Permeability-controllable Membranes. Part 8.[1] Electrical Redox Sensitive Permeation through a Multibilayer-immobilized Film Containing a Ferrocenyl Group as a Redox Site

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Multibilayer-immobilized films containing a ferrocenyl group as a redox site at a hydrophobic alkyl chain ($FeC_{11}C_{18}N^+$) or as a hydrophilic head group of bilayer-forming dialkylammonium amphiphiles ($2C_{16}N^+Fc$) have been prepared by ion-complexation with poly(styrenesulphonate) (PSS^-). Redox reactions of ferrocene groups in multibilayer films ($FcC_{11}C_{18}N^+/PSS^-$ and $2C_{16}N^+Fc/PSS^-$) have been shown to occur only in the fluid state of bilayer matrices above their phase-transition temperatures (T_c = 35 and 26 °C, respectively), as confirmed by cyclic voltammetry. The permeability of water-soluble, non-ionic fluorescent probes through the multibilayer film cast on a platinium minigrid sheet is enhanced by a factor of 2–5 by the electrochemical oxidation of a ferrocenyl unit in the fluid multibilayer film above its T_c, compared with that of the reduced form. Permeability could be changed reversibly over at least 20 cycles by repeated redox reactions in the case of the $2C_{16}N^+Fc/PSS^-$ film containing an Fc unit near the hydrophilic moiety. In contrast, the change in permeability of the $FcC_{11}C_{18}N^+/PSS^-$ film with a redox site in the hydrophobic dialkyl chain became irreversible under continuous redox reactions.

Recently, various types of permeability-controllable synthetic membranes have been developed in connection with a study of transport mechanisms in biological membranes.[2] In biological membranes, electrical signals play an important role in membrane transport. At synapses, transmitter substances are released from the presynaptic terminal membrane in response to a change in the neuron potential. We have prepared various electrical-signal receptive, permeability-controllable artificial lipid membranes: a bilayer-corked nylon capsule membrane,[3,4] a monolayer-immobilized porous glass plate,[5,6] and a polyion-complex-type multibilayer-immobilized cast film,[7,8] in which lipid monolayers or multibilayers are immobilized on a physically stable support membrane. Permeability across these lipid-immobilized membranes can be changed reversibly by switching on and off an external electric field; this is due to orientation changes in the lipid bilayers.[4,7,8] Burgmeyer and Murray reported that the permeability of chloride anions through a polypyrrole film deposited on a gold grid sheet could be changed by the formation of positive charge on the film (by an electrochemical oxidation).[9]

In this paper, we describe the preparation of two kinds of polyion-complex type, multibilayer-immobilized cast films containing a ferrocene group as a redox site at the end of the hydrophobic alkyl chain or near the hydrophilic head group of amphiphiles, and demonstrate the reversible permeation changes through the film due to the temperature-dependent electrochemical redox reactions of the ferrocenyl group. A schematic illustration of the apparatus used for permeation measurements and film structures is shown in Figure 1. The multibilayer-immobilized films containing a ferrocenyl group ($FeC_{11}C_{18}N^+/PSS^-$ and $2C_{16}N^+Fc/PSS^-$) were cast on a platinum minigrid sheet and the permeation of a water-soluble, nonionic probe (**1**) across the film was followed fluorophotometrically by switching on and off an electric potential applied to a Pt grid/film. A non-ionic, fully water-soluble permeant probe was chosen to avoid electrostatic and hydrophobic interaction with the polyion-complex film and electrodes.

Saji and co-workers have reported the reversible formation and disruption of aqueous micelles,[10,11] the formation of a thin film on a substrate,[12] and the control of ionophore activity in liquid membranes[13] using ferrocene compounds in electrochemical redox reactions.

Experimental

Materials.—The structures and purities of product compounds were confirmed by t.l.c. with a flame ionization detector, n.m.r. and i.r. spectroscopy, and elemental analysis (C, H, and N). Sodium poly(styrenesulphonate) (PSS^-) was obtained by radical polymerization of the respective monomer in aqueous solution and purified by dialysis in aqueous solution using a cellulose tube [$M = (1–5) \times 10^6$]. Preparation of the freely water-soluble, non-ionic fluorescent permeation probe, 2,6-bis(1,3,4,5-tetrahydroxcyclohexanecarboxamidomethyl)naphthalene (**1**), has been reported elsewhere.[14]

Ferrocene containing, bilayer-forming amphiphiles were prepared in the following manner.

11-Ferrocenylundecyl(dimethyl)octadecylammonium bromide ($FcC_{11}C_{18}N^+ Br^-$).—11-Bromoundecanoyl ferrocene was prepared by Friedel–Crafts acylation, in the presence of $AlCl_3$ (14.7 g, 0.11 mol), from ferrocene (18.6 g, 0.10 mol) and 11-bromodecanoyl chloride (31.2 g, 0.11 mol) in dry methylene dichloride (300 cm^3) at room temperature. The product was a dark yellow powder (from methanol) (14.7 g, 34%), m.p. 53–55 °C. This compound (14.0 g, 0.032 mol) was subjected to a Clemmensen reduction with zinc powder (80 g, 1.22 mol) and $HgCl_2$ (6 g, 0.022 mol) in acidic aqueous solution, to give a yellow powder (13.3 g, 98%), m.p. 33 °C. 11-Ferrocenylundecyl bromide (4.7 g, 0.011 mol) was quaternized with dimethyl-(octadecyl)amine (3.34 g, 0.011 mol) in ethanol under reflux for 72 h to give a pale yellow powder from ethyl acetate (twice) (5.2 g, 66%), R_F 0.8 (benzene); $\delta_H(CDCl_3)$ 0.9 (t, 3 H, Me), 1.3–1.7 (m, 50 H, CH_2), 2.4 (t, 2 H, CH_2Fc), 3.4 (s, 6 H, CH_3-N^+), and 4.2 (s, 9 H, ferrocenyl).

