Large-Area (600 mm^2) Bimolecular Film in Aqueous Solutions. The Effect of an Amine-Ni^{2+} Complex on the Stability of *cis*-9-Octadecenylamine Bimolecular Film

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A large, planar, bimolecular film (bilayer, 600 mm^2) of *cis*-9-octadecenylamine (oleylamine) was found to be formed in an $NiCl_2$ solution. From the effect of the added metal ions on the proton magnetic resonance spectrum of octadecenylamine, it appears that the complex between the octadecenylamine molecule and the metal ion increases the stability of the bilayer. The thickness of the bilayer depended strongly on the kind of solvent in the film-forming solution. When decane, a normal alkane solvent, was used, the thickness of the bilayer was greatest (4.6 nm), and the temperature effect was smallest. The experimental results are explained in terms of (i) the influence of the solvent on the conformation of the octadecenyl chain and (ii) the amount of solvent remaining in the film.

A previously reported bilayer of 1-tetradecanol in an aqueous solution was stable, but not large (1 mm^2).[1,2] An increase in the bilayer size is essential to the improvement of the accuracy of electrical and optical measurements. Using a dipping technique, Tien *et al.* have prepared the largest planar bilayers thus far produced (*ca.* 100 mm^2).[3] Spherical bilayer has also been produced with areas up to *ca.* 100 mm^2 using a bubble technique.[4] For most measurements, however, a spherical bilayer is not as appropriate as a planar bilayer.

In order to prepare a large bilayer, it is necessary to increase the layer stability. The bonds between the constituent molecules and their surroundings may have a stabilizing effect on the bilayer. For example, it is expected that complex formation between the hydrophilic group of molecules in the bilayer and multivalent ions in the aqueous solution increases the stability of the bilayer.

On the other hand, it has been found empirically that no stable bilayer can be prepared from a dilute film-forming solution (surface-active molecule/organic solvent).[2]

Therefore, the molecules of an unsaturated hydrocarbon chain are more suitable than those of a saturated chain in view of the solubility. As a system which satisfies the above factors governing stability, a *cis*-9-octadecenylamine bilayer was formed in an $NiCl_2$ solution.

Experimental

Materials. The distilled *cis*-9-octadecenylamine (purity$>$99%) used in this study was purchased from the Tokyo Kagaku Seiki Co., Ltd. The aqueous solution surrounding the bilayer were prepared from redistilled water and guaranteed reagents (Wako Pure Chemical Industries, Ltd.). All solvents were of reagent grade.

Preparation of Large Bilayer Films. Bilayers were prepared by two methods (I and II), using the dipping technique. Method I: (1) Two compartments (F_1 and F_2) of the same volume were separated by a Teflon plate A (thickness: 2 mm) having a unique hole (width: 13 mm, length: 60 mm) at its center (Fig. 1). A 30 mM* $NiCl_2$ solution was introduced into both compartments through tubes (T_1 and T_2) until the water levels were raised to a point just below the hole in the Teflon plate (Fig. 1a). A small volume D (*ca.* 0.1 cm^3) of an octadecenylamine/alkane solution [*i.e.*, the film-forming solution, 0.2/1 (v/v)] was then delivered onto the rim around

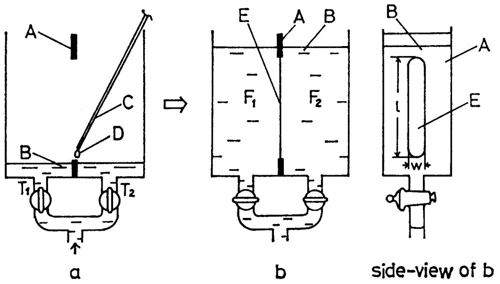

Fig. 1. Method (I) for the preparation of large bilayer. A: Teflon plate, B: aqueous solution, C: Teflon tube, D: a drop of the octadecenylamine/decane solution, E: large bilayer (width w = 12 mm, length l = 60 mm), T_1 and T_2: tube.

