The Electrocapillary Curves of the Phosphatidylcholine Monolayer at the Polarized Oil-Water Interface. I. Measurement of Interfacial Tension Using a Computer-Aided Pendant-Drop Method

Takashi Kakiuchi, Masatoshi Nakanishi, and Mitsugi Senda*
Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606

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The conditions for obtaining the electrocapillary curves of the dilauroylphosphatidylcholine (DLPC) monolayer formed at the polarized nitrobenzene-water interface have been studied using the pendant drop method. To facilitate the measurement, the system for the interfacial tension measurement, including the computer software for the data handling, has been developed based on digital image processing of the video image of a pendant drop. The 95% confidence interval of the measured interfacial tension value was ± 0.06 mN m^{-1}. The time required to measure an electrocapillary curve with 30 points was 30 min, while the time necessary to convert the image data to the interfacial tension was 1 min. The potential drop across the monolayer has been demonstrated to influence the stability of the monolayer strongly. The DLPC markedly lowered the interfacial tension by forming a stable monolayer when the interface was polarized so that the aqueous phase has a negative potential with respect to the nitrobenzene phase. On the other hand, when the aqueous phase became positively polarized, the adsorbed DLPC molecules started to desorb from the interface, giving rise to the disruption of the monolayer. The electrocapillary curves only in the former potential range were found to be thermodynamically meaningful.

The structure of the electrical double layer in the vicinity of the surface of lipid bilayers, monolayers, and biological membranes is important in understanding various surface phenomena associated with the biological as well as biomimetic membranes and, in general, with oil-water interfaces. The electrically polarizable oil-water interface is a useful means to study the electrical phenomena at the oil-water interface, in that the potential drop across the interface is accurately controlled externally.[1] This type of system has been used successfully to elucidate the double layer structure of ionic surfactant monolayers at oil-water interfaces,[2,3] aside from a wide range of applications to analytical chemistry and the electrochemistry of ion-transfer reactions.[4] If one introduces phospholipids to this type of interface, one may expect to be able to study in detail the interfacial and electrochemical properties of the phospholipid monolayer which are not readily accessible by other methods, e.g., the properties of the electrical double layer and the kinetics of ion-transfer reaction across the monolayer, by using the estahlished method of the analysis of the electrocapillary and electrochemical data.[4,5] Along this line, several groups have reported on the electrochemical characteristics of the phospholipid monolayers at the polarized oil-water interface: a retardation of the ion-transfer reaction from water to oil,[6,7] the differential capacitance of the phospholipid monolayers at the nitrobenzene-water interface[8] and at the dichloroethane-water interface,[9] and the electrocapillarity measurements at the dichloroethane-water interface in the presence of a phospholipid monolayer.[10] To analyse the double layer structure, the basic properties of the monolayer, e.g., the potential of zero charge and the excess charge density, must be evaluated. These quantities can be obtained by measuring the electrocapillary curve of the interface. Since phospholipids are highly surface active, the very dilute concentration of the μmol dm^{-3} order is enough to make up a saturated monolayer at the interface.[8] Such a low concentration of phospholipid requires a long time to reach the adsorption equilibrium. Accordingly, to obtain a thermodynamically meaningful electrocapillary curve, a method that is capable of measuring the equilibrium value of interfacial tension is desirable. For this purpose, the pendant-drop method and the sessil-drop method are the most suitable. Girault and Schiffrin have recently developed a new pendant-drop method by applying a video-image digitizer.[11] They later improved this method by introducing a video-image digitizer with a higher resolution specifically designed for the electrocapillary measurements.[10] In this study, we wish to report a similar apparatus also based on the video-image digitizing technique but with a higher accuracy and faster data handling using a commercially available video image digitizer in combination with computers. By using this new apparatus, the conditions for obtaining the equilibrium electrocapillary curve of the phosphatidylcholine monolayer at the polarized nitrobenzene-water interface have been studied.

