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# SURFACE PROPERTIES OF SYNTHETIC PHOSPHOLIPIDS

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SUMMARY

1. Three kinds of dipalmitoyl DL-1-phosphatidyl-alkanolamines were synthesized in a pure state and the monolayer properties of these compounds and of the synthetic phosphatidylcholine were measured. Their conformations in monolayer in relation to the effects of the chemical structures of polar groups upon their surface parameters were discussed.

2. Distinct differences in limiting areas were observed in the phosphatidylethanolamine group. This could be interpreted by assuming the parallel orientation of the polar moieties to the interface as predicted by M. M. STANDISH AND B. A. PETHICA (*Trans. Faraday Soc.*, 64 (1968) 1113). It was also suggested that in phosphatidyl choline the polar moiety was more inclined to the interface at least at ordinary room temperature and near the isoelectric point.

3. These conformations, however, are largely altered by and dependent on temperature. For example, phosphatidylethanolamine at 40° behaves in monolayer like phosphatidyl choline at 25°.

4. Effects of $UO_2^{2+}$ and $Th^{4+}$ on $\pi$–$A$ curves and $\Delta V$–$A$ curves were interpreted by assuming the above conformation for polar moieties in monolayer.

## INTRODUCTION

Recently, both natural and synthetic phospholipids have been extensively studied in monolayer at air–water or oil–water interfaces. Most of these studies have been performed with an aim to exploring their functional analogy to living membranes. However, these types of monolayers are ultimately artificial and are considered to differ from natural membranes in many ways, such as composition or molecular arrangement. Monolayer study must be exclusively understood to offer one method of learning the properties of the membrane materials in the state of monolayer. From this point of view, deforming the molecules synthetically gives a relevant basis for study that can be expected to produce many advantages. Thus, many studies have been performed on the synthesis of phospholipids, as well as monolayer studies using these synthesized materials in which chain length and degree of unsaturation of acyl groups of the phospholipids were widely altered. These studies have offered many suggestions concerning the effects of non-polar

groups on monolayer properties. However, attention was not necessarily paid to the contribution of the chemical structure of polar groups to the properties of the films, in spite of the fact that the question of the conformation and orientation of the head groups in membranes still remains open. We, therefore, synthesized dipalmitoyl glycerylphosphorylalkanolamines containing various polar groups and observed their surface properties in monolayer.

## MATERIALS AND METHODS

Dipalmitoyl DL-1-phosphatidylethanolamine, dipalmitoyl, DL-1-glycerylphosphoryl-3-amino-1-propanol, dipalmitoyl DL-1-glycerylphosphoryl-DL-1-amino-2-propanol were synthesized, details of which are described elsewhere[1]. Purities of these substances were checked by means of thin-layer chromatography. Dipalmitoyl DL-phosphatidylcholine was a commercial product (Sigma) and was purified by means of preparative thin-layer chromatography so as to give one spot on silica gel thin-layer plates.

Surface pressure ($\pi$) measurements were performed by the hanging plate method using a glass plate of 4.8-cm perimeter. Changes in surface tension were picked up by an electro-microbalance, the output from which was fed to an X–Y recorder (Y-axis) through an amplifier. The positions of the barrier for compression on the trough, that is, the area axis, were detected by a resistance wire through which a constant current (5.5 mA) was fed by a mercury battery. Electrical potentials at the contactor sliding on the resistance wire with the barrier were recorded relative to the ground using the X-axis of the recorder as the area axis. The sensitivity of the electro-microbalance used here is 1 % of full scale, so that the errors introduced in the measured surface pressure are between 0.1 and 0.4 dyne/cm depending on the measuring ranges. Estimations of molecular areas were carried out within 1 % of measuring error.

Surface potentials ($\Delta V$) were measured by the ionization method, consisting of Ra-226 (5 $\mu$C) which was suspended at a fixed position above the film (about 5 mm). Potentials between the air electrode and the reference calomel electrode immersed in the subphase were fed to the electrometer (Keithley 610 B), the input impedance of which was $10^{14}$ $\Omega$. The output of the electrometer was recorded on the Y-axis of another X–Y recorder. The X-axis was driven by electric signals from the barrier in the same way as in the $\pi$ measurement. Monolayers were made by delivering 20–50 $\mu$l of spreading solution with a micropipette. A Teflon trough was used, the dimension of which was 45 cm $\times$ 10 cm in surface area. Chloroform–methanol (8:2 v/v) and hexane–ethanol (8:2, v/v) solutions were used as spreading solvents. The subphase solutions consisted of 0.1 M NaCl and 0.01 M buffer, together with the additives.

