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Ion Interactions and Electrostatic Effects on TMPyP/DMPA Monolayers

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In this work, the influence of the different ionic strengths in the aqueous subphase on the monomer–dimer equilibrium of a water-soluble tetracationic porphyrin, TMPyP, in a complex monolayer containing an insoluble lipid with negatively charged group, DMPA, has been investigated. LiCl, KClO₄, and tetraethylammonium perchlorate, TEAP, have been used. Surface pressure–area, π – A , and surface potential–area, ΔV – A , isotherms have been measured in the presence of salts in the subphase. As reference, the monolayer of DMPA in absence of TMPyP was studied by π – A and ΔV – A isotherms on those subphases. Reflection spectroscopy, ΔR , was also used to infer the arrangement of the porphyrin in the cospread monolayer of TMPyP/DMPA, molar ratio 1:4, on different aqueous salt solutions. A decrease of ΔR with increasing salt concentration independent of the type of salt is obtained indicating a loss of porphyrin molecules to the subphase, which magnitude is dependent on the electrolyte. Further, a slight red shift of λ_{max} with respect to that at 420 nm obtained in absence of salt is observed. The analysis of reflection spectra at the air–water interface in the presence of Li⁺ and K⁺ ions in the subphase proposes a new semidimer phase for the porphyrin molecules in the mixed monolayer with a dimer fraction of $\alpha_D \approx 0.5$. The model cannot be applied to the effect of TEAP molecules on the cospread monolayer due to the big size of TEA⁺ ion.

Introduction

The architecture and molecular engineering of supermolecular assemblies at surfaces or interfaces are controlled by intermolecular interactions of different nature. The various interactions in monolayers containing amphiphilic components as well the external surface pressure determine the structure of an organized monolayer at the air–water interface when the film is compressed. Different methods to fabricate mixed monolayers containing an amphiphilic component and a water-soluble nonamphiphilic component have been developed either by adsorption of the nonamphiphilic component from the aqueous subphase to the lipid monolayer^{1,2} or by cospreading of the mixture amphiphilic–nonamphiphilic components.^{3–8} The organization of this kind of monolayers is based on, e.g., electrostatic interactions between the lipid matrix and the water-soluble adsorbate with a charge of opposite sign. The electrostatic interactions in monolayers of ionic components may lead to a variety of phenomena, e.g., nonspecific effects produced by the

formation of an ionic atmosphere adjacent to the surface film (diffuse double layer), effects on the ionization of components of the monolayer, or specific effects caused by the formation of ion pairs between molecules of a monolayer.

In this work, we present results of the investigation of the electrostatic interactions between a cationic water-soluble porphyrin, 5,10,15,20-tetrakis(1-methyl-4-pyridyl)-21*H*,23*H*-porphine, TMPyP, and a phospholipid, dimyristoylphosphatidic acid, DMPA, with negatively charged headgroup that are organized in a complex monolayer at the air–water interface. The structures and area per vertical DMPA and flat TMPyP molecules are drawn in Chart 1.

The molecular organization of the TMPyP/DMPA system has been described in a previous paper.⁸ The optimal molar ratio of TMPyP/DMPA is 1:4 according to the stoichiometry and to the high monolayer stability observed. The orientation for the porphyrin ring was always parallel to the interface. At a surface pressure of ≈ 8 mN/m the area per porphyrin spread is 3.2 nm² corresponding to a dense-packed monolayer of porphyrin underneath the lipid matrix of DMPA in an expanded state. If no porphyrin is dissolving in the subphase, a further compression of the complex monolayer leads to the formation of the phase of porphyrin dimers coexisting with a phase of porphyrin monomers. In this phase of dimers, a layer of porphyrin molecules is directly bound to the negative charges of the lipid matrix with a second layer of porphyrin molecules still having access to the negative charges due to a 45° twist with respect to the porphyrin molecules of the first layer. In Scheme 1, the configuration of two molecules of TMPyP in the dimer

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Scheme 1. In-plane Configuration of Two Stacked Molecules of Porphyrin in a Monolayer of TMPyP/DMPA (1:4 Molar Ratio)

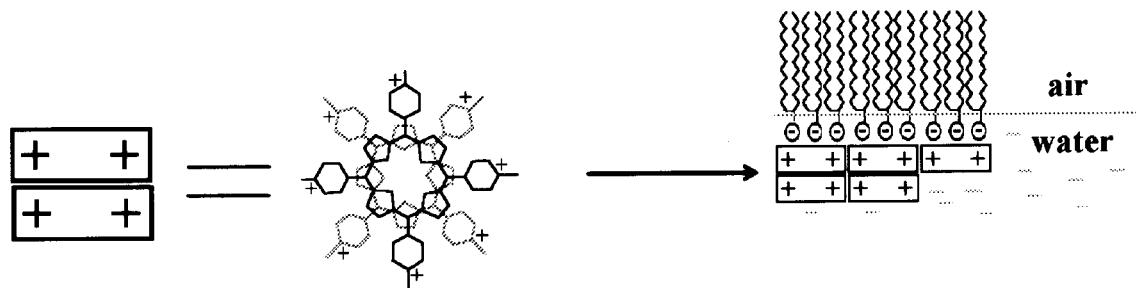
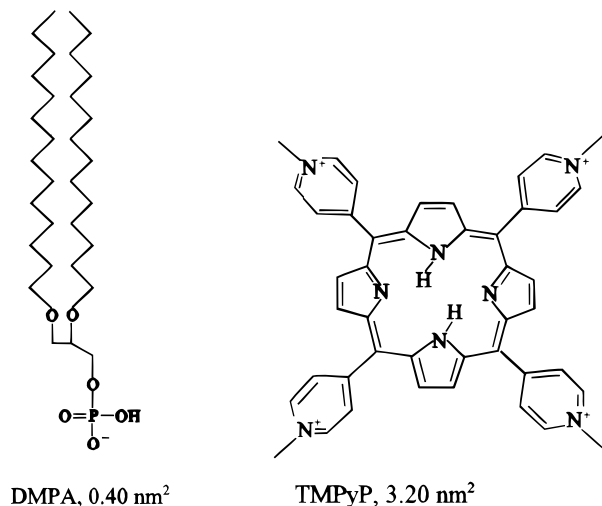


Chart 1. Structures and Areas of DMPA (vertical section) and TMPyP (in flat orientation) Molecules



phase at the air–water interface is shown. The different intensity of molecules in the scheme indicates the top and lower planes of the dimers.

