

# Langmuir–Blodgett Monolayers at a Liquid–Liquid Interface

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One of enduring problems of phospholipid adsorption at the interface between two immiscible electrolyte solutions (ITIES) has been the inability to determine and control the exact nature of the adsorbed monomolecular layer. In the present study, this problem has been overcome by the use of the well-known Langmuir–Blodgett technique. It has been shown that reproducible layers of known surface pressure can be deposited at the interface and that the deposition surface pressure has a great influence on the behavior of the layer. Interfacial capacitance data has been extracted and electrocapillary curves have been drawn. The noted shift in the electrocapillary minimum and the lowered interfacial capacitance have been explained by use of a simple electrostatic model. Probe ion transfer studies show no change in the interfacial kinetics or in the Gibbs free energy of transfer.

## Introduction

There is continuing interest in phospholipid monolayers at the interface between two immiscible electrolyte solutions. This arises from their possible application as simple models of biological membranes. However, the molecular environment of phospholipid monolayers at the interface between two immiscible electrolyte solutions (ITIES) is somewhat different from the naturally occurring bilayer structures. The main difference is the presence of organic solvent within the monolayer. The solvent molecules screen the attractive interactions between the hydrocarbon tails of the phospholipids, thus making the layer more expanded.<sup>1–3</sup> In the majority of the studies on phospholipid monolayers at ITIES to date, the layer has been formed by adsorption of the lipid from the bulk organic phase. However, there is an uncertainty as to the exact state of the layer due to the inability to control the surface pressure.<sup>4–10</sup> It would therefore be highly desirable to be able to control the state of the monolayer by external means. Two conceivable methods would be either to control the surface pressure in situ or to transfer a monolayer

already in the desired state to the interface. The former case has been realized in the combination of the Langmuir technique and electrochemical control over the interface by Grandell and Murtomäki.<sup>11–13</sup> However, their design suffers from the exceedingly large interfacial area hampering the electrochemical measurements, difficulties with monolayer loss to the bulk organic phase, and large volumes of toxic organic solvent used. This paper studies the second possibility of transferring a monolayer in certain state onto a liquid–liquid interface by use of the Langmuir–Blodgett technique.

The study of monolayers at ITIES in the past has focused on two different aspects: the phase behavior and the ion permeability. The phase behavior of surfactant molecules can be assessed through interfacial capacitance or surface tension measurements. At ITIES, the effect of potential drop across the monolayer can be studied, in addition to the variables affecting the complex phase behavior of phospholipids (pH, temperature, ion concentration, etc.). The results obtained previously for phosphatidylcholine (PC) monolayers adsorbed at ITIES showed two different types of behavior. At negative Galvani potential differences (water vs organic phase,  $\Delta_o^w\phi = \phi_w - \phi_o$ ), the interfacial capacitance is low, indicating formation of a compact monolayer. The absolute value of the capacitance then depends on the length of the hydrocarbon tails and the bulk concentration of the phospholipid.<sup>7,8</sup> On the other hand, at positive Galvani potential differences, the capacitance approaches that of a bare interface, especially for shorter phospholipids.<sup>8</sup> This has been attributed to a nonequilibrium process, namely reorientation of the lipid followed by partial desorption,<sup>7,12</sup> i.e., attainment of electroadsorption equilibrium. Surface tension increases rapidly at positive potentials.<sup>14–16</sup> Girault and Schiffrin

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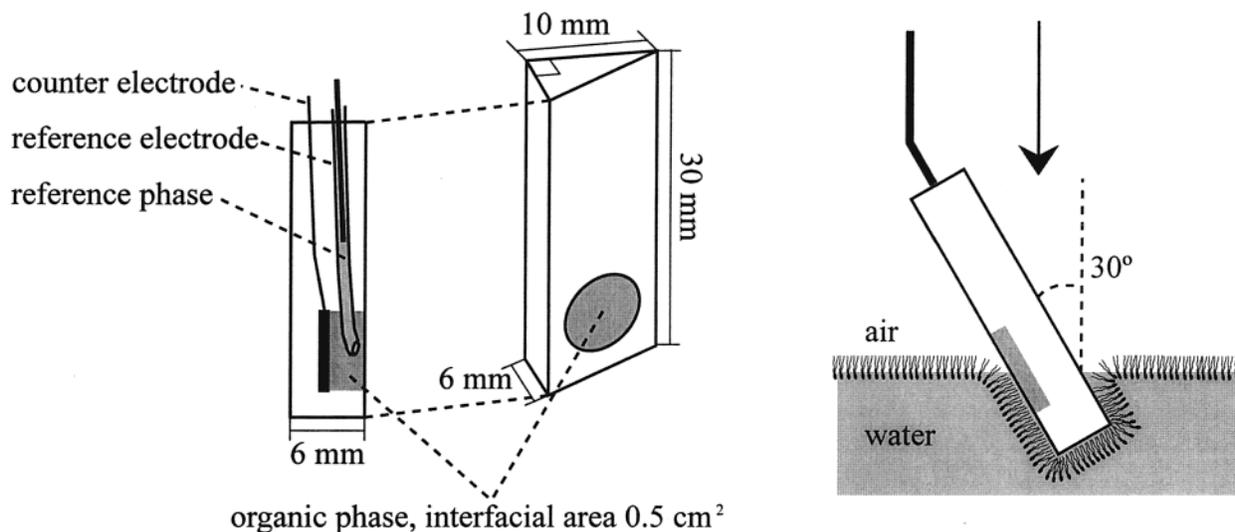
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**Figure 1.** Schematic drawing of the electrochemical cell and the dipping procedure.

explained it in terms of protonation of the phosphate headgroup followed by a surface reorientation of the PC layer.<sup>14</sup> It has also been argued that it is difficult to endow any definite thermodynamic meaning to the surface tension data at these extreme positive potentials.<sup>16</sup> These earlier studies have been carried out either at water–1,2-dichloroethane (DCE) or water–nitrobenzene (NB) interface.