Surface viscosities were observed using the canal method[2–4], in which a canal 1 mm in width, 50 mm in length and 5 mm in depth was prepared on the Teflon barrier. Measurements were performed with the aid of a special device by which the barrier was driven to retain automatically a constant $\pi$ during measurement. The inclination of the straight line obtained on the recorder between areas and time gives surface viscosity. The trough was placed in a steel chamber thermostated with circulating water from a thermostat, the temperature inside the chamber being controlled to within $\pm$ 0.1°.

RESULTS

$\pi$–$A$ curves and $\Delta V$–$A$ curves were measured on the films of dipalmitoyl phosphatidylethanolamine, -*i*-propanolamine, -*n*-propanolamine and -choline at pH's ranging from 2 to 10, the subphase containing 0.1 M NaCl. Fig. 1 shows the results obtained at pH 5.4. It can be seen that the films are expanded in the order dipalmitoyl phosphatidylcholine > -*n*-propanolamine > *i*-propanolamine > -ethanolamine, and also that dipalmitoyl phosphatidylcholine gives a liquid expanded film at low $\pi$. It can also be seen that dipalmitoyl phosphatidylethanolamine, -*i*-propanolamine and -*n*-propanolamine are in the state of liquid condensed or solid films under the same conditions at this temperature and pH, these states having been confirmed by viscosity measurements. This difference between the ethanolamine and choline group coincides well with most of the results that are reported in the literature[5,6]. Distinct phase transitions, however, were observed in the $\pi$ –$A$ curves of dipalmitoyl phosphatidylcholine at this temperature irrespective of the pH examined here[2–12]. Fig. 2 shows the $\pi$–$A$ and $\Delta V$–$A$ curves at pH 9.5, as examples of behavior at a high pH. Dipalmitoyl phosphatidyl-*i*-propanolamine and -*n*-propanolamine show the remarkable expanded $\pi$–$A$ curves at this pH, while dipalmitoyl phosphatidylethanolamine and -choline are scarcely affected by the change of pH from 5.4 to 9.5. The films of dipalmitoyl phosphatidylethanolamine, -*i*-propanolamine and -*n*-propanolamine are increasingly expanded in relation to increase in pH and the same order of expansion noted above is found. Dipalmitoyl phosphatidylcholine expands only slightly, as seen in Fig. 3.

In Fig. 4 it can be seen that the dipalmitoyl phosphatidylcholine film is remarkably contracted by the presence of $UO_2(NO_3)_2$, while dipalmitoyl phosphatidylethanolamine, -*i*-propanolamine and -*n*-propanolamine are all shifted to the

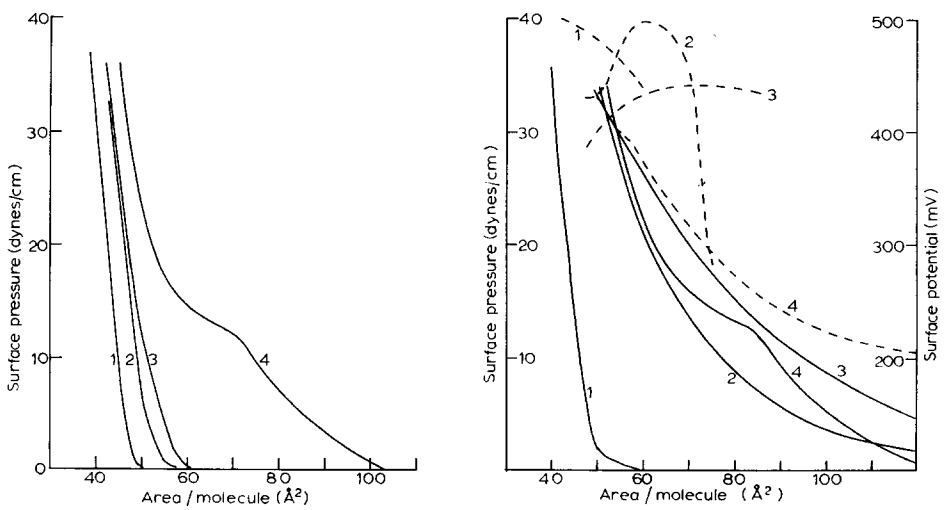


Fig. 1. $\pi$–A curves at pH 5.4 (25°). 1, dipalmitoyl phosphatidylethanolamine; 2, dipalmitoyl phosphatidyl-*i*-propanolamine; 3, dipalmitoyl phosphatidyl-*n*-propanolamine; 4, dipalmitoyl phosphatidylcholine.

