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Short communication

Double layer potential and degree of dissociation in charged lipid monolayers

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Abstract

One of the contributions to the surface potential in charged phospholipid monolayers at air–water interfaces is the double layer potential. In this note several misconceptions found in the literature concerning the relationship between the double layer potential and the degree of dissociation of the lipid polar headgroups are critically analyzed. The deviations of the double layer potential measurements from the Gouy–Chapman theory observed by several authors are explained by taking into account the dependence of the degree of dissociation with concentration, area per lipid molecule and pH.

Keywords: Lipid monolayer; Double layer potential; Degree of dissociation; Surface potential

Surface potential measurements in lipid layers at the air–water or air–aqueous electrolyte solution interface provide valuable information about the structure of the interface. The nature of this potential is not yet fully understood, and its theoretical estimation is still a matter of discussion. Different experimental techniques have been used to identify the two contributions to this potential: the dipole potential and the double layer potential (Brockman, 1994; Moncelli et al., 1998). The dipole potential is the potential drop across the lipid layer and is evaluated as a sum of contributions from the water molecules, the lipid headgroups and the hydrophobic tails. The double layer potential is the potential drop in the electrical double layer that is formed due to the polar nature of the phospholipids. While the dipole potential has been the subject of numerous studies, the double layer potential is usually evaluated introducing severe approximations for the degree of dissociation of headgroups (Morgan et al., 1988; Taylor et al., 1989; Oliveira et al., 1992; Prieto et al., 1998). The objective of this note is to avoid these approximations and to clarify, in

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terms of the Gouy–Chapman (GC) theory (McLaughlin 1989), the dependence of the double layer potential and the degree of dissociation on the ionic environment of the lipid headgroups (pH, salt concentration, ion binding if any, etc.).

The surface charge density of a polar lipid monolayer depends on the molecular packing of the lipid (i.e. the area per lipid molecule), and the ionization state of headgroups. The degree of dissociation α of the lipid headgroups is determined by the intrinsic pK_a of the lipid and the interfacial pH, pH_σ, according to

$$\frac{\alpha}{1-\alpha} = 10^{pH_\sigma - pK_a} = 10^{pH_b - pK_a}\, e^{F\psi_o/RT} \qquad (1)$$

where pH_σ has been expressed in terms of the bulk pH, pH_b, and the electric potential drop between the interface and the bulk solution, ψ_o. Symbols R, F, and T denote the gas constant, the Faraday constant, and the absolute temperature, respectively. K_a is defined as the equilibrium constant of the reaction $AH \rightleftharpoons A^- + H^+$, where AH denotes a headgroup in neutral form and A^- a headgroup in ionized form.

The potential drop ψ_o is not experimentally accessible and the simplest approach for its calculation is the use of the GC theory. This is a mean force potential theory where the average screening produced by ions in solution depends on the total ion concentration, $2c + 10^{-pH_b} + 10^{pH_b - 14}$, where the first term accounts for the electrolyte ions and the others for the water ions (i.e. H^+ and OH^-). In the case of lipid headgroups bearing one negative charge per molecule, the expression for ψ_o is

$$\psi_o = \frac{2RT}{F}\operatorname{arcsinh}(f\alpha),$$
$$f = -e[A^2 4RT\varepsilon\varepsilon_o(2c + 10^{-pH_b} + 10^{pH_b - 14})]^{-1/2} \qquad (2)$$

where e is the elementary charge, A is the area per lipid headgroup, ε is the dielectric constant, and ε_o is the vacuum permittivity. The substitution of Eq. (2) into Eq. (1), using the logarithmic form of the arcsinh function, yields the following transcendental equation

$$\frac{\alpha}{1-\alpha} = 10^{pH_b - pK_a}(f\alpha + \sqrt{1 + (f\alpha)^2})^2, \qquad (3)$$

which allows the evaluation of α. Eq. (3) shows that α depends on A, pH_b, and c (Healy and White, 1978), but this fact has not always been considered in the study of the surface potential (Morgan et al., 1988; Prieto et al., 1998). The dependence of α on bulk concentration c has been recognized sometimes a posteriori, i.e. as a result of the discrepancies found between the experimental results and the approximate theoretical expressions used (Taylor et al., 1989). Furthermore, if binding of cations to negative lipid headgroups is considered, Eq. (1) needs to be rewritten as