The investigation of ion transfer across monolayers initially concentrated on natural mixtures of phospholipids at ITIES. These studies usually showed a retarding effect on ion transfer.<sup>4–6</sup> On the contrary, however, later studies with pure phospholipids have revealed no such effect. In fact, the apparent rate constant has been observed to increase in the presence of adsorbed phospholipids for certain ions.<sup>8,10</sup> This enhancement has been explained in terms of double-layer effects arising from the orientation of the zwitterionic headgroups of phosphatidylcholine molecules.<sup>8,17</sup> In addition, specific interactions between transferring ions and the monolayer have also been reported.<sup>13</sup>

This paper describes a new method of producing phospholipid monolayer at ITIES based on the combination of the Langmuir–Blodgett technique and an electrochemical cell with an immobilized liquid–liquid interface. The organic phase in this study is nitrophenyl octyl ether (NPOE) immobilized with poly(vinyl chloride). A distearoyl phosphatidylcholine (DSPC) monolayer deposited at different surface pressures has been characterized using four-electrode cyclic voltammetry in order to show that the state of the monolayer can be controlled by the deposition conditions. To explain qualitatively the observed capacitance behavior, a simple electrostatic model is presented. The ion permeability of the monolayer has been tested by using a probe ion (tetraethylammonium cation, TEA<sup>+</sup>) which transfers within the potential window.

### Method

The Langmuir–Blodgett technique has been used extensively to produce mono- and multilayer films on solid substrates. The method involves first assembling a monolayer at the air–water interface and then transfer-

ring it onto a substrate. Multilayers can be built up by repeating this procedure the desired number of times. The resulting mono- or multilayer can be characterized by various spectroscopic and electrochemical techniques.<sup>18,19</sup> The present study differs in the choice of the substrate. Instead of conventional solid substrate, for example a glass slide, an electrochemical cell has been dipped through the monolayer at the air–water interface. A schematic drawing of the cell is shown in Figure 1. The organic phase was immobilized by the use of a gelling agent (PVC). The cell is made of hydrophobic polytetrafluoroethene (PTFE, Teflon). When it is immersed into the subphase, the whole cell, including the interface, is covered by a monolayer. The monolayer can then be characterized using standard ITIES electrochemistry. The procedure will be presented in more detail in the following paragraph.

The lipid solution, typically 40  $\mu\text{L}$ , was pipetted onto an aqueous subphase. The solvent (chloroform) was then left to evaporate; the time allowed for this was usually 10 min. Using movable barriers, the surface pressure was adjusted to the desired value. The rate of compression was 5 mm min<sup>-1</sup>. The monolayer was left to reorientate and relax for 20 min. Subsequently, the electrochemical cell was dipped through the monolayer at an angle of 30 degrees, with the organic phase facing down (see Figure 1). The rate of dipping was 2.0 mm min<sup>-1</sup>. This position was found to be optimum for the transfer of the monolayer. The quality of the transfer was determined both from the subsequent electrochemical measurements and the so-called transfer ratio, with values close to unity corresponding to successful transfer. The triangular shape of the cell was designed to facilitate future spectroscopic measurements performed in a cuvette. Prior to beginning the electrochemical measurements, the excess lipid was suctioned off the air–water interface with the aid of a suction pump, and the aqueous counter and reference electrodes were introduced into the trough.

Three different lipids were examined: DSPC, dipalmitoyl phosphatidylcholine (DPPC), and dioleoyl phosphatidylcholine (DOPC). The first two are known to form rigid monolayers and were chosen in order to study the effect

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of chain length. On the other hand, DOPC forms more expanded monolayers due to a double bond in the hydrocarbon tail. It was possible to transfer monolayers of all these three phospholipids. However, DSPC proved to be experimentally easiest and was chosen for more detailed investigation.

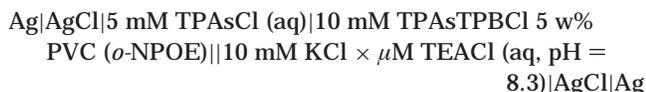
### Experimental Section

**Apparatus.** The Langmuir trough (KSV Instruments Ltd., Helsinki) was made of PTFE and equipped with a well at the bottom. The dimensions of the trough were 150 mm (width)  $\times$  300 mm (length). The barriers were made of Delrin. The trough was thermostated using a computer-controlled Lauda thermostat (C6 CS, Lauda-Königshofen, Germany). The surface pressure was measured with a Wilhelmy plate made of sand blasted platinum. The entire apparatus was placed in a grounded Faraday cage and computerized with an IBM-compatible (Intel 486 processor) personal computer via an interface unit (KSV Instrument Ltd., Helsinki) connected to the serial port. KSV Instruments Ltd. also provided the control software.