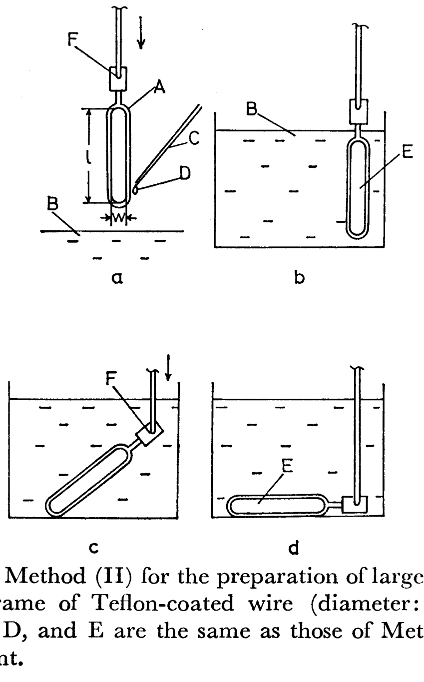

Fig. 2. Method (II) for the preparation of large bilayer. A: Frame of Teflon-coated wire (diameter: 2 mm), B, C, D, and E are the same as those of Method (I), F: joint.

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* Throughout this paper, 1 M = 1 mol/dm^3.

the hole with the help of a Teflon tube C (diameter: 1 mm) attached to a syringe. (2) The 30 mM $NiCl_2$ soultion was gradually added to both compartments. Two water levels were maintained at equal heights. The water levels were then raised until they reached *ca.* 5 mm above the hole (Fig. 1b). By this procedure, a colored film formed over the hole in the Teflon plate. After *ca.* 10 min, the colored film became black. Method II: A frame of Teflon-coated wire (diameter: 2 mm, Fig. 2a) was used in place of the Teflon plate. (1) A small volume D of the film-forming solution was delived onto the frame A in a manner similar to that described for Method I. (2) By lowering the frame into the aqueous solution (30 mM $NiCl_2$), a colored film formed on the frame (Fig. 2b). (3) After the frame had been further lowered, the major axis of the frame was rotated around the joint F through 90 ° (Figs. 2c and 2d). Thus, a longer film formed in the lateral direction, as well as in the vertical direction.

After *ca.* 10 min, these colored films became black. In order to improve the stability of the black film, care was taken to protect the film from vibration. The stability of the film was also increased by prepainting the frame with the sample solution.

Measurements. In order to estimate the thickness of the black film, the electrical capacitance of the film was measured by means of an AC bridge (Ando Electric Co., Ltd., Model TR-IC). Measurements were carried out in the frequency range from 30 Hz to 3MHz. The resistance of the black film was also obtained by the transient DC method. The potential across the film was measured using an electrometer of high-input impedance (Takeda Riken Industry Co., Ltd., Model TR-8651) by means of a pair of reversible Ag/AgCl electrodes. The area of the film was estimated from a photograph. The temperature of the film and its surrounding solution was maintained constant to within an accuracy of ±0.5 °C by thermostatically-controlled water circulation. Proton magnetic resonance (PMR) spectra of metal octadecenylamine complexes were obtained at 60 MHz and 35 °C, using a Hitachi R-24 spectrometer. Monolayer characteristics of the octadecenylamine at the air–aqueous solution interface were obtained using the Wilhelmy method.

Results and Discussion

The black film formed by Method I is suitable for the measurement of electric properties, while that formed by Method II is suitable for the measurement of optical properties. The stability of the films was greatly influenced by the kind of solvent in the film-forming solution. When octane and dodecane were used as film-forming solution solvents, planar black films with areas up to 600 mm^2 were formed in the 30 mM $NiCl_2$ solution. In the case of decane as the solvent, the largest bilayer (5000 mm^2) was obtained. The films persisted over a period of one hour. When hexane, tetradecane, and hexadecane were used as solvents, however, the areas of the black films were less than 10 mm^2.

The stability of the bilayer was also influenced by the cation in the aqueous solution. The order of the stabilities of large black films was $Ni^{2+} > Co^{2+} > Mn^{2+}$. On the other hand, stable black films were not formed in NaCl, KCl, $CaCl_2$, and $MgCl_2$ solutions (30—100 mM). The addition of Ni^{2+} ions changed the PMR spectrum of the octadecenylamine (Fig. 3). The $-NH_2$ signal o octadecenylamine was no longer observable, as can be seen in Fig. 3b. The $-CH_2N<$ signal is shifted downfield by 0.3 ppm and is also broadened. When ethyl glycinate was mixed in a 0.1 mM Cu^{2+} ion solution, the glycine–CH_2– signal changed in a manner similar to that for the $-CH_2N<$ signal.[5] The change in the glycine–CH_2– signal is accounted for by the proximity of the paramagnetic Cu^{2+} ion. Therefore, it appears that the proximity of the paramagnetic Ni^{2+} ion to the nitrogen atom of the octadecenylamine molecule is responsible for the change in the PMR spectrum (Fig. 3b). The magnitude of change in the $-CH_2N<$ signal was dependent on the kind of metal ion in the aqueous solution (Fig. 4). In particular, the addition of Ni^{2+} ions results in a remarkable change in the $-CH_2N<$ signal. The order of the change in magnitude of the $-CH_2N<$ signal was compatible with the Irving-Williams series ($Ni^{2+} > Co^{2+} > Mn^{2+}$), with respect to the stability of the complexation. The order of the stabilities of black films also agree with the Irving-Williams series.

