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Monolayer Formation of Dilauroylphosphatidylcholine at the Polarized Nitrobenzene-Water Interface

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Double layer capacitance of the polarized nitrobenzene-water interface has been measured in the presence of the adsorption of L-α-dilauroylphosphatidylcholine (DLPC). The double layer capacitance was lowered with the increase of the DLPC concentration in the negative branch of the potential range where the aqueous phase was in negative potential with respect to the nitrobenzene phase. A saturated monolayer of DLPC was formed at a concentration of DLPC above 20 μmol dm^{-3}. The area occupied by a DLPC molecule at the saturated monolayer was 0.73 nm^2 which was estimated by comparing the capacitance data with those obtained from a spread monolayer of DLPC at the interface. In the negative branch the monolayer was stable over 100 mV of the applied potential across the monolayer. The adsorption was described by the Frumkin adsorption isotherm. The interaction between the adsorbed DLPC molecule was weak, corresponding to the penetration of nitrobenzene molecules and, possibly, ions between the hydrocarbon chains of DLPC. This penetration also explains the high capacitance value of 11 μF cm^{-2} for the saturation monolayer. In the potential range where the aqueous phase is positive, the capacitance increased with the DLPC concentration.

Recent advances in electrochemistry of the oil-water interface have established the basic experimental methods and the relevant theories for studying the ion-transfer processes taking place at the interface.[1-4] Based on this recent achievement a quantitative study of the effect of an adsorbed monolayer of phospholipids and related compounds on ion transfer processes across the oil-water interface has now become possible. It is expected to provide fundamental information for a better understanding of the mass-transfer processes occurring at biological as well as artificial membranes. Koryta et al.[5] have reported that the ion-transfer reaction at the nitrobenzene-water interface became irreversible in the presence of lecithin in the nitrobenzene phase. Similar retardation of ion-transfer reaction has also been found for the tetraethylammonium ion transfer across the 1,2-dichloroethane-water interface in the presence of egg yolk lecithin.[6] However, in these studies the state of the adsorbed lecithins at the interface was not characterized enough to investigate in depth the mechanism of the retardation of the ion-transfer rate. Even the formation of a monolayer at the interface has not been confirmed. The purpose of the present communication is to establish the condition of the monolayer formation of phosphatidylcholine at the nitrobenzene-water interface, which is essential to the detailed study of the double layer structure and ion-transfer processes across lipid monolayers. We have studied the monolayer formation using L-α-dilauroylphosphatidylcholine (DLPC). This phospholipid monolayer is also expected to serve as a model particularly useful for studying ion-phospholipid interactions, since at polarized oil-water interfaces the structure of the electrical double layer can be studied in detail by electrocapillary measurements. Watanabe et al. have studied the adsorption of phospholipids at the methyl isobutyl ketone-water interface and have elucidated interesting qualitative features of phospholipid adsorption including the effect of pH on the aqueous solution side.[7-9] Their studies are mainly concerned with the effect of foreign substances as well as solution pH rather than the adsorption properties of the phospholipid monolayer itself. Recently, Girault and Schiffrin[10] have reported the adsorption behavior of phosphatidylcholine and phosphatidylethanolamine at the polarized 1,2-dichloroethane-water interface by measuring electrocapillary curves. In the present study, we have measured the differential capacitance of the polarized nitrobenzene-water interface in the presence of phosphatidylcholine monolayers formed by the two methods: i.e. adsorbed monolayer and spread monolayer.

Experimental

DLPC was obtained from Sigma Ltd. and was used without further purification. Tetrapentylammonium tetraphenylborate (TPnATPB) was prepared from tetrapentylammonium iodide and sodium tetraphenylborate and was twice recrystallized from acetone-ethanol mixture. Aqueous solution of reagent grade tetrapentylammonium chloride (TPnACl) was treated with silver chloride to remove trace iodide ion. Lithium chloride monohydrate (a Merck's Spurapur grade) was dissolved in water to prepare the stock solution. The concentration of TPnACl and LiCl solutions were determined by potentiometric titration with a standard silver nitrate solution. Nitrobenzene was distilled under reduced pressure. The middle 60% of the distillate was shaken with active alumina and then equilibrated with water after filtrating off the precipitates. Triply distilled water was used throughout the measurements. All other chemicals used were of reagent grade.

