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# Divalent Cation-Induced Phase Transition of Phosphatidylserine Monolayer at the Polarized Oil-Water Interface and Its Influence on the Ion-Transfer Processes

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The phase properties of dipalmitoylphosphatidylserine(DPPS) monolayer at a polarized nitrobenzene–water interface have been studied by measuring the double-layer capacitance of the interface, $C_{dl}$, under precise control of the potential drop across the interface. When a saturated DPPS monolayer was in contact with an aqueous 0.1 mol dm$^{-3}$ LiCl solution, the $C_{dl}$ took a value of 9.5 μF cm$^{-2}$. The presence of $Ca^{2+}$ or $Mg^{2+}$ at greater than 2 mmol dm$^{-3}$ in the aqueous phase induced a decrease in $C_{dl}$ down to 1.5 μF cm$^{-2}$, corresponding to the phase transition of the monolayer from a liquid-expanded to a condensed phase. The condensed DPPS monolayer was stable against a change in the applied potential across the interface between $\Delta_o^w\phi$=—0.14 and 0.10 V, where $\Delta_o^w\phi$ is the potential of the aqueous phase with respect to that in the nitrobenzene phase. Cyclic voltammetric measurements of ion transfer showed that the DPPS monolayer in the liquid-expanded state decreased the rate of $ClO_4^-$ transfer from the aqueous to the nitrobenzene phase, but did not affect the rate of tetramethylammonium ion, $TMA^+$, transfer. This indicates the importance of the electrostatic interaction between the negatively charged DPPS monolayer and the transferring ions in determining the rate of ion transfer across the monolayer. In contrast, the condensed monolayer significantly diminished the rates of ion transfer for both $TMA^+$ and $ClO_4^-$ ions, suggesting that the presence of the condensed monolayer altered the rate-determining step and that the condensed monolayer exerted an additional hydrodynamic friction on the ion-transfer processes.

The phospholipid monolayer formed at the polarized oil–water interface is a suitable model system for studying the electrical aspects of biological and artificial membranes, e.g., double-layer structure in the vicinity of the membrane-solution interface, specific interaction of ions with membranes, and, in particular, the charge-transfer processes across membranes. The advantage of the monolayer formed at the polarized oil–water interface is that it is possible to accurately control the potential drop across the interface and, hence, to apply electrochemical techniques to characterize the phenomena associated with the electrified interface.[1,2] The electrochemical phenomena at oil–water interfaces had been seen as being a model of biological membranes already in the earliest stage of electrochemistry at oil–water interfaces.[3,4] Although some pioneering work on the electrocapillarity at the oil–water interface in the presence of phospholipid adsorption had been reported,[5–7] electrochemically well-defined data on the phospholipid monolayers at the interface have become available only during the last decade, after the establishment of the theoretical concept and experimental methods of the polarized oil–water interface.[1,8,9] Since then, there have been several attempts to study the effect of phospholipid monolayers on the ion-transfer reaction across the interface.[10–12] However, the properties of the monolayers themselves formed at the polarized interface are not sufficiently understood to correctly interpret the observed behavior. Recent studies of the adsorbed monolayer of phosphatidylcholines have revealed the fundamental properties of the monolayers at the polarized oil–water interfaces by using interfacial tension[13,14] and double-layer capacitance measurements.[15,16] Phosphatidylserine is one of the important constituents of biological membranes and, hence, has been the subject of extensive studies,[17–30] especially in relation to the specific interaction of the phosphatidylserine head group with cations, which can cause a drastic change in phosphatidylserine-containing lipid assemblies. The purpose of the present work was to electrochemically characterize the phosphatidylserine monolayer at the polarized oil–water interface and to elucidate the effect of the divalent-cation induced phase transition of the monolayer on the ion-transfer processes across the interface. The present communication reports on the results obtained from the double-layer capacitance and cyclic voltammetry measurements at the polarized nitrobenzene–water interface in the presence of an adsorbed DL-α-dipalmitoylphosphatidyl-L-serine, DPPS, monolayer.

## Experimental

DPPS 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. An aqueous solution of reagent-grade tetrapentylammonium chloride (TPnACl) was treated with silver chloride to remove trace iodide ion. LiCl·$H_2O$, $CaCl_2$·$4H_2O$ (Merck, Spurapur grade) and $MgCl_2$·$6H_2O$ (Merck, Pro Analysis) were dissolved in water to prepare the stock solution. The concentration of TPnACl solution was determined from 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 out 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$^{-3}$ TPnATPB | (0.1−2$a$) mol dm$^{-3}$ LiCl | | |
| Ag | AgCl | TPnACl | $x$ μmol dm$^{-3}$ DPPS | $a$ mol dm$^{-3}$ $MCl_2$ | AgCl | Ag |
| | | (W) | (NB) | (W) | | |