Experimental

Reagents. Reagent-grade nitrobenzene was distilied and purified as has been described elsewhere.[1] The nitrobenzene and the nitrobenzene solution were equilibrated with water or an aqueous solution of lithium chloride at 25.0 °C overnight to ensure mutual saturation. Aqueous solutions of lithium chloride and tetrapentylammonium chloride (TPnACl), and a nitrobenzene solution of tetrapentylammonium tetraphenylborate (TPnATPB) were prepared as has been described elsewhere.[13] Reagent grade dichloro-

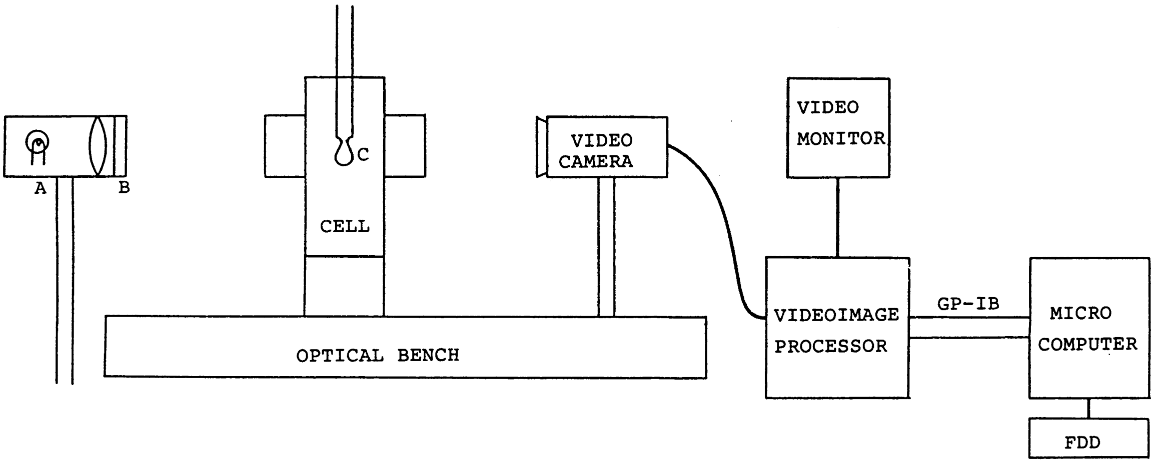

Fig. 1. Block diagram of the apparatus for interfacial tension measurement applying video image processing technique to the pendant drop method. (A) Light source, (B) filter, (C) pendant drop.

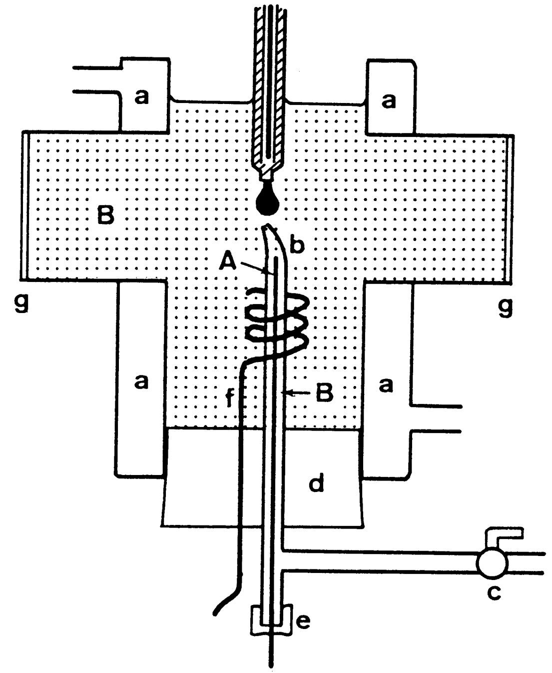

Fig. 2a. Diagram of the cell for the measurement with Configuration I. (a) Water jacket, (b) Luggin capillary, (c) stop cock, (d) silicone rubber stopper, (e) silicone rubber cap, (f) Pt electrode, (g) optically flat Pyrex glass, (A) Ag/AgCl electrode, (B) aqueous solution.