The organic reference electrode system was contained in a thin PTFE tube, see Figure 1. A platinum plate at the back of the cavity in the cell acted as the organic counter electrode. The potential difference was controlled by means of a four-electrode potentiostat (Electronics Department, Southampton University, U.K.) equipped with positive feedback IR compensation. The potential function was provided by a waveform generator PPR1 (Hi-Tek Instruments, U.K.), and the resulting current was measured with a Tektronix TDS420A four channel digitizing oscilloscope (Tektronix Inc., USA).

**Materials.** 2-Nitrophenyl octyl ether (*o*-NPOE) (Fluka, Selectophore) was used as received and utilized as the nonaqueous solvent. Poly(vinyl chloride) (PVC) (Sigma, very high molecular weight) was used as a gelling agent. The mixture of PVC (5 w-%) and NPOE with the base electrolyte was heated to 110 °C and then simply cast into the cell.<sup>20</sup> The aqueous solutions were prepared from Milli-Q treated water. Chloroform (Sigma-Aldrich, HPLC grade) was used to prepare the lipid stock solutions.

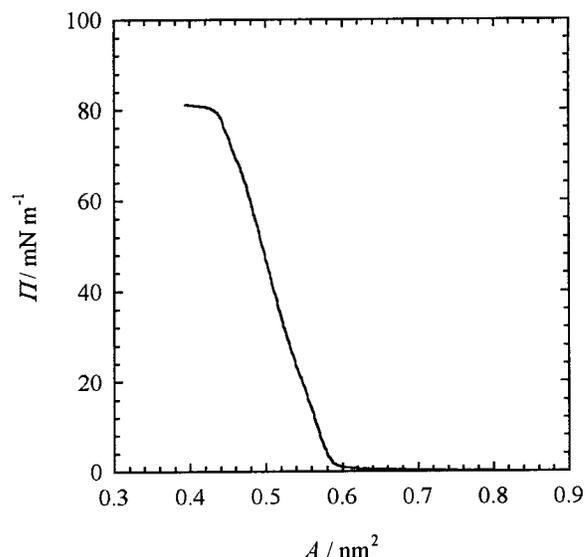
Potassium chloride (KCl) (Merck, p.a.) was used as the aqueous supporting electrolyte. The aqueous phase was buffered with potassium dihydrogen phosphate and sodium monohydrogen phosphate (J. T. Baker B. V., 'Baker Analyzed'<sup>®</sup>). The organic base electrolyte tetraphenylarsonium tetrakis-4-chlorophenylborate (TPAsTPBCl) was prepared by metathesis of tetraphenylarsonium chloride (TPAsCl) (Sigma, p.a.) and potassium tetrakis-4-chlorophenylborate (KTPBCl) (Aldrich, p.a.) as described elsewhere.<sup>21</sup> The probe tetraethylammonium cation was added as the chloride salt (TEACl) (Sigma) to the aqueous phase. The cell used was as follows:



Monolayers were prepared from 1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine (distearoyl phosphatidylcholine, DSPC, >99%) purchased from Sigma in powder form and used without further purification. All of the lipid stock solutions (in chloroform) were stored at  $-5$  °C.

### Results and Discussion

Figure 2 shows a plot of the surface pressure,  $\Pi$ , as a function of the mean molecular area,  $A$ , for DSPC at 20 °C. The isotherm shows that monolayer collapse is visible at surface pressures of slightly below 80  $\text{mN m}^{-1}$ . The mean molecular area at collapse is  $0.43 \text{ nm}^2$ . The mean molecular area is slightly higher in this case with the phosphate buffer than with the subphase containing divalent cations (e.g.,  $\text{Ca}^{2+}$ ).<sup>11</sup> The isotherm does not



**Figure 2.** Isotherm of DSPC at air–water interface. The contents of the subphase: 10 mM KCl and a phosphate buffer, pH 8.3. Temperature 20 °C.

display a fluid phase, the surface pressure at the transition from an expanded to a condensed phase is very low. It should be noted that the phase behavior of phospholipids is different at air–water and at organic solvent–water interfaces. At water–hydrocarbon interfaces, the fluid phase of dipalmitoyl phosphatidylethanolamine (DPPE) is stabilized if the organic solvent can mix with the hydrocarbon tails of the phospholipid. A pure two-dimensional lipid phase is then only obtained at higher surface pressures.<sup>3</sup> The same effect has been noted at water–*n*-heptane interfaces with various phosphatidylcholines (including DSPC)<sup>1,2</sup> and at water–DCE interfaces with DSPC.<sup>11</sup> Grandell and Murtoimäki also noted that the organic base electrolyte TPAsTPBCl can be accommodated within the monolayer. In general, it can be said that monolayers at a water–organic solvent interface are more disordered and expanded than the corresponding monolayers at an air–water interface.<sup>2,11,22</sup>

Base electrolyte voltammograms for the system are shown in Figure 3 for both the absence and presence of a deposited monolayer. The monolayer present in (b) was deposited at a surface pressure of 60  $\text{mN m}^{-1}$ . As can be seen, the capacitive current is greatly reduced upon the addition of a monolayer. The small peak in the middle of the potential window in the presence of phospholipid is due to transfer of a trace impurity (most probably of organic origin). In the absence of a monolayer, the larger capacitive current disguises the transfer of this impurity.