(I)

where M denotes $Ca^{2+}$ or $Mg^{2+}$. 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, $E$, was controlled by means of a laboratory-made potentiostat similar in circuit design to that used by Osakai et al.[31] In Cell (I) the potential of zero charge, $E_{pzc}$, was 0.34 V.[14] The potential region positive (negative) to $E_{pzc}$ will hereafter be referred to as the positive (negative) branch. Since the surface potential at the pzc is small,[9] the potential referred to $E_{pzc}$ can be taken as a convenient measure of the inner potential difference between the two phases, $\Delta_O^W\phi$, where the potential of the nitrobenzene phase is taken as a reference. Then, $\Delta_O^W\phi>0$ in the positive branch and vise versa. A residual iR drop was compensated for by a positive-feedback method. In impedance measurements, the d.c. potential was scanned from 0.20 to 0.44 V at 5 mV s$^{-1}$ using a function generator (Hokuto Denko Ltd., HB-104). An a.c. voltage of 5 mV peak-to-peak and 50 Hz from an RC Oscillator (Kikusui, ORC-11) was superimposed on the d.c. voltage. The real and imaginary parts of the output of a lock-in amplifier (NF Circuit Design Block Ltd., LI-574A) were fed to a micro-computer (Fujitsu, FM-8), via a 12-bit A/D converter equipped with a GP-IB interface (ADTEK System Sci., R-488AD type II). The double-layer capacitance was calculated from the imaginary component of the a.c. impedance.[15]

The cell used for the a.c. impedance and cyclic voltammetry measurements was of the two-electrode type illustrated in Fig. 1. The electrodes were silver-silver chloride electrodes. A polarized nitrobenzene-water interface was formed at the upper part of the narrowed cylindrical portion in the middle of the cell. To obtain a flat interface, only the part of the inner surface of the cell lower than this interface was made hydrophobic by applying dimethyldichlorosilane vapor.[32] The geometrical area of the interface was 0.196 cm$^2$. One advantage of this type cell is that aqueous solution in the upper part of the cell can be renewed after the formation of the interface, even after the formation of a phospholipid monolayer. An aqueous solution of 0.05 mol dm$^{-3}$ tetrapentylammonium chloride, TPnACl, was made to contact with the nitrobenzene phase through a glass frit at the bottom of the cell. All measurements were made at 25.0±0.05 °C.

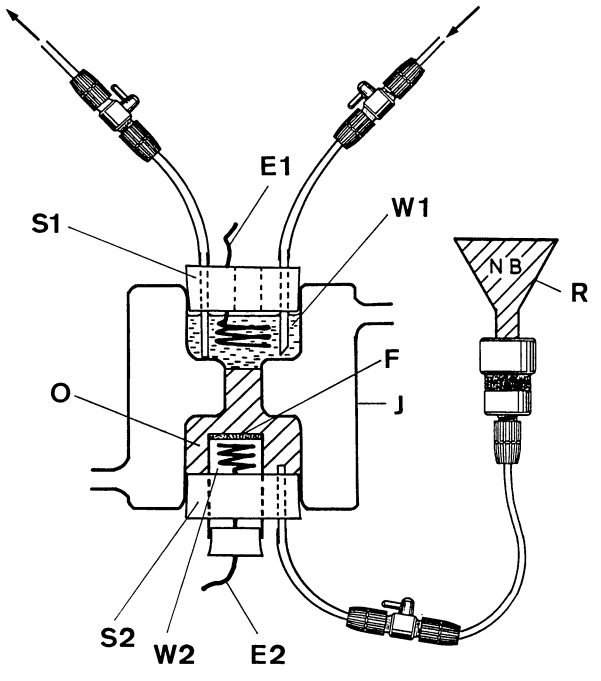


Fig. 1. Cell design used for impedance and cyclic voltammetry measurement. W1: aqueous solution, W2: aqueous solution containing TPnACl, O: nitrobenzene solution, E1 and E2: Ag-AgCl electrodes, F: glass frit, S1 and S2: silicone rubber stoppers, J: water jacket, R: nitrobenzene solution reservoir.

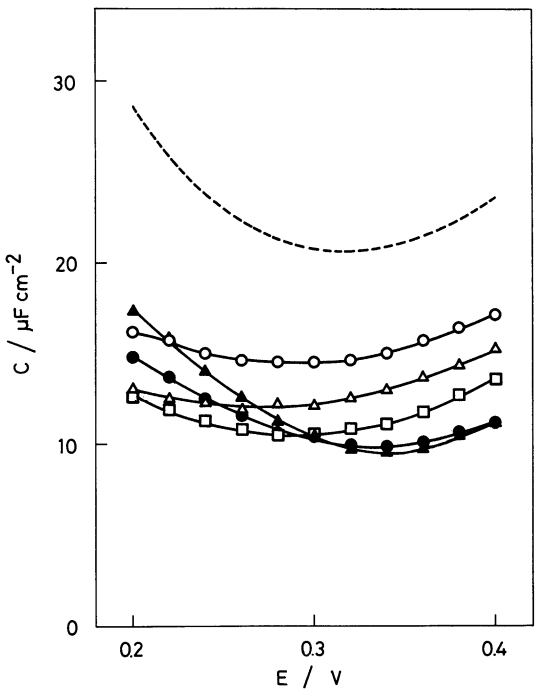


Fig. 2. Double layer capacitance vs. potential curves for the interface between 0.1 mol dm$^{-3}$ LiCl and nitrobenzene solution containing 0 (----), 1 (○), 2 (△), 5 (□), 10 (●), and 20 (▲) μmol dm$^{-3}$ dipalmitoylphosphatidylserine.