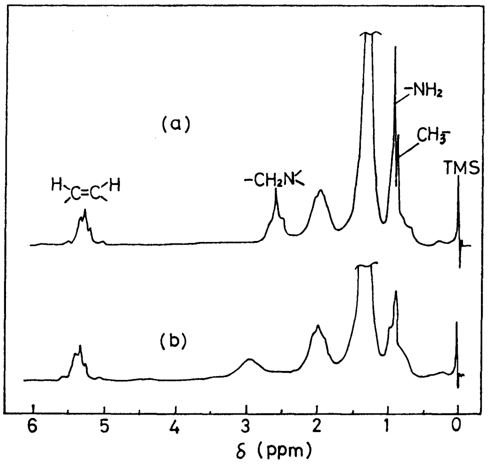

Fig. 3. Effect of added Ni^{2+} ion on the PMR spectrum of octadecenylamine; (a) octadecenylamine, (b) 1 dm^3 octadecenylamine plus 4 mM $NiCl_2 \cdot 6H_2O$. Internal reference: TMS.

From the above argument, it can be assumed that the interaction (complex formation) between the octadecenylamine molecule and metal ion (particularly,

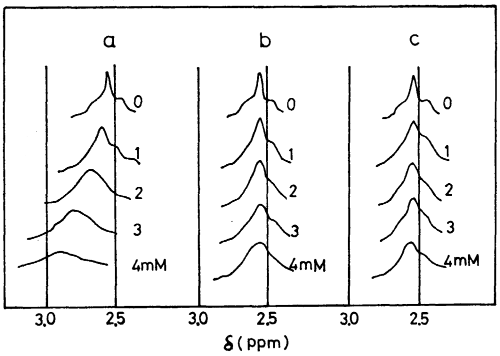

Fig. 4. Effect of the concentration of added metal ions on the $-CH_2N<$ signal of octadecenylamine; (a) $NiCl_2 \cdot 6H_2O$ plus octadecenylamine, (b) $CoCl_2 \cdot 6H_2O$ plus octadecenylamine, (c) $MnCl_2 \cdot 4H_2O$ plus octadecenylamine. Internal reference: TMS.

the Ni^{2+} ion) increases the stability of black film and that black films of large area are thus formed in aqueous solutions.

In accordance with the suggestion of Hanai *et al.*,$^{6)}$ the static capacitance C_t (0 Hz) of the entire system (*i.e.*, the black film and its surrounding aqueous solution) is equal to the capacitance C_m of the black film itself, $C_t(0\ \text{Hz}) = C_m$. For several systems it was found that the static capacitance and the capacitance at 1 kHz agree with each other, $C_t(0\ \text{Hz}) = C_t(1\ \text{kHz})$. Thus, the capacitance of the black film itself can be approximated by the observed capacitance of the entire system at 1 kHz. This approximation is the same as that which was used for 1-tetradecanol bilayers.$^{1)}$ The capacitance of the black film itself depends strongly on the kind of hydrocarbon solvent used in its formation. In Fig. 5, it is seen that with decane as the film-forming solution solvent, the capacitance is smaller than that for any other alkane. In CoCl$_2$ solutions, as well as in NiCl$_2$ solutions, decane minimizes the capacitance.

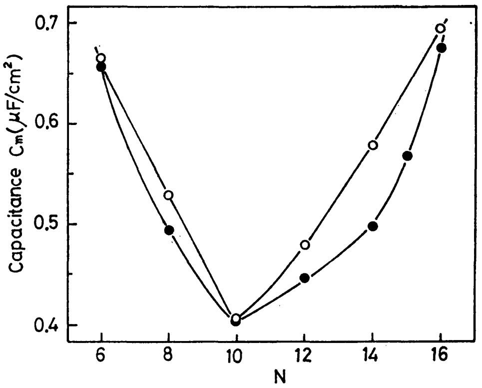

Fig. 5. Capacitance per unit area of black films of octadecenylamine dissolved in various alkanes. *N* is the number of carbon atoms of alkane used as solvent. ○: In 30 mM NiCl$_2$ solution, ●: in 30 mM CoCl$_2$ solution.