(11-Ferrocenylundecyl)trimethylammonium Bromide[10] ($FeC_{11}N^+ Br^-$).—A single-chain amphiphile was prepared by quaternization of 11-bromoundecylferrocene (13.6 g, 0.033 mol)

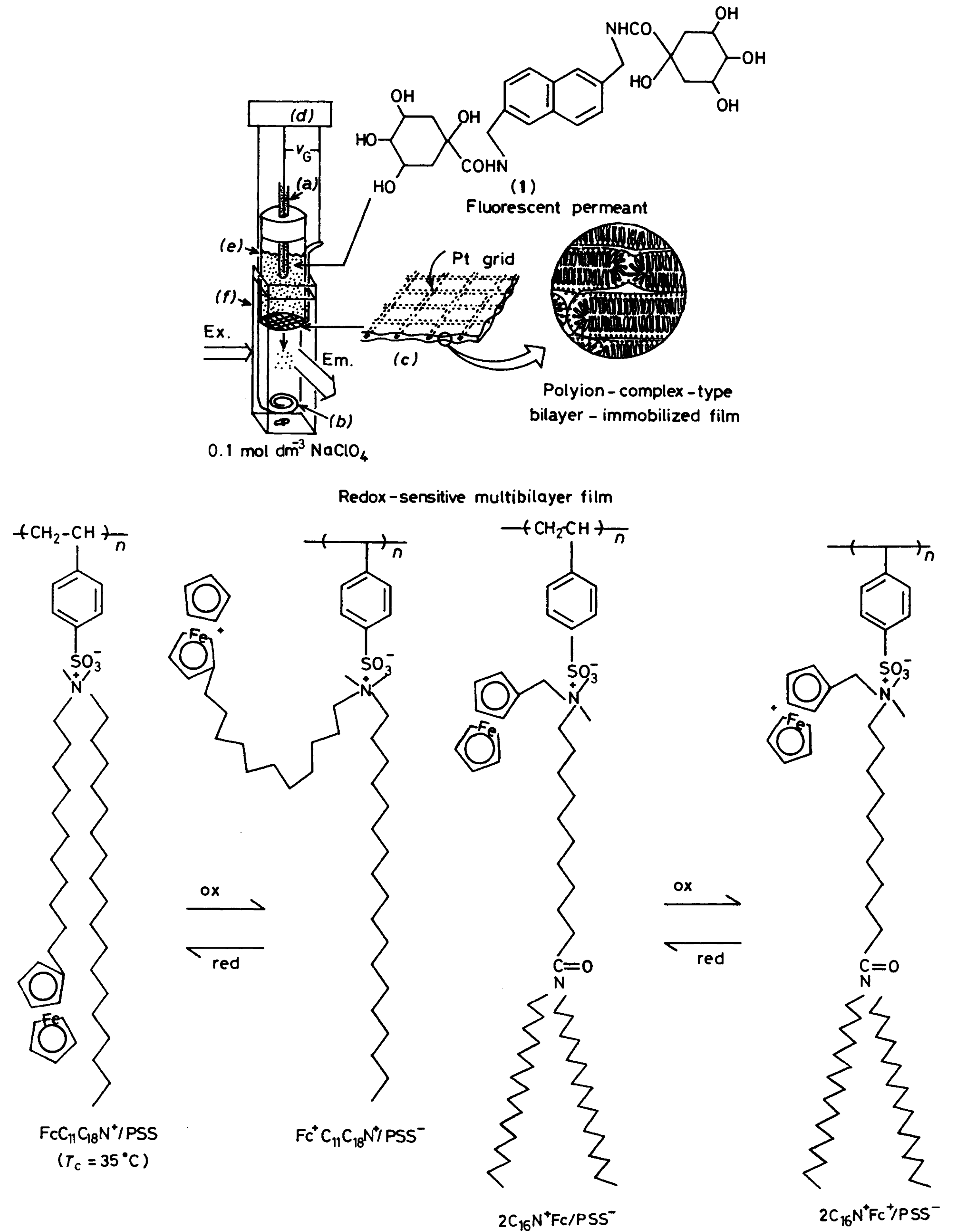


Figure 1. Experimental set-up for the permeation of fluorescent probes (**1**) through redox-type multibilayer films cast on a Pt minigrid sheet: (*a*) reference electrode, Ag/AgCl in saturated KCl (−0.05 V *vs.* s.c.e.); (*b*) Pt wire for counter electrode; (*c*) Pt minigrid sheet (100 mesh, 70 μm thick, 28 mm^2 area) embedded in redox-type bilayer films; (*d*) potentiostat; (*e*) polyethylene tube; (*f*) 1 cm quartz cell.

with excess trimethylamine in tetrahydrofuran (THF) in a pressure bottle at 50 °C for 20 h. The product was a pale yellow powder (from ethyl acetate) (7.14 g, 46%), R_F 0.8 ($CHCl_3$–MeOH, 9:1); δ_H($CDCl_3$) 1.3–1.5 (m, 18 H, CH_2), 2.4 (t, 2 H, CH_2Fc), 3.4 (s, 9 H, CH_3N^+), 3.6 (t, 2 H, CH_2N^+), and 4.3 (s, 9 H, ferrocenyl).

10-(Dihexadecylaminocarbonyl)decyl(ferrocenylmethyl)dimethylammonium Bromide ($2C_{16}N^+Fc\ Br^-$).—10-(Dihexadecylaminocarbonyl)decyl bromide was prepared from *N,N*-dihexadecylamine (10.0 g, 0.022 mol) and 11-bromoundecanoyl chloride (8.6 g, 0.03 mol) which were kept in dry chloroform in the presence of triethylamine at 0 °C for 10 h. The product formed white needles from ethyl acetate (12.2 g, 80%), m.p. 38 °C. The bromo compound so obtained (10.3 g, 0.014 mol) was quaternized with (ferrocenylmethyl)dimethylamine (3.6 g, 0.015 mol, b.p. 85–90 °C/0.2 mmHg) in ethanol under reflux for 120 h, to give a dark-yellow powder from ethyl acetate (3.5 g, 37%), m.p. 37–50 °C (liquid crystalline behaviour), R_F 0.1 ($CHCl_3$–NH_4OH, 99:1), δ_H($CDCl_3$) 0.9 (t, 6 H, Me), 1.3–1.5 (m, 62 H, CH_2), 2.3 (t, 2 H, CH_2CO), 3.2 (t, 4 H, CH_2N), 3.5 (s, 6 H, CH_3N^+), 3.6 (s, 2 H, CH_2N^+), and 4.5 (s, 9 H, ferrocenyl).

Polyion-complex Films.—An aqueous dispersion (20 cm^3) of bilayer-forming amphiphiles, $FeC_{11}C_{18}N^+\ Br^-$ (0.36 g, 0.5 mmol) or $2C_{16}N^+Fc\ Br^-$ (0.48 g, 0.5 mmol), and an aqueous solution (20 cm^3) of sodium poly(styrenesulphonate) (PSS^-, 0.36 g, 0.5 mmol monomer) were mixed at 70 °C (at which temperature the amphiphiles exist in a fluid liquid-crystalline state above their T_c). The yellow precipitates formed were washed, dried, and purified by reprecipitation from chloroform with methanol. The powder so obtained (recovery 90%) was confirmed by elemental analysis (C, N, and S). Halide counterions exchanged to PSS^-, and inorganic salts (Na^+ and Br^-) were not detected to within analytical error. The polyion-complex powder ($FeC_{11}C_{18}N^+/PSS^-$ and $2C_{16}{}^+Fc/PSS^-$) was dissolved in chloroform (3 cm^3) and cast on a platinum minigrid sheet (100 mesh, 70 µm thick). The film was transparent, physically strong, and water-insoluble. The film thickness was estimated to be 100 µm from the scanning electron micrograph (s.e.m.) observation.