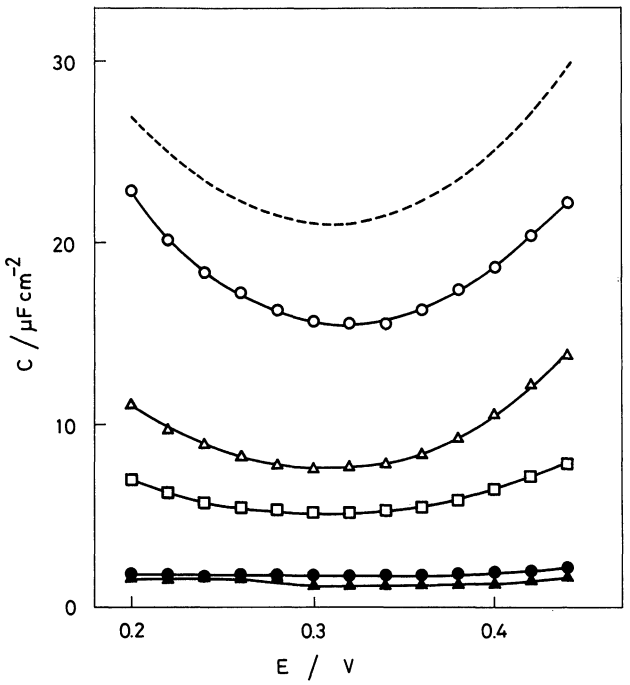


Fig. 3a. Double layer capacitance vs. potential curves for the interface between 0.05 mol dm$^{-3}$ $MgCl_2$ and the nitrobenzene solution containing 0 (----), 1 (○), 2 (△), 5 (□), 10 (●), and 20 (▲) μmol dm$^{-3}$ dipalmitoylphosphatidylserine.

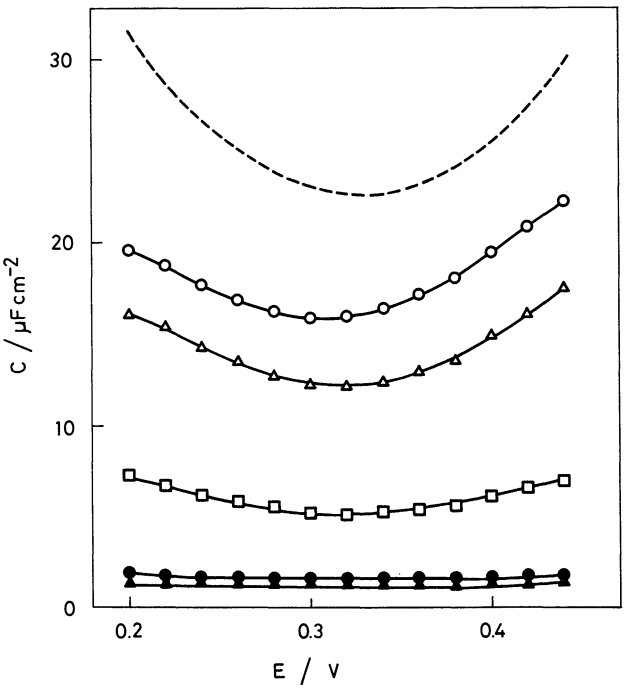


Fig. 3b. Double layer capacitance vs. potential curves for the interface between 0.05 mol dm$^{-3}$ $CaCl_2$ and nitrobenzene solution containing 0 (----), 1 (○), 2 (△), 5 (□), 10 (●), and 20 (▲) μmol dm$^{-3}$ dipalmitoylphosphatidylserine.

## Results

The double-layer capacitance, $C_{dl}$, vs. $E$ curves at several concentrations of DPPS, $c_{DPPS}$, in Phase IV at $a$=0 in Cell (**I**) are shown in Fig. 2. It typically took 2 h to obtain the time-invariant value of $C_{dl}$, which was considered to indicate the adsorption equilibrium. The $C_{dl}$ value was lowered with an increase in $c_{DPPS}$ and reached a saturation at $c_{DPPS}$=20 μmol dm$^{-3}$. The minimum of the $C_{dl}$ vs. $E$ curve was shifted from $E$=0.29 V to 0.34 V by the formation of a saturated monolayer. The $C_{dl}$ value at this minimum was 9.5 μF cm$^{-2}$. The $C_{dl}$ vs. $E$ curves are presented in Figs. 3a and 3b at five different concentrations of DPPS, when the aqueous solution contained 0.05 mol dm$^{-3}$ $MgCl_2$ or 0.05 mol dm$^{-3}$ $CaCl_2$. The $C_{dl}$ values at two different compositions of the aqueous phase, 0.1 mol dm$^{-3}$ LiCl and 0.05 mol dm$^{-3}$ $MgCl_2$, are compared at $E$=0.30 V in Fig. 4. As can be seen from these figures, the $Mg^{2+}$ and $Ca^{2+}$ ions drastically lowered the double-layer capacitance down to 1.5 μF cm$^{-2}$ when $c_{DPPS}$=20 μmol dm$^{-3}$. Moreover, $C_{dl}$ became almost indenpendent of $E$ over the 200 mV span ($-0.14\ V \leqq \Delta_o^w\phi \leqq 0.10\ V$) of the polarization range, when $c_{DPPS}$ was greater than 10 μmol dm$^{-3}$. The $C_{dl}$ vs. $E$ curves for $Ca^{2+}$ and $Mg^{2+}$ were almost identical with each other. To check the effect of the divalent ion concentration on the $C_{dl}$ value, $C_{dl}$ was measured upon changing the concentration of $CaCl_2$ or $MgCl_2$, while the total concentration of $Cl^-$ ions was kept constant by adding an appropriate amount of LiCl. Figure 5 shows the $C_{dl}$ values at $E$=0.30 V. The $C_{dl}$ value was sharply decreased with divalent cation concentration up to 2 mmol dm$^{-3}$ and remained unchanged with a further increase beyond 2 mmol dm$^{-3}$; the critical concentration for giving a lower $C_{dl}$ value was found to be 2 mmol dm$^{-3}$ for both $Ca^{2+}$ and $Mg^{2+}$.

