
2C$_n$N$^+$2C$_1$



C$_8$AzoC$_{10}$N$^+$



heparin



chondroitin sulfate

The bilayer characteristics of the polyion complexes were examined by thermal and spectral methods. Differential scanning calorimetry(DSC) has been used extensively for characterizing the phase transition behavior of synthetic bilayers.12) Figure 1 demonstrates DSC thermograms of bilayer complexes. The endothermic peak of a $2C_{16}N^{+}2C_{1}$/chondroitin sulfate sample suspended in water is located at 35 °C which is 8 ° higher than the phase transition temperature($T_c$ = 27 °C at the peak top) of aqueous $2C_{16}N^{+}2C_{1}$ bilayer. A similar result was found for a complex of $2C_{12}$-L-Glu-ph$C_4N^{+}$ and heparin, $T_c$ of the complex being higher by 8 ° than that of the aqueous bilayer alone. When the latter bilayer was complexed with chondroitin sulfate(charge ratio, 0.43), the endothermic peak was observed at 41 °C. The extents of $T_c$ rise in the complexed bilayers are similar to those observed in other immobilized bilayer systems.5,7)

Figure 2 shows absorption spectra of a cast complex of an azobenzene bilayer ($C_8AzoC_{10}N^{+}$) and heparin. The absorption maximum situated at 300 nm at 15 °C becomes broader with the rise in temperature up to ca. 60 °C, and then moves to 340 nm at 79 °C. The spectrum was reverted by lowering temperature.

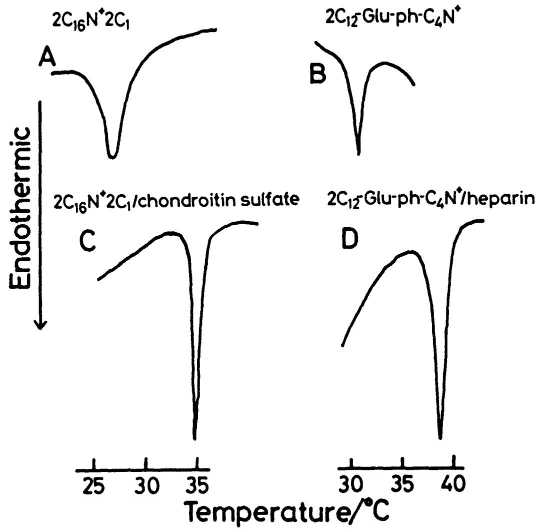


Fig. 1. DSC thermograms of aqueous bilayers(A and B) and polyion complexes(C and D). Charge ratio of the complex: 0.5 for C and 3.3 for D. Instrument Seiko Electronics SSC/560. The behavior is not affected by small changes in the charge ratio.

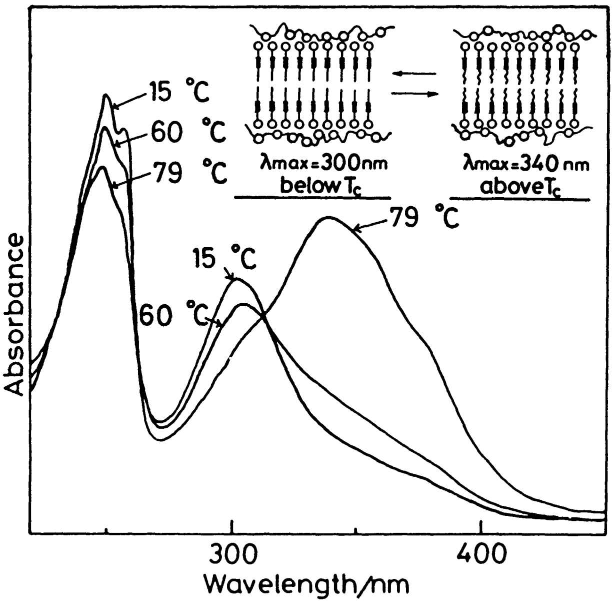


Fig. 2. Spectral change due to phase transition of the bilayer.
$C_8AzoC_{10}N^{+}$/heparin = 0.24.

These changes are very similar to those of the aqueous bilayer($T_c$ = 64 °C) which have been explained by different orientations of the azobenzene chromophore.13) This spectral coincidence suggests that the bilayer organizations in the two systems are identical.

The azobenzene amphiphile($C_8AzoC_{10}N^+$) is molecularly dispersed in the liquid crystalline matrix(10 fold excess) of the aqueous $2C_{18}N^+2C_1$ bilayer. The $\lambda_{max}$ in this case is at 355 nm, in agreement with that in organic media where no bilayers are formed. When the matrix bilayer is converted to the crystalline state($T_c$ = 42 °C), the azobenznee amphiphile segregates as clusters, giving rise to a new $\lambda_{max}$ at 302 nm.

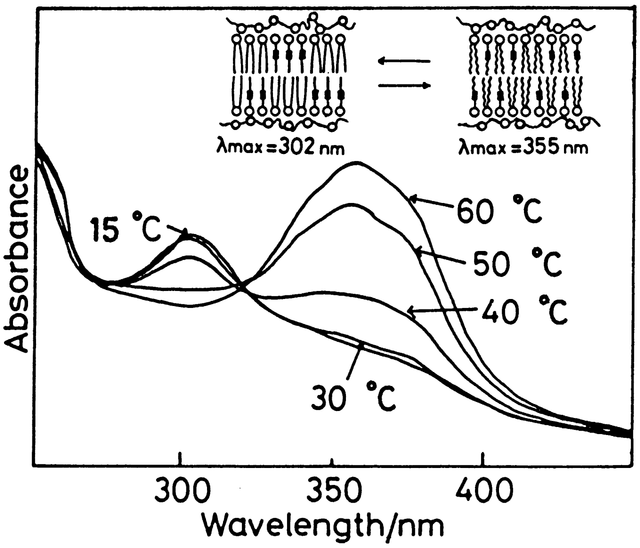


Fig. 3. Phase separation of bilayer in a cast film of $2C_{18}N^+2C_1/C_8AzoC_{10}N^+$/heparin.

The same situation was found for a polyion complex prepared from 3 x $10^{-5}$ mol of $2C_{18}N^+2C_1$, 3 x $10^{-6}$ mol of $C_8AzoC_{10}N^+$, and 6.6 x $10^{-5}$ anion unit mol of heparin (recovery, 90%). As shown in Fig. 3, a cast film of this three-component complex gives $\lambda_{max}$ at 302 nm at low temperatures, and $\lambda_{max}$ shifts to 355 nm at high temperatures. The spectral change is most pronounced at 40 - 50 °C, in accordance with $T_c$(44 °C) of the bilayer in the complex. The phase separation phenomenon of the polyion complex is intrinsically the same as that of the aqueous bilayer.

The above mentioned series of experiments clearly establish that aqueous bilayers are immobilized in the form of polyion complexes with retention of fundamental bilayer characteristics such as molecular orientaiton, phase transition, and phase separation. This is possible because of pronounced stability of the bilayer assemblage which is not destroyed by ion pairing. This technique is applicable to other combinations of ammonium bilayers and anionic polymers. In addition, polyion complexes were formed from negatively charged bilayers of phospholipids and synthetic amphiphiles and polymeric cations.14) It is concluded that formation of polyion complexes is a quite general technique for immobilizing bilayers.

## References

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