The electrochemical cell is represented by:

I	II	III	IV	V	VI	VII
		50 mmol dm^{-3}	0.1 mol dm^{-3}	0.1 mol dm^{-3}		(I)
Ag	AgCl	TPnACl	TPnATPB	LiCl	AgCl	Ag
		(W)	(NB)	(W)		

The interface between Phases IV and V is the polarized nitrobenzene-water interface. The potential of the right-hand side of the cell with respect to the left is denoted as E^{W-}_{O+}, where super- and subscripts, W− and O+, indicate that the right-hand and left-hand side reference electrodes are reversible to an anion, Cl^- ion in Phase V, and a cation, $TPnA^+$ ion in Phase IV, respectively.

A flat polarized nitrobenzene-water interface was formed at the orifice of a glass tube of 0.548 cm inner diameter. Other details of the measuring cell have been described elsewhere.[11] The double layer capacitance was measured by phase-selective ac polarography. The principle of the method and the type of measuring cell have been described previously.[11] An ac voltage of 50.0 Hz and 5 mV^{p-p} was superimposed on the dc voltage. In the adsorption method ac polarograms were usually recorded with a scan rate of 5 $mV\,s^{-1}$ from E^{W-}_{O+}=0.15 to 0.48 V. No change was observed in the shape of the polarogram if recorded with a scan rate of 0.5 $mV\,s^{-1}$ or if the sweep direction was reversed. In the spread method, the impedance was measured at a fixed potential as a function of time. All measurements were made at 25.0±0.5 °C.

A monolayer of DLPC at the interface between Phases IV and V was made in two ways: The formation of the monolayer by adsorption of DLPC from nitrobenzene phase (the adsorption method) and by placing a known amount of DLPC as a toluene-chloroform solution on the interface (the spread method). In the adsorption method a nitrobenzene solution of DLPC was prepared by adding a needed amount of 10 $mmol\,dm^{-3}$ chloroform solution of DLPC to 0.1 $mol\,dm^{-3}$ TPnATPB nitrobenzene solution with a micro syringe. The mixture was then sonicated at 27 °C to dissolve the added DLPC completely. Without this sonication treatment small particles persisted in the solution and sometimes gave rise to a characteristic peak in the far positive potential range in a capacitance vs. potential curve. In the spread method phosphatidylcholine was first dissolved in 3 : 2 (v/v) toluene-chloroform mixture to prepare the stock solutions of various concentrations. The 3 : 2 toluene-chloroform mixture has a density of 1.1, which is smaller than that of the nitrobenzene solution, 1.18, and larger than that of the aqueous solution, 1.0, and was used as a spreading solvent to avoid undesirable mixing due to density difference. This mixed solvent itself introduced no detectable change in the double layer capacitance. The DLPC solutions for the spreading method were stored in a decicator containing the solvent of the same composition in the bottom compartment. On spreading DLPC at the interface the solution of 1×10^{-6} dm^3 was applied through an L-shaped fine glass capillary of 0.55 mm inner diameter which was connected to a 1×10^{-5} dm^3 micro syringe mounted on a micro manipulator. The tip of the capillary was drawn out to a sharp conical shape with an orifice of 0.15 mm inner diameter. First the capillary was inserted in the nitrobenzene solution beneath the interface. Then the tip was brought up through the interface into the aqueous solution side just above the interface and a few tenths of 1×10^{-6} dm^3 DLPC solution were squeezed out of the tip of a micro syringe to form a small "bubble" at the tip. The tip was then brought down slowly to a position just beneath the interface. The "bubble" spontaneously spread away over the interface while the tip was passing through the interface. This procedure was repeated several times to complete the spreading of 1×10^{-6} dm^3 DLPC solution. The time needed for the spreading procedure was typically 30 s. The time at the completion of the final spreading was taken as t=0 for the later analysis of the data. After the spreading, the interface was brought down in close proximity to the glass frit of the reference electrode compartment. The shape of the interface was then readjusted to obtain a flat interface. The solution resistance was then measured promptly. This resistance measurement was made every time before an impedance measurement. Usually it took 0.5—1.5 min from the completion of the spreading to the start of the impedance measurement.