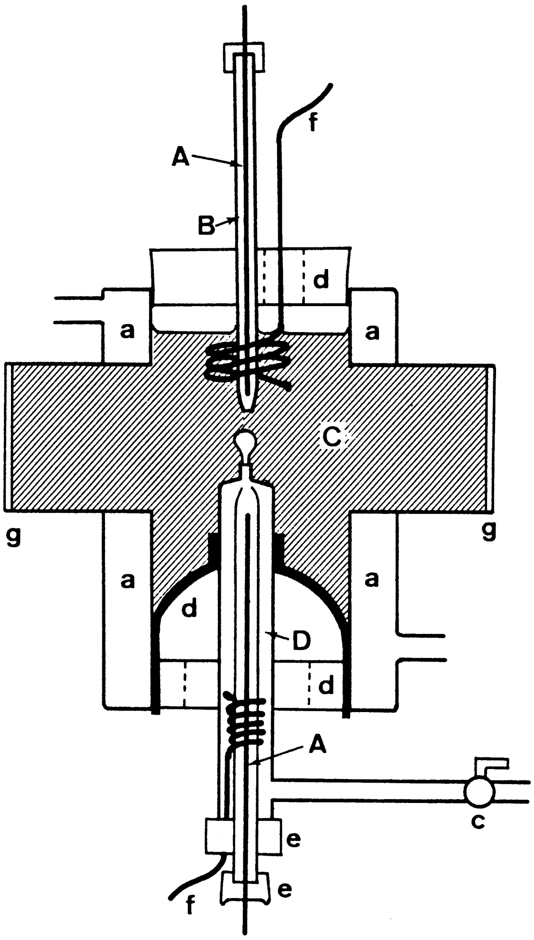

Fig. 2b. Diagram of the cell for the measurement with Configuration II. (a)—(g) and (A) are same to Fig. 2a. (B) Aqueous TPnACl solution, (C) nitrobenzene solution, (D) LiCl aqueous solution.

methane (DCM) and 1,2-dichloroethane (DCE) were purified as follows.[14] These solvents were washed three times with 10% $NaHCO_3$ to remove the HCl. The solvents were then washed with water. After they had then been dried with $CaCl_2$, the solvents were distilled. The fractions of bp 39 °C (DCM) and 83.5 °C (DCE) were collected and stored in the dark. These solvents were equilibrated with water at 25 °C overnight before use in the interfacial-tension measurements. The water used to prepare aqueous solutions for the interfacial tension measurements was first distilled and then redistilled in the presence of potassium permanganate after having been passed through ion-exchange resin and the Organic Removal Filter (Barnstead). The L-α-dilauroylphosphatidylcholine, DLPC, (Sigma) was dissolved in a 3 : 2 toluene-chloroform solution and then stored in a refrigerator until use. All the other chemicals were of a reagent grade.

Interfacial Tension Measurements. The block diagram of the apparatus used for the interfacial tension measuremts is shown in Fig. 1. A glass cell and a video camera were set on an optical bench (Sigma Koki Co. Σ-06-(3)) 80 cm long and supported by two legs (Σ-02 and Σ-03) on rubber dampers. The cell (Fig. 2) had two windows consisting of optically flat borosilicate glass disks 3 cm in diameter (Fig. 2g), and it was installed in a cell holder mounted on a coarse-fine rotation stage (Σ-401-(2)). Two scale-etched Plexiglas plates mounted on a carrier beneath the rotation stage so that the two plates were parallel with the axis of the optical bench and sandwiched the glass cell were used to locate the position of an object in the cell. Two types of cell configurations were used in the measurement. In Configuration I, the cell (Fig. 2) was filled with an aqueous solution. In Fig. 2a, A is a platinum wire which served as an auxiliary electrode, while b is a reference electrode of a Luggin-capillary type in which an aqueous LiCl solution and an Ag/AgCl electrode were incorporated. A glass tube (Fig. 3) filled with a nitrobenzene solution was inserted into the solution in the cell. In Fig. 3, e and h are the platinum wire and the Luggin capillary used as the counter electrode and the reference electrode respectively in the nitrobenzene phase. The tip of this glass tube (i in Fig. 3) has an outer diameter of 2 mm and an inner diameter of 1 mm. This glass tube was mounted on a tip holder set on an assembly of a X,Y tilt stage (Σ-501), a X,Y precision translation stage (Σ-206), and a rack-pinion type X-block (Narisige, M-6). In Configuration II, a nitrobenzene solution was placed in the cell. In this case, a drop of the aqueous solution was formed in the nitrobenzene solution from the tip of the glass tube (Fig. 2b). The tip for this configuration was made of poly(tetrafluoroethylene) tubing 1 mm in outer diameter and 0.5 mm in inner diameter. Below, the interfacial tension was measured in Configuration I unless otherwise stated. The video camera, a CCD type with a picture element of 381(h)×492(v) (Tokyo Electronic Industry Co., CS3300), was secured with a lens holder (Σ-41-(3)) mounted on a set of a X,Y tilt stage (Σ-501) and a coarse-fine rotation stage (Σ-401-(2)). The television lens (Asahi Precision Co., Cosmicar 50 mm F1.4) was mounted on the CCD camera with an extension tube (Cosmicar, 40 mm). The standard tungsten bulb light source unit (Nikon) with a filter (GIF, Nikon) and a diffuse light mat was used to obtain the green diffuse light, which was shed into the flat window of the glass cell. The video signal from the camera was digitized by means of a video-image processor (Nippon Avionics Co., Image Mate) to 640(h)×482(v) pixels, each of which had 256 gray levels. The conversion can be made every 1/30 s. This image processor was controlled by a micro-computer (Fujitsu Co., FM-11AD2) through the GP-IB interface.

