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The Electrocapillary Curves of the Phosphatidylcholine Monolayer at the Polarized Oil-Water Interface. II. Double Layer Structure of Dilauroylphosphatidylcholine Monolayer at the Nitrobenzene-Water Interface

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Electrocapillary curves have been measured at the polarized nitrobenzene-water interface in the presence of dilauroylphosphatidylcholine (DLPC) in the nitrobenzene phase. DLPC formed a saturated monolayer when the concentration of DLPC was above 10 μmol dm^{-3}. The area occupied by a DLPC molecule in the saturated monolayer was 0.93 nm^2, indicating that the monolayer was in the liquid-expanded state. The stability of the monolayer was strongly affected by the electrical potential difference across the interface. The monolayer was stable when the interface was negatively polarized, i.e., the potential in the aqueous phase was negative with respect to that in the oil phase. The potential of zero charge shifted to a negative potential as the adsorption of DLPC increased, which indicated the specific adsorption of lithium ions in the aqueous phase to the head group of the DLPC molecules exposed to the aqueous phase. When the interface was positively polarized, the adsorption of DLPC abruptly decreased and the monolayer appeared to be disrupted.

Phospholipid monolayers formed at the air-water interface and at the oil-water interface have been extensively used to study the surface properties of the monolayers, which are expected to represent, to some extent, the surface properties of phospholipid bilayers as well as those of biological membranes.[1,2] Among those monolayers, the monolayer formed at the polarizable oil-water interface is particularly suitable for studying the electrochemical properties of phospholipid monolayers, since the potential drop across the interface can be accurately controlled externally at this interface.[3] Thus, the monolayer at the polarized oil-water interface enables us to study its electrochemical properties in detail, e.g., the double layer structure in the vicinity of the monolayer and the ion-transfer reaction across the monolayer. The electrochemical study of the retardation of the ion transfer across the oil-water interface in the presence of a phospholipid monolayer has been reported by Koryta et al.,[4] Girault and Schiffrin,[5] and Cunnane et al.[6] However, the properties of the monolayer, itself, formed at these interfaces are not yet fully understood. To make full use of the advantage of the monolayer at the polarized interface, it is important to elucidate the properties of the phospholipid monolayers formed at the polarized interface. For example, knowledge regarding the double layer structure seems indispensable to correctly interpret the measured change of the rate constant of the ion-transfer reaction, e.g., taking account of the Frumkin correction.[7] Several attempts have been made to characterize the phospholipid monolayer at the polarized oil-water interface. We reported the conditions of the monolayer formation of L-α-dilauroylphosphatidylcholine (DLPC) at the nitrobenzene-water interface after measuring the double layer capacitance of the interface.[8] Although double layer capacitance is a sensitive measure which can be used to detect any structural change of the interface, the basic properties of the interface, such as the absolute amount of adsorbed substances and the excess surface charge density, can be estimated thermodynamically only through electrocapillary curve measurements. Girault and Schiffrin have studied the electrocapillary curves at the 1,2-dichloroethane-water interface in the presence of egg york phosphatidylcholine and phosphatidylethanolamine.[9] They have also studied the effect of pH in the aqueous side of the interface on the electrocapillary curves at different concentrations of phosphatidylcholine.[10] However, a detailed analysis of the electrocapillary curves has not yet been reported. In Part I of this study, we found that an equilibrium electrocapillary curve can be obtained for the DLPC monolayer at the polarized nitrobenzene-water interface in the potential range where the potential of the aqueous phase was negative with respect to that in the nitrobenzene phase.[11] In the present communication, we will focus our attention mainly on the electrocapillary curves in this potential region of the interface recorded at various concentrations of DLPC.

Experimental

The interfacial tension was measured at 25±0.05 °C using a computer-aided pendant drop method.[12,13] Details of this method were described in Part I.[11] In the measurement, the drop of an aqueous solution was formed in the nitrobenzene phase to make sure that the amount of the DLPC was sufficient to make up a saturated monolayer. A laboratory-made four-electrode potentiostat of the type proposed by Samec et al.[14] was used to control the potential drop across the polarized interface. The nitrobenzene used as a solvent of the oil phase was distilled and then treated with active alumina. DLPC was purchased from Sigma and was used without further purification. DLPC was dissolved in a 3:2 toluene-chloroform mixture as a stock solution. The nitrobenzene solution of DLPC for the measurement was prepared so that the final concentration of a 3:2 toluene-chloroform mixture in nitrobenzene was 10% (v/v). The methods for the preparation of tetrapentylammonium tetraphenylborate (TPnATPB) and of the tetrapentylammonium chloride (TPnACl) solution have been described elsewhere.[15] All other chemicals were of reagent grade. The nitrobenzene solution was equilibrated with an aqueous solution of lithium chloride prior to a measurement.