Fig. 2. $\pi$–$A$ and $\Delta V$–$A$ curves at pH 9.5 (25°). Solid lines, $\pi$; dashed lines, $\Delta V$. Designation of numbers, see Fig. 1.

larger area and are gathered together into a narrow area range. Fig. 5 shows the $\Delta V$'s of dipalmitoyl phosphatidylethanolamine, -*i*-propanolamine, -*n*-propanolamine and -choline with and without $UO_2^{2+}$. In every case, $\Delta V$ is raised by $UO_2^{2+}$ and the curves for dipalmitoyl phosphatidylethanolamine, -*i*-propanolamine and -*n*-propanolamine are closer together and more simplified. The same characteristics are more clearly seen at lower pH, as indicated in Fig. 6.

Similar trends are also observed in the effects of $Th^{4+}$ on $\pi$–$A$ and $\Delta V$–$A$ curves.

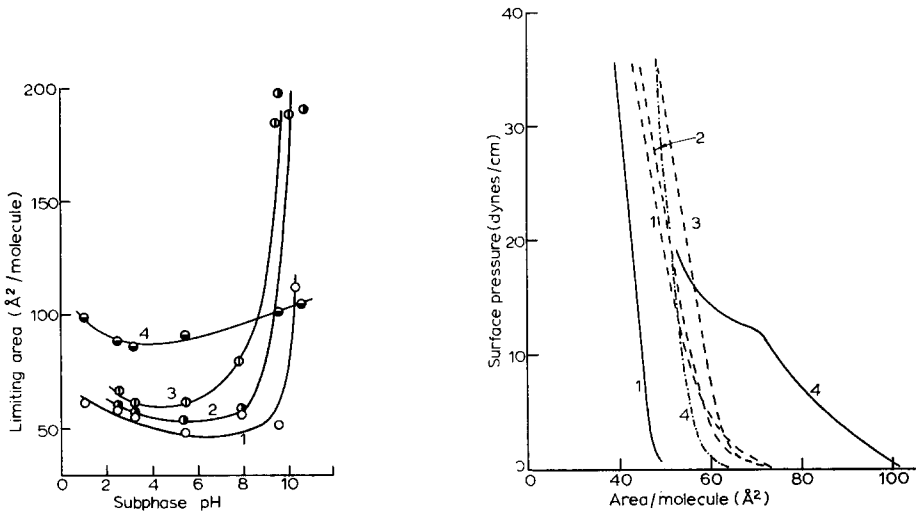


Fig. 3. Relations between limiting areas and subphase pH's at 25°. Designation of numbers, see Fig. 1.

Fig. 4. Effects of $UO_2^{2+}$ on $\pi$–$A$ curves at pH 5.0 (25°). Solid lines, no additive; dashed lines, 1.4 mM $UO_2(NO_3)_2$. Designation of numbers, see Fig. 1.

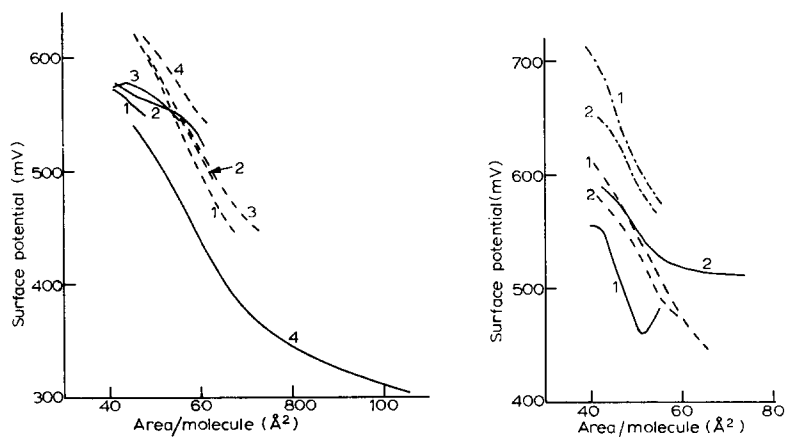


Fig. 5. Effects of $UO_2^{2+}$ on $\Delta V$–$A$ curves at pH 5.0 (25°). Solid lines, no additive; dashed lines, 1.4 mM $UO_2(NO_3)_2$. Designation of numbers, see Fig. 1.