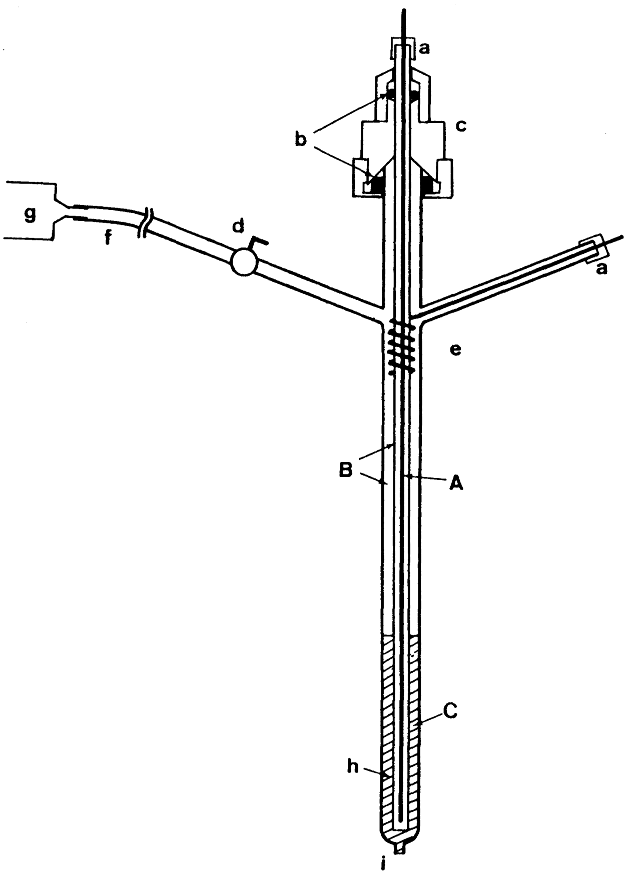

Fig. 3. Diagram of an upper part of the cell for Configuration I. (a) Silicone rubber cap, (b) O-ring, (c) connector, (d) stop cock, (e) Pt electrode, (f) silicone rubber tubing, (g) gas tight syringe, (h) luggin capillary, (i) tip to form a pendant drop, (A) Ag/AgCl electrode, (B) aqueous TPnACl solution, (C) nitrobenzene solution.

Alignment of the Video Camera: First, the rotation angle of the camera was adjusted so that the image of a vertically hanging string was seen horizontally on the screen of a monitor TV (Tokyo Eletronic Industry Co., 9M10A). Then, the position of the focal plane was found as follows. A micrometer with a mesh of 0.5 mm/div. (Kyoto Kogaku) was hung over from the glass tube holder into the position where the glass cell was otherwise located. The position and the angle of the micrometer were adjusted so that the plane of the micrometer was vertical to the axis of the optical bench, which the light beam was introduced in parallel with. The X,Y stage under the glass tube holder was adjusted to bring the micrometer to the focal plane. A high-precision steel ball 5.000±0.001 mm in diameter was hung from the glass tube holder into the focal plane. The number of pixels in the digitized image corresponding to the diameter of the ball in horizontal direction (a pixels) was recorded. The angle of the camera in the vertical direction was adjusted similarly using the micrometer. The number of pixels corresponding to the diameter of the steel ball was again recorded (b pixels). The angle of the camera was adjusted so that the plane of the CCD sensor in the camera was normal to the axis of the optical bench. The micrometer was hung into the position of the focal plane. The camera was turned, step by step, by rotating the rotation stage, and the number of pixels corresponding to the horizontal interval of the micrometer mesh was recorded. The position where the number of pixels was greatest, $L_{\max}$, was found by plotting the number of bits against the rotation angle. The angle of the elevation of the camera was adjusted using the X,Y tilt stage, so that the $L_{\max}a/b$ quantity was at its maximum.