The data presented in Figure 3 can be used to calculate a pseudo electrocapillarity curve. Current is the sum of both faradaic and capacitive components. In the case of a potential sweep, the capacitive current is the interfacial capacitance,  $C$ , times the sweep rate  $v$ . The faradaic part is proportional to the square root of the sweep rate if the process can be considered reversible or irreversible. In the absence of redox species, the faradaic current at ITIES results from ion transfer which has been found to be reversible. The current can thus be expressed in mathematical form as follows<sup>23</sup>

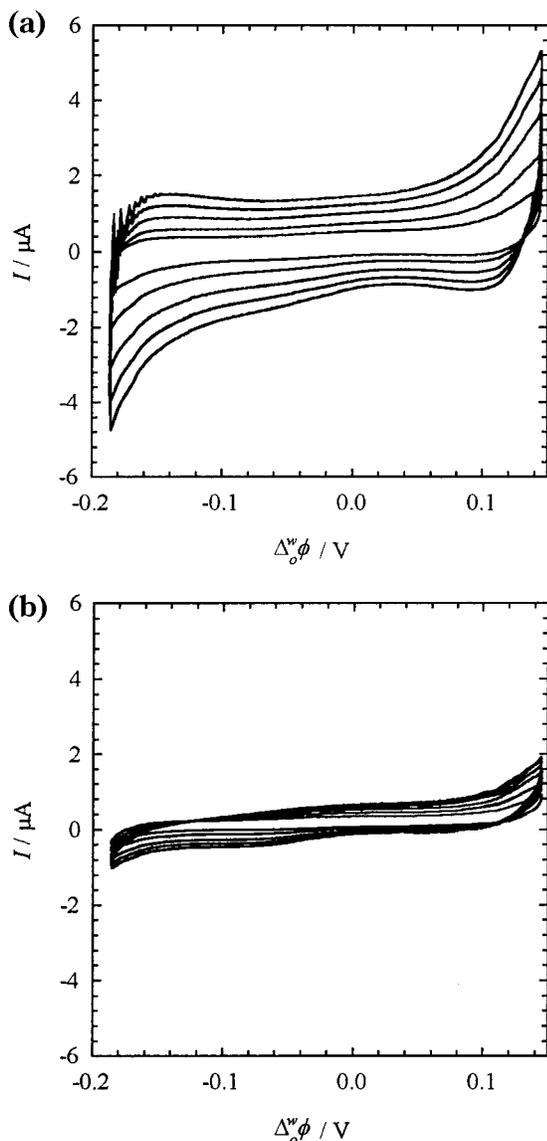
$$I = Cv + Mv^{1/2}$$

where  $M$  is a constant. Following this method, pseudo

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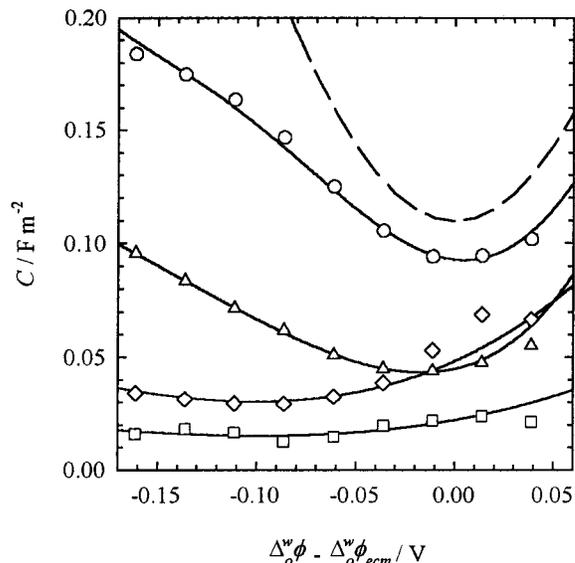
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**Figure 3.** Base electrolyte voltammograms at sweep rates of 10, 25, 50, 75, and 100  $\text{mV s}^{-1}$  in the absence (a) and presence (b) of a DSPC monolayer deposited at the surface pressure of 60  $\text{mN m}^{-1}$ .

electrocapillary curves presented in Figure 4 were calculated for different deposition pressures. It is important to note that the values for the bare interface are in reasonable agreement with the values obtained by Samec et al. from impedance measurements.<sup>24</sup>

In Figure 4, three different types of behaviors are evident. With the bare interface, the capacitance follows the expected Verwey–Niessen behavior.<sup>25</sup> The dashed line shows the capacitance calculated from the Verwey–Niessen model. A monolayer deposited at a surface pressure of 30  $\text{mN m}^{-1}$  lowers the capacitance. At a deposition pressure of 40  $\text{mN m}^{-1}$ , a distinct difference is visible: the minimum of the electrocapillary curve has shifted by 90 mV and also the capacitance has dropped appreciably. The curve appears to be asymmetric. This may be only an illusion since more negative potentials