Fig. 6. Effects of $UO_2^{2+}$ and $Th^{4+}$ on $\Delta V$ of dipalmitoyl phosphatidylethanolamine (1) and -*n*-propanolamine (2) at pH 2.4 (25°). Solid lines, no additive; dashed lines, 1.4 mM $UO_2(NO_3)_2$; dot-and-dashed lines, 1 mM $Th(NO_3)_4$.

In this case, all $\pi$–$A$ curves are contracted in the presence of $Th^{4+}$, and the surface potentials are raised more than with $UO_2^{2+}$ (Figs. 6 and 7).

$\pi$–$A$ curves undergo a remarkable change due to temperature, as shown in Fig. 8. In this figure, it can be seen that both dipalmitoyl phosphatidylethanolamine and -choline films show liquid expanded films at a temperature higher than their respective characteristic temperatures, which can be estimated by the rheological properties of the respective films. At a temperature lower than these characteristic ones, both the dipalmitoyl phosphatidylethanolamine and -choline films are condensed and the $\Delta V$ curve of dipalmitoyl phosphatidylcholine becomes identical to that of dipalmitoyl phosphatidylethanolamine; that is, the discrepancy between the polar groups of these compounds disappears at this low temperature.

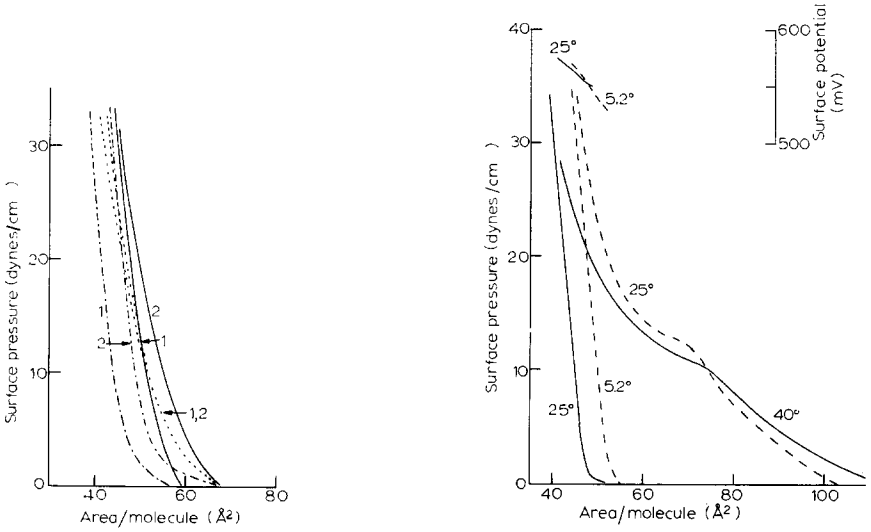


Fig. 7. Effects of $UO_2^{2+}$ and $Th^{4+}$ on $\pi$–$A$ curves at pH 2.4 (25 °). Solid lines, no additive; dotted lines, 1.4 mM $UO_2(NO_3)_2$; dot-and-dashed lines, 1 mM $Th(NO_3)_4$. 1, dipalmitoyl phosphatidylethanolamine; 2, dipalmitoyl phosphatidyl-*n*-propanolamine.

Fig. 8. $\pi$–$A$ and $\Delta V$–$A$ curves of dipalmitoyl phosphatidylethanolamine and -choline at different temperatures. Solid lines, dipalmitoyl phosphatidylethanolamine; dashed lines, dipalmitoyl phosphatidylcholine.