Results and Discussion

Adsorbed Monolayer. The double layer capacitance of the interface was measured in the potential range between E^{W-}_{O+}=0.15 and 0.48 V for ten different concentrations of DLPC from c=0.1 to 100 $\mu mol\,dm^{-3}$. The time required to attain adsorption equilibrium was checked by measuring the capacitance as a function of time. Figure 1 illustrates the time dependence of the capacitance at c=5 $\mu mol\,dm^{-3}$ and at E^{W-}_{O+}=0.20 V after the formation of a new nitrobenzene-water interface at the orifice of the glass tube. In this case, Fig. 1 shows that at least 60 min was needed to reach a time invariant value. We recorded ac polarograms to obtain the capacitance (C) vs. potential (E^{W-}_{O+}) curves after a sufficiently long time, usually 60 min, had elapsed. Some of the results are shown in Fig. 2. The vertical bars in the figure show the standard errors for the triplicate measurements. The capacitance decreased with the concentration of DLPC in the range of E^{W-}_{O+} between 0.15 and 0.35 V as shown in Fig. 2, indicating the strong adsorption of DLPC at the interface. At a concentration higher than 20 $\mu mol\,dm^{-3}$, the capacitance showed no further decrease. This probably corresponds to the formation of a saturated monolayer in this potential range. The minimum capacitance value for this saturated monolyer was 11 $\mu F\,cm^{-2}$ at E^{W-}_{O+}=0.24 V. This is significantly higher than the value, ca. 1 $\mu F\,cm^{-2}$ found in bilayer lipid membrane.[12] If the monolayer is only a half of a bilayer lipid membrane, the capacitance would be at least one fifth of the measured value. The observed high capac-

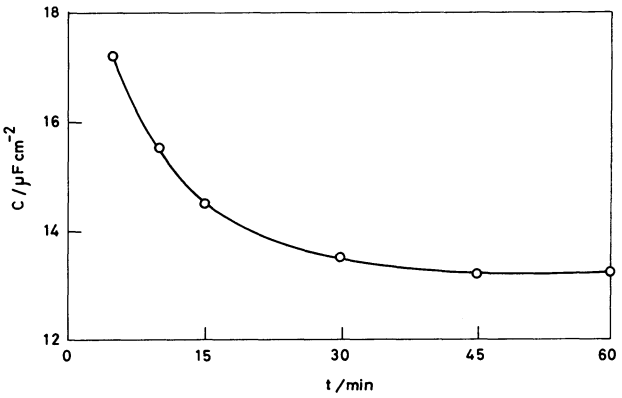

Fig. 1. Change of the double layer capacitance with time for 5 $\mu mol\,dm^{-3}$ DLPC at E^{W-}_{O+}=0.20 V.

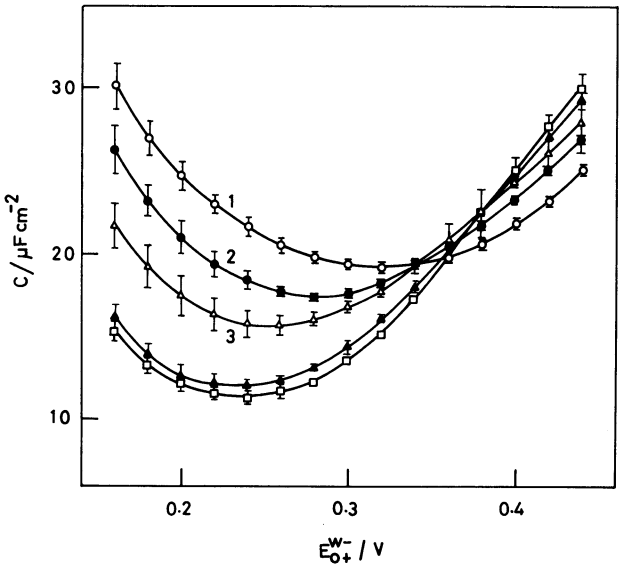

Fig. 2. Equilibrium double layer capacitance vs. applied potential curves recorded by the adsorption method for a given concentration of DLPC: 0(○), 1(●), 2(△), 10(▲), and 50(□) μmol dm^{-3} in nitrobenzene.

itance presumably reflects the penetration of solvent nitrobenzene molecules and, possibly, $TPnA^+$ and TPB^- ions into the hydrocarbon chain part of the monolayer, resulting in the higher effective dielectric constant of the monolayer.