The influence of different salts in the aqueous subphase on the monomer–dimer equilibrium of the porphyrin has been investigated. LiCl, KClO₄, and tetraethylammonium perchlorate, TEAP, have been used at 10^{−3}, 10^{−2}, and 0.1 M concentrations. Surface pressure–area, π – A , and surface potential–area, ΔV – A , isotherms, and reflection spectroscopy, ΔR , were used to infer the arrangement of the porphyrin in the mixed TMPyP/DMPA monolayer in the presence of salts in the subphase.

Experimental Section

Materials. 5,10,15,20-Tetrakis(1-methyl-4-pyridyl)-21H,23H-porphine (TMPyP, Aldrich) and 1- α -dimyristoylphosphatidic acid (DMPA, Sigma) were used without further purification. Tetraethylammonium perchlorate (TEAP) was supplied by Fluka. Lithium chloride (LiCl) and potassium perchlorate (KClO₄) were from Merck and used as supplied. A mixture of trichloromethane and methanol, ratio 3:1 (v/v), was used as spreading solvent. The pure solvents were obtained without further purification from Panreac (Spain) and Baker Chemicals (Germany). The water for the subphase was prepared with a Millipore Milli-Q- Plus system.

Monolayers at the Air–Aqueous Subphase Interface. Details of experimental conditions to prepare the TMPyP/DMPA monolayers and for measurements of π – A isotherms and of reflection spectra at normal incidence have been described elsewhere.⁸ The ΔV – A isotherms were determined in a home-made trough supplied with a system that detects the difference in surface potential, ΔV , of the monolayer-covered aqueous subphase and the bare aqueous surface by the vibrating plate method.⁹ All isotherms were experimented at least three times.

Results and Discussion

1. π – A and ΔV – A Isotherms. Aqueous Subphase with LiCl. The π – A and ΔV – A isotherms obtained for monolayers of DMPA (parts A and C of Figure 1) and of TMPyP/DMPA (parts B and D of Figure 1), ratio 1:4, at the air–water interface with several LiCl concentrations, 0–0.1 M, are shown in Figure 1.

The π – A isotherms of the DMPA monolayer clearly show an effect of the presence of LiCl in aqueous subphase. A pronounced expansion at low π and convergence at high π with respect to that on pure water (Figure 1A) with increasing of salt concentration is observed. Also, a liquid expanded–liquid condensed (LE–LC) phase transition appears, and its area depends on the salt concentration. This behavior is similar to that found for DMPA in the presence of NaCl.¹⁰

In Figure 1B, π – A isotherms for mixed monolayers of TMPyP/DMPA, molar ratio 1:4, on aqueous subphases with LiCl at several concentrations are shown. In this case, it seems that the area values at low surface pressure are independent of the salt concentration. The most remarkable effect in these isotherms is the decrease of the surface pressure for the LE–LC phase transition with increasing LiCl concentration. At 0.1 M LiCl, the isotherms for DMPA (Figure 1A) and TMPyP/DMPA (Figure 1B) tend to converge. This suggests that the porphyrin is partially removed from the lipid matrix with increasing salt concentration.

The ΔV – A isotherms for DMPA monolayers (Figure 1C) on several LiCl–water subphases show a potential near 0 mV at high area values in contrast to that observed on pure water, $\Delta V = -100$ mV.⁶ The value of $\Delta V = 0$ mV obtained in the presence of LiCl is due to the compensation of the negative charges of DMPA headgroups with positive Li⁺ ions from aqueous subphase. The abrupt rise of the potential upon compression is shifted to larger surface areas with increasing salt concentration. Moreover, the potential at low area values increases with increasing of LiCl concentration, i.e., 35 mN/m ΔV values of 315, 352, 399, and 450 mV are obtained for 0, 10^{−3}, 10^{−2}, and 0.1 M LiCl, respectively, in the subphase.

The ΔV value for a DMPA monolayer on water pH = 0.5 is equal to 530 mV at 35 mN/m, where the phospholipid is completely protonated.⁶ This means that any difference of potential value with respect to 530 mV at the same DMPA surface density could be related to an incomplete compensation of the negative lipid charges by the monocations in the subphase disregarding lateral effects such as the intercalation of ions in the hydrophobic part or specific interactions. Therefore, the rise of ΔV with