**Figure 4.** Electrocapillary curves calculated from the base electrolyte voltammograms at different deposition pressures of the monolayer. (○) bare interface, (Δ) 30  $\text{mN m}^{-1}$ , (◇) 40  $\text{mN m}^{-1}$ , and (□) 50  $\text{mN m}^{-1}$ . The solid lines are only as a guide to the eye. The dashed line is calculated from the Verwey–Niessen theory.

were not investigated (due to limitations of the available potential window). The two highest values of deposition pressure, 50 and 60  $\text{mN m}^{-1}$  (not shown), are characterized by very low and approximately constant interfacial capacitance. The points corresponding to the deposition pressure of 60  $\text{mN m}^{-1}$  are not shown as the interfacial capacitance was below the detection limit of the method. Thus, it can only be concluded that the interfacial capacitance was very low, of the order of 0.01  $\text{F m}^{-2}$ . The lowered value of interfacial capacitance in the presence of a phospholipid monolayer has been attributed to both the replacement of interfacial (unstructured) water with polar phospholipid headgroups (plus structured water)<sup>14</sup> and thus, lowering of the dielectric constant in the interfacial region<sup>26</sup> and, generally, to the formation of a compact layer of low dielectric constant.

The shift in the minimum of the electrocapillarity curve (electrocapillary minimum, ecm) has been observed before for a variety of phosphatidylcholines.<sup>7,8,12,14,15</sup> Three main explanations for this shift have been reported. First, Girault and Schiffrin<sup>14</sup> proposed that the reason is the protonation of the phosphate group followed by a reorientation of the lipid. The interfacial  $\text{pH}^\sigma$  can differ significantly from the aqueous bulk value  $\text{pH}^b$  (according to  $\text{pH}^\sigma = \text{pH}^b + F/(2.303RT)(\phi(0) - \phi(w))$ ). Although the  $\text{p}K_a$  value for the phosphate group in a phosphatidylcholine is 1.7,<sup>27</sup> Girault and Schiffrin observed that protonation readily occurs at pH 5. However, in the present study, the bulk pH was 8.3. Besides, the possible protonation would increase the potential at the interface, thus increasing the interfacial pH (compared to no protonation). Thus, the possibility of protonation of the headgroup can be ruled out.

A second possibility would be the orientation of the zwitterion at an angle with the surface. As the phosphate group is directly attached to the glyceride part of the phosphatidylcholine and the ternary ammonium group is

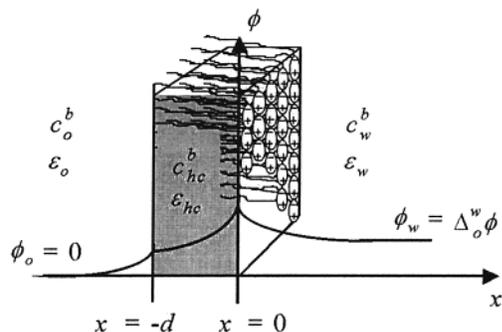
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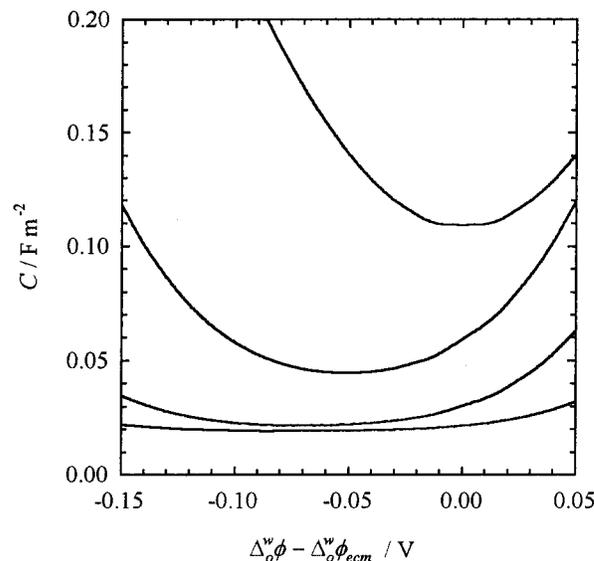


**Figure 5.** Three-layer model used for the interfacial region.

away from it, the deviation from the parallel alignment should occur so that the positive charge would be toward the water phase.<sup>15,28</sup> Even though the dipole of the PC zwitterion is large ( $22 D^{28}$ ), a small departure from parallel orientation would not displace the electrocapillary curve significantly. However, such a departure would result in a shift of ecm in a positive direction, which is at odds with the experimental results.