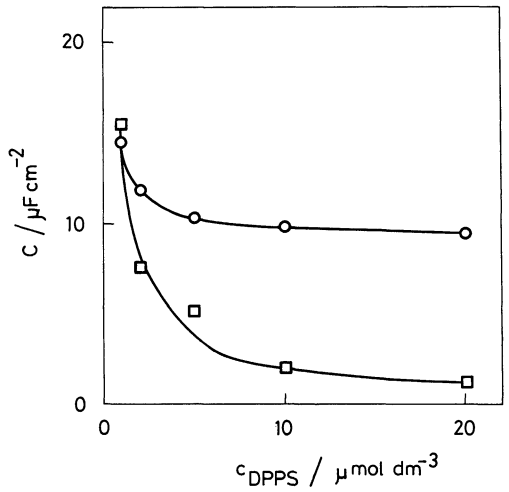


Fig. 4. Change in double layer capacitance with dipalmitoylphosphatidylserine concentration at $E$=0.30 V, when the aqueous phase is 0.1 mol dm$^{-3}$ LiCl (○) or 0.05 mol dm$^{-3}$ $MgCl_2$ (□).

The effect of the presence of a DPPS monolayer on the ion-transfer processes was examined by measuring the ion-transfer rate constants for the tetramethylammonium ion, $TMA^+$, and the $ClO_4^-$ ion transfer from the aqueous to the nitrobenzene phase. For this measurement, after the formation of the monolayer, Phase V was replaced with an aqueous solution containing 0.5 mmol $dm^{-3}$ TMACl or $LiClO_4$ by sucking an old solution and filling a new solution. This procedure was repeated five times. It was confirmed from the shape of the $C_{dl}$ vs. $E$ curves before and after this procedure that this exchange of the solution did not destroy the DPPS monolayer. The cyclic voltammograms for $TMA^+$ ion transfer in the absence and presence of the DPPS monolayer in contact with 0.05 mol $dm^{-3}$ $CaCl_2$, are shown in Figs. 6a and 6b, respectively. There was no significant difference in the midpoint potentials before and after the introduction of the monolayer to the interface. The standard rate constant of ion tranfer was estimated from the variation of the peak separation with the sweep rate (10—200 mV $s^{-1}$) using a method proposed by Nicholson for quasi-reversible charge-transfer reactions.[33,34] Since the positive feedback method employed in the present study ensured that the uncompensated resistance was less than 10 Ω[35] and the current was always smaller than 50 μA, the contribution of the uncompensated resistance to the estimated

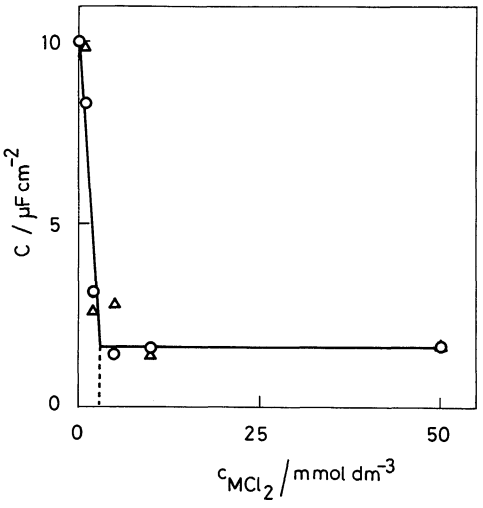


Fig. 5. Effect of the divalent cation concentration on the double layer capacitance at $E$=0.30 V. $MgCl_2$ (△) and $CaCl_2$ (○).

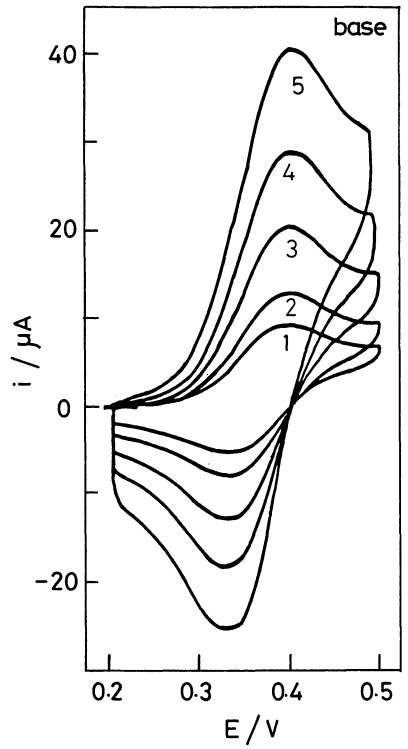


Fig. 6a. Cyclic voltammograms for the transfer of tetramethylammonium ion from the aqueous phase to the nitrobenzene phase in the absence of the dilauroylphosphatidylserine monolayer. The aqueous solution contains 0.05 mol $dm^{-3}$ $CaCl_2$ and 0.5 mmol $dm^{-3}$ tetramethylammonium chloride. Scan rate is 10(1), 20(2), 50(3), 100(4), and 200(5) mV $s^{-1}$.