## DISCUSSION

There have been many discussions about the conformations of the polar groups of phospholipids in monolayers. It has been generally inferred that these compounds are in a discharged state between molecules in membranes because of the presence of a fully ionized charged pair under biological conditions[6,7]. SHAH AND SCHULMAN[8–10] deduced the intramolecular salt linkage between phosphate and quaternary ammonium ions in phosphatidylcholine molecules in monolayer. On the other hand, the intermolecular linkage was also discussed in dipalmitoyl lecithin monolayer, and it was suggested that the positive and negative charges were coplanar in a plane parallel to the interface[12–14]

In Fig. 1 it can be seen that dipalmitoyl phosphatidylethanolamine, -*i*-propanol-

amine and -*n*-propanolamine (phosphatidylethanolamine group) give liquid condensed or solid films at room temperature (about 25°), while dipalmitoyl phosphatidylcholine gives a liquid expanded film under the same conditions. This shows that the conformation modes of the monolayers are markedly different between the phosphatidylethanolamine group and dipalmitoyl phosphatidylcholine, at least at this temperature and pH. In the present investigation, all phospholipids have the same acyl chain, so that the observed differences in monolayer behaviors among them obviously arise from the divergence in the polar moieties in the molecules which dominate their respective structures. Among dipalmitoyl phosphatidylethanolamine, -*i*-propanolamine and -*n*-propanolamine, there is an order of dipalmitoyl phosphatidylethanolamine < -*i*-propanolamine < -*n*-propanolamine in molecular areas. Fig. 3 shows a pH dependence of the limiting areas at the respective zero pressures ("lift-off" of the $\pi$–$A$ curves).

STANDISH AND PETHICA[15] calculated the contributions of the dipole moment of the phosphorylethanolamine group to the overall surface dipole moment of dipalmitoyl phosphatidylethanolamine at the interface, and concluded that it oriented on a plane parallel to the interface. They also argued that the phosphorylcholine group of dipalmitoyl phosphatidylcholine was rather inclined in monolayer and that the positive group of dipalmitoyl phosphatidylcholine was deeper in the subphase than that of dipalmitoyl phosphatidylethanolamine relative to the respective phosphate groups. This argument was used to interpret the expanded $\pi$–$A$ curves of dipalmitoyl phosphatidylcholine.

The results of the $\pi$–$A$ curves obtained in the present study may be explained in the same way as the above-cited argument, that is, the observed limiting areas may be deduced as follows. The limiting areas of dipalmitoyl phosphatidylethanolamine and -*n*-propanolamine at the respective zero pressures are 48 and 58 $A^2$/molecule, respectively. The difference between them (10 $A^2$) is thought to be caused by the difference in distance between the positive and negative charges in dipalmitoyl phosphatidylethanolamine and -*n*-propanolamine, that is, one methylene. From an X-ray study of hydrated egg lecithin it was calculated that the phosphorylcholine moiety of this molecule had the dimensions 25.5 $A^2 \times 8$ $A$ as a cylindrical shape in a fully extended state[16]. The cross-sectional area of 25.5 $A^2$ of a cylinder corresponds to about 6 $A$ in diameter of the circular cross-section. If this figure is assumed to be approximately equal to the polar moiety of dipalmitoyl phosphatidylethanolamine, the limiting area of dipalmitoyl phosphatidylethanolamine of 48 $A^2$ ($= 6 \times 8$) can be deduced from the parallel orientation of the polar group. According to this orientation, the difference in limiting areas obtained between dipalmitoyl phosphatidylethanolamine and -*n*-propanolamine (10 $A^2$) is calculated to be approx. 1.7 $A$ in length along the polar chain; this difference must correspond to the length of one methylene. This figure is reasonable as the distance of the C–C bond[17,18].

The limiting area of dipalmitoyl phosphatidyl-*i*-propanolamine is 55 $A^2$/molecule, and the difference from that of dipalmitoyl phosphatidylethanolamine is about 7 $A^2$. This difference is calculated to correspond to an increment of about 0.7 $A$ in diameter of the cross-section of the cylinder which represents the polar moiety of dipalmitoyl phosphatidyl-*i*-propanolamine. Their limiting areas are revealed by the horizontal rectangular cross-sections of the cylinders lying parallel to the interface. This increment in area, therefore, must obviously be caused by one methyl side chain

in the polar group of dipalmitoyl phosphatidyl-*i*-propanolamine. The above assumption would lead to a prediction that their films have rigid structures which consist of networks due to the intermolecular ionic linkage; these facts are clearly seen from the viscosity measurements. It was observed in this study that all members of the phosphatidylethanolamine group had extremely high surface viscosities at temperatures below 30°. In our laboratory it was also observed that these highly viscous rigid structures were disrupted and the monolayers became fluid as the temperature was elevated. Consequently, the above argument regarding conformation is restricted to ordinary room temperature range. This may be understood by the following observations.