Determination of the Conversion Factor and Correction of Distortion: To obtain the coordinates of the edge of the pendant drop from the digitized image, the relation between the true value of the coordinate and its corresponding value in the digitized image was determined by measuring an object having a known shape and dimensions. The possible distortion of the image was corrected by using the micrometer described above and a standard image processing technique. As was to be expected from the advantage of a CCD type video camera free from bulge distortion, the distortion of a video image was found to be negligibly small. The vertical and horizontal size factors of the measuring system were determined by measuring the size of a steel ball immersed in the cell (Fig. 2a) filled with 0.05 mol dm^{-3} LiCl.

Measurement of the Shape of a Pendant Drop: The drop was formed at the tip, after the glass tube had been brought to the position of the focal plane. To detect the edge of the drop, the change in the gray level was checked against the transverse distance across the drop image (Fig. 4). The gradient of this plot exhibited maxima at either two (Fig. 4) or four points. The gray level —at these two points in the former case and at the outer two points in the latter case— were used as the threshold level of the thresholding procedure.

Data-Processing: The thresholded data were transferred to the microcomputer through GP-IB and then compressed to generate run-length data before the data storage. The overall data acquisition and transfer required 30 s for 1 image data. The data were further processed by a computer (Fujitsu, FACOM M382) in the Kyoto University Data Processing Center. After the noises on the margin of a plane of the image and those due to dust stuck on the cell surfaces had been removed by a software technique, the coordinate for the circumference of the drop was extracted. The position of the apex was found, assuming that the drop had an axial symmetry.

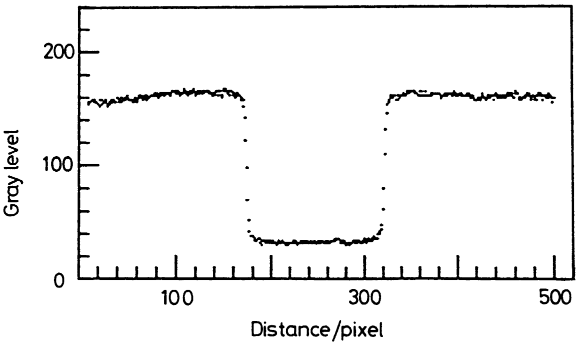

Fig. 4. Change of the gray level on the transverse distance across the drop.

Calculation of Interfacial Tension from the Drop Shape: The theoretical curve was obtained by solving numerically the Bashforth-Adams equation[15] in the following form:

$$\frac{d\theta}{ds} = 2 - \frac{\sin\theta}{x} + \beta y$$

$$\frac{dx}{ds} = \cos\theta$$

$$\frac{dy}{ds} = \sin\theta$$

for the initial conditions, $dx/ds=1$, $dy/ds=0$, and $d\theta/ds=1$ when $s=0$, where

$$\beta = -\frac{\Delta\rho g b^2}{\gamma}$$

In these equations, $x=X/b$, $y=Y/b$, and $s=S/b$. X and Y represent the Cartesian coordinate of a point P on the edge of the drop, S is the distance from the apex to P along the circumference of the drop, and b is the radius of the curvature at the apex. The origin of the coordinate was taken as the apex. θ is the angle between a tangent to the surface at P and the horizontal, $\Delta\rho$ is the density difference, g is the acceleration of gravity, and γ is the interfacial tension. An initial value for b was found from the curvature around the apex obtained by fitting the experimental points around the apex to a third-order polynomial. An initial value for β was set equal to -0.5. The calculated curve obtained using the Runge-Kutta method was fitted into the experimental curve based on the least square principle using the SALS (FORTRAN subroutine for regression analysis).[16] Since the drop shape had an axial symmetry, half of the experimental points were used for the regression