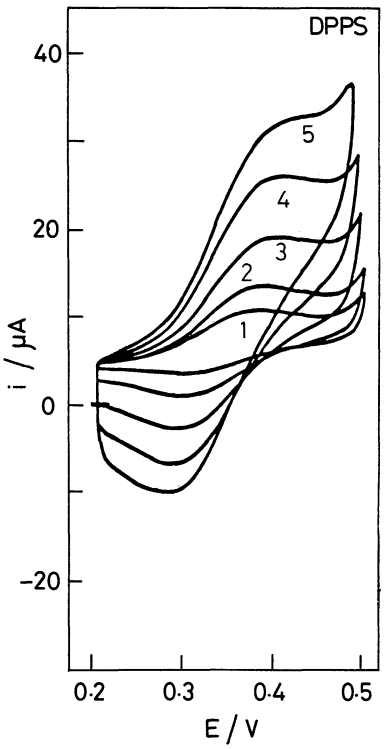


Fig. 6b. Cyclic voltammograms for the transfer of tetramethylammonium ion from the aqueous to the nitrobenzene phase in the presence of a saturated monolayer of dipalmitoylphosphatidylserine. The aqueous solution contains 0.05 mol $dm^{-3}$ $CaCl_2$ and 0.5 mmol $dm^{-3}$ tetramethylammonium chloride. Scan rate is 10(1), 20(2), 50(3), 100(4), and 200(5) mV $s^{-1}$.

Table 1. Effect of $Ca^{2+}$-Induced Phase Transition of Dipalmitoylphosphatidylserine Monolayer on the Standard Rate Constants of Ion Transfer Across the Nitrobenzene–Water Interface at 25 °C

| Ion | $k_s \times 10^2$/cm $s^{-1}$ | | | |
|---|---|---|---|---|
| | 0.1 M LiCl | | 0.05 M $CaCl_2$ | |
| | Free | DPPS | Free | DPPS |
| $TMA^+$ | 1.9 | 1.7 (0.9)[a] | 1.9 | 0.3 (0.2) |
| $ClO_4^-$ | 1.3 | 0.4 (0.3) | 1.4 | 0.4 (0.3) |

a) The number in parenthesis indicates the ratio of the $k_s$ in the presence of DPPS monolayer over that in the absence of the monolayer.

values of the standard rate constants is likely to be negligible. In the evaluation of the values of the standard rate constants, we assumed that the transfer coefficient was 0.5 and that the ratio of the diffusion coefficient of the ions in the aqueous phase was twice as large as that in the nitrobenzene phase. The standard rate constants obtained are summarized in Table 1.

## Discussion

The $C_{dl}$ value at the minimum of the $C_{dl}$ vs. $E$ curve for a saturated monolayer in contact with 0.1 mol $dm^{-3}$ LiCl, 9.5 μF $cm^{-2}$, is comparable with that of the dilauroylphosphatidylcholine, DLPC, monolayer at the nitrobenzene–water inteface,[15] suggesting that the DPPS monolayer is also in a similar liquid-expanded state. This significantly high $C_{dl}$ value, in comparison with that of bilayer lipid membranes, appears to be due to the penetration of solvent molecules, and, possibly, ions into the hydrocarbon chain part of the adsorbed DPPS monolayer,[15] and may be seen as being a common feature of the monolayer in a liquid-expanded monolayer at oil–water interfaces. On the other hand, DPPS forms a condensed monolayer at the air–water interface at room temperature.[30] The difference in the phase behavior of DPPS monolayers at the air–water and nitrobenzene–water interfaces is attributable to a diminished cohesion between the hydrocarbon chains of the adsorbed DPPS molecules at the nitrobenzene–water interface, as is generally observed in the adsorption of lipids at oil–water interfaces.[36,37] Moreover, the DPPS monolayer probably bears a net negative charge, since the pH in the aqueous solution of Phase V in Cell (**I**) was typically 5.8, at which DPPS molecules are known to be negatively charged.[38] In other words, the DPPS molecules should behave as anionic surfactants. This is, in fact, reflected by the shift of the capacity minumum on the $C_{dl}$ vs. $E$ curves to the more positive potential (Fig. 2), i.e., the adsorption of negatively charged DPPS molecules is stabilized in the positive branch. The increase in $C_{dl}$ in the negative branch is likely to be attributable to the adsorption pseudocapacitance associated with the partial desorption and/or reorientation of DPPS molecules from the interface. A similar increase in $C_{dl}$ due to the desorption of phospholipids from the nitrobenzene–water interface has been observed for phosphatidycholine monolayers in contact with an aqueous LiCl solution[15,16] and a dilauroylphosphatidylethanolamine, DLPE, monolayer in contact with an aqueous solution at pH=12.5.[39] The electrostatic repulsion between the negatively charged DPPS molecules would also be responsible for the monolayer being in a liquid-expanded state. Although $Li^+$ ions are known to induce a gradual crystallization of the phosphatidylserine bilayers,[40–43] there appears to be no such condensation in the monolayer in the present system.