Results

The electrochemical cell employed in the present study was

I	II	III	IV	V	VI	VII
Ag	AgCl	0.02 M[†] TPnACl	0.1 M TPnATPB + $x\mu$M DLPC	0.05 M LiCl	AgCl	Ag
		(W)	(NB)	(W)		

The interface between Phases IV and V was a polarized nitrobenzene-water interface. The other nitrobenzene-water interface between Phases III and IV was a nonpolarized interface.[15] The potential of the right hand side of cell with respect to the left is denoted as E^{W-}_{O+}, where the super- and subscripts, W− and O+, indicate that the right-hand side and left-hand side reference electrodes are reversible to an anion (Cl^-) in Phase V and a cation ($TPnA^+$) in Phase IV, respectively. The electrocapillary curves were recorded between $x=0$ and 50 in the cell two hours after the formation of an interface in order to ensure the establishment of adsorption equilibrium.[11]

The potential of zero charge, E_{pzc}, of this system was found to be 0.32 V (Fig. 2) when $x=0$. Since at E_{pzc} the Galvani potential difference between the nitrobenzene and the aqueous phases is very small in the absence of specific adsorption,[16] the potential referring to E_{pzc} at $x=0$ can be taken as a convenient measure of the Galvani potential difference, $\Delta^W_O\phi$. As we have shown in Part I, the DLPC monolayer gives an equilibrium electrocapillary curve when $\Delta^W_O\phi<0$, i.e., in Region I (see Fig. 1). In the more positive potential range, Region II, the interfacial tension, γ, abruptly increased and the data in this region were much less reproducible (Fig. 1). Below, we mainly deal with the electrocapillary curves measured in Region I.

The electrocapillary curves at $x=0$, 0.5, 1, 2, 5, 10, 20, and 50 are shown in Fig. 2. Each point represents the average value of triplicate measurements. For the electrocapillary curves at the four higher concentrations, only data from Region I are plotted. In Region I, the interfacial tension decreased as the concentration of DLPC was increased. Here, γ is plotted against the logarithm of the concentration of DLPC in the nitrobenzene phase, $\ln c_{DLPC}$, at different values of E^{W-}_{O+} (Fig. 3). At a higher concentration of DLPC, the γ vs. $\ln c_{DLPC}$ curve at any value of E^{W-}_{O+} gave a straight line. This

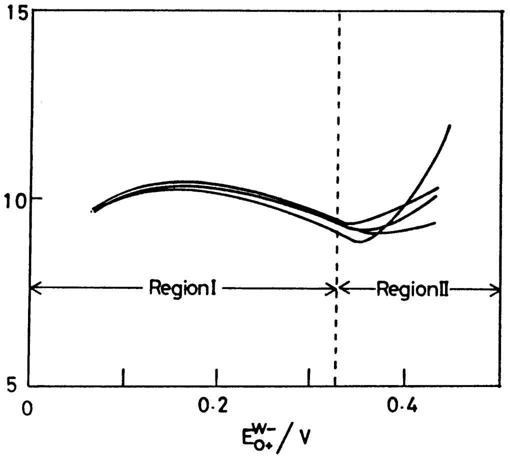

Fig. 1. The reproducibility of the electrocapillary curve when $c_{DLPC}=10$ μmol dm^{-3}.