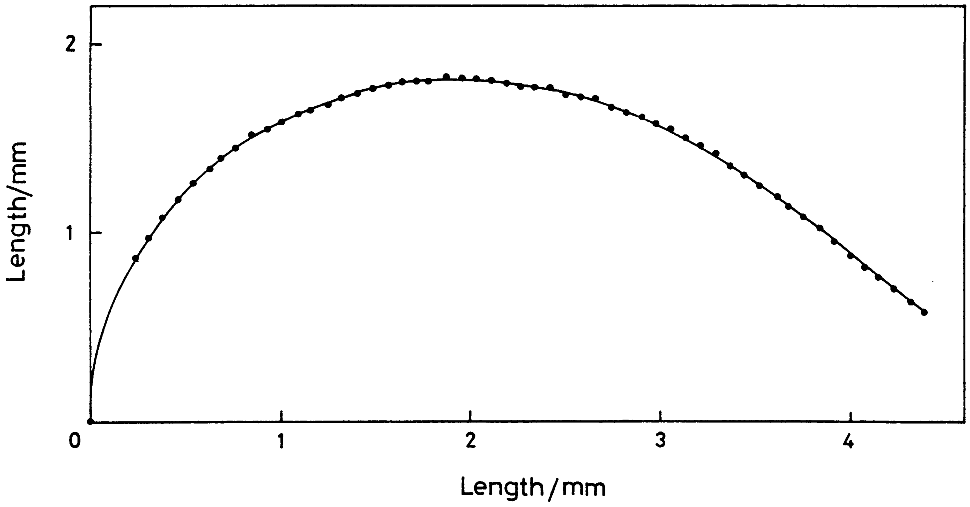

Fig. 5. An example of the result of the least square curve fitting of the numerical solution of the Bashforth-Adams equation to the experimental data; (—●—), experimental; (——), regression curve. The data is for the nitrobenzene–water interface at 25 °C.

Table 1. Values of Interfacial Tension for the 1,2-Dichloroethane-Water, Dichloromethane-Water, and Nitrobenzene-Water Interfaces at 25 °C

Interface	Interfacial tension/mN m^{-1}	
	Present method	Literature data
1,2-Dichloroethane/water	27.93±0.09[a]	28.33[b] (0.19)[d]
Dichloromethane/water	27.93±0.03[a]	28.06[b] (0.22)[d] 28.0[c]
Nitrobenzene/water	25.20±0.07[a]	25.20[c]

a) 95% confidence interval. b) Ref. 10. c) Ref. 18. d) Standard deviation.

analysis. In the calculation with SALS, the Marquardt method and the biweight method were employed for the non-linear regression and the adjustment of the weight attached to each point respectively.[16] The cpu time required to evaluate an interfacial tension value from the run length data for each drop comprised of 250 points for half of the drop image was typically 2 s. Figure 5 shows an example of the curve fitting by the nonlinear regression analysis. The software for the control of the video-image processor and the data processing was written in Basic09 and the machine language on the operating system OS-9.[17] FORTRAN77 was used for data processing on FACOM M-382.

Control of the Pointial Differences. The potential drop across the interface was controlled with a laboratory-made four-electrode potentiostat similar to the type in Ref. 18. The solution resistance was compensated for by using a positive feedback method.[18]

All the measurements were made at 25.00±0.05 °C. The density of the solutions was measured as has been described elsewhere.[1]

Results and Discussion

The Interfacial Tension Measurement for the Pure Organic Solvent-Water Interface. First, the interfacial tensions between water and pure organic solvents were measured. The interfacial tensions for the DCE-water, DCM-water, and nitrobenzene-water interfaces at 25 °C are given in Table 1, with a 95% confidence interval. In general, the present results are in excellent agreement with the literature data given in the second column of Table 1. The 95% confidence interval of the present method is 0.06 on the average, and the precision is better than those obtained by Girault et al.[12] although their video image digitizer had a higher resolution, 1066(h)×575(v). The difference is probably to be ascribed to the method of calculating the interfacial tension from the drop image. They employed the inflection plane method[12] to minimize the computational time. The precision of this method[12] depends greatly on how accurately the point of inflection is determined. The curvature change is not always large enough to determine this point with a high accuracy, even when the curve-fitting technique with a higher polynomial is used. Moreover, the requirement that the inflection point be on the drop shape limits the applicability of this method. The advantage of the direct regression of the data to the Bashforth-Adams equation, which does not require the presence of the inflexion point, seems obvious. This method was first utilized by Girault et al.[11] for the pendant drop data with less resolution.

Table 2. Comparison of Two Computational Methods for Obtaining an Interfacial Tension Value from a Pendant Drop Image

Method	Interfacial tension[a)]
	mN m^{-1}
Curve fitting method	25.34±0.15[b)]
Selected plane method	25.03±0.99[b)]

a) Interface between a nitrobenzene solution of 0.1 mol dm^{-3} TBATPB and an aqueous solution of 0.05 mol dm^{-3} LiCl at E^{W-}_{O+}=0.310 V. b) 95% confidence interval.