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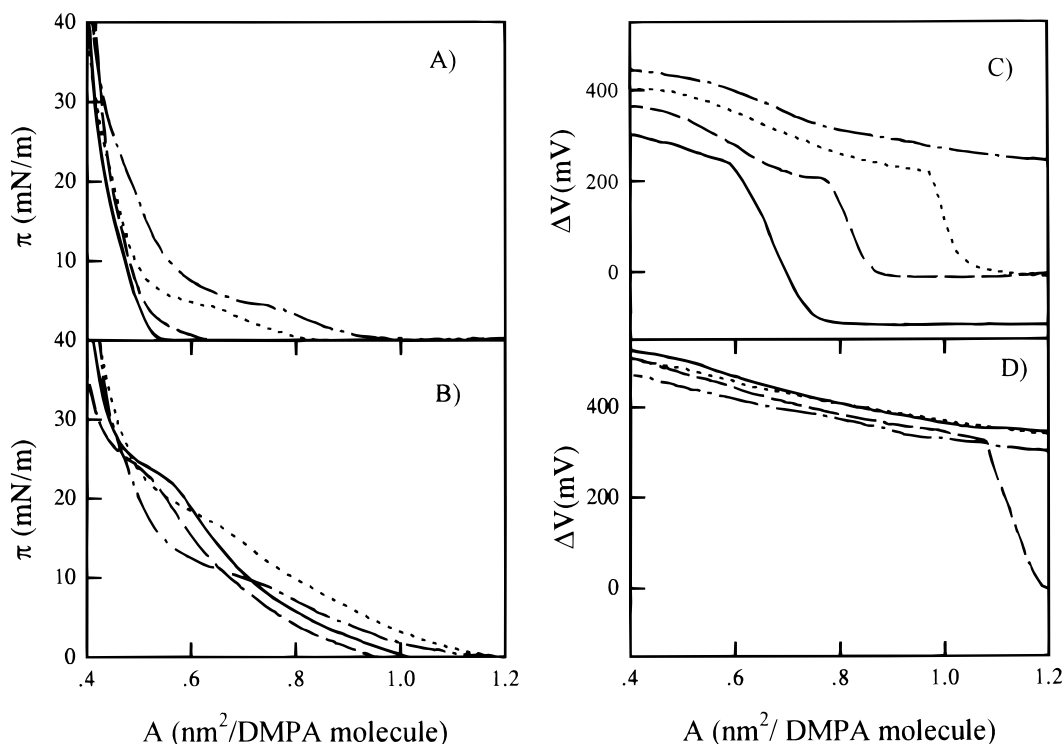


Figure 1. π - A and ΔV - A isotherms of DMPA monolayers (A and C, respectively) and of TMPyP/DMPA = 1:4 (B and D, respectively) at the air-water interface for different concentrations of LiCl in aqueous subphase: 0 M, solid line; 10⁻³ M, dashed line; 10⁻² M, dotted line; 0.1 M dotted-dashed line. $T = 21^\circ\text{C}$.

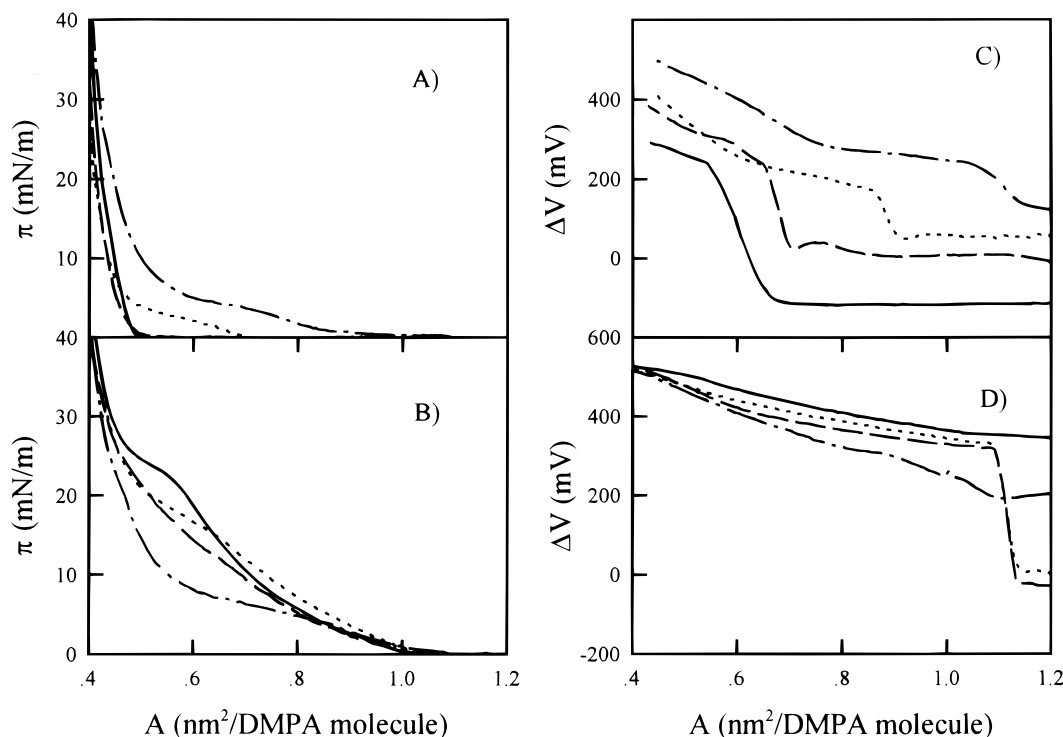


Figure 2. π - A and ΔV - A isotherms of DMPA monolayers (A and C, respectively) and of TMPyP/DMPA = 1:4 (B and D, respectively) at the air-water interface for different concentrations of KClO₄ in aqueous subphase: 0 M, solid line; 10⁻³ M, dashed line; 10⁻² M, dotted line; 0.1 M dotted-dashed line. $T = 21^\circ\text{C}$.

increasing LiCl concentration indicates a high degree of compensation of the negative charge of DMPA by Li⁺ ions.

In monolayers of TMPyP/DMPA, ratio 1:4 (see Figure 1D), ΔV values at 35 mN/m are 527, 510, 510, and 461 for 0, 10⁻³, 10⁻², and 0.1 M LiCl, respectively. The value of ΔV in the absence of LiCl is close to that obtained for DMPA monolayers at pH = 0.5, i.e., 530 mV.⁶

Likewise, if a concentration of 0.1 M LiCl is used, the values of ΔV for monolayers with and without porphyrin, 450 and 461 mV, respectively, are very similar. As mentioned above, this behavior suggests that the porphyrin is partially replaced by Li⁺ in the monolayer of DMPA with increasing salt concentration. This phenomenon requires a quantitative analysis.