Figure 5 clearly shows that the presence of $Ca^{2+}$ or $Mg^{2+}$ in the aqueous solution at higher than 2 mmol $dm^{-3}$ induces a phase transition. The low $C_{dl}$ value of 1.5 μF $cm^{-2}$ is twice as large as the capacitance value of bilayer lipid membranes,[44] which is presumably two-times thicker than the monolayer. Hence, this low $C_{dl}$ value of the DPPS monolayer indicates that the nitrobenzene molecules and the ions are excluded from the monolayer and that the monolayer is in a liquid-condensed or even a solid crystalline phase.[27] There have been numerous studies on the divalent cation-induced phase transition of phosphatidylserine assemblies,[45–47] e.g., monolayers at air–water interface,[25,48–51] bilayer membranes,[29] and vesicles.[52–54] It has been found that the interaction of phosphatidylserine assemblies with $Ca^{2+}$ ions is usually stronger than with $Mg^{2+}$ ion.[29,47,55–57] However, the present results have shown that the effects of $Ca^{2+}$ and $Mg^{2+}$ on the phase transition of the DPPS monolayer were of the same magnitude, suggesting that the phase transition is essentially governed by an electrostatic interaction between the DPPS molecules and the divalent ions at the interface. This is in harmony with the observation that $Mg^{2+}$–DPPS and $Ca^{2+}$–DPPS complexes form similar bilayer structures.[29] The threshold level of the divalent cation concentration necessary for causing the phase transition, 2 mmol $dm^{-3}$, agrees well with the fact that phosphatidylserines interact strongly with divalent metals at low concentrations ($10^{-4}$—$10^{-3}$ mol $dm^{-3}$) in the presence of a physiological concentration of monovalent salts ($10^{-1}$ mol $dm^{-3}$).[46] The stability of the condensed monolayer against the applied potentials conforms to the fact that the negative charges on DPPS molecules were effectively neutralized by the adsorbed divalent cations by forming a 2:1 complex.[24,58] If the divalent cations and DPPS molecules form a 1:1 complex at the interface, the monolayer would be positively charged and a desorption of the complex would occur at the positive end of the positive branch, as is the case of cationic surfactants.[59]

Table 1 shows that the influence of the DPPS monolayer on the ion-transfer processes clearly depends on the state of the monolayer. In the liquid-expanded state, the contribution of the hydrocarbon chain part of the monolayer to the rate-determining step seems to be very small, since the presence of the monolayer did not change the $k_s$ value for $TMA^+$ ion transfer, the Stokes radius of which is grater than that of $ClO_4^-$ (0.204 and 0.135 nm, respectively, calculated from the limiting ionic conductances in water at 25 °C.) If the ion-migration process through the monolayer is a rate-determining step, the friction from the hydrocarbon part of the monolayer, if any, on $TMA^+$ ions would be larger than on $ClO_4^-$ ions. In fact, the results obtained from the DLPC monolayer have shown that in the liquid-expanded state having the capacitance of ca. 10 $\mu F\ cm^{-2}$, the area occupied by a phospholipid molecules at the interface is of the order of 0.7—0.9 $nm^2$;[14,15] the solvent molecules occupy about one half of the area of the interface. Therefore, there is at the interface a free space large enough to pass the transferring ions without exerting any appreciable hydrodynamic drag on the ions. Then, the rate-determining step is probably the dehydration process of the transferring ions, as is the case of the ion transfer in the absence of the monolayer,[35] and $k_s$ would not be very much affected by the presence of adsorbed DPPS molecules.

In contrast, more than a three-fold decrease in $k_s$ was observed for $ClO_4^-$ transfer, even when the DPPS monolayer was in the liquid-expanded state (Table 1). A most probable interpretation for this fact is the electrostatic repulsion between the transferring anion and the negatively charged DPPS monolayer, i.e., so called Frumkin's double-layer effect.[60] Upon transferring, $ClO_4^-$ ions probably experience a repulsion from the negatively charged interface, resulting in a decrease in the effective surface concentration at the aqueous side of the interface, where a certain reaction plane presumably resides. A similar decrease in $k_s$ has been found in $ClO_4^-$ transfer across a negatively charged DLPE monolayer.[39] One may expect the acceleration of cation transfer at the same monolayer, if there exists the Frumkin effect on the ion-transfer processes across the monolayer. Unfortunately, owing mainly to the uncompensated solution resistance, the $k_s$ values in Table 1 approach an upper limit of the rate constant measurable in the cyclic voltammetry method and the possible acceleration may not have been detected for the $TMA^+$ ion transfer in the present study. The double-layer effect on the ion-transfer reaction across bilayer lipid membranes has been studied mainly from a rather macroscopic point of view, e.g., the overall shape of current vs. potential curves.[61] In our opinion, the double-layer effect exhibits its significance primarily on the kinetic aspects of the ion-transfer processes, as is the case of the electron-transfer processes at electrode-solution interfaces.[60]