The capacitance of the black film is influenced both by the thickness and the dielectric constant of the hydrocarbon core of the film. Since the volume fraction of the solvent in core is not known, the dielectric constant of the core cannot be unequivocally estimated. Thus, the thickness of the black film cannot be accurately calculated from the capacitance measurement. Assuming, however, that the dielectric constant of the core has an intermediate value between that of the solvent of the sample solution and that of the octadecenyl chains, the thickness of the core may be estimated, as is shown in Fig. 6. The thickness of the film has a maximum value in the case of decane. The variation in thickness suggests that the hydrocarbon solvent greatly affects the conformation of the octadecenyl chains.

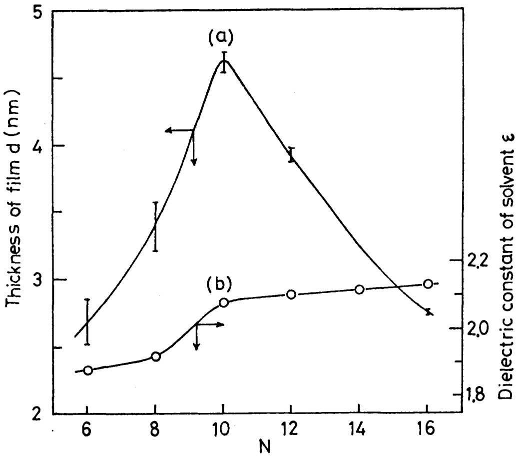

Fig. 6. Curve (a): calculated thickness of black film in the 30 mM NiCl$_2$ aqueous solution. *N* is the number of carbon atoms of alkane (solvent of the sample solution). The upper end of vertical line indicates the calculated value, assuming the dielectric constant of the core of black film to be equal to the value in curve (b) (*i.e.* the dielectric constant of normal alkane in the same solution). The lower end of vertical line indicates the calculated values, assuming the dielectric constant of the core to be that 1-octadecene, 2.15. The curve shows the mean value.

Fettiplace *et al.*$^{7)}$ have reported the for hydrocarbon solvents of chain lengthgreater than decane, the thickness of black films (of glycerol monooleate or of lecithin) decrease with increasing solvent chain length. In addition, the following fact was observed. For solvents of chain length shorter than decane, the thickness of the octadecenylamine films decreases markedly with decreasing length of the hydrocarbon chain of the solvent (Fig. 6). This experimental, result can be accounted for both by (i) the influence of the solvent on the conformation of octadecenyl chain and (ii) that of the amount of solvent remaining in the film as described below. (i) The influence of the solvent on the conformation of the octadecenyl chain: The monolayer of octadecenylamine on the NiCl$_2$ solution (30 mM) is not condensed, but expanded. As a result, it appears that the molecular cavity (vacancy) between octadecenyl chains is larger than that between octadecyl chains, but that the octadecenyl chains are contiguous with each other due to the Van der Waals force which minimizes the molecular cavity. This property of octadecenylamine may appear also in black films of this molecule (note the properties of monolayers and bilayers of tetradecanoic acid$^{1)}$). Therefore, the effective length of a octadecenyl chain in a black film (*i.e.*, the thickness of the film) becomes shorter than the length of the fully extended form. Since the alkane molecules used as solvents occupy the molecular cavity of the octadecenyl chain, the conformation of the octadecenyl chain should be influenced by both the kind and the amount of solvent remaining in the film. In other words, the effective length of the octadecenyl chain (*i.e.*, the thickness of the black film) should be increased by the solvent remaining in the black film. (ii) The influence of the amount of solvent remaining in the film on the film thickness: Bewing and Zisman$^{8)}$ have estimated the amount of normal alkanes in alkylamine–normal alkane mixed films adsorbed on platinum. Their conclusion is as follows. When both chains contain the same number of carbon atoms, the largest quantities of alkanes remain in the film. When the length of the alkane molecule is shorter or longer than that of the alkylamine molecule, the molar fraction of the alkane remaining in the films decreases. Their conclusion was made on the basis of the alkane remaining in saturated hydrocarbons. On the other hand, Shah and Schulman[9] have suggested from the additivity of the surface areas of mixed lecithin–cholesterol monolayers that the stereochemical dimensions of cholesterol are appropriate for occupying the molecular cavities in lecithin monolayers. In the present case, it appears from curve (a) in Fig. 6 that decane rather than other normal alkanes tends to remain among the octadecenyl chains. The decrease in the molar fraction of alkane in the film increases the conformational change of the octadecenyl chains, and the film thus becomes thinner. Consequently, decane as a solvent results in maximum film thickness.