The third explanation attributed the shift partly to the orientation of C=O bond in the carbonyl groups and partly to the adsorption of aqueous cations.<sup>15</sup> The adsorption of total positive charge onto the zwitterionic surface has also been found with dimyristoyl phosphatidylcholine, DMPC, liposomes in KCl solution below the transition temperature with electrophoretic mobility measurements.<sup>29</sup> It should also be stressed that potassium and sodium cations bind to the zwitterionic of phosphatidylcholines more strongly than chloride and phosphate anions, which exhibit negligible affinity for the PC headgroup.<sup>30</sup>

To explain both the shift in the electrocapillary minimum and the lowered value in the interfacial capacitance, two different aspects need to be accounted for. As outlined above, the shift in the ecm appears therefore to be caused by cation binding. On the other hand, the lowered value of capacitance can be explained by modified dielectric properties of the interfacial region. To explain these observations, the interfacial region has been modeled by splitting it into three distinct layers. The situation is schematically pictured in Figure 5. A brief outline of the theory is given here, details are given in the Appendix. The theory consists of an electrostatic picture based on the solution of the Poisson–Boltzmann equation. It consists of three different layers: the organic phase, the hydrocarbon domain of the monolayer, and the aqueous phase. It was realized that simply modifying the dielectric constant of the monolayer phase does not result in capacitances approaching the geometrical capacitance ( $\epsilon_{hc}/d$ , which for a monolayer of DSPC gives a capacitance of  $0.01 \text{ F m}^{-2}$ , where  $\epsilon_{hc}$  is the dielectric constant of the hydrocarbon phase). Therefore, only partial ion exclusion in the monolayer phase takes place, except in the case of the deposition at  $60 \text{ mN m}^{-1}$ . In the model, the partition coefficient  $K_b$  governs the ionic concentration inside the monolayer. The shift of the ecm to more negative potentials results from cations binding with the monolayer. This results in positive surface charge  $\sigma$ , which is of course potential dependent. This effect is incorporated in the binding constant  $K_b$ , which is in reality a composite



**Figure 6.** Theoretical electrocapillary curves. The uppermost curve is calculated from the Verwey–Niessen theory, others according to the present model. Parameter values  $c_w = 100 \text{ mM}$ ,  $c_o = 10 \text{ mM}$ ,  $\epsilon_w = 78$ ,  $\epsilon_o = 24.2$ ,  $d = 20 \text{ \AA}$ ,  $K_b = 50 \text{ dm}^3 \text{ mol}^{-1}$ . Other parameters, from bottom to the top,  $K_p = 1/100, 1/20, 1/5$ , and  $\epsilon_{hc} = 5, 5, 10$ .

quantity including contributions from all of the ions in the system. In addition, the dielectric permittivity of the monolayer and the area per one phospholipid molecule appear as parameters in the general model (see eq 11 in the Appendix). It should also be noted that the two phenomena incorporated in the model have qualitatively different effects on the capacitance. The purpose of the study is not to extract values of mean molecular area, permittivity, partition, and binding constants. The point is to show that ion binding to the headgroups and partial ion exclusion from the hydrocarbon domain of the monolayer can be used to explain the observed behavior.

Calculated electrocapillary curves are shown in Figure 6. The values of the various parameters were taken as follows. The bulk concentrations were  $100 \text{ mM}$  and  $10 \text{ mM}$  in the aqueous and organic phases, respectively. The relative permittivities were  $24.2$  and  $78$  for NPOE and water, respectively.<sup>31</sup> The thickness of the hydrocarbon domain was taken to be  $20 \text{ \AA}$ . The dielectric permittivity and the partition coefficient were varied. The binding constant was taken to be  $50 \text{ dm}^3 \text{ mol}^{-1}$ , as this gave the appropriate shift in ecm. The mean molecular area of the phospholipid was varied. The shift in the minimum was mainly determined by the binding constant, the mean molecular area has only a minor effect. The relative permittivity and thickness of the hydrocarbon layer and the partition coefficient are linked; the capacitance is lowered with lowering the dielectric constant, making the layer thicker, or lowering the partition coefficient. They all should be determined by the state of the monolayer. Compact monolayer implies low partition coefficient and low permittivity ( $\epsilon_{hc} \rightarrow 2$ ). On the other hand, the more expanded the monolayer is, the closer the partition coefficient is to 1 and the permittivity  $\epsilon_{hc}$  to the permittivity of the solvent. It is conceivable to have a partition coefficient greater than one if there are specific interactions between the monolayer and the organic base electrolyte.

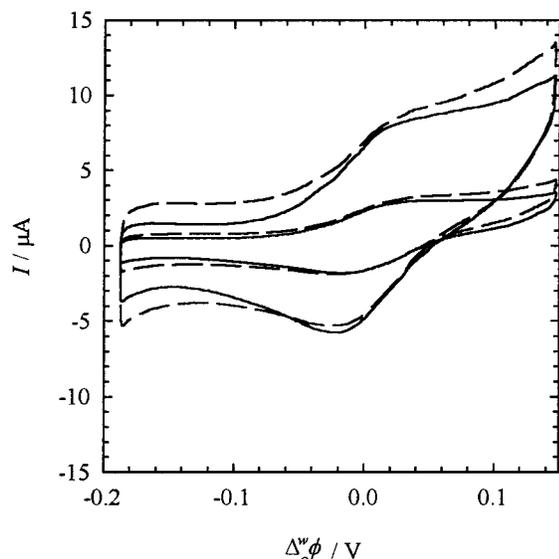
The topmost curve in Figure 6 is the Verwey–Niessen capacitance. The other curves were calculated using values

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**Figure 7.** Effect of presence (solid line) of a DSPC monolayer on TEA<sup>+</sup> transfer. Corresponding voltammograms in the absence of a monolayer (dotted line). Sweep rates 10 and 100 mV s<sup>-1</sup>.