Contrary to the case of the liquid-expanded monolayer, the condensed DPPS monolayer slowed down the transfer of both $TMA^+$ and $ClO_4^-$ ions(4th and 5th columns in Table 1). Since the monolayer is neutralized by the adsorbed divalent cations, the double-layer effect is obviously of minor importance. One of the most probable sources that influences the ion transfer, irrespective of the charge sign on a transferring ion, is ion transfer through the hydrocarbon chain part of the monolayer. In the course of the phase transition from the liquid-expanded to the liquid-condensed phase induced by divalent cations, the solvent molecules are squeezed out of the monolayer, as discussed above. Therefore, in this case the transferring ions must creep into the layer of hydrocarbon chains. In this migration process, the hydrodynamic interaction of transferring ions with the hydrocarbon chains, which is in a condensed or crystalline state, over a certain distance should become significant and, therefore, the size, rather than the charge sign, of the ion would be a determining factor. In fact, as can be seen in Table 1, the ratios of the rate constants in the absence and presence of the DPPS monolayer (Table 1), which may be seen as representing the degree of retardation of ion transfer, was significantly smaller for $TMA^+$ ions than for $ClO_4^-$ ions, when the monolayer was in a condensed state.

The observed change in the mechanism of the ion-transfer processes seems to be of fundamental importance in reference to studies concerning the permeation processes of charged components through liposome membranes.[62,63] The difference in the permeability of simple ions through bilayer lipid membranes has usually been discussed in terms of the partition coefficient of the ions between the lipid phase and aqueous solution, e.g., using the Born equation for solvation.[64,65] It is to be noted that the difference in the partition coefficients is generally reflected in the equilibrium properties of ion transfer, i.e., the standard Gibbs energy of transfer between the two phases, which can be correlated with the experimentally observed midpoint potential in Figs. 6a and 6b. The present result, that neither the liquid-expanded nor the condensed DPPS monolayer caused the appreciable shift of the midpoint potential, strongly argues that the kinetic barrier of the monolayer against $TMA^+$ and $ClO_4^-$ ion transfer cannot be explained in terms of the equilibrium partion properties of these ions. It has recently been found that the change in the rate-determining step of ion transfer was induced by a change in the membrane fluidity of a liposomes composed of dimyristoylphosphatidylcholine and cholesterol,[66] which is in line with the present results.

## References

1) J. Koryta, *Electrochim. Acta*, **24**, 293 (1979); **29**, 445 (1984); **33**, 189 (1988).