Characterization of Films.—The microstructure of polyion-complex type films, $FcC_{11}C_{18}N^+/PSS^-$ and $2C_{16}N^+Fe/PSS^-$, were observed by a Hitachi H-600 transmission electron microscope (t.e.m.). The film was soaked in aqueous 2% uranyl acetate (negative staining agents) at 60 °C for 1 h and cut to an ultrathin section applied to t.e.m. observations.

X-Ray diffraction measurements using Ni-filtered Cu-K_α radiation were carried out by means of a flat camera (Rigaku Denki, Rotaflex RU-200).

Differential scanning calorimetry (d.s.c.) of multibilayer-immobilized cast films was carried out with a Daini-Seikosha Model SSC-560 instrument. A small piece of the film was sealed with 50 mm^3 of an aqueous solution in a silver sample pan and heated from 5 to 90 °C at rate of 2 °C min^{-1}. Phase-transition temperatures (T_c) of the reduced (Fc) and oxidized form (Fe^+) of the multibilayer films were measured in nitrogen-purged, distilled water and in an aqueous solution of cerium(IV) ammonium nitrate, respectively.

Cyclic voltammetric studies of the ferrocene-containing films cast on a Pt working electrode were carried out in a nitrogen purged 0.1 mol dm^{-3} $NaClO_4$ aqueous solution at various temperatures using a three-electrode potentiogalvanostat (Nikko Keisoku Co., model NPGFZ-2501). A platinum wire and Ag/AgCl in saturated KCl (−0.05 V *vs.* s.c.e.) were used as the counter and reference electrodes, respectively.

Permeation Measurements.—Freely water-soluble non-ionic fluorescent probes (**1**) were dissolved in aqueous $NaClO_4$ (0.1 mol dm^{-3}) and stored in the upper cell (Figure 1). Permeation through the film/grid (area 28 mm^2, thickness 100 µm) was followed fluorophotometrically in the lower cell at 340 nm (excited at 280 nm) in a 0.1 mol dm^{-3} aqueous $NaClO_4$. The aqueous solution was oxygen degassed and nitrogen purged before each experiment. The relative permeation rate, *P* (cm^2 s^{-1}) was obtained from equation (1),[5–8] where *d* and *S* are a

$$P = Jd/C_0S \qquad (1)$$

thickness and an area of the film, respectively, and *J* and c_0 are the permeation flux and the initial concentration of the probe in the upper cell, respectively.

The permeation experiments were carried out after having applied a potential +0.5 and 0 V *vs.* s.c.e. to the Pt grid/film in order to oxidize and reduce a ferrocene unit in the film, respectively. The reference electrode (Ag/AgCl/aq. KCl) and the counter Pt wire were set in the upper and the lower aqueous solution in the cell, respectively, as shown in Figure 1. The same potentiogalvanostat was used as that for cyclic voltammetric studies. The decomposition and quenching of fluorescent probes were not detected upon application of a potential of −0.5 to +0.7 V on the Pt grid/film during permeation experiments for 5 min. Since the reference electrode and the counter electrode are on opposite sides of the dividing minigrid sheet, the potential of the working electrode may not show the correct value.

When the Pt counter electrode was set in the upper solution with the reference electrode, however, the fluorescence intensity was markedly reduced and permeation of probes was not constant, probably because the fluorescence was quenched or its lifetime shortened. This was proved by a separate experiment in which the fluorescence intensity and spectrum of the probe decreased and changed with time. However, reliable and reproducible permeation results were obtained when three electrodes were set up, as shown in Figure 1. It is not clear why changing the position of the counter electrode should affect the fluorescence intensity.

Results

Characterization of Films.—Figures 2(*a*) and 2(*b*) show the s.e.m. of an intersectional view of the $2C_{16}N^+Fc/PSS^-$ film cast on the Pt minigrid sheet and its enlargement, respectively. The s.e.m. observation shown in Figure 2(*b*) shows the multilayer structures which pile up parallel to the film plane. In order to clarify the microstructure of the multilayer film, transmission electron microscopy and *X*-ray analysis of the film were carried out. Figure 2(*c*) is a t.e.m. photograph of an enlargement of the intersectional view of the $2C_{16}N^+Fc/PSS^-$ film stained negatively, and shows the distinct multibilayer structure of which the mean thickness of one white-line is estimated to be *ca.* 4.0 nm. *X*-Ray diffraction patterns of the $2C_{16}N^+Fc/PSS^-$ film are shown in Figure 2(*d*), in which the incident beam was exposed parallel to the film plane (edge view). A series of strong reflection arcs with 3.8 nm spacing parallel to the film plane was clearly observed, which consisted of both a bimolecular length of $2C_{16}N^+Fc$ amphiphiles (3.8–4.2 nm, estimated from Corey–Pauling–Kolton molecular models) and one white-line width [Figure 2(*c*)]. When the *X*-ray beam was exposed perpendicular to the film plane, reflections of a long spacing of bilayer thickness were not observed.

These findings indicate that $2C_{16}N^+Fc$ amphiphiles exist as well-oriented multibilayers complexing with polyanions, which pile up parallel to the film plane, as illustrated in Figure 1. Similar t.e.m. observations and *X*-ray reflections were obtained in the case of the $FcC_{11}C_{18}N^+/PSS^-$ film, in which Fc groups are located at the end of alkyl chains. These structure data are also consistent with those of other polyion complex cast films from simple dialkylammonium amphiphiles and polyanions reported by Kunitake and co-workers.[15,16]

D.S.C. Measurements.—The liquid-crystalline nature is one of the fundamental physicochemical properties of synthetic or