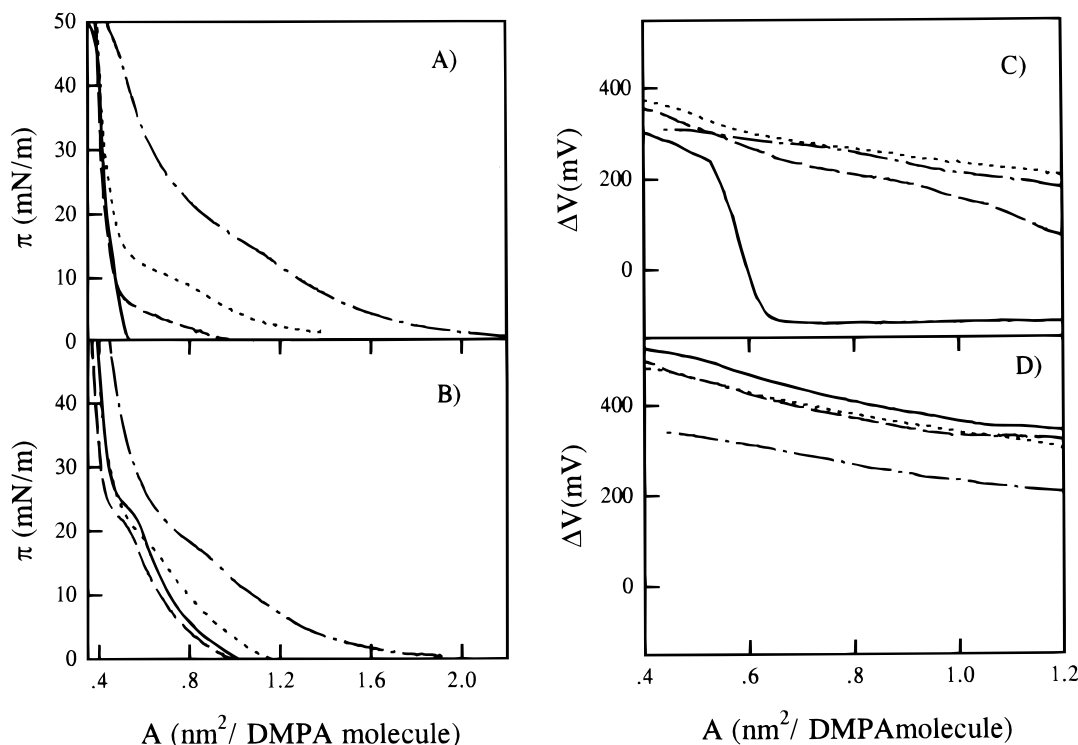


Figure 3. π - A and ΔV - A isotherms of DMPA monolayers (A and C, respectively) and of TMPyP/DMPA = 1:4 (B and D, respectively) at the air-water interface for different concentrations of TEAP in aqueous subphase: 0 M, solid line; 10⁻³ M, dashed line; 10⁻² M, dotted line; 0.1 M dotted-dashed line. $T = 21^\circ\text{C}$.

Aqueous Subphase with KClO_4 . Figure 2 shows the π - A and ΔV - A isotherms of DMPA (parts A and C of Figure 2) and TMPyP/DMPA (parts B and D of Figure 2) monolayers with several KClO_4 concentrations. The π - A isotherms of DMPA (Figure 2A) show a behavior similar to that described above for LiCl (see Figure 1A). In fact, the surface pressure values of the LE-LC phase transition and the expansion of the area at low surface pressure increase with increasing salt concentration for a DMPA monolayer. Regarding the surface potential (Figure 2C), the measured values increase with increasing surface pressure as well as increasing salt concentration in the subphase.

In the case of the mixed TMPyP/DMPA monolayer, the LE-LC phase transition (Figure 2B) appears at lower surface pressure values and the molecular areas at low surface pressure increase when the salt concentration is raised with respect to the TMPyP/DMPA monolayer on water. Further, the surface potential (Figure 2D) increases with increasing surface pressure and moreover it is practically independent of KClO_4 concentration at 35 mN/m. It is worth noting that the ΔV value at 35 mN/m is very close to the value mentioned above for a completely protonated DMPA monolayer, i.e., 530 mV.⁶

Subphase with TEAP. In the same way as before, π - A and ΔV - A isotherms were measured on TEAP-aqueous subphase for both monolayers. The results are shown in Figure 3. At high concentrations of TEAP (10⁻² and 0.1 M in parts A and B of Figure 3, respectively) a clear expansion of π - A isotherms for both monolayers is observed. In both cases with 0.1 M TEAP, the isotherms also show an expansion at high surface pressure. Thus, the area per molecule of DMPA at $\pi = 35$ mN/m increases by 0.17 nm² for the lipid monolayer (see Figure 3A) and by 0.08 nm² for the TMPyP/DMPA monolayer (see Figure 3B) with respect to the

area in absence of TEAP in the subphase. This phenomenon could be due to lateral separation of the headgroups of the DMPA matrix because of the approach of TEAP ions.

The π - A isotherm for a monolayer of TMPyP/DMPA (see Figure 3B) shows a slight reduction of molecular area, ~ 0.03 nm² at low TEAP concentration, i.e., 10⁻³ M, with respect to the reference isotherm on pure water. On the other hand, the phase transition LE-LC for DMPA and for TMPyP/DMPA monolayers follows the tendency described above for LiCl and KClO_4 , i.e., an increase of the surface pressure of LE-LC phase transition for the lipid monolayer and an decrease of this surface pressure for the mixed monolayer, respectively, with increasing salt concentration.

Parts C and D of Figure 3 show plots of ΔV versus A for DMPA and TMPyP/DMPA monolayers, respectively. In the former case, the value of potential at 35 mN/m increases with increasing concentration of TEAP, i.e., 356 and 375 mV for 10⁻³ and 10⁻² M, respectively; however, the value decreases for 0.1 M (318 mV). This value is in agreement with that obtained in the absence of salt (315 mV). Otherwise, ΔV is always decreasing with increasing salt concentration in the presence of porphyrin, i.e., 505, 498, and 314 mV for 10⁻³, 10⁻², and 0.1 M, respectively, as compared to 527 mV for 0 M. It is remarkable that the ΔV of both monolayers is similar on the 0.1 M TEAP subphase.