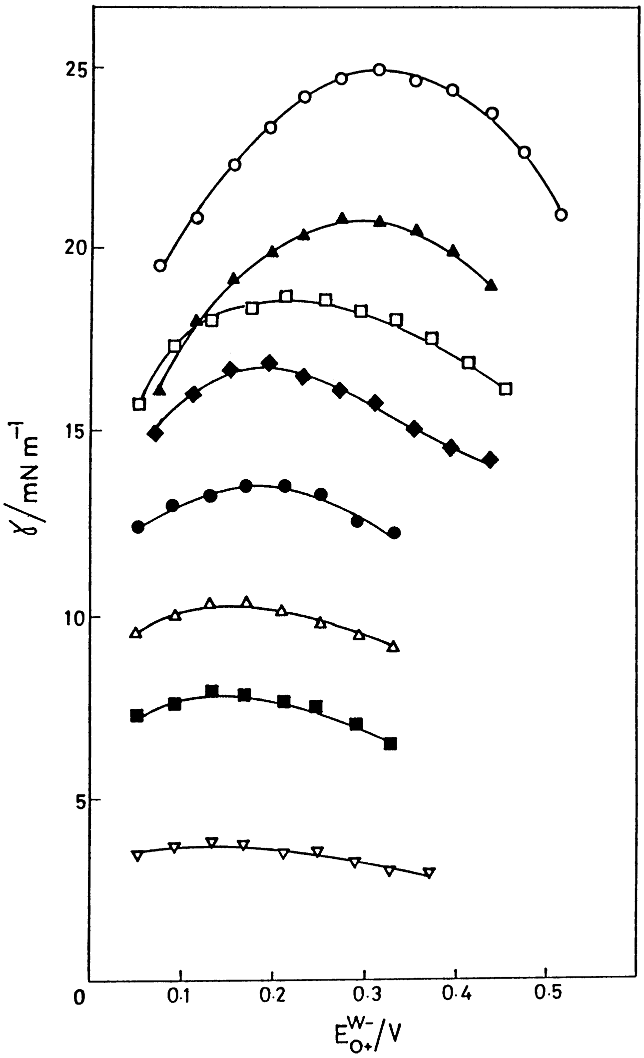

Fig. 2. The electrocapillary curves at 25 °C for the interface between the nitrobenzene solution of 0.1 mol dm^{-3} TPnATPB and the aqueous solution of 0.05 mol dm^{-3} LiCl in the presence of x μmol dm^{-3} DLPC in the nitrobenzene phase: x=0(○), 0.5(▲), 1(□), 2(◆), 5(●), 10(△), 20(■), and 50(▽).

[†] 1 M=1 mol dm^{-3}.

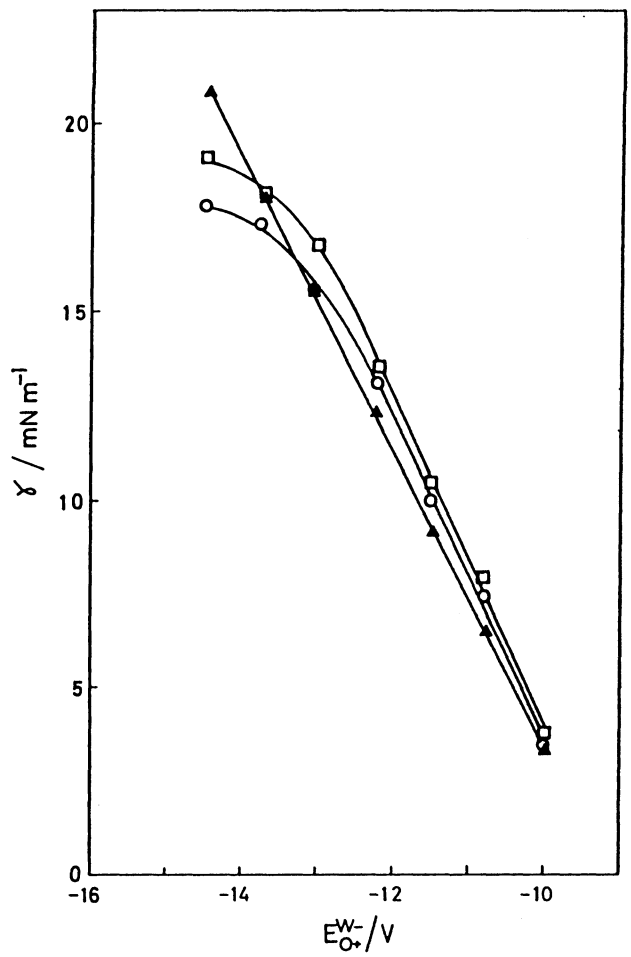

Fig. 3. γ vs. ln $c_{\rm DLPC}$ curves at $E^{\rm W-}_{\rm O+}$=0.09(▲), 0.15(□), and 0.33(○) V.

linear dependence indicates the formation of a saturated monolayer of DLPC at the interface. The possibility of micelle formation of the DLPC in the nitrobenzene phase is excluded within the concentration range studied, because of the lack of a break point in the curves. The adsorbed amount of DLPC was calculated from the slope of the curves in Fig. 3 using the thermodynamic relation based on the electrocapillary equation at the polarized oil-water interface:[17]

$$\Gamma_{\rm DLPC} = -\frac{1}{RT}(\partial\gamma/\partial \ln a^{\rm NB}_{\rm DLPC})_{E^{\rm W-}_{\rm O+},\mu_i}, \qquad (1)$$