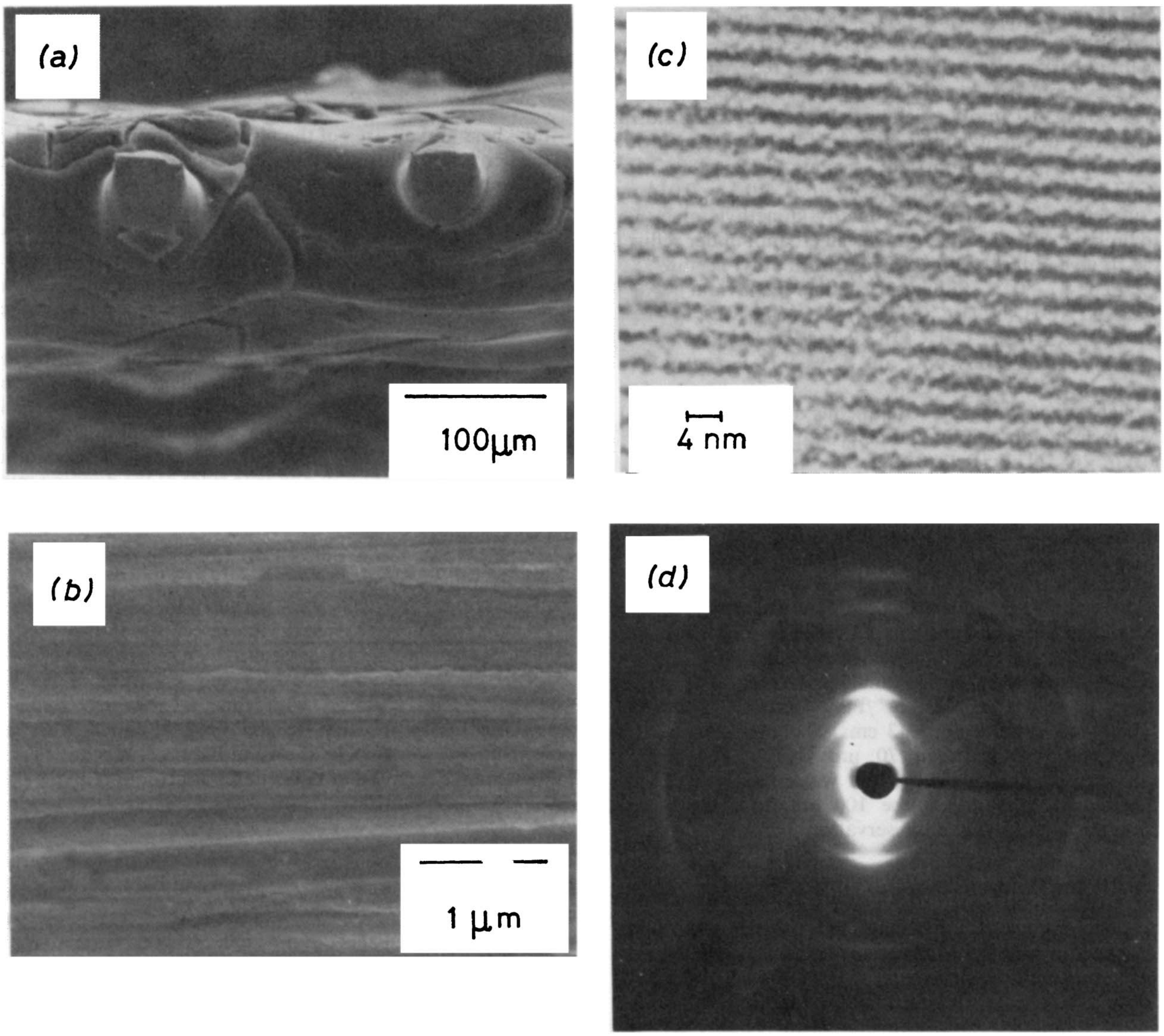


Figure 2. Scanning electron micrographs (*a*) and (*b*), transmission electron micrograph (*c*), and *X*-ray diffraction pattern (*d*) of the intersectional view of the $2C_{16}N^+Fc/PSS^-$ multibilayer film cast on a Pt grid sheet.

natural lipid bilayers. The results of d.s.c. measurements of redox-type multibilayer films are shown in Figure 3. The reduced form of the $FcC_{11}C_{18}N^+/PSS^-$ film showed a sharp endothermic peak at 35 °C (ΔH = 6.5 kcal mol^{-1}) in distilled water, indicating a phase transition from the solid to the liquid-crystalline state undergone by the multibilayers in the film. In contrast, the film in an aqueous solution of cerium(IV) ammonium nitrate (oxidizing agent) showed no endothermic peaks in the range 5–90 °C. After re-reduction of the film by aqueous $Na_2S_2O_4$, the peak area (ΔH) at the T_c did not revert to the original one as shown in the dotted line of Figure 3. The results indicate that in $FcC_{11}C_{18}N^+$ amphiphiles, the Fc group located at the end of the dialkyl chain is unable to assume the oxidized Fc^+ form in the film, which when re-reduced cannot reproduce the original well-packed multibilayer structures.

The $2C_{16}N^+Fc/PSS^-$ film, containing an Fc group near a hydrophilic head group, showed sharp endothermic peaks at 26 °C (ΔH = 8.5 kcal mol^{-1}) and 16 °C (ΔH = 6.5 kcal mol^{-1}) in the reduced form (in distilled water) and in the oxidized form [in aqueous Ce(IV) ion solution], respectively. Thus, the phase-transition temperature (T_c) of $2C_{16}N^+Fc$ bilayers in the film changed from 26 to 16 °C with slightly decreasing ΔH values during the oxidation. After re-reduction of the oxidized film by aqueous $Na_2S_2O_4$ solution, a d.s.c. peak at 26 °C was observed similar to that in the original reduced film. This indicates that the oxidation of an Fc group in the hydrophilic part of the $2C_{16}N^+Fc/PSS^-$ film occurs with a slight disordering of the multibilayer structures and the bilayer structure can be completely restored by re-reduction, in contrast to the $FcC_{11}C_{18}N^+/PSS^-$ film.

Cyclic Voltammetry.—Figure 4 shows cyclic voltammetric curves of the $FcC_{11}C_{18}N^+/PSS^-$ film cast on a Pt wire in nitrogen-purged 0.1 mol dm^{-3} aqueous $NaClO_4$ and indicates the reversible one-electron oxidation and reduction steps with a scan rate of 50 mV s^{-1}: E_{pa} = +0.52 V *vs.* s.c.e. in the anodic region (Fc to Fc^+) and E_{pc} = +0.30 V *vs.* s.c.e. in the cathodic region (Fc^+ to Fc). Peak-top currents in both the anodic and cathodic regions largely depended on temperature. The shapes of cyclic voltammetric curves did not change for at least 5 min under these sweep rates at each temperature. In the case of the $2\,C_{16}N^+Fc/PSS^-$ film, E_{pa} and E_{pc} were similarly observed at +0.51 and +0.30 V *vs.* s.c.e., respectively, and the magnitude of