of partition coefficient of 1/5, 1/20, 1/100, permittivity of the monolayer 10, 5, 5, and a mean molecular area of 100, 60, 50 Å<sup>2</sup>. The last values in each case would correspond to a very compact monolayer and first values to a largely expanded monolayer. Similar changes to those found in the experimental curves are observed. It would appear that the monolayer undergoes a phase change from an expanded to a condensed phase between the deposition pressures of 40 and 50 mN m<sup>-1</sup>. This phase change results in a change in the partition coefficient. At the highest deposition pressure, the resulting monolayer is in a fully condensed state. The monolayer deposited at 30 mN m<sup>-1</sup> would appear to be in a highly expanded state. Comparison between Figures 4 and 6 demonstrates that using this kind of approach gives general agreement between the experimentally and theoretically derived curves in terms of the shift in the value of the ecm and the values of the interfacial capacitance.

Even though DSPC does not show a transition between expanded and condensed phases at the air–water interface, there is evidence that this transition occurs at a liquid–liquid interface.<sup>1</sup> This would also imply rearrangement of lipids during deposition. However, it is unlikely that the resulting film would be inhomogeneous because of the rather high deposition surface pressures used. This view is supported by the fluorescence micrographs of phospholipids at a liquid–liquid interface where the film is homogeneous at high surface pressures.<sup>3</sup>

Probe ion transfer across the monolayer was also studied, a typical example is shown in Figure 7 where the monolayer was deposited at the surface pressure of 60 mN m<sup>-1</sup>. The slightly irregular shape of the voltammograms (both in the absence and in the presence of the monolayer) is probably due to the cell geometry. The cell was designed to be suitable for the transfer of a monolayer and possible spectroscopic studies. In the end, the main point was to compare different deposition surface pressures.

As can be seen from Figure 7, the peak separation has stayed close to 60 mV and the position of the peaks has not shifted, indicating that the process is diffusion controlled and that the Gibbs free energy of transfer has not been affected. The main point of difference is the reduced capacitance in the presence of the monolayer.

However, since the total peak current is the same in both cases, there seems to be an increase in the faradaic component. This is in agreement with the previous work involving ion transfer at a lipid covered interface.<sup>10</sup>

### Conclusions

It has been shown, for the time, that the Langmuir–Blodgett technique can be used to assemble monolayers of phosphatidylcholine molecules at the interface between two immiscible electrolyte solutions in a controlled and reproducible manner. The monolayer was characterized by four-electrode cyclic voltammetry, which yielded both capacitance data and information on ion permeability of the monolayer. The deposition surface pressure had a great influence on the behavior of the monolayer. At the lower deposition pressures, the capacitance was low at negative potentials, but approached that of bare interface at positive potentials. At the higher deposition pressures, the capacitance was very low and approximately constant. The minimum of the electrocapillary curve shifted in the negative direction. These observations were interpreted by a simple electrostatic model. In this model, aqueous cation binding to the zwitterionic phospholipid headgroup is responsible for the shift in the ecm and the lowered dielectric constant, and the organic electrolyte concentration in the hydrocarbon domain of the monolayer causes the lower values of the interfacial capacitance. Initial studies on probe ion transfer indicated an enhancement in the rate of ion transfer.

This technique of controlling the surface pressure offers the possibility of studying biologically important reactions within a greatly improved mimetic system. Ionizable drug transfer will form the focus of a future publication.

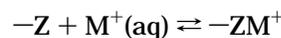
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### Appendix

The interface is divided into three layers shown in Figure 5. The organic and aqueous bulk phases have bulk concentrations and relative permittivities of  $c_o^b$ ,  $c_w^b$ , and  $\epsilon_o$ ,  $\epsilon_w$ , respectively. The zwitterions of the phospholipid headgroup are assumed to be parallel to the plane  $x = 0$ . The layer between  $x = -d$  and  $x = 0$  is representing the hydrocarbon domain of the monolayer with relative permittivity  $\epsilon_{hc}$ . The concentration in the hydrocarbon domain in the absence of potential difference,  $c_{hc}^b$ , is related to the bulk concentration in the organic phase through the standard chemical potentials in both phases

$$c_{hc}^b = c_o^b \exp\left(\frac{\mu_o^0 - \mu_{hc}^0}{RT}\right) = K_p c_o^b \quad (1)$$

where  $K_p$  is the partition coefficient between the organic phase and the hydrocarbon domain of the monolayer. The surface charge  $\sigma$  is assumed to be due to aqueous cations binding with the phosphonic groups



where an aqueous cation  $M^+(\text{aq})$  binds to the zwitterionic headgroup of the phospholipid,  $Z$ , with 1:1 stoichiometry. The law of mass action written for this reaction gives

$$K_b = \frac{\alpha}{(1-\alpha)c_w^b \exp[f(\Delta_o^w \phi - \phi(0))]} \quad (2)$$

where  $K_b$  is the equilibrium (binding) constant,  $\alpha$  is the degree of binding, and the surface concentration of the cation is related to the bulk concentration through the Boltzmann factor  $\exp[f(\Delta_o^w \phi - \phi(0))]$  where  $f = F/RT$ ,  $\Delta_o^w \phi$  is the Galvani potential difference between the water and organic phases, respectively, and  $\phi(0)$  is the potential at  $x = 0$ . If the binding energy is not a function of the degree of binding and the micropotential due to the zwitterion is either small or constant, the use of law of mass action is justified.<sup>32</sup> The surface charge due to the bound cations follows then from the assumption of 1:1 binding stoichiometry

$$\sigma = \frac{\alpha e}{A} \quad (3)$$

where  $e$  is the elementary charge and  $A$  is the mean molecular area of phospholipids in the monolayer.