Analysis of Surface Pressure-Area (π - A) and Surface Potential-Area (ΔV - A). The dependence of the surface potentials of the monolayer of DMPA on the concentration of salt in the aqueous subphase seems to agree with the Gouy-Chapman model, see eq 1,¹¹ at least for LiCl and KClO_4

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$$\Delta V = 4\pi n\mu - \frac{2.303kT}{e} \log \left(\frac{134^2 \cdot 4}{A^2 C_i} \right) \quad (1)$$

where ΔV is the surface potential, n the dipole number per cm^2 , μ_d the contribution of those dipoles to the surface potential, k Boltzmann's constant, T the temperature in K, e the charge of one electron, A the surface area per DMPA molecule, and C_i the concentration of salt in bulk.

The required conditions to apply the theory of Gouy are not strictly fulfilled for molecular assemblies such as monolayers. However, it is possible to apply it since, at relatively short distances of the interface, the lines of force of the charged groups are equidistant and perpendicular to the interface as required by the Gouy model.¹¹

The value of the slope in a plot of ΔV versus $\log C$ for both systems according to eq 1 will provide important information about the interactions between the positive ions in the subphase and the negative charges of the phospholipid in the monolayer.

Figure 4 shows ΔV versus $\log C$ of the different salts in the aqueous subphase (TEAP ●; KClO_4 , ■; LiCl , ▲) for DMPA monolayers, part A, and for TMPyP/DMPA monolayers, part B, at 35 mN/m.

In the case of DMPA monolayer, this plot for KClO_4 is linear with a slope of 54.5 mV per decade, which is in good agreement with the value expected from eq 1, i.e., 58.6 mV/decade with $T = 21^\circ\text{C}$. The potential value with 0.1 M KClO_4 is near 530 mV, corresponding to that for a DMPA monolayer with complete negative charges compensated.⁶

Also, the plot of LiCl is linear; however, the slope is equal to 49 mV/decade, slightly smaller than that expected from eq 1 (58.6 mV/decade). In this case, the system is not totally fitted to the Gouy–Chapman model. Complete compensation of charges at the interface is not achieved when a concentration of 0.1 M LiCl is used. A potential value of 450 mV with 0.1 M LiCl and at 35 mN/m was obtained, i.e., 80 mV less than that expected for a completely neutralized DMPA monolayer (530 mV).⁶

DMPA monolayers on TEAP–aqueous subphases show a behavior very different from the others. A strong decrease of ΔV value at high TEAP concentration, i.e., 0.1 M as compared to 0.01 M, is observed. This phenomenon could be attributed to the big size of the TEA^+ ion. The area projection for this cation when approaching the headgroups of DMPA has been estimated to $\sim 0.53 \text{ nm}^2$. This is larger than the surface area per DMPA molecule at 35 mN/m (0.40 nm^2). Therefore, the approach of the TEA^+ ion to the headgroup of DMPA causes an expansion of the matrix as illustrated in Chart 2. Then, a correction of the experimental value, ΔV_{exp} , at high surface pressures, 35 mN/m, with 0.1 M TEAP is necessary that takes this expansion into account.

$$\Delta V_{\text{corrected}} = \Delta V_{\text{experimental}} \frac{A_{\text{DMPA,TEAP}}}{A_{\text{DMPA,WATER}}} \quad (2)$$

Thus, $\Delta V_{\text{corrected}}$ is equal to 426 mV (Figure 4A, open circle and dashed line), which is in the range of the value expected from eq 1. This correction was also taken account for LiCl and KClO_4 both for DMPA and TMPyP/DMPA monolayers and no change was observed.

The three straight lines in Figure 4A (including the correction in case of TEAP) have nearly the same slope but are shifted to less positive potential in the sequence $\text{K}^+ > \text{Li}^+ > \text{TEA}^+$. This indicates decreasing charge compensation of the lipid matrix in this sequence due to increasing ionic radius (with hydration sphere for Li^+).

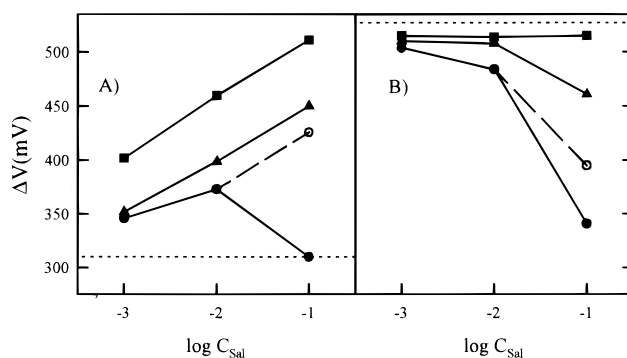
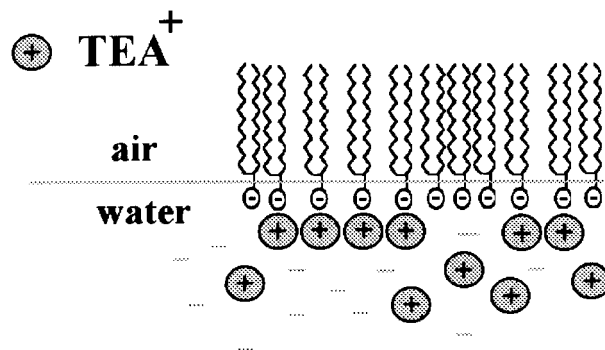


Figure 4. Values of ΔV of DMPA (A) and of TMPyP/DMPA (B), monolayers at 35 mN/m plotted versus logarithm of the concentration of different salts in an aqueous subphase: TEAP, ●; KClO_4 , ■; LiCl , ▲. The dotted line in (A) corresponds to the ΔV value of the monolayer of DMPA on an aqueous subphase with pH = 5.5 (315 mV), and that in (B) is the ΔV value of the lipid monolayer at an aqueous subphase with pH = 0.5 (530 mV).