where $\Gamma_{\rm DLPC}$ is the relative surface excess of DLPC, $a^{\rm NB}_{\rm DLPC}$ the activity of the DLPC in the nitrobenzene phase, R the gas constant, and T the absolute temperature. The subscripts of the parenthesis in the right-hand side of Eq. 1 indicate that the derivative must be taken while keeping $E^{\rm W-}_{\rm O+}$ and the chemical potentials of components except for DLPC constant. In the calculation, $a^{\rm NB}_{\rm DLPC}$ was taken to be equal to the concentration of DLPC in the nitrobenzene phase, $c_{\rm DLPC}$. Since the concentration of the DLPC in the nitrobenzene phase is small, $\Gamma_{\rm DLPC}$ can be equated to the adsorbed amount of DLPC at the interface. The adsorption isotherm of DLPC at $E^{\rm W-}_{\rm O+}$=0.210 V is shown in Fig. 4. The maximum adsorption of DLPC, $\Gamma^{\rm m}_{\rm DLPC}$, calculated from the limiting slope of the γ vs. ln$c_{\rm DLPC}$ curves was plotted as a function of $E^{\rm W-}_{\rm O+}$ in Fig. 5. $\Gamma^{\rm m}_{\rm DLPC}$ was independent of $E^{\rm W-}_{\rm O+}$ between $E^{\rm W-}_{\rm O+}$=0.08 and 0.280 V; the monolayer was stable in this potential range. This is in accordance with our previous results, obtained from differential capacitance measurements of the DLPC monolayer at the same interface.[8] The $\Gamma^{\rm m}_{\rm DLPC}$ in this potential range was found to be 1.76×10^{-10} mol cm^{-2}, which corresponds to the value of the occupied area of a DLPC molecule (0.93 nm^2). The isotherm fitted well to the Frumkin isotherm (Fig. 4). The solid curve in Fig. 4 is a regression curve using the Frumkin isotherm:[18]

$$Bc_{\rm DLPC} = \frac{\theta}{1-\theta}\exp(-2a\theta), \qquad (2)$$

where B is the adsorption coefficient, $\theta=\Gamma_{\rm DLPC}/\Gamma^{\rm m}_{\rm DLPC}$, and a is the interaction parameter. In the regression

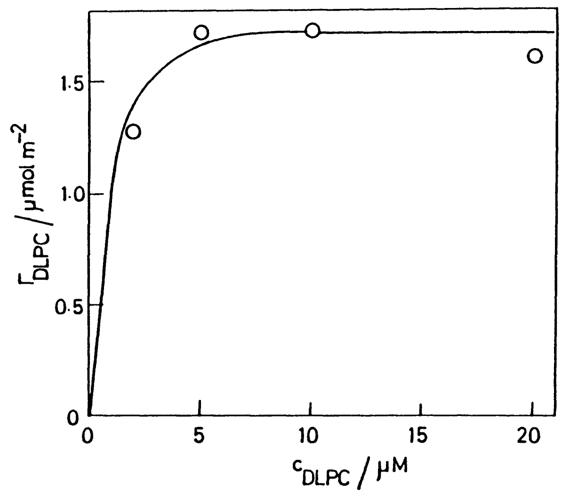

Fig. 4. Adsorption isotherm of DLPC at $E^{\rm W-}_{\rm O+}$=0.210 V. The solid line is the regression curve using the Frumkin isotherm.

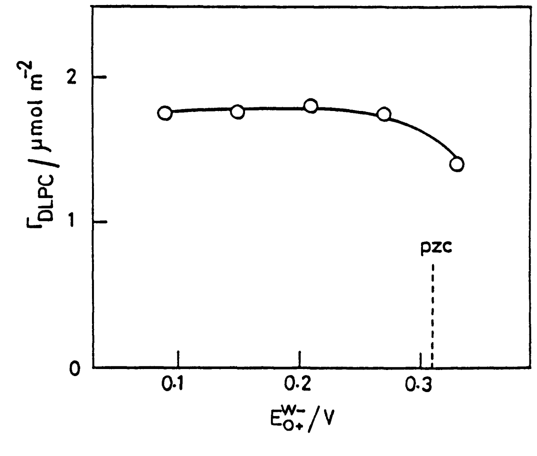

Fig. 5. Dependence of $\Gamma_{\rm DLPC}$ at $c_{\rm DLPC}$=10 μmol dm^{-3} on the applied potential.

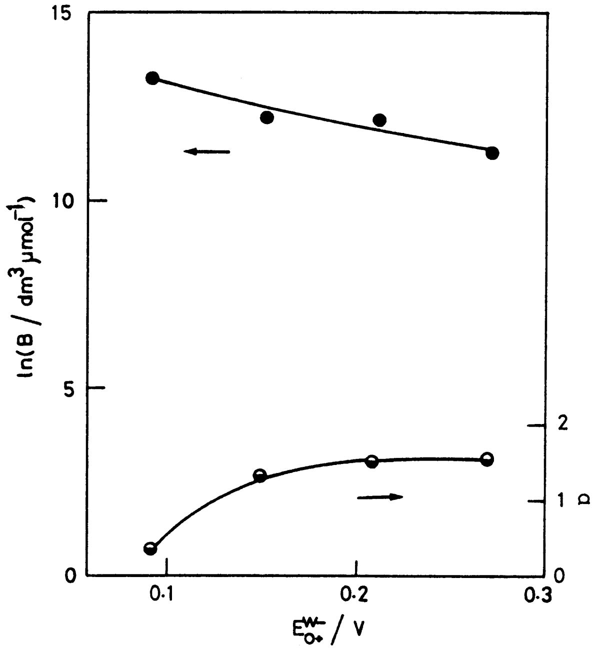

Fig. 6. Dependence of the logarithm of the adsorption coefficient, ln*B*, and the interaction parameter, *a* on the applied potential.