From the above argument, the change in the film thickness may be interpreted on the basis of the solvent in the film.

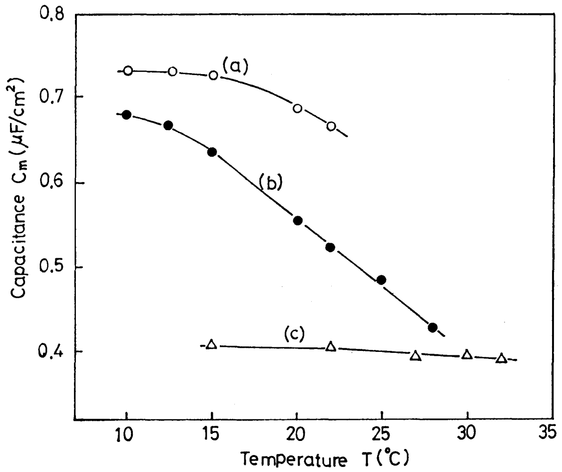

Fig. 7. Effect of temperature on the capacitance of black films in the 30 mM $CoCl_2$ solution.
(a): Film formed from octadecenylamine/hexane solution, (b): Film formed from octadecenylamine/tetradecane solution, (c): Film formed from octadecenylamine/decane solution.

Figure 7 shows the influence of temperature on the capacitance of the films. For films formed from an octadecenylamine/hexane or tetradecane solution, a marked effect of temperature on the capacitance of the film is found. It appears that the temperature dependence of the capacitance is associated with the molar fraction of the solvent in the films. That is to say, the molar fraction of the solvent in the films decreases with decreasing temperature, and then the octadecenyl chains in the film approach each other in order to reduce the molecular cavity from which the solvent molecules emerged. Consequently, the capacitance of the film increases with decreasing temperature. Since the capacitance of octadecenylamine and hexane films remains constant below 15 °C, it appears that hexane is almost completely excluded from the film. On the other hand, for black films produced in octadecenylamine/decane solutions, the capacitance also remains constant. However, the reason for the constancy of curve c in Fig. 6 differs from that for the flat part of curve a. As described above, it may be assumed from curve a that decane rather than other alkanes tends to remain in the octadecenyl chains. Therefore, since decane molecules in the film are hardly excluded, in spite of the decrease in temperature (from 32 to 15 °C), no temperature dependence of the capacitance is observed. The experimental results shown in Fig. 7 is, therefore, consistent with the assumption for curve a in Fig. 6.

For the film formed in an octadecenylamine/decane solution, the resistance of the $NiCl_2$ solution (30 mM) was in the range from 4 to $8 \times 10^6\ \Omega\ cm^2$. For films formed from octadecenylamine dissolved in other solvents, their resistances were from 1 to $5 \times 10^6\ \Omega\ cm^2$. In view of the uncertainties in the experimental results, it cannot be asserted that films formed with decane had higher resistances. The above result, however, suggests that a change in the film thickness has no significant effect on the film resistance. In other words, it appears that the rate-determining step for the permeation of ions across the black film is not the stage at which the ions move through the hydrocarbon core of the film, but the stage at which the ions enter from the aqueous solution into the hydrocarbon core of the film.

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References

1) M. Okuyama, T. Yoshida, T. Tanii, M. Kunugi, and I. Ishizaki, *Bull. Chem. Soc. Jpn.*, **48**, 191 (1975).

2) M. Okuyama and T. Yoshida, *Maku* (*Membrane*), **1**, 263 (1976).

3) H. T. Tien, S. Carbone, and E. A. Dawidowicz, *Kolloid-Z.*, **212**, 165 (1966).

4) R. Pagano and T. E. Thompson, *Biochim. Biophys. Acta*, **144**, 666 (1967).

5) R. Mathur and N. C. Li, *J. Am. Chem. Soc.*, **86**, 1289 (1964).

6) T. Hanai, D. A. Haydon, and J. Taylor, *Proc. R. Soc. London, Ser. A*, **281**, 377 (1964).

7) R. Fettiplace, D. M. Andrews, and D. A. Haydon, *J. Membr. Biol.*, **5**, 277 (1971).

8) K. W. Bewig and W. A. Zisman, *J. Phys. Chem.*, **67**, 130 (1963).

9) D. O. Shah and J. H. Schulman, *J. Lipid Res.*, **8**, 215 (1967).