Chart 2. Effect of the TEAP Ions on the DMPA Monolayer at High Surface Pressures and with a Salt Concentration of 0.1M



In the case of TMPyP/DMPA monolayers, eq 1 cannot be applied since the bulk solution contains no porphyrin. In Figure 4B, the values of ΔV for the cospread monolayer at $\pi = 35 \text{ mN/m}$ on the different subphases (TEAP, ●; KClO_4 , ■; LiCl , ▲) are plotted versus $\log C_{\text{salt}}$.

ΔV , obtained at 35 mN/m using several concentrations of KClO_4 , is constant and very close to the value found for a completely protonated DMPA monolayer (530 mV, dashed line in Figure 4B). In this mixed monolayer, a replacement of porphyrin molecules bound to the lipid matrix by K^+ ions is possible when the salt concentration is high without a significant variations of ΔV , since $\Delta V = 527 \text{ mV}$ for a monolayer of TMPyP/DMPA in the absence of salt, and $\Delta V = 512 \text{ mV}$ for a DMPA monolayer in the presence of 0.1 M KClO_4 .

The effect of LiCl at low concentrations on the mixed monolayer is similar to that produced by KClO_4 . However, a substantial decrease of surface potential with 0.1 M LiCl is noted. This may be attributed to a removal of porphyrin due to the presence of Li^+ .

In the case of TEAP, a decrease of the ΔV values at 35 mN/m with increasing of salt concentration is observed. Also, a considerable expansion at high surface pressures is noted in the isotherm (Figure 3B, dotted and dashed line); however, this is slightly smaller than that obtained for the monolayer without porphyrin. The expansion can be interpreted in the same way as above for DMPA due to the big size of TEA^+ (see Chart 2). Therefore, the surface potential should be corrected by eq 2 as before taking account of the variation of phospholipid density with respect to the system in the absence of TEAP, and the

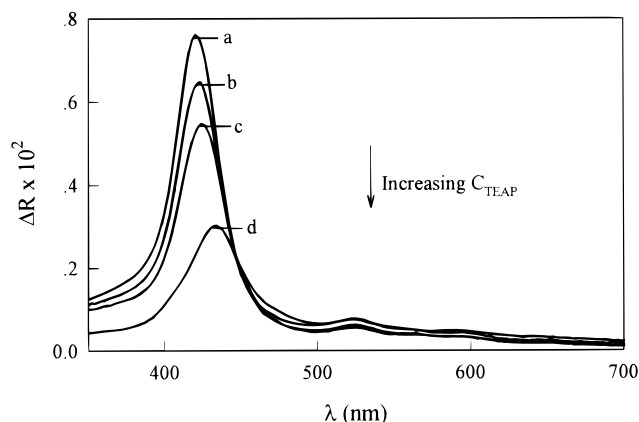


Figure 5. Reflection spectra of a monolayer of TMPA/DMPA = 1:4 formed by cospreparing at the air–water interface with different concentrations of TEAP: 0 M, curve a; 10^{-3} M, curve b; 10^{-2} M, curve c; 0.1 M, curve d. $\pi = 35$ mN/m; $T = 21$ °C.

Table 1. Values of ΔR_{\max} , λ_{\max} , Γ and α_D of a Monolayer of TMPyP/DMPA Compressed at 35 mN/m on Several Aqueous Subphases

| water subphase | | $\Delta R_{\max} \times 10^2$ | λ_{\max} (nm) | $\Gamma \times 10^{11}$ (mol/cm ²) | α_D |
|-------------------|--------|-------------------------------|-----------------------|--|------------|
| water | | 0.729 | 420 | 8.90 | 0.84 |
| TEAP | 1 mM | 0.630 | 422 | 7.61 | 0.68 |
| | 10 mM | 0.556 | 424 | 6.57 | 0.48 |
| | 100 mM | 0.305 | 430 | 3.24 | 0.26 |
| KClO ₄ | 1 mM | 0.474 | 426 | 5.38 | 0.55 |
| | 10 mM | 0.401 | 425 | 4.67 | 0.50 |
| | 100 mM | ~0.033 | 424 | ~0.40 | |
| LiCl | 1 mM | 0.555 | 425 | 6.57 | 0.51 |
| | 10 mM | 0.492 | 425 | 5.76 | 0.49 |
| | 100 mM | ~0.021 | 428 | ~0.26 | |

corrected value of ΔV at 35 mN/m and 0.1 M TEAP is 395 mV (Figure 4B, open circle and dashed line).

2. Reflection Measurements. Reflection spectroscopy at the air–water interface can be used to infer the molecular organization of molecules incorporated in insoluble monolayers.^{12,13} Therefore, reflection spectra (ΔR) of complex monolayers of TMPyP/DMPA, molar ratio 1:4, have been measured at several surface pressures from 5 to 35 mN/m on different aqueous salt solutions.

Figure 5 shows the reflection spectra for a monolayer of TMPyP/DMPA = 1:4 at $\pi = 35$ mN/m using different concentrations of TEAP on the aqueous subphase, 0–0.1 M. A decrease of ΔR with increasing of TEAP is clearly obtained indicating a loss of porphyrin molecules to the subphase. This effect is observed for all of the three salts used. In Table 1, the average values of ΔR_{\max} and λ_{\max} values for the TMPyP/DMPA monolayer at 35 mN/m on different salt–aqueous subphase are listed. Further, a slight red shift of the maximum of Soret band (λ_{\max}) with respect to that at 420 nm obtained in the absence of TEAP with increasing salt concentration (Figure 5, curve a) is observed. Again, this effect is common for all salts used and will be analyzed later on.