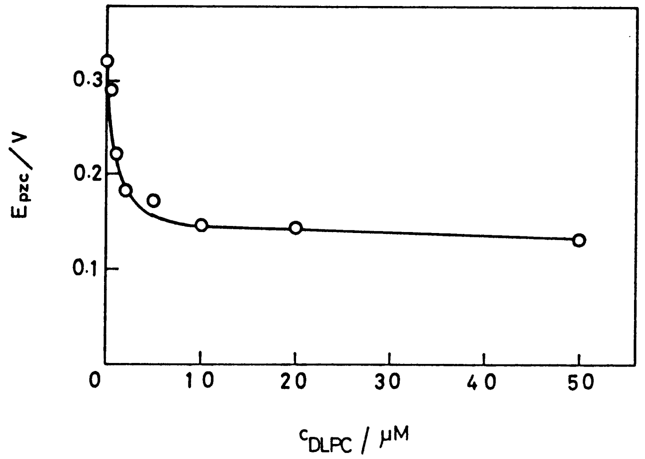

Fig. 7. Shift of the potential of zero charge, E_{pzc}, with the increase of the DLPC concentration.

analysis, the value of Γ^m_{DLPC}, estimated from the limiting slope of the γ vs. $\ln c_{DLPC}$ plot, was employed. The adsorption isotherm in Fig. 4 ensures the absence of multi-layer formation at the interface. The logarithm of B and a are plotted in Fig. 6 as a function of E^{w-}_{o+}. As can be seen in Fig. 2, the potential of zero charge, E_{pzc}, shifted to a negative potential with the increase of c_{DLPC} (Fig. 7). The shift reached 170 mV when the saturated monolayer was formed.

Discussion

Figures 3, 4, and 5 clearly manifest the formation of a saturated DLPC monolayer at the polarized nitrobenzene-water interface. The monolayer was stable when the $\Delta^w_o\phi$ was negative. This confirms our previous conclusion concerning the monolayer formation of DLPC obtained from capacitance measurement.[8] The value of the area occupied by a DLPC molecule in the saturated monolayer, 0.93 nm^2, is 20% greater than the value estimated from the capacitance measurements using the spread monolayer technique.[8] If we take account of the uncertainty intrinsic to the spread method, i.e., the necessary extrapolation of the capacitance value to the value at t=0,[8] the values from the two independent methods agree with each other and suggest that the saturated monolayer is in the liquid-expanded state.[19] The ln B was weekly dependent on the applied potential (Fig. 6) and decreased with a positive increase in the potential. The value of ln B at E^{w-}_{o+}=0.22 V was 12.5, corresponding to an adsorption free energy of 31 kJ mol^{-1}. This is in agreement with the value estimated from the capacitance measurement at the same polarization.[8] Wandlowski et al. reported a similar value from the capacitance measurements.[20] The a parameter was also varied with the applied potential, yet the value of a was always positive.