A condensed pressure–area curve is obtained with the dipalmitoyl phosphatidylcholine film at 5.2° that is almost the same as that of the dipalmitoyl phosphatidylethanolamine film at 25° (Fig. 8). The surface potentials of dipalmitoyl phosphatidylcholine at this low temperature are nearly identical to those of dipalmitoyl phosphatidylethanolamine at 25°. In contrast the dipalmitoyl phosphatidylethanolamine film at high temperature resembles the dipalmitoyl phosphatidylcholine film at room temperature (25°). It is unquestionable from their relatively high melting points (approx. 200°) as compared to fatty acids for example, that they contain in part an ionic lattice structure in the anhydrous solid state. It was reported that there were thermotropic phase transitions in dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanolamine at 41 and 65°, respectively, in the presence of more than 20 % water[19–21]. In monolayer state, some kind of thermotropic phase transitions might be allowed for. The above mentioned facts observed in this study might be correlated to this kind of phase change. It is suggestive that monolayer conformations of this kind of substance are dominated by these phase transitions.

The effects of subphase pH on the $\pi$–$A$ curves indicate that the weakness of the network is in the order of the chain length of the polar moieties in dipalmitoyl phosphatidylethanolamine, -*i*-propanolamine and -*n*-propanolamine; namely, the longer the chain length, the weaker the network structure. The same order of weakness in monolayer structure was also observed in the effect of temperature on the fluidity of the monolayers, that is, in the temperatures at which the respective monolayers became fluid (unpublished observations)

All the results mentioned above support the idea that the phosphatidylethanolamine group is in a parallel orientation relative to the interface in the polar moieties and forms a two-dimensional network structure at 25° and near the isoelectric point. Dipalmitoyl phosphatidylcholine, however, does not form this structure under the same conditions. These conformations of the phospholipids examined here are considered not to be incompatible with the effects of additives as follows.

In the presence of 1.4 mM $UO_2(NO_3)_2$ in subsolution, $\pi$–$A$ curves of dipalmitoyl phosphatidylethanolamine, -*i*-propanolamine and -*n*-propanolamine shift to the larger area, whereas that of dipalmitoyl phosphatidylcholine contracts greatly. All the $\pi$–$A$ curves are positioned nearer than those in the absence of $UO_2^{2+}$, especially in a low surface pressure region. This fact would lead to a consideration that the discrepancy in structure of the polar moieties of these compounds has mostly disappeared in their monolayer conformations. Since $UO_2^{2+}$ probably binds quantitatively to the phosphate groups of phospholipids[22–24], the negative sites of the monolayers are discharged by binding $UO_2^{2+}$. This makes the film positively charged

as a result. This may disrupt the network structures originally presented and the molecular areas may be shifted to the larger areas by a penetration of $UO_2^{2+}$ into the $\pi$–surface. The distinct contractile effect of $UO_2^{2+}$ on the dipalmitoyl phosphatidylcholine film might arise from the fact that the repulsive forces are reduced by discharge of the phosphate group, allowing formation of another structure between phosphate and $UO_2^{2+}$. The dipalmitoyl phosphatidylcholine film binding $UO_2^{2+}$ gives a condensed $\pi$–$A$ curve (Fig. 4), but the area at a higher $\pi$ is larger than that of pure dipalmitoyl phosphatidylcholine, probably due to penetration of $UO_2^{2+}$.

The relationships between surface potentials and areas are relatively complex in the phosphatidylethanolamine group (Figs. 5 and 6), while those for dipalmitoyl phosphatidylcholine are all simple. When $UO_2^{2+}$ is present, these relationships in the phosphatidylethanolamine-group are simplified as seen in these figures. It is inferred from these results that the network structure of the phosphatidylethanolamine group undergoes some distortion by compression so as to change the surface dipole moments irregularly, but in the presence of $UO_2^{2+}$ this effect of compression disappears because of a disruption of the network, causing it to become more compressible. This is more clearly seen at lower pH (Fig. 6).

The effects of $Th^{4+}$ seems to be different from that of $UO_2^{2+}$ as seen in Figs. 6 and 7. Their precise mechanism is not yet known, but these two cations showed extremely strong effects on both the $\pi$–$A$ and $\Delta V$–$A$ relations with the various cations examined in this study.

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