In the absence of salts, the reflection spectra for a monolayer of TMPyP/DMPA = 1:4 showed a Soret band shifted to blue with increasing surface pressure⁸ from 430 nm at $\pi = 5$ mN/m to 420 nm at $\pi = 35$ mN/m. This shift was attributed to the formation of porphyrin dimers where the TMPyP molecules formed a stacking configuration with a twist of the parallel lying rings of 45°. With this

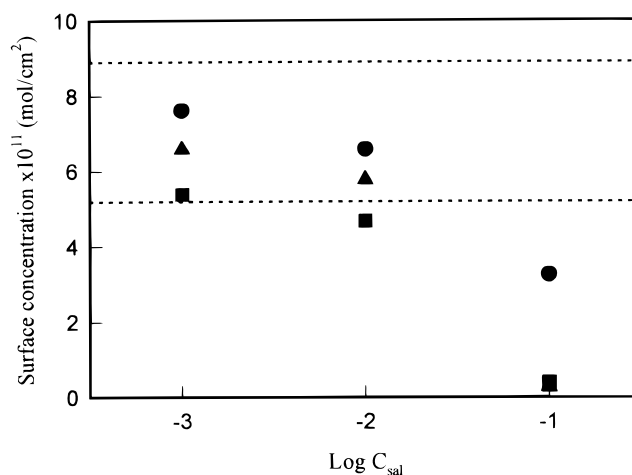


Figure 6. Surface concentration of porphyrin, Γ in mol/cm², at 35 mN/m plotted versus logarithms of salt concentration: TEAP, ●; KClO₄, ■; LiCl, ▲. The dotted top line corresponds to the surface concentration of TMPyP in the absence of salts (8.9×10^{-11} mol/cm², see Table 1), and that in bottom is the Γ value of a dense-packed monolayer of the porphyrin molecules in monomer phase and flat orientation underneath the lipid matrix in an expanded state (5.19×10^{-11} mol/cm²).

configuration,⁸ the porphyrin molecules located in the second layer have access to headgroups of the lipid matrix (see Scheme 1).

On the basis of this model,⁸ $\lambda_{\max} = 430$ nm when there are only monomers of the porphyrin (phase I) at the interface. However, if nearly all of the porphyrin are in the dimer (phase II) at the air–water interface, $\lambda_{\max} = 420$ nm and the intermediate values of λ_{\max} indicate the coexistence of both phases. These phases have been directly observed by Brewster angle microscopy (BAM).¹⁴

In the presence of salts in the aqueous subphase, the reflection spectra of the mixed TMPyP/DMPA monolayers are modified depending on the cation. As shown above, λ_{\max} is shifted to red with increasing the concentration of TEAP (see Table 1) suggesting a disappearance of the dimer phase. For the other salts, LiCl and KClO₄, the maximum wavelength is practically constant (426 ± 2 nm) independent of the salt concentration indicating a constant molar fraction of dimer at the air–salt aqueous subphase. However, the interpretation of this phenomenon requires a quantitative determination of dimer and monomer at each surface pressure.

With this aim, the reflection spectra of TMPyP/DMPA monolayers in the presence of salts have been analyzed in detail by the monomer–dimer model described for this monolayer in the absence of salts.⁸ The monolayer reflection at any wavelength, ΔR_λ , can be expressed as

$$\Delta R_\lambda = 0.322 f(\epsilon_I \Gamma_I + \epsilon_{II} \Gamma_{II}) \quad (3)$$

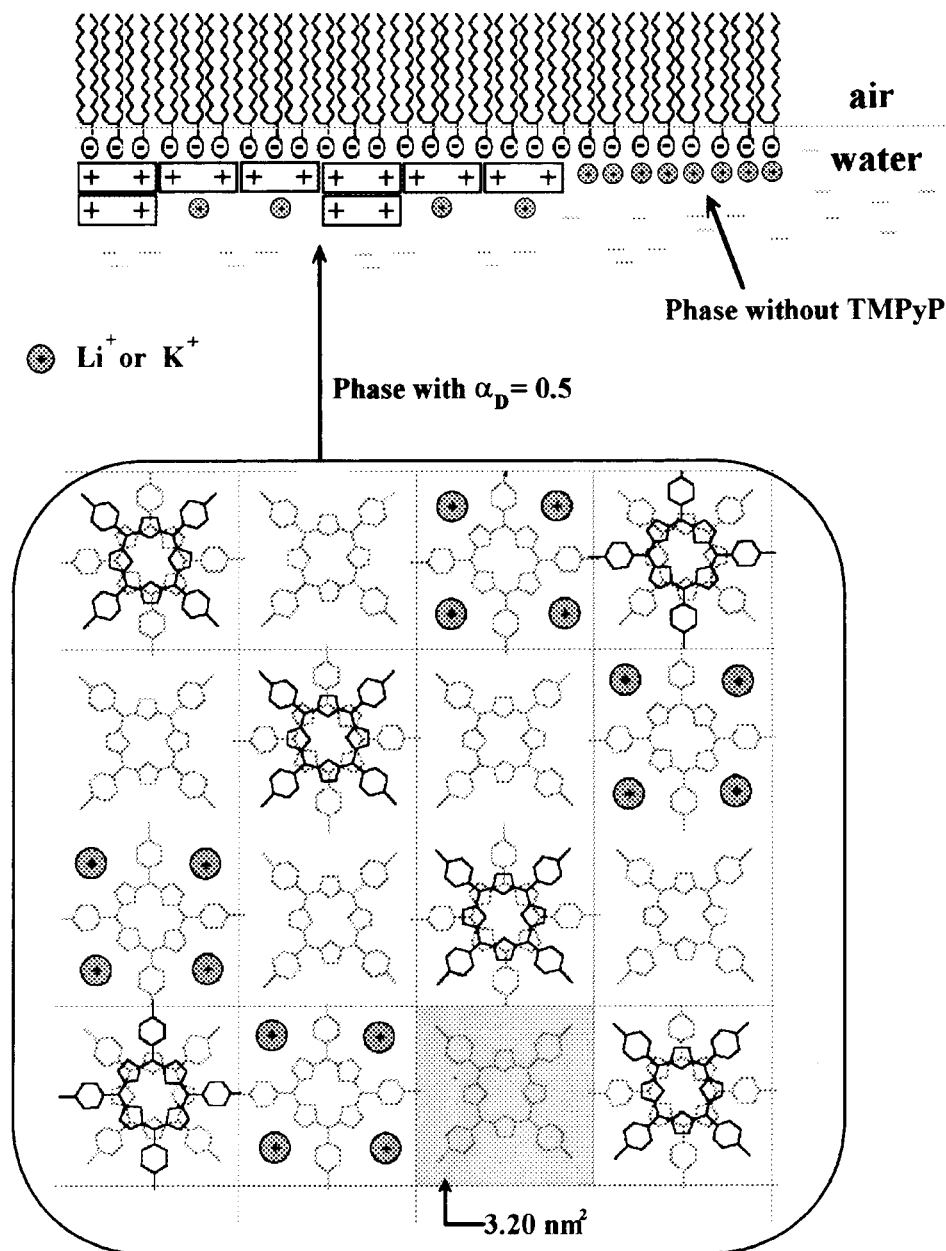
where $f = 1.5$ is the orientation factor of a flat configuration of the porphyrin with respect to the random orientation in solution; the factor $0.322 = 2.303(R)^{1/2}$, where $R \approx 0.2$ is the reflectivity of a bare air–water interface;² ϵ is the extinction coefficient in solution ($L \text{ mol}^{-1} \text{ cm}^{-1}$); and Γ is the surface concentration of TMPyP (mol cm^{-2}). Subscripts I and II correspond to monomer and dimer phases, respectively. ϵ_I and ϵ_{II} have been determined for $\lambda = 430$ and $\lambda = 420$ nm from the data shown in ref 8 and are $\epsilon_{I(430)} = 2 \times 10^5$, $\epsilon_{I(420)} = 1.66 \times 10^5$, $\epsilon_{II(430)} = 1.24 \times 10^5$, and $\epsilon_{II(420)} = 1.7 \times 10^5 L \text{ mol}^{-1} \text{ cm}^{-1}$.