2) H. H. J. Girault and D. J. Schiffrin, "Electroanalytical

Chemistry," ed by A. Bard, Marcel Dekker, New York (1989), Vol. 15, p. 1.
3) P. Kandidow, *Z. Phys. Chem.*, **83**, 587 (1913).
4) R. Beutner, *J. Am. Chem. Soc.*, **36**, 2045 (1914).
5) A. Watanabe, M. Matsumoto, H. Tamai, and R. Gotoh, *Kolloid Z. Z. Polym.*, **228**, 58 (1980).
6) A. Watanabe, A. Fujii, Y. Sakamori, K. Higashitsuji, and H. Tamai, *Kolloid Z. Z. Polym.*, **243**, 42 (1971).
7) A. Watanabe, H. Tamai, and K. Higashitsuji, *J. Colloid Interface Sci.*, **43**, 548 (1973).
8) C. Gavach, *Compt. Rend.*, **C266**, 1196 (1968).
9) T. Kakiuchi and M. Senda, *Bull. Chem. Soc. Jpn.*, **56**, 1322, 1753 (1983).
10) J. Koryta, Le Q. Hung, and A. Hormanova, *Stud. Biophys.*, **90**, 25 (1982).
11) H. H. J. Girault and D. J. Schiffrin, "Charge and Field Effect in Biosystems," ed by M. J. Allen and P. N. R. Usherwood, Abacus Press, England (1984), p. 171.
12) V. J. Cunnane, D. J. Schiffrin, M. Fleischman, G. Geblewicz, and D. Williams., *J. Electroanal. Chem. Interfacial Electrochem.*, **243**, 455 (1988).
13) H. H. J. Girault and D. J. Schiffrin, *J. Electroanal. Chem. Interfacial Electrochem.*, **179**, 277 (1984).
14) T. Kakiuchi, M. Nakanishi, and M. Senda, *Bull. Chem. Soc. Jpn.*, **61**, 1845 (1988); **62**, 403 (1989).
15) T. Kakiuchi, M. Yamane, T. Osakai, and M. Senda, *Bull. Chem. Soc. Jpn.*, **60**, 4223 (1987).
16) T. Wandlowski, V. Mareček, and Z. Samec, *J. Electroanal. Chem. Interfacial Electrochem.*, **242**, 277 (1988).
17) D. Papahadjopoulos and N. Miller, *Biochim. Biophys. Acta*, **135**, 624 (1967).
18) C. Newton, W. Pangborn, S. Nir, and D. Papahadjopoulos, *Biochim. Biophys. Acta*, **506**, 281 (1978).
19) M. Eisenman, T. Gresalfi, T. Riccio, and S. McLaughlin, *Biochemistry*, **18**, 5213 (1979).
20) H. H. Hauser and M. C. Phillips, *Progr. Surf. Membr. Sci.*, **13**, 297 (1979).
21) M. J. Hope and P. R. Cullis, *Biochem. Biophys. Res. Commum.*, **92**, 847 (1980).
22) G. Cevc, A. Watts, and D. Marsh, *Biochemistry*, **20**, 4955 (1981).
23) D. L. Holwerda, P. D. Ellis, and R. E. Wuthier, *Biochemistry*, **20**, 418 (1981).
24) S. Ohki and R. Kurland, *Biochim. Biophys. Acta*, **645**, 170 (1981).
25) A. C. McLaughlin, *Biochemistry*, **21**, 4879 (1982).
26) R. A. Dluhy, D. G. Cameron, H. H. Mantsch, and R. Mendelsohn, *Biochemistry*, **22**, 6318 (1983).
27) S. Ohnishi and T. Ito, *Biochemistry*, **13**, 881 (1974).
28) T. Ito, S. Ohnishi, M. Ishinaga, and M. Kito, *Biochemistry*, **14**, 3064 (1975).
29) H. Hauser and G. G. Shipley, *Biochemistry*, **22**, 2171 (1983); *Biochemistry*, **23**, 34 (1984); *Biochim. Biophys. Acta*, **813**, 343 (1985).
30) R. A. Demel, F. Paltauf, and H. Hauser, *Biochemistry*, **26**, 8659 (1987).
31) T. Osakai, T. Kakutani, and M. Senda, *Bull. Chem. Soc. Jpn.*, **57**, 370 (1984).
32) T. Kakutani, T. Osakai, and M. Senda, *Bull. Chem. Soc. Jpn.*, **56**, 991 (1983).
33) R. S. Nicholson, *Anal. Chem.*, **37**, 1351 (1965).
34) Z. Samec, V. Mareček, and M. Weber, *J. Electroanal. Chem. Interfacial Electrochem.*, **100**, 841 (1979).
35) T. Kakiuchi, J. Noguchi, M. Kotani, and M. Senda, *J. Electroanal. Chem. Interfacial Electrochem.*, in press.
36) J. T. Davies, *Proc. Roy. Soc. London, Ser. A*, **208**, 224 (1951).
37) J. Mingins, J. A. G. Taylor, B. A. Pethica, G. M. Jackson, and B. Y. T. Yue, *J. Chem. Soc., Faraday 1*, **78**, 323 (1982).
38) T. Seimiya and S. Ohki, *Biochim. Biophys. Acta*, **298**, 546 (1973).
39) T. Kakiuchi, M. Kotani, and M. Senda, in preparation.
40) H. Hauser and G. G. Shipley, *J. Biol. Chem.*, **256**, 11377 (1981).
41) H. Hauser and G. G. Shipley, *Biochemistry*, **22**, 2171 (1983).
42) G. Cevc, J. M. Sedden, and D. Marsh, *Biochim. Biophys. Acta*, **814**, 141 (1985).
43) H. L. Casal and H. H. Mantsh, *Biochemistry*, **26**, 4408 (1987).
44) H. T. Tien, "The Chemistry of Biosurfaces," ed by M. L. Hair, Marcel Dekker, New York (1971), Vol. 1, Chap. 6, p. 239.
45) See for review, J. -F. Tocanne and J. Teissie, *Biochim. Biophys. Acta*, **1031**, 111 (1990).
46) D. Papahadjopoulos, *Biochim. Biophys. Acta*, **163**, 240 (1968).
47) S. McLaughlin, N. Mulrine, T. Gresalfi, G. Vais, and A. McLaughlin, *J. Gen. Physiol.*, **77**, 445 (1981).
48) S. Ohki and R. Sauve, *Biochim. Biophys. Acta*, **511**, 377 (1978).
49) B. M. Abraham and J. B. Ketterson, *Langmuir*, **2**, 801 (1986).
50) T. Seimiya and S. Ohki, *Biochim. Biophys. Acta*, **274**, 15 (1972).
51) T. Handa and M. Nakagaki, *Colloid Polym. Sci.*, **257**, 374 (1979).
52) S. Ohki, S. Roy, H. Ohshima, and K. Leonards, *Biochemistry*, **23**, 6126 (1984).
53) S. Ohki, *Biochim. Biophys. Acta*, **689**, 1 (1982).
54) J. R. Silvius and J. Gagné, *Biochemistry*, **23**, 3232, 3241 (1984).
55) S. Ohki and N. Durzgues, *Biochim. Biophys. Acta*, **552**, 438 (1979).
56) C. Newton, W. Pangborn, S. Nir, and D. Papahadjopoulos, *Biochim. Biophys. Acta*, **506**, 281 (1978).
57) N. Gresh, *Biochim. Biophys. Acta*, **597**, 345 (1980).
58) S. Nir, C. Newton, and D. Papahadjopoulos, *Biochemistry*, **5**, 116 (1978).
59) T. Kakiuchi, M. Kobayashi, and M. Senda, *Bull. Chem. Soc. Jpn.*, **60**, 3109 (1978); **61**, 1545 (1988).
60) A. N. Frumkin, *Z. Phys. Chem., Abt. A*, **164**, 121 (1933).
61) A. H. Hainsworth and S. B. Hladky, *Biophys. J.*, **51**, 27 (1987) and references cited therein.
62) S. Doniach, *J. Chem. Phys.*, **68**, 4912 (1978).
63) J. F. Nacle and H. L. Scott, *Biochim. Biophys. Acta*, **513**, 236 (1978).
64) J. P. Dilger, S. G. A. McLaughlin, T. J. McIntosh, and S. A. Simon, *Science*, **206**, 1196 (1979).
65) J. Dilger and S. McLaughlin, *J. Membr. Biol.*, **46**, 359 (1979).
66) E. Sada and M. Terashima, *Hyomen*, **27**, 313 (1989).