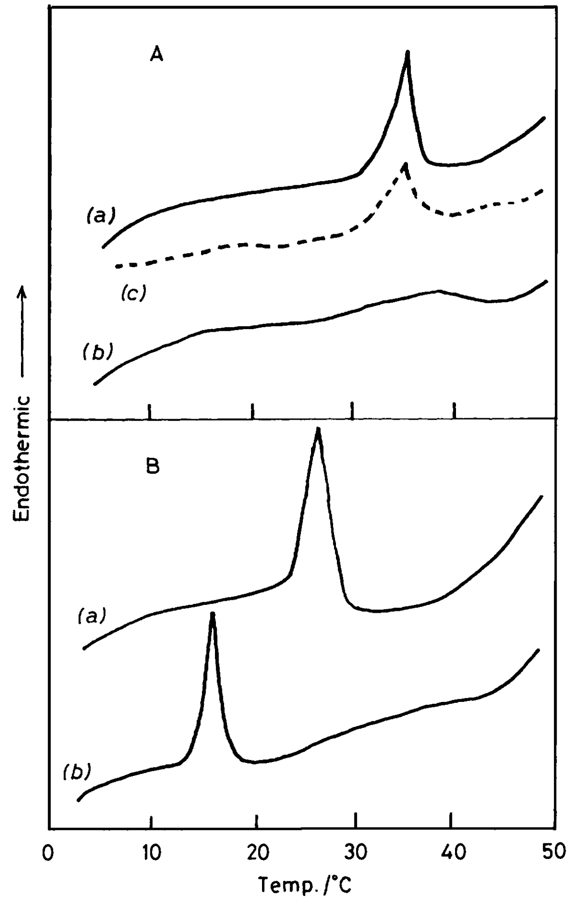


Figure 3. Differential scanning calorimetric curves of A: $FeC_{11}C_{18}N^+$ /- PSS^- films in (*a*) distilled water (the reduced form); (*b*) in aqueous Ce^{VI} ion (the oxidized form); (*c*) in aqueous $Na_2S_2O_4$ (the re-reduced form); B: $2C_{16}N^+Fc/PSS^-$ films in (*a*) distilled water (the reduced form) and (*b*) aqueous Ce^{VI} (the oxidized form). Scan rate; 2 °C min^{-1}.

both peak-top currents increased with increasing temperature as did those of the $FcC_{11}C_{18}N^+/PSS^-$ film.

Figure 5 shows the temperature dependence of the anodic (I_{pa}) and cathodic (I_{pc}) peak currents of the $FcC_{11}C_{18}N^+/PSS^-$ and $2C_{16}N^+Fc/PSS^-$ films. The I_{pa} and I_{pc} values of the $FcC_{11}C_{18}N^+/PSS^-$ film increased significantly above 35 °C, which is consistent with phase transitions of bilayers. Thus, the oxidation and reduction of Fc groups in the film occurred only in the fluid liquid-crystalline state above the T_c, but not in the solid state below the T_c of the reduced bilayers. The resistance of the $FcC_{11}C_{18}N^+/PSS^-$ and $2C_{16}N^+Fc/PSS^-$ films was measured with two platinum electrodes across the cast film in 0.1 mol dm^{-3} aqueous NaCl. The membrane resistance was (1–2) × 10^6 Ω in the solid state below the T_c and decreased to (2–4) × 10^5 Ω in the fluid liquid-crystalline state above T_c. The relatively large resistance of the film may cause the difference in potential between the oxidation and reduction peaks in the cyclic voltammetric curves. Thus, the temperature effects of cyclic voltammetry can be explained on the grounds that the counter anions (ClO_4^-) compensating for the formation of Fc^+ cations in the film cannot penetrate into the solid state of the multibilayer film below the T_c.

Similarly, the redox peak currents of the $2C_{16}N^+Fc/PSS^-$ film increased above 26 °C, T_c for the reduced form of bilayers (see Figure 5). This can be also explained by the decrease in the membrane resistance and the increase in penetration of counter anions in the fluid film above the T_c.

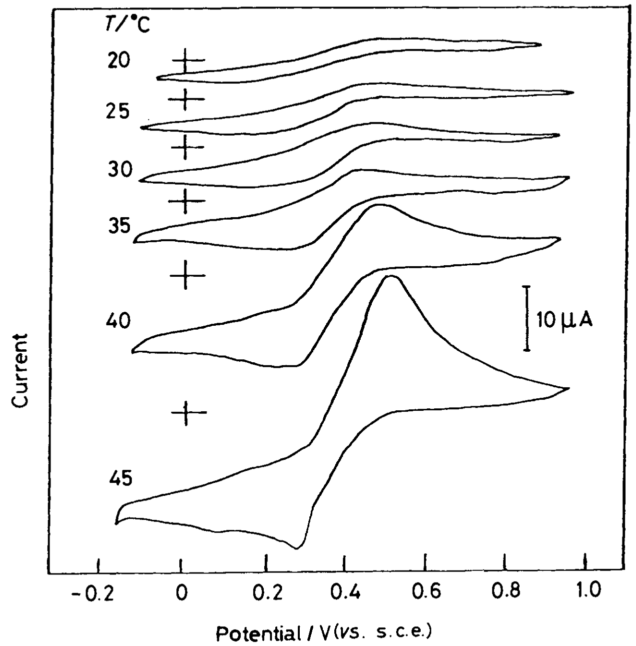


Figure 4. Cyclic voltammograms of the $FcC_{11}C_{18}N^+/PSS^-$ film cast on a Pt wire in 0.1 mol dm^{-3} aqueous $NaClO_4$ at various temperatures. Sweep rate; 50 mV s^{-1}.

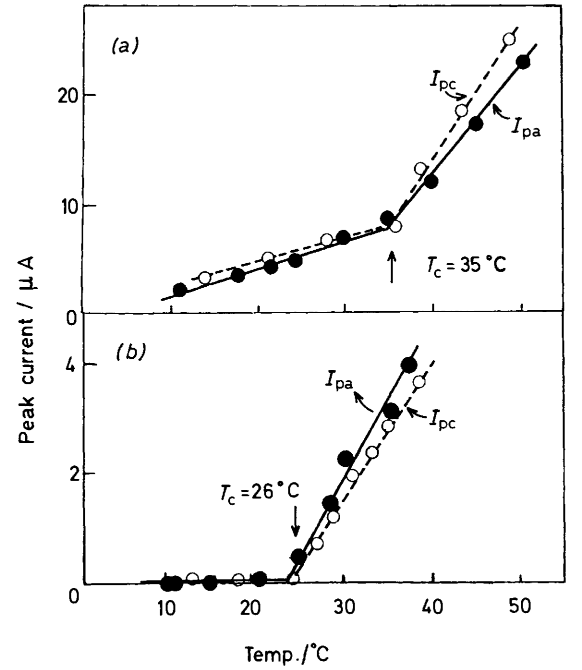


Figure 5. Temperature dependence of the cathodic (I_{pc}) and anodic peak currents (I_{pa}) of (*a*) the $FcC_{11}C_{18}N^+/PSS^-$ film and (*b*) the $2C_{16}N^+Fc/PSS^-$ film. Phase-transition temperatures (T_c) in the reduced form of each film obtained by d.s.c. measurements are shown as arrows.