At potentials positive to 0.35 V, the capacitance increased with the DLPC concentration. Since the potential of zero charge for the base solutions, $E^{W-}_{O+,pzc}$, is 0.34 V,[13] the results in Fig. 2 indicate that DLPC exhibits asymmetrical adsorption behavior with respect to the potential drop across the interface. There is a decrease of the capacitance with increasing concentration of DLPC in the negative branch, that is, at $E^{W-}_{O+} < E^{W-}_{O+,pzc}$, but an increase of the capacitance with DLPC concentration in the positive branch, that is at $E^{W-}_{O+} > E^{W-}_{O+,pzc}$ Such a dependence of the adsorption of phospholipid at the oil-water interface may be of importance in relevance to the dependence of physicochemical properties of biological and artificial membranes on the trans-membrane potential. Change of the adsorbed state of phosphatidylcholines with the applied voltage has also been observed at the mercury-solution interface.[14,15]

Spread Monolayer. To confirm the formation of a DLPC monolayer at the interface, we have measured the double layer capacitance after spreading a known amount of DLPC at the interface. After the spreading of the DLPC solution, the capacitance in the negative branch continuously increased with time owing to the desorption of DLPC molecules from the interface to the nitrobenzene phase. The capacitance value at E^{W-}_{O+}=0.24 V is plotted against the square root of time in Fig. 3. The straight line in Fig. 3 up to 900 s indicates that the desorption of DLPC is diffusion

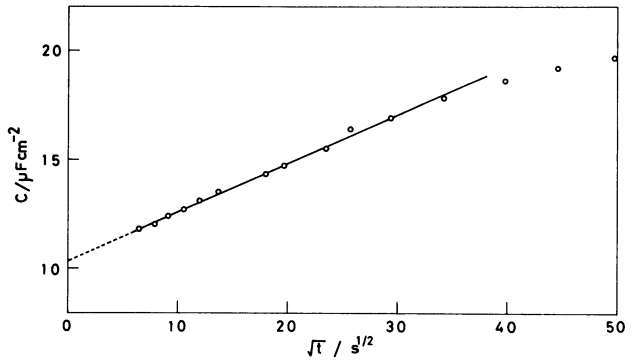

Fig. 3. Change of the double layer capacitance with time at E^{W-}_{O+}=0.240 V after spreading 1×10^{-6} dm^3 of 80 μmol dm^{-3} DLPC in 3:2 toluene-chloroform mixture.

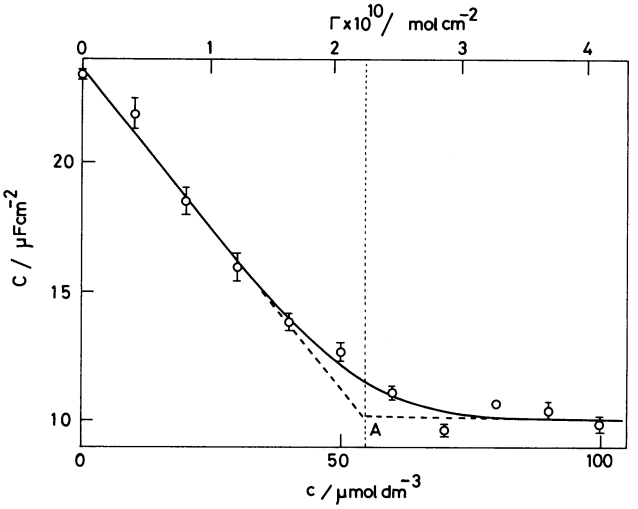

Fig. 4. Extrapolated double layer capacitance at t=0 and at E^{W-}_{O+}=0.240 V as a function of the concentration of DLPC in spreading solution at the interface. Upper scale indicates the calculated amount of DLPC to be present at the interface.