The negative shift of the E_{pzc} with an increase in the DLPC adsorption (Fig. 7) means that there exists a Galvani potential difference at the E_{pzc}, such that the inner potential in the aqueous phase becomes more negative with respect to the inner potential in the nitrobenzene phase. There are four possible sources for this shift of the E_{pzc}: the nitrobenzene and the water molecules at the interface, the head group and the carbonyl group of the adsorbed DLPC molecules, and the specific adsorption of ions in the presence of the monolayer. The contribution from the first factor would be very small, because the $\Delta^w_o\phi$ at the E_{pzc} in the absence of the specific adsorption of ions at the interface is very small.[16] The second and third factors are the permanent dipole moments of the DLPC molecules oriented at the interface. The phosphatidylcholine molecule has a zwitterion group, whose isoelectric point is at pH=5.5.[19] The pH of the aqueous solution studied was typically 5.8, and, hence, all the head groups exposed to the aqueous side of the interface were in zwitterionic form. The dipole moment of the zwitterion group is so large that even its minute orientation normal to the interface should give rise to large dipole potential. However, neutron diffraction studies of the phosphatidylcholine bilayer have demonstrated a coplaner arrangement of the choline segment, i.e., with the P-N dipole approximately parallel to the plane of the bilayer.[21] A similar conclusion has been reached in a study of the phosphatidylcholine monolayer at the air-water interface.[22] Moreover, since the phosphate group is directly attached to the glyceride part of the phosphatidylcholine and the trimethylammonio group is away from it, the deviation of the orientaion of the zwitterionic group from the parallel position, if any, should result in a shift of the pzc to the positive direction, which is at variance with the present results. On the other hand, the C=O bond of the carbonyl group in the phosphatidylcholine monolayer at the air-water interface is oriented with its oxygen atom pointing toward the aqueous phase and with the angle of C=O axis being within 20° with respect to the interface.[23] If the carbonyl group is oriented similarly in the polarized nitrobenzene-water interface, the contribution of this oriented dipole is estimated to be 70 mV at the saturation coverage. This contribution to the surface potential, however, can explain only part of the shift of the E_{pzc} observed experimentally. Consequently, the above-mentioned three possible contributions arising from the oriented dipoles cannot fully account for the observed shift of 170 mV. The remaining factor that can account for the observed change of the E_{pzc} is the specific adsorption of an Li^+ ion in the aqueous phase to the hydrophilic head group of the adsorbed monolayer. This specific adsorption should result in a potential difference in the aqueous phase, such that the potential at the inner Helmholtz plane (IHP) where the Li^+ ion is specifically adsorbed, ϕ_1, is positive with respect to the bulk of the aqueous phase. This direction of the shift is in harmony with the observed trend of E_{pzc}. The specific adsorption of Li^+ ions to the phospholipid surface has been found in the liposomes made of phosphatidylcholine.[24] Pospisil et al. reported the specific effect of Li^+ ion on the phase transition of dipalmitoylphosphatidylcholine adsorbed at the mercury-solution interface.[25] The specific binding of Li^+ ion to a lipid bilayer made of a phosphatidylserine membrane was found to significantly affect its physicochemical properties and physiological functions.[26]

If the specific adsorption of Li+ does occur at the interface, the shift of E_{pzc} is expected to depend on the concentration of Li^+ ion in the aqueous phase. Figure 8 shows that the shift of E_{pzc} with the LiCl concentration exceeds the shift attributable to a change in the potential of the reference electrode in the aqueous phase (dotted line). The latter change was calculated using the Davies equation[27] for an evaluation of the mean ionic activity of LiCl. The observed large shift of E_{pzc} with the Li^+ ion concentration (solid line) confirms the presence of the specific adsorption of Li^+. If we make a simplified assumption, that the distribution of the nonspecifically adsorbed ions in the aqueous phase follows the Gouy-Chapman theory, we can estimate the degree of the Li^+ ion binding to the interface. The shift of E_{pzc}, after subtracting the potential drop due to the oriented carbonyl group, which was assumed to be 70 mV irrespective of the $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi$ and of the LiCl concentration, should correspond to ϕ_1. Although ϕ_1 is in general larger than the potential at the outer Helmholtz plane (OHP), ϕ_2, which passes through the center of the nonspecifically adsorbed Li^+ ion at its closest approach to the interface, the error would be small if we equate ϕ_1 with ϕ_2, because some of the nonspecifically adsorbed ions can enter beyond the OHP due to the sparse distribution of the adsorbed DLPC molecules(cf. Figs. 9a and 9b). Then, by using the Gouy-Chapman theory, the surface charge density corresponding to the ϕ_2 potential at a given concentration of LiCl can be calculated. This surface charge density may be taken as the specifically adsorbed amount of Li+ ions, since the potential is at the E_{pzc} and there is no excess surface charge density in the nitrobenzene phase. The specifically adsorbed amount of Li^+ ion, ${}^{i}\Gamma_{\mathrm{Li}}$, and the degree of the counterion binding, $\alpha={}^{i}\Gamma_{\mathrm{Li}}/\Gamma_{\mathrm{DLPC}}$, thus obtained, are listed in Table 1. The Γ_{DLPC} was assumed to be constant over the concentration range of LiCl studied.

In the case of the phosphatidylcholine bilayer, lipophilic anions are much more strongly adsorbed at the membrane surface than lipophilic cations.[28] Flewelling and Hubbell attributed this asymmetric adsorbability to the potential drop across the membrane surface and then estimated for the egg yolk phosphatidylcholine membrane that the potential at the membrane surface is 240±70 mV positive with respect to the bulk solution.[29] It is interesting that this magnitude of the membrane potential is comparable with the amount of the shift of the E_{pzc} found in the present study. The double-layer structure of the nitrobenzene-water interface in the presence of the satu-