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Chart 3. Model of a New Ordered and Homogeneous Phase for a Monolayer of TMPyP/DMPA, in Molar Ratio of 1:4, under the Influence of the Different Salts, KClO_4 and LiCl , in Aqueous Subphase



The reflectivities ΔR_{430} and ΔR_{420} were determined experimentally. Using eq 3, we obtain two equations with two unknown quantities, Γ_I and Γ_{II} , which can be determined directly. The total concentration of porphyrin at the interface will be $\Gamma = \Gamma_I + \Gamma_{II}$ and the molar fraction dimer is defined as $\alpha_D = \Gamma_{II}/\Gamma$. The average values of Γ and α_D obtained in this way are shown in Table 1.

The surface concentration of porphyrin, Γ , is plotted versus $\log C_{\text{salt}}$, in Figure 6. Clearly, the surface density of porphyrin Γ decreases with the increasing of salt concentration independent of the type of salt, although the magnitude of the effect depends on the electrolyte. Thus, porphyrin molecules are removed from the interface by competition with the cation of the subphase as has been indicated by the surface potential values. In the presence of TEAP, the molar fraction of dimer, α_D , decreases with increasing salt concentration. This may indicate that the TMPyP molecules of the second layer are bound less strongly to the DMPA matrix than those of the first layer. On the other hand, α_D is nearly constant

($\alpha_D \approx 0.5$) when LiCl or KClO_4 are used independent of the concentration (see Table 1). With 0.1 M KClO_4 or LiCl the reflection values are very small and affected by experimental error, and therefore α_D cannot be determined with sufficient precision. In contrast, λ_{max} is well defined with small experimental error, and similar values are found at high and low concentrations. This indicates that α_D values are almost constant at 0.5 for KClO_4 and LiCl .

It is amazing that the molar fraction of dimer is kept almost constant and close to 0.5 in the presence of LiCl or KClO_4 , moreover when the total surface concentration of porphyrin decreases strongly with increasing salt concentration. This value of α_D is obtained also at 20 mN/m (data not show) for the mentioned salts. This behavior could be explained by the formation of the new phase with $\alpha_D \approx 0.5$ that is stabilized by the presence of Li^+ or K^+ ions, including all the porphyrin molecules at the interface. This phase could coexist with a phase where there are no porphyrin molecules and the charges of the

headgroups of DMPA are compensated by the cations from the aqueous subphase. This model is shown in Chart 3.

In the proposed organization of the a new semidimer phase, Chart 3, the porphyrin of the second layer is redistributed in a less dense organization and well ordered keeping constant a value of $\alpha_D \approx 0.5$; however, porphyrin molecules are lost to the subphase due to the presence of ions in the subphase. Also, the structure presents holes through which Li^+ or K^+ ions have access to the headgroups of DMPA. The amount of cations bound to the monolayer will be determined by the surface density of DMPA at each surface pressure.

A comparison of the structure observed by BAM¹⁴ of the mixed monolayer of TMPyP/DMPA, in a molar ratio of 1:4, at the air–water interface without salts in the subphase, with the semidimer phase in the presence of salts has to be done. In the former case, a high dipole density has been formed between dimer and monomer phases (I and II) due to the different distribution of the DMPA molecules in the domain regions, 8 molecules of DMPA per 3.2 nm^2 (area per a flat molecule of porphyrin),

dense phase, and in the homogeneous regions where there are only 4 molecules of DMPA per 3.2 nm^2 , expanded phase, and therefore an increasing electrostatic energy is required for the morphology observed.¹⁴ In contrast, the semidimer phase is energetically favored in the presence of salts by the disappearance of the part of that increasing dipole density produced by the monomer–dimer structure described elsewhere.⁸ The new phase proposed here will be homogeneous.

Chart 3, however, is not valid for the effect of the TEAP molecules on the lipid matrix. Due to a bigger size of TEA^+ , these cations approaching the negative charges of DMPA molecules (see Chart 2), the holes drawn in Chart 3 cannot be filled. Thus, such an ordered phase with $\alpha_D \approx 0.5$ is not expected for TEAP.

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