We have also compared the present method of data-analysis with the selected-plane method, which has been commonly used as a convenient method for calculating the interfacial tension from a drop shape.[19] A comparison of the two methods for the same data is given in Table 2. This table indicates that the selected-plane method gives a comparable interfacial tension value, but with a confidence interval 6 times wider. This result is expected, for the selected-plane method makes use of only a few out of the many experimentally obtained points in the calculation.

Electrocapillary Measurements. Electrocapillary curves in the absence of phospholipid adsorption were first recorded at intervals of 20 mV between 0.1 V and 0.5 V for the system represented by the cell:

I	II	III	IV	V	VI	VII	
Ag	AgCl	0.02 M† TPnACl (W)	0.1 M TPnATPB (NB)	0.05M LiCl (W)	AgCl	Ag	(I)

The interface between Phases IV and V is the polarized nitrobenzene-water interface. The potential of the right-hand-side of Cell(I) with respect to the left is denoted as E^{W-}_{O+}, where the super- and subscripts indicate that the reference electrodes in the aqueous and nitrobenzene phases are reversible to the anion (Cl$^-$) and the cation (TPnA$^+$) respectively. The nitrobenzene phase contained a 10% 3:2 toluene-chloroform mixture. The results for the triplicate measurements are shown in Fig. 6 (Curve A), with error bars for standard deviations. The average standard deviation of the interfacial tension was 0.25 mN m^{-1}, larger than the deviation for the pure solvents (Table 1) because of the fluctuation of the potential in the electrocapillarity measurements.

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† 1 M=1 mol dm^{-3}.

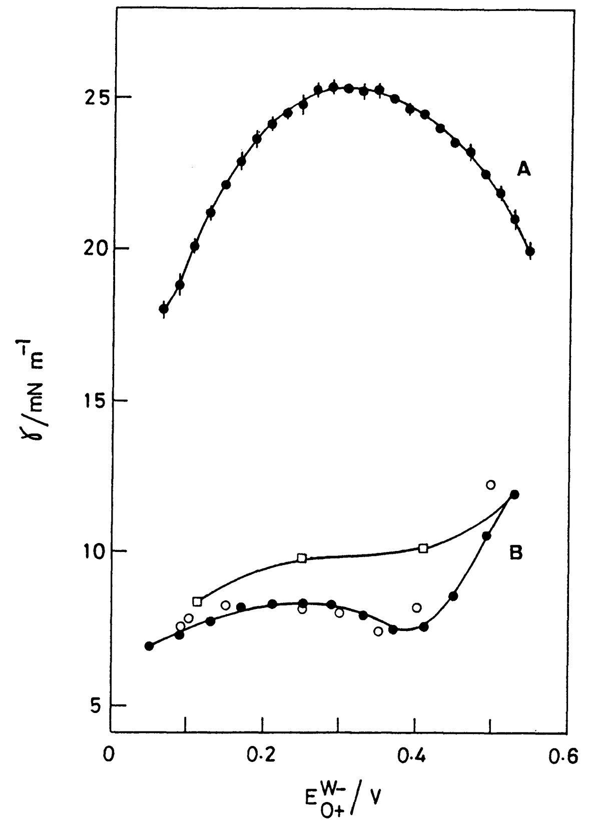

Fig. 6. (A) Electrocapillary curve for the interface between 0.1 mol dm^{-3} TPnATPB and 0.05 mol dm^{-3} LiCl at 25 °C. 20 μmol dm^{-3} DLPC and 0.05 mol dm^{-3} LiCl at 25 °C (—●—). (B) After the adsorption equilibrium was established under open circuit condition, the potential was first scanned from 0.05 to 0.5 V (—●—) and then reversed (—□—). These data are compared with the data obtained through the plot method (—○—).

The electrocapillary curves in the presence of the DLPC monolayer were measured in order to obtain the basic properties of the electrocapillarity of the phospholipid monolayers at the polarized nitrobenzene-water interface. For this measurement, Configuration II of the cell was used to ascertain that the amount of DLPC molecules in the bulk was enough to make up a monolayer at the interface. DLPC was dissolved in the nitrobenzene phase, which contained a 10% 3:2 toluene-chloroform mixture, to facilitate the dissolution of DLPC. The time variation in the interfacial tension was followed after the drop had been squeezed out of the tip at a constant potential. At least one hour was needed for the establishment of adsorption equilibrium, when the concentration of DLPC in the nitrobenzene phase, $c_{\rm DLPC}$, was 1 μmol dm^{-3}. In the following measurements, the interfacial tension was measured 2 h after the formation of the interface.