limited.[16] By extrapolating the initial straight line in Fig. 3 to t=0 we obtained the capacitance value at which a known amount of DLPC was supposed to be spread at the interface. The capacitance at t=0 thus obtained at E^{W-}_{O+}=0.24 V is plotted in Fig. 4 as a function of the DLPC concentration in the spreading solution. The upper scale in Fig. 4 indicates the spread amount of DLPC, Γ, in mol cm^{-2} unit calculated by assuming that the whole DLPC molecules contained in the spreading solution participated in the monolayer formation. The vertical bars show the standard errors for the triplicate measurements. The capacitance first decreased linearly with Γ and then reached a saturation value of 10.5 μF cm^{-2} at c=80 μmol dm^{-3} of the spreading solution. This linear change of the capacitance is in accord with Eq. 1,[17] which is based on the Frumkin's parallel plate condenser model:

$$C = C'\theta + (1-\theta)C^0 \quad (1)$$

where C^0 and C' are the C values at θ=0 and 1, respec-

tively, and θ is the surface coverage. At the concentrations of DLPC in the spreading solution above 80 μmol dm^{-3} there was no further decrease in the capacitance, indicating the formation of a saturated DLPC monolayer at the interface. If Eq. 1 holds at any value of θ, the two straight parts of the curve in Fig. 4 should cross each other at Point A. The deviation of the experimental curve from Point A indicates that at higher coverage certain portion of the spread DLPC molecules escaped from the interface and did not take part in the monolayer formation. Only after an excess of DLPC was applied, did the surface seem fully covered with the DLPC molecules as envisaged by the saturation of the capacitance value in Fig. 4. Hence, it would be reasonable to adopt a value of Γ at Point A in Fig. 4 for estimating the packing density of the saturated monolayer. The Γ value at Point A is 2.3×10^{-10} mol cm^{-2}, which corresponds to the area occupied by a DLPC molecule being 0.73 nm^2. This value is close to that for a lecithin bilayer in a liquid crystal phase. In the case of a bilayer composed of dipalmitoylphosphatidylcholine in the presence of excess water, the area was found to be 0.7 nm^2 at temperatures above the critical temparature, T_c=41 °C, whereas the area becomes 0.48 nm^2 at temperatures below T_c.[18] On the other hand, when the area per molecule in the monolayer at the air-water interface is 0.70 nm^2/molecule, didecanoyl- and dimyristoylphosphatidylcholines take on a liquid-expanded state at 25 °C.[19] The present result, therefore, indicates that the saturated DLPC monolayer formed at the nitrobenzene-water interface is in the liquid-expanded state and the DLPC molecules in the monolayer are as dense as the packing of the phosphatidylcholine molecules in the bilayer above T_c. It has been established that in a mesomorphic lamellar phase above T_c, the hydrocarbon chains are in a melted and liquid-like state.[20] This is in harmony with the interpretation for the high capacitance value of DLPC monolayer described above. In the liquid-expanded monolayer the hydrocarbon chains are melted and in a highly fluid and mobile state.[20] In contrast, a more dense liquid-condensed or solid monolayer would give a much lower capacitance value, since in such a membrane the solvent molecules and ions can not be accommodated in the hydrocarbon part which is in a crystaline state. Phillips[21] has recommended the phosphatidylcholine monolayer at the air-water interface occuping 0.70 nm^2/molecule as the most appropriate for membrane-oriented experiments using the monolayer. In this respect, the DLPC monolayer at the polarized nitrobenzene water interface can be used to study the properties, e.g., transfer of ions across the lipid-water interface, of biological as well as artificial lipid membranes.