Chemical and electrochemical oxidation of Fc groups in the film were also confirmed by electronic spectroscopy: the oxidized film turned from dark yellow to dark blue (λ_{max} 470 and 630 nm) which agrees with the absorption spectrum for Fc^+ ions.[17]

Permeation through the $FcC_{11}C_{18}N^+/PSS^-$ *Film*.—Figure 6 shows typical time courses for the water-soluble non-ionic

Table. Permeation rate constants of redox-sensitive multibilayer-immobilized films.[a]

		10^9 P/cm^2 s^{-1}		
Film	Temp./°C	Reduced form (Fc)	Oxidized form (Fc$^+$)	Re-reduced form (Fc)
$FcC_{11}N^+/PSS$	45	9.8	—	—
$FcC_{11}C_{18}N^+/PSS^-$ [b]	20	1.2	1.1	1.2
	45	2.0	10	3.8
$2C_{16}N^+Fc/PSS^-$ [c]	20	0.25	0.27	0.26
	45	0.50	1.6	0.51

[a] Average of three experiments: experimental error of ±10%. [b] T_c of the reduced form = 35 °C. [c] T_c of the reduced form = 26 °C.

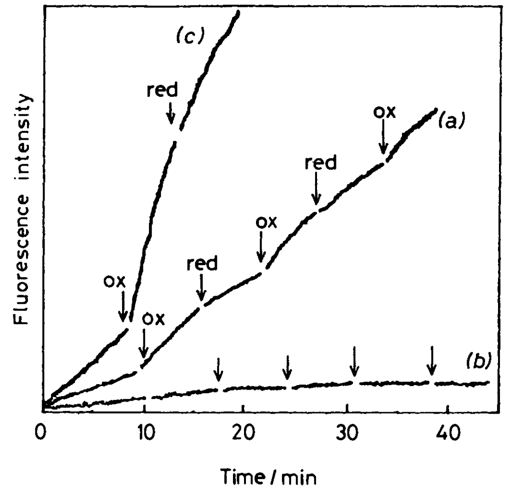


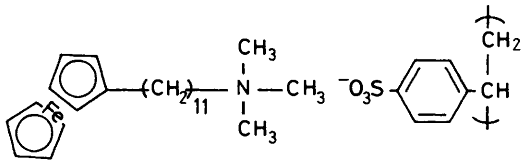
Figure 6. Permeation changes of the probe (**1**) through (*a*) the $FcC_{11}C_{18}N^+/PSS^-$ film at 45 °C (above T_c); (*b*) the $FcC_{11}C_{18}N^+/PSS^-$ film at 20 °C (below T_c); (*c*) the single-chain-type $FcC_{11}N^+/PSS^-$ film at 45 °C. The potential of +0.5 V and 0 V *vs.* s.c.e. were applied to the Pt grid/film at ox and red, respectively.

fluorescent probe (**1**) (1.0×10^{-3} mol dm^{-3}) through the $FcC_{11}C_{18}N^+/PSS^-$ film cast on a Pt minigrid sheet in nitrogen-purged aqueous $NaClO_4$ (0.1 mol dm^{-3}). The permeation of the probe was followed fluorophotometrically (see Figure 1). Permeation was measured three times in each experiment (deviations ±10%). The average permeation rate constant, P (cm^2 s^{-1}), was calculated from the slope using equation (1) and is summarized in the Table.

At 45 °C, permeation was relatively slow ($P = 2.0 \times 10^{-9}$ cm^2 s^{-1}) in the reduced form of the film. When a potential of +0.5 V *vs.* s.c.e. was applied to the Pt grid/film, the film turned from dark yellow to dark blue within 30 s which indicates oxidation of the neutral Fc to the cationic Fc$^+$ amphiphiles; permeability then increased by a factor of 5 ($P = 1.0 \times 10^{-8}$ cm^2 s^{-1}). When the potential on the Pt grid/film was switched off to 0 V, after 10 min in the oxidized form the dark blue Fc$^+$ film was again reduced to a dark-yellow film within 30 s and permeation of the probes decreased. Permeability, however, did not revert completely to the original slow rate ($P = 3.8 \times 10^{-9}$ cm^2 s^{-1}). After further redox reactions of the film, the degree of change in permeation decreased and the permeability increased gradually with time although a clear colour change in the film was observed at each redox reaction. Thus, the permeability change of the $FcC_{11}C_{18}N^+/PSS^-$ film by redox reactions was not reversible.

At 20 °C, permeation did not increase and the colour of the film did not change on application of a potential to the $FcC_{11}C_{18}N^+/PSS^-$ film. Thus, oxidation of the film does not occur in the solid state below a T_c of 35 °C of the reduced film, because counter ions compensating the Fc$^+$ cation cannot penetrate into the solid film, and permeability does not change.

When the polyion-complex film was prepared from a single-chain amphiphile ($FcC_{11}N^+$) and polyanions (PSS^-), the permeability of the film was relatively fast ($P = 9.8 \times 10^{-9}$ cm^2 s^{-1}) and was drastically increased on oxidation of the film at all temperatures in the range 15–60 °C. The permeability did not decrease on reduction of the film (see Figure 6). The single-chain-type $FcC_{11}N^+/PSS^-$ film was physically weak and the membrane resistance across the film was very low (5×10^3 Ω) compared with those of double-chain-type amphiphile films [(1–2) × 10^6 Ω].



Fc-C_{11}-N$^+$/PSS$^-$

In order to clarify the effect of temperature on the permeability changes of the $FcC_{11}C_{18}N^+/PSS^-$ film, permeation rates of the probe (**1**) both with and without the applied voltage of 0.5 V *vs.* s.c.e. on the Pt grid/film were obtained at various temperatures. Arrhenius plots are shown in Figure 7. The permeability of the reduced Fc film in the absence of a potential on a Pt grid showed an inflexion near 35 °C, which corresponds to the phase-transition temperature of the reduced form (T_c = 35 °C). Thus, the permeation of probes (**1**) increased simply in the fluid liquid-crystalline state compared with the solid state below the T_c. The permeability of the oxidized Fe$^+$ film increased drastically above 35 °C at the T_c for the reduced form on application of the potential. The increased permeability did not revert completely to the slow rate on re-reduction of the oxidation film (see the Table).

The results of the cyclic voltammogram and the Arrhenius plot of permeability indicate that oxidation of the Fc groups buried in the hydrophobic dialkyl chains of the $FcC_{11}C_{18}N^+/PSS^-$ film occurs only in the fluid state above T_c for the reduced film and permeability changes due to redox reactions are observed above 35 °C. Oxidation of the film would produce largely disordered structures because of the formation of hydrophilic Fc$^+$ ions at the end of the alkyl chains. Since permeability was not reduced by re-reduction, the reformation of the well-packed bilayer structure by the re-reduction of Fc groups buried in the hydrophobic part seems to be difficult. This is confirmed by d.s.c. measurements: the phase transition of lipid bilayers disappeared in the oxidized film and the enthalpy change at T_c for the re-reduced film did not revert to that of the original reduced form.