In the case of 1:1 electrolytes, the Poisson–Boltzmann equation for phase  $\beta = o, hc, w$  can be written as follows

$$\frac{d^2 \phi}{dx^2} = \frac{2Fc_\beta^b}{\epsilon_\beta} \sinh[f(\phi(x) - \phi_\beta)] \quad (4)$$

where  $c_\beta^b$ ,  $\epsilon_\beta$ , and  $\phi_\beta$  are the bulk concentration, dielectric permittivity, and electric potential of phase  $\beta$ , respectively. For the present case, the boundary conditions are

$$\phi_o = \phi_{hc} = \phi(x \rightarrow -\infty) = 0 \quad (5a)$$

$$\phi_w = \phi(x \rightarrow \infty) = \Delta_o^w \phi \quad (5b)$$

$$\left. \frac{d\phi}{dx} \right|_{x=\pm\infty} = 0 \quad (5c)$$

Integrating eq 4 once yields the electric potential gradient at the boundaries between different regions

$$\left. \frac{d\phi}{dx} \right|_{x=0+} = \left( \frac{8RTc_w^b}{\epsilon_w} \right)^{1/2} \sinh \left[ \frac{f}{2} (\Delta_o^w \phi - \phi(0)) \right] \quad (6a)$$

$$\left. \frac{d\phi}{dx} \right|_{x=-d-} = \left( \frac{8RTc_o^b}{\epsilon_o} \right)^{1/2} \sinh \left[ \frac{f}{2} \phi(-d) \right] \quad (6b)$$

$$\left( \left. \frac{d\phi}{dx} \right|_{x=-d+} \right)^2 - \left( \left. \frac{d\phi}{dx} \right|_{x=0-} \right)^2 = \frac{4RTc_{hc}^b}{\epsilon_{hc}} (\cosh[f\phi(-d)] - \cosh[f\phi(0)]) \quad (6c)$$

The boundary conditions for the electric displacement are

(32) Healy, T. W.; White, L. R. *Adv. Colloid Interface Sci.* **1978**, *9*, 303.

$$\epsilon_o \left. \frac{d\phi}{dx} \right|_{x=-d-} = \epsilon_{hc} \left. \frac{d\phi}{dx} \right|_{x=-d+} \quad (7a)$$

$$\epsilon_{hc} \left. \frac{d\phi}{dx} \right|_{x=0-} = \epsilon_w \left. \frac{d\phi}{dx} \right|_{x=0+} + \sigma \quad (7b)$$

Here the ion size is neglected or it can be considered that the plane of adsorption is equal to the plane of zwitterions. Equations 6 and 7 can be used to relate  $\phi(-d)$  to  $\phi(0)$

$$(c_o^b \epsilon_o - c_{hc}^b \epsilon_{hc}) \cosh[f\phi(-d)] = (c_o^b \epsilon_o - c_{hc}^b \epsilon_{hc} \cosh[f\phi(0)]) + \left( (2c_w^b \epsilon_w)^{1/2} \sinh \left[ \frac{f}{2} (\Delta_o^w \phi - \phi(0)) \right] + \frac{\sigma}{(4RT)^{1/2}} \right)^2 \quad (8)$$

The procedure to solve these equations numerically by the so-called shooting method is as follows: First, guess a value of  $\phi(0)$ . Second, calculate  $\phi(-d)$  from eq 8. Third, solve the Poisson–Boltzmann equation between 0 and  $-d$ . Fourth compare the values obtained from the second and fourth steps, and repeat the cycle until they agree. Step 3 was done using a variable order Adams–Bashford–Moulton integration with adaptive step size implemented in Matlab 5.2. The integration was started using the fourth-order Runge–Kutta method.<sup>33</sup> The system as a whole is electroneutral

$$Q_o + Q_{hc} + \sigma + Q_w = 0 \quad (9)$$

where  $Q_\beta$  is the charge in the phase  $\beta$ . The interfacial capacitance  $C$  is defined as follows<sup>25</sup>

$$C = \frac{\partial Q}{\partial \Delta_o^w \phi} \quad (10)$$

where  $Q$  is the charge flowing to the interface when its area is increased by unit amount. In this case, it is defined as follows

$$Q = -Q_o - Q_{hc} = Q_w \sigma = \int_0^\infty \rho dx + \sigma = 8RTc_w^b \epsilon_w \sinh \left[ \frac{f}{2} (\Delta_o^w \phi - \phi(0)) \right] + \sigma \quad (11)$$

In eq 11, the surface charge  $\sigma$  is also a function of the total potential drop and the interfacial potential  $\phi(0)$  (the degree of binding is naturally potential dependent). In practice, the capacitance was calculated by fitting a polynomial to the calculated values of charge as a function of the Galvani potential difference followed by analytical differentiation of the polynomial.

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