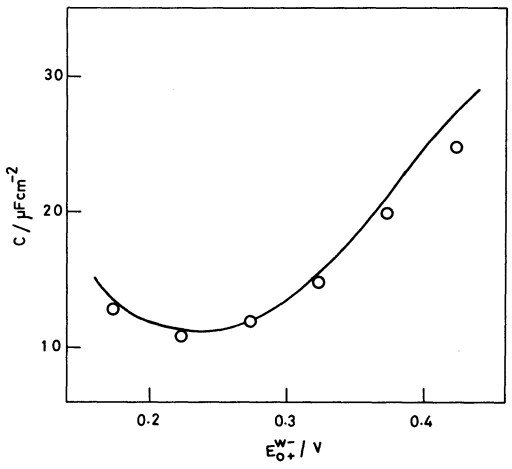

Fig. 5. Comparison of the capacitance data obtained from the adsorption method (—) at 20 μmol dm^{-3} DLPC and the spread method (O). For the spread method, 1×10^{-6} dm^3 of 100 μmol dm^{-3} DLPC was applied to the interface.

In order to check if the monolayer formed by the spread method is the same as that formed in the adsorption method at the higher concentration of DLPC, we compared in Fig. 5 the C vs. E^{W-}_{O+} curve at c=20 μmol dm^{-3} recorded by the adsorption method with the C values at several E^{W-}_{O+} obtained by extrapolating C values to t=0 for the spread monolayer when a DLPC molecule has an area of 0.73 nm^2. The agreement between the values from the two methods is excellent except at the positive extreme of E^{W-}_{O+}. It is clear from this figure that the saturated monolayer formed in the adsorption method is essentially the same as that formed in the spread method. Consequently, a condensed monolayer of DLPC whose occupied area is 0.73 nm^2 can be formed also by the adsorption method, provided that the concentration of DLPC in nitrobenzene is sufficiently high.

Adsorption Isotherm in the Negative Branch. The surface coverage can be estimated using Eq. 1, though this equation is strictly valid only when the change of θ with the electrical potential is negligible,[22] which we assume in the present analysis. This assumption would become inadequate in the positive branch where $d\theta/dE^{W-}_{O+}$ is relatively large. The adsorption isotherm obtained at E^{W-}_{O+}=0.24 V is shown with error bars in Fig. 6. In the calculation of θ we took the C values

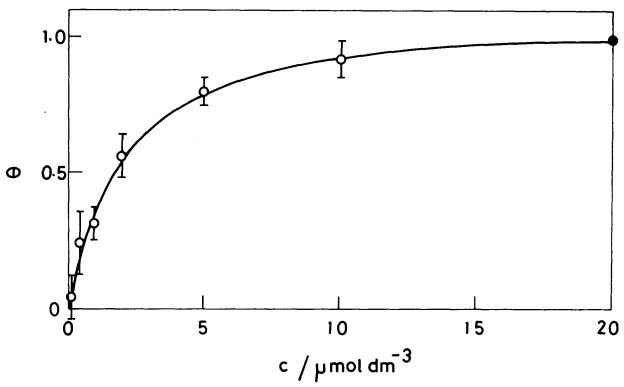

Fig. 6. Adsorption isotherm for DLPC at E^{W-}_{O+}= 0.24 V.

for c=20 mol dm^{-3} as C' in Eq. 1. Similarly, isotherms were obtained at the potentials between E^{w-}_{o+}=0.18 and 0.26 V. These isotherms were analyzed by using the Frumkin isotherm of the form:[17]