Permeation through the $2C_{16}N^+Fc/PSS^-$ *Film.*—Figure 8 shows typical time courses for permeation of the probe (**1**)

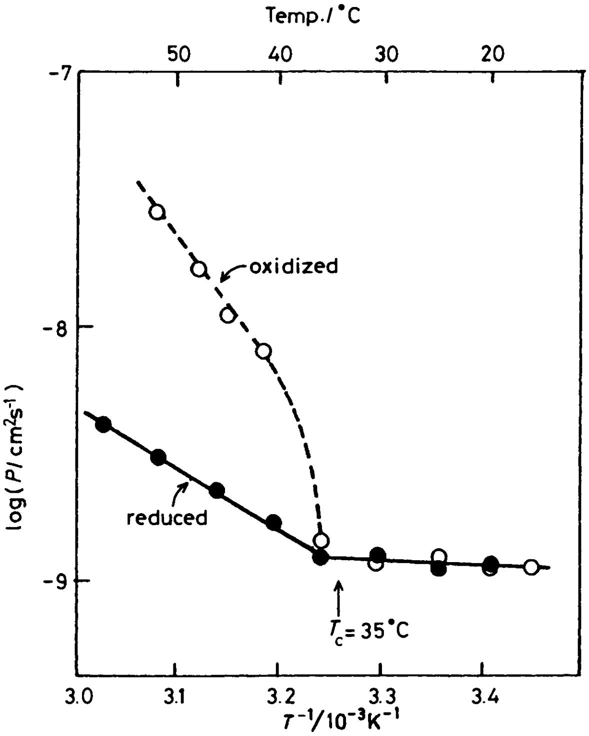


Figure 7. Arrhenius plots of permeation rates through the reduced and oxidized $FcC_{11}C_{18}N^+/PSS^-$ films. An arrow shows the T_c of the reduced film obtained by d.s.c. measurements.

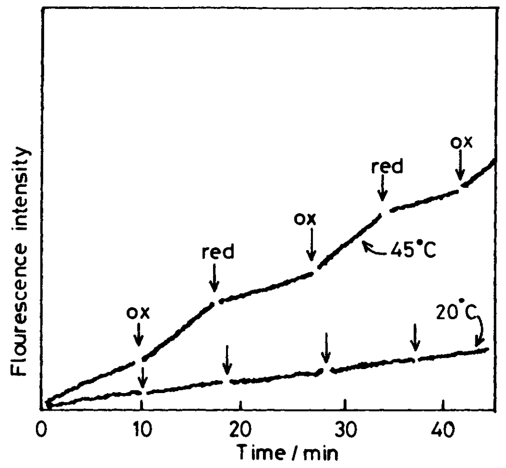


Figure 8. Permeation changes of the probe (**1**) through the 2 $C_{16}N^+$ Fc/PSS^- film. The potential of +0.5 V and 0 V *vs.* s.c.e. were applied to the Pt grid/film at ox. and red., respectively.

through the $2C_{16}N^+Fc/PSS^-$ film containing a ferrocenyl group near the hydrophilic head group. At 45 °C, application of a potential 0.5 V *vs.* s.c.e. to the Pt grid/film, increased the permeability by a factor of 3.2 [$P = (0.50–1.6) \times 10^{-9}$ cm^2 s^{-1}] within 30 s, accompanied by a colour change in the film to dark blue. The permeation enhancement could be observed with a voltage of 0.3–0.7 V *vs.* s.c.e. on the Pt grid: oxidation of the film and permeability enhancement did not occur with the application of a voltage <0.2 V and the permeability change became irreversible with a voltage >0.8 V, owing to damage to the redox sites in the film. Permeability reverted completely to the original slow rate on reduction of the film ($P = 0.51 \times 10^{-9}$ cm^2 s^{-1}). These permeability changes could be reproduced repeatedly for at least 20 cycles without damaging the film, in contrast with those of the $FcC_{11}C_{18}N^+/PSS^-$ film (Figure 6). At 20 °C, the permeability did not increase and the colour of the film did not change, on application of a potential (0.3–0.8 V) to the film on a Pt grid sheet.

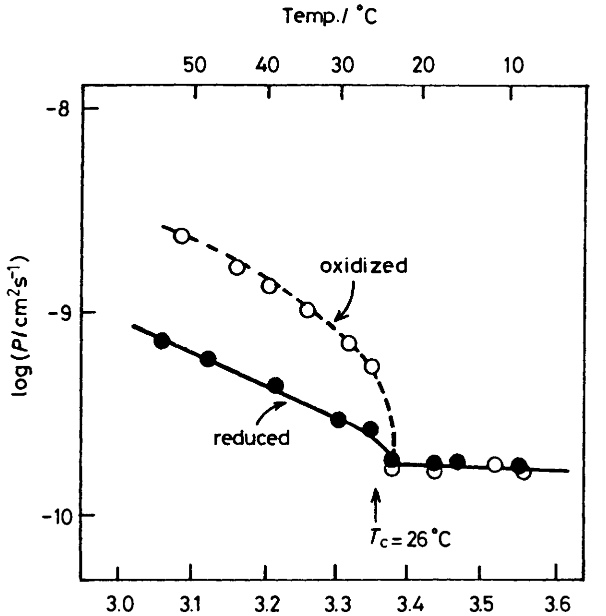


Figure 9. Arrhenius plots of permeation rates through the reduced and oxidized 2 $C_{16}N^+Fc/PSS^-$ films. An arrow shows the T_c of the reduced film obtained by d.s.c. measurements.

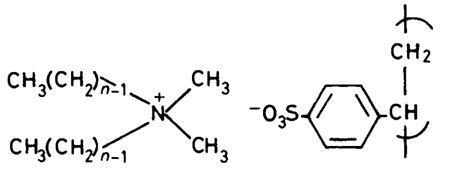
Figure 9 shows Arrhenius plots of the temperature dependent permeations of probe (**1**) through the $2C_{16}N^+Fc/PSS^-$ film. The permeability of the reduced form showed an inflexion at T_c = 26 °C of the reduced film and increased relatively in the fluid liquid crystalline state above the T_c. Oxidation of the film occurred above 26 °C and permeability increased significantly compared with that of the reduced film. Permeation changes between the reduced and oxidized form above 26 °C were reversible. In the case of the $2C_{16}N^+Fc/PSS^-$ film containing a redox site near the hydrophilic head group, oxidation seems to cause a slight structure change in bilayer orientations (decreases in T_c from 26 to 16 °C and ΔH from 8.5 to 6.5 kcal mol^{-1}), and then permeability across the film increases. The structure change of multibilayers in the film by redox reactions is not so large and the permeability can be controlled reversibly by continuous redox reactions.