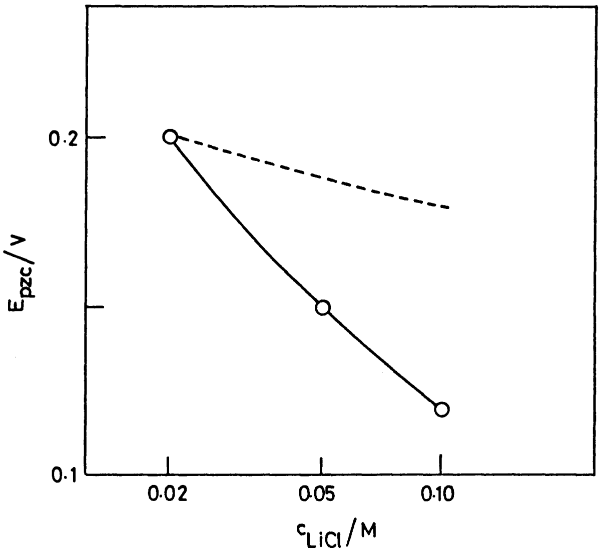

Fig. 8. Shift of the potential of zero charge with the concentration of DLPC in the nitrobenzene phase. The dotted line is the expected curve in the case of no specific adsorption.

Table 1. Specific Adsorption of Li^+ Ion to the Dilauroylphosphatidylcholine Monolayer

$c(\mathrm{LiCl})$	ΔE_{pzc}	$F\,{}^{i}\Gamma_{\mathrm{Li}}$	α
$\mathrm{mol\,dm^{-3}}$	mV	$\mu\mathrm{C\,cm^{-2}}$	%
0.02	55	2	12
0.05	100	9	50
0.10	100	13	74

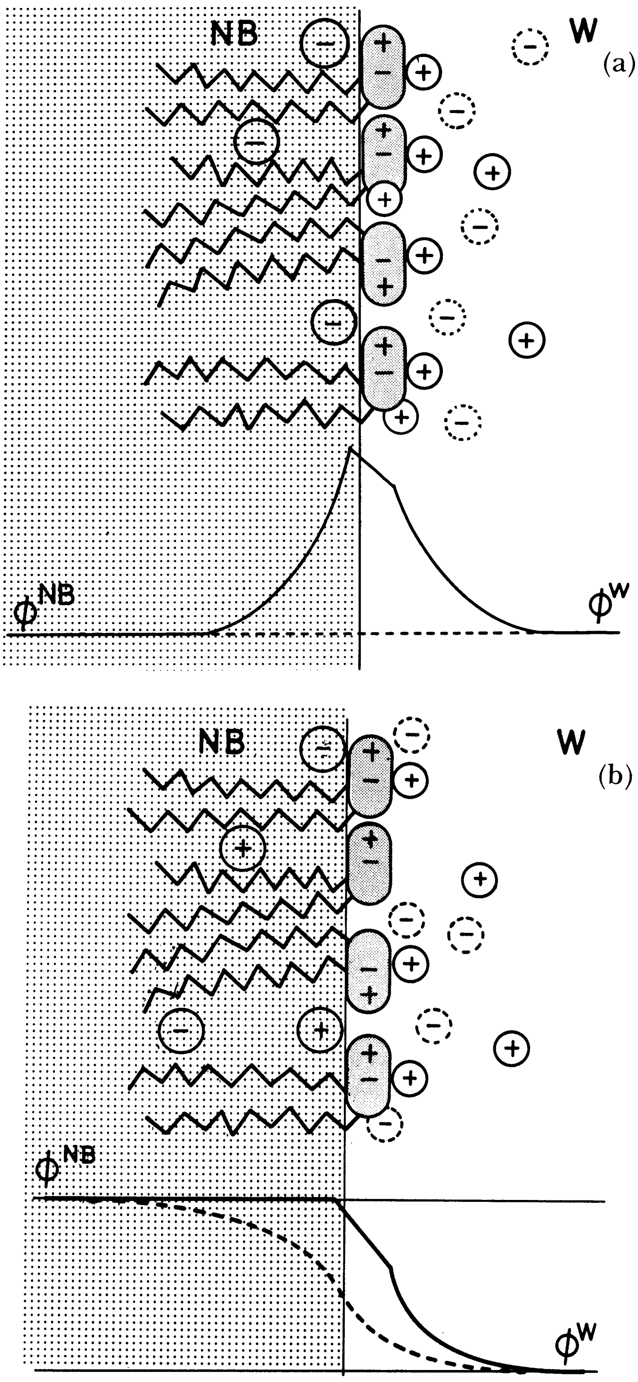

Fig. 9. Schematic representation of the double layer structure and the potential profile of the interface between the nitrobenzene solution of TPnATPB and the aqueous solution of LiCl in the presence (——) and in the abscence (----) of DLPC adsorption: a) at the E_{pzc} for the base electrolyte, b) at the E_{pzc} for the saturated DLPC monolayer.

rated DLPC monolayer, deduced from the present analysis, is illustrated in Fig. 9a at $E^{W-}_{O+}=0.32$ V ($\Delta^{W}_{O}\phi \simeq 0$) and in Fig. 9b at the E_{pzc} for the saturated DLPC monolayer, respectively. The inner Helmholtz plane in the aqueous phase was postulated to lie on the line passing through the centers of the specifically adsorbed Li^+ ions. Also, schematically illustrated are the penetration of $TPnA^+$ and TPB^- ions in the nitrobenzene phase into the hydrocarbon part of the DLPC monolayer and the random orientation of the zwitterion group in the plane parallel to the interface.