The lower curve in Fig. 6 shows the electrocapillary curve recorded at $c_{\rm DLPC}$=20 μmol dm^{-3} in Cell(I) after the DLPC had been allowed to adsorb 2 h in the open circuit. From the differential capacitance measurements of the same system, the saturated monolayer of DLPC, whose density at the interface was 0.75 nm^2 per DLPC molecule, was found to be formed at this concentration.[8] The measurement was first made at intervals of 40 mV by changing the potential from 0.05 V to 0.55 V (—●— in Curve B in Fig. 6). The interfacial tension was greatly suppressed by the strong adsorption of DLPC. The electrocapillary curve showed a convex shape between $E^{\rm W-}_{\rm O+}$=0.05 and 0.35 V. Beyond $E^{\rm W-}_{\rm O+}$=0.35 V, the interfacial tension abruptly increased with the increase in the potential. When the potential was reversed from the positive extreme of the potential to sweep in the negative direction, the interfacial tension gave a much higher value (—□— in Fig. 6) as compared with the value obtained initially at the same $E^{\rm W-}_{\rm O+}$ value. This hysteresis probably reflects the slow diffusion process of DLPC and substantiates the desorption of the DLPC molecules from the interface in the positive extreme range of the potential. The concomitant increase in the differential capacitance in this potential region has also been observed.[8] When the potential was reversed at a potential less than 0.35 V, no such hysteresis was observed.

To check if the applied potential during the monolayer formation influences the properties of the monolayer after the adsorption equilibrium had been established, the monolayer was formed while the applied potential was kept at a constant value during the formation of the monolayer: the plot method. The open circles in Fig. 6 were recorded after the interface had been kept at a given value of $E^{\rm W-}_{\rm O+}$ during the course of adsorption. The agreement of the results in Fig. 6 obtained from the two methods suggests that the electrical potential difference across the interface does not introduce any significant difference to the monolayer formed eventually at the interface, provided that the potential is less than 0.35 V, Region I. The discrepancy between the values obtained by the two methods in the potential range positive to $E^{\rm W-}_{\rm O+}$=0.35 V, Region II, indicates the slow processes associated with the desorption of DLPC molecules and possibly their reorientation within the monolayer. Correspondingly, the interfacial tension values obtained in Region I were reproducible, whereas the values in Region II were much less reproducible. At this positive extreme of the potential, fine, small, emulsion-like particles were sometimes observed coming spontaneously out of the tip. This is another piece of evidence of complicated non-equilibrium processes occurring in this potential range. Thus, only the data in Region I seem to be thermodynamically meaningful.

Figure 6 shows that the electrocapillary maximum, i.e., the potential of the zero charge was shifted by 100 mV to the negative direction accompanied by the formation of the saturated DLPC monolayer (Curve B). The reduced curvature of Curve B in Fig. 6 compared with the curve without the DLPC monolayer (Curve A in Fig. 6) suggests a decrease in the surface charge density caused by the DLPC adsorption at a given value of the potential drop across the interface. The curvature, i.e., the second derivative of the interfacial tension with respect to E^{W-}_{O+} in Region I, gave the capacitance value of 11.5 μF cm^{-2} for the DLPC monolayer. This is in accord with the value of 11 μF cm^{-2} obtained from the differential capacitance measurements[8)] for the same monolayer. This agreement confirms the thermodynamic consistency[1)] of the two results in Region I. An electrocapillary curve similar to the curve in Fig. 6 has been obtained by Girault and Schiffrin[10)] for an egg-yolk phosphatidylcholine monolayer at the DCE-water interface. They interpreted the positive slope in Region II (Region B in Ref. 10) as showing the positive excess charge density on the aqueous solution side, originating from the positively charged head group of adsorbed phosphatidylcholine molecules due to the protonation of the phosphate group of the phosphatidylcholine. However, the present results indicate that it is difficult to endow the electrocapillary curve in this region with any definite thermodynamic meaning. For the same reason, it is doubtful if we can estimate the surface charge density from the differential capacitance vs. the potential curve in the presence of phosphatidylcholine adsorption by the back-integration of the curve from the far positive potential in Region II, as was claimed by Wandlowski et. al.[9)] for the dipalmitoylphosphatidylcholine adsorption at the DCE-water interface. The subsequent paper will treat the electrocapillary curves at different concentrations of DLPC in an attempt to analyze the double-layer structure of the adsorbed DLPC monolayer at the nitrobenzene-water interface.

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