It should be mentioned that the observed permeability enhancement is due to an increase in either the mobility or the solubility of probes in the oxidized form, since the permeability constant (*P*) is composed of diffusion constants (*D*) and solubility constants (*S*). Sada and co-workers have reported[18] that permeation rates of water-soluble dye probes through the composite membrane containing phospholipid multibilayers increase drastically near the T_c of incorporated lipid bilayers, and this permeability enhancement has been explained by the increase in diffusion constants in the fluid bilayers because the solubility constant hardly changed over the wide temperature range spanning the T_c. In our work, the solubility of the water-soluble non-ionic probe (**1**) in the hydrophobic bilayer matrix should be very low and would not change because of the formation of Fc^+ cations in the film by redox reactions. Therefore, observed permeability enhancement can be explained by the increase in the diffusibility of probes due to the disordering of multibilayer film on oxidation.

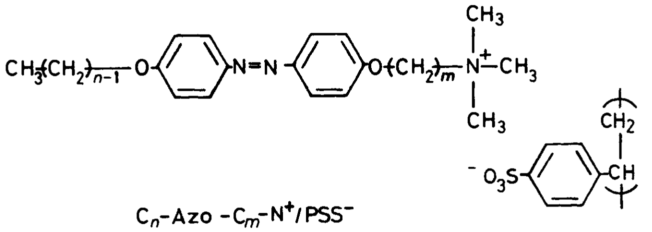
Discussion

As a means of preparing highly ordered multilayer films from amphiphiles, the Langmuir–Blodgett (LB) technique is well-known, transferring monolayers at the air–water interface onto a substrate by dipping processes.[19,20] However, this technique does not give a large area and larger thickness (more than a few micrometers) of a self-supporting, water-insoluble film within a limited time. Recently, a much simpler technique of preparing self-standing multibilayer cast films (Y-type LB membranes) has been developed by several methods: (*i*) casting of a polymer solution of amphiphiles with slow evaporation of solvent,[21–26] (*ii*) corking amphiphiles into a porous filter membrane by dipping the filter into the amphiphile solution and drying it,[3,18,27] (*iii*) polymerization of an aqueous cast film of amphiphile multibilayers,[16,28] and (*iv*), prepaing a polyion complex from cationic bilayer-forming amphiphiles and polyanions, casting a chloroform solution of the complex on a substrate, and drying.[7,8,15] Of these methods, the polyion-complex method gives a stable multibilayer-immobilized film suitable for permeability measurements in aqueous solution over a wide temperature range, and each amphiphile is immobilized with a polymer chain at the head group by ion interactions and still retains the mobility of dialkyl chains in the film.

We have reported that the permeation rate through the polyion-complex multibilayer-immobilized film from a simple dialkyl amphiphile ($2C_nN^+2C_1/PSS^-$) or an azobenzene-containing amphiphile (C_nAzoC_m-N^+/PSS^-) can be changed reversibly by switching on and off an electric field parallel[8] or perpendicular[7] to the membrane, respectively. In previous papers,[1,29] we have reported the permeation control of a viologen-containing, polyion-complex-type multibilayer-immobilized film ($2C_{16}V^{2+}/PSS^-$) by electrochemical redox reactions. The electrochemical reduction of the film from dicationic $2C_{16}V^{2+}$ to radical cationic $2C_{16}V^{+\bullet}$ bilayers on a Pt minigrid sheet raised the phase-transition temperature of the bilayers from 24 to 38 °C. Thus, at 30 °C, the permeability of the oxidized $2C_{16}V^{2+}/PSS^-$ film is fast due to the fluid liquid-crystalline state at the low T_c (24 °C) of the $2C_{16}V^{2+}$ bilayers, and the permeability decreases by the reduction of the film because the reduced $2C_{16}V^{+\bullet}$ bilayers have a high T_c at 38 °C and exist in the solid state at 30 °C. Thus, in this film, an electrochemical energy is converted into the transition at the T_c (fluidity change) of multibilayer films.

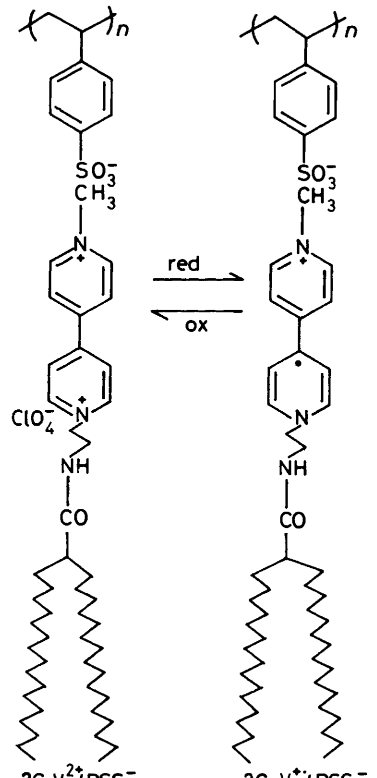


$2C_nN^+2C/PSS^-$



C_n-Azo-C_m-N^+/PSS^-

In this paper, we have described the increases in permeability through the ferrocene-containing multibilayer film ($FcC_{11}C_{18}N^+/PSS^-$ and $2C_{16}N^+Fc/PSS^-$) due to the oxidation of Fc groups only in the fluid state of the reduced bilayer film above the T_c. The membrane resistance across the film is also decreased by oxidation. Thus, the permeation enhancement by the oxidation can be explained by the structure change of the bilayers (decrease of T_c and ΔH) or a change in the hydrophobic nature of the film (formation of cationic Fc^+ groups), both of which cause the increase of the diffusion of fluorescent probes through the film.



$2C_{16}V^{2+}/PSS^-$ (T_c = 24 °C)

$2C_{16}V^{+\bullet}/PSS^-$ (T_c = 38 °C)

Permeability of chloride anions through polypyrrole films deposited on a gold grid sheet has been reported by Murray and co-workers to be increased by positive-charge formation on the film due to oxidation.[9] The release of various anions from a polypyrrole film deposited on an electrode has also been reported to be changed by electrochemical redox reactions.[30,31] These ion-gate films of polypyrrole, however, showed slow responses, requiring 15–30 min for the permeation change, and poor reproducibility because of the morphological damage of the polymer film by repeated redox reactions. In contrast, our ferrocene- or viologen-containing multibilayer-immobilized films showed high physical stability and a quick response to permeation changes. The fluid bilayer structure is important for the achievement of reversible, signal-receptive permeation changes.

In conclusion, the polyion-complex-type, amphiphile-immobilization method gives us physically stable, water-insoluble, self-supporting films which nevertheless retain the fluidity of bilayer structures, and the ferrocene-containing multibilayer-immobilized film showed an electrochemically reversible permeability change in redox reactions. This film should be an interesting model for synaptic systems in which a nerve impulse (electric signal) initiates the rapid release of a chemical intermediary such as acetylcholine.

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