$$\beta c = \frac{\theta}{1-\theta}\exp(-2a\theta) \qquad (2)$$

where β is the adsorption coefficient, c is the concentration of phosphatidylcholine, and a is the interaction parameter. Figure 7 illustrates $\ln[\theta/\{c(1-\theta)\}]$ vs. θ plot at E^{w-}_{o+}=0.24 V to test this isotherm. Vertical bars in the figure show the standard error which was calculated from the experimental error in the capacitance measurements taking account of the error propagation. The solid line in Fig. 7 is the curve calculated through the weighted linear regression. The experimental isotherm can thus be fitted well to the Frumkin isotherm. From the intercept and the slope of this regression curve, $\ln\beta$ and the interaction parameter were found to be -1.1 ± 0.3 and -0.28 ± 0.05, respectively, where the intervals designate standard errors. Similar fittings were obtained for the data between E^{w-}_{o+}=0.18 and 0.26 V. The $\ln\beta$ and a values are plotted in Fig. 8 as a function of E^{w-}_{o+}. The vertical bars in the figure indicate the standard errors. In the potential range examined, neither $\ln\beta$ nor a depend strongly on the potential. In other words, DLPC forms a stable monolayer in the potential range of about 100 mV between E^{w-}_{o+}=0.18 and 0.26 V, irrespective of the change of the electrical potential difference across the interface. This greatly simplifies the interpretation of the data when studying the ion-transfer reaction across the DLPC monolayer in this potential range. The average value of $\ln\beta$, -1.1, is equivalent to the standard adsorption energy of 37 kJ mol^{-1}, if we choose 1 mol dm^{-3} and θ=1 for the standard states in the nitrobenzene and in the adsorption layer, respectively. This value of the standard adsorption energy is approximately four times greater than that for the adsorption of hexadecyltrimethylammonium ion at the nitrobenzene-water interface in similar polarization potential range.[23] The small α value, 0.25 on average, means a weak attractive interaction between the adsorbed DLPC molecules. The observed weak interaction is probably due to the penetration of nitrobenzen molecules between the hydrocarbon chains of the phosphatidylcholine molecules resulting in reduced cohesion between the chains. The reduced interaction between hydrocarbon chain is manifested by the behavior of phosphatidylcholines at the oil-water interface: Mingins et. al.[24] have found that diacylphosphatidylcholines having hydrocarbon chains shorter than C_{14} do not show a characteristic phase transition at the heptane-water interface between 5 and 20 °C.

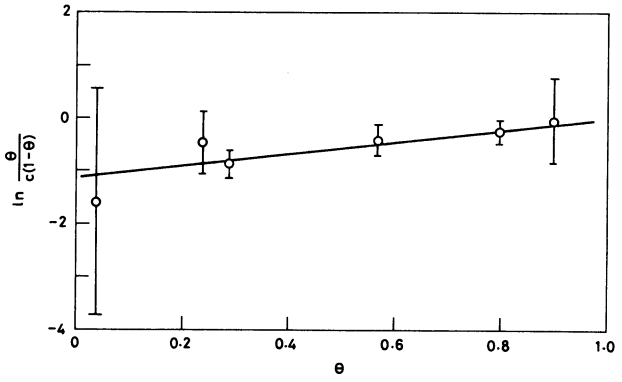

Fig. 7. Test of the Frumkin isotherm for the adsorption of DLPC at E^{w-}_{o+}=0.24 V.

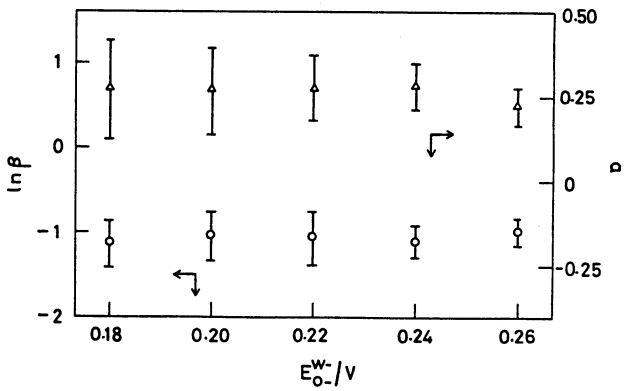

Fig. 8. Logarithm of the adsorption coefficient (○) and the interaction parameter (△) of the Frumkin isotherm for the adsorption of DLPC as a function of the electrical potential.

Adsorption in the Positive Branch. According to our prelimimary results of the interfacial tension measurements, the interfacial tension in this potential region showed a tendency to recover from the extremely low value in the more negative potential range, but still remains lower than the interfacial tension for the base solution. A similar change of the interfacial tension with the applied potential has been observed by Girault and Schiffrin[6,10] at the 1,2-dichloroethane-water interface. Consequently, the observed increase of the capacitance in Fig. 2 may be ascribed to the change of the orientation of adsorbed DLPC molecules, possibly accompanied by a partial desorption with the change of the electrical potential difference across the interface. The concomitant increase of the real part of the addmittance was observed in the positive branch, suggesting the presence of non-equilibrium processes at the interface.

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