The abrupt increase in the interfacial tension (Fig. 1) when the potential was increased from Region I to II would predict the appearance of a peak on the corresponding equilibrium capacitance vs. potential curve. However, experimentally no such peak at this potential was observed on the differential capacitance vs. potential curves.[8,20,30] In the presence of the adsorption of a surface active substance, the capacitance, $C=(\partial q/\partial E)_c$, is generally composed of two terms:[31]

$$\left(\frac{\partial q}{\partial E}\right)_c = \left(\frac{\partial q}{\partial E}\right)_\Gamma + \left(\frac{\partial q}{\partial \Gamma}\right)_E \left(\frac{\partial \Gamma}{\partial E}\right)_c , \tag{3}$$

where q is the excess surface charge density, E the electrical potential difference, c the bulk concentration of the adsorbate, and Γ the surface excess of the adsorbate. The first term on the right-hand side of Eq. 3 is a quantity called "true capacitance" and the second term is the "adsorption pseudocapacitance." In Region I, the Γ_{DLPC} is practically independent of E^{W-}_{O+} (Fig. 5) and, hence, the second term makes no significant contribution to the total capacitance. On the other hand, in Region II, the desorption of DLPC from the interface certainly takes place, judging from the recovery of the interfacial tension (Fig. 1). Then, the $(\partial\Gamma/\partial E)_c$ term in Eq. 3 is not generally negligible. When the adsorption is a diffusion limited process, which is likely to be the case for the DLPC adsorption, because of the low value of c_{DLPC}, the second term in Eq. 3 may be rewritten as[31]

$$\left(\frac{\partial q}{\partial \Gamma}\right)_E \left(\frac{\partial \Gamma}{\partial E}\right)_c = \left(\frac{\partial q}{\partial \Gamma}\right)_E \left(\frac{\partial \Gamma}{\partial E}\right)_{c,\omega=0} \frac{b+2}{(b+1)^2+1}, \tag{4}$$

where $(\partial\Gamma/\partial E)_{c,\omega=0}$ is $(\partial\Gamma/\partial E)_c$ at $\omega \to 0$, $\omega=2\pi f$ is the angular frequency, and f is the ac frequency employed in a capacitance measurement. The b is given by

$$b=(2\omega/D)^{1/2}(\partial\Gamma/\partial c)_E, \tag{5}$$

where D is the diffusion coefficient of the adsorbate. Only when b is small, can the measured capacitance be seen as being the thermodynamic capacitance.[32] In the case of the Frumkin isotherm,

$$(\partial\Gamma/\partial c)_E = \frac{\Gamma_m}{c} \frac{\theta(1-\theta)}{1-2a\theta(1-\theta)} . \tag{6}$$

If we assume that the diffusion coefficient of the DLPC in the nitrobenzene is the same as that of dilauroylphosphatidylethanolamine, 3.8×10^{-6} cm^2 s^{-1},[33] we obtain $b=6.38\sqrt{\omega}$ when $c=10$ µmol dm^{-3}, $a=1$, and $\theta=0.5$. Accordingly, even at $f=1$ Hz the last factor of the right hand side of Eq. 4 is significantly smaller than 1, resulting in the disappearance of the expected peak on a measured capacitance vs. potential curve. If the adsorption-desorption process is sluggish giving a "frozen" monolayer, the second term in Eq. 3 is again smaller than the equilibrium value. Thus, the measured capacitance in Region II is in any case smaller than the equilibrium capacitance and the integration of the measured capacitance vs. potential curve would not give the excess surface charge density.

The abrupt increase of the interfacial tension in the adsorption of egg yolk phosphatidylcholine at the 1,2-dichloroethane-water interface has been suggested to be due to the protonation of the phosphatidylcholine molecules at the interface.[9] In the experimental conditions employed in the present study, this protonation model is not applicable. The pK_a of the phosphate group of phosphatidylcholines at the air-water interfaces is of the order of 3.0.[34] On the other hand, in the present study the ϕ_2 potential in the aqueous phase does not exceed 30 mV,[16] even in the positive extreme of Region II, because of the relatively high concentration of LiCl. Accordingly, the surface pH is not lower than 4.5 and, hence, practically all the DLPC molecules at the interface should be in a zwitterionic form,[34] even when the change of pH in the vicinity of the interface is taken into account. A probable explanation of the abrupt rise of γ would be a desorption of the DLPC molecules from the interface accompanied by the process of the facilitated Li^+ ion transfer in Region II.

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