$$\frac{\alpha}{1-\alpha} = (10^{pK_a - pH_b} + K_b c)^{-1}\, e^{F\psi_o/RT} \qquad (4)$$

where K_b is the binding constant of cations to negative headgroups. The term $K_b c$ can be comparable in magnitude to $10^{pK_a - pH_b}$ when pH_b is much higher than pK_a even in diluted solutions. For instance, if $pH_b = 7$, $pK_a = 3$, $K_b = 0.6\ M^{-1}$ and $c = 0.01$ M, the term $10^{pK_a - pH_b} + K_b c$ takes the value $(1 + 60) \times 10^{-4}$, and Eq. (4) yields a value of α which is one third of that obtained from Eq. (1). Therefore, when computing the surface potential at neutral pH and moderate ion concentrations, the possibility of ion binding should be examined.

In a recent study (Prieto et al. 1998), the surface potential of DMPA monolayers at the air–LiCl aqueous solution interface is evaluated as

$$\Delta V = 4\pi n\mu - 2.303\frac{RT}{F}\ln\left(\frac{134^2 \times 4}{A^2 c}\right) \qquad (5)$$

where the first term is the dipole contribution to the surface potential and the second corresponds to the double layer potential. The dipole potential of a lipid monolayer changes with the lipid density (Cseh and Benz, 1999) and it is not simply given by the first term of Eq. (5). However, we analyze here only the double layer potential. In particular, the second term of Eq. (5) is an approximate expression of the GC potential that is only valid for large values of the argument of the logarithm. Prieto et al. (1998) claim that the experimental data do not fit well to the GC theory because the experimental slope of the plot of ΔV versus $\log_{10}(c)$ is 49 mV/decade, and not 60 mV/decade as predicted by Eq. (5) (see Fig. 4a in

Prieto et al., 1998). However, this discrepancy can be explained by considering the variation of α with concentration c (note that A and pH are kept constant in these experiments). In fact, if α and ψ_o are evaluated from Eqs. (3) and (2), respectively, a theoretical slope of 47 mV/decade can be found (see Fig. 1), in agreement with the experimental observations. In Fig. 1, a neutral pH has been assumed (no pH information is available in Prieto et al. 1998) and pK_a has been taken as 2.5. The inset in Fig. 1 shows the variation of α with concentration.

Still another example of reported deviations from the GC theory can be found in a study of condensed stearic acid monolayers at the air–NaCl aqueous solution by Taylor et al., (1989). Apparently, the theoretical expression of the double layer potential is not in agreement with the experimental values measured at different concentrations. However, no disagreement is obtained when using the correct expression for ψ_o (i.e. taking into account how α changes with c) instead of assuming a fixed value for α as the authors do ($\alpha = 0.05$ for all concentrations). Fig. 2 shows the difference between ψ_o using $\alpha = 0.05$ and ψ_o using $\alpha(c)$. The slope of the logarithmic plot of ψ_o versus c using $\alpha(c)$ is 19 mV/decade, in good agreement with the 20 mV/decade observed in Fig. 1 of Taylor et al. (1989).

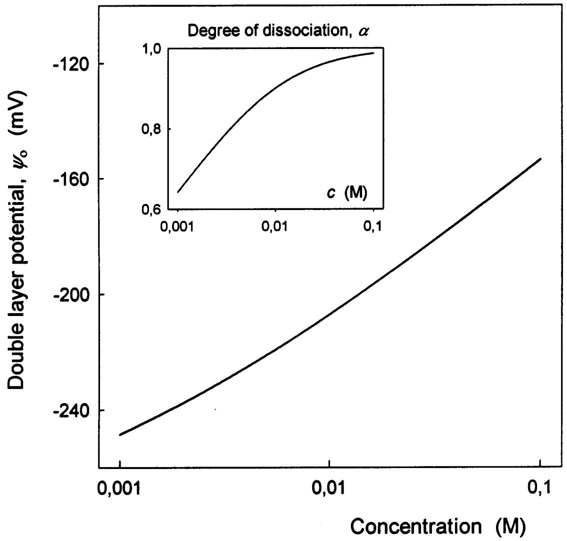

Fig. 1. Variation of the double layer potential with bulk ion concentration in a lipid monolayer at the air–1:1 aqueous electrolyte solution interface. The slope is 47 mV/decade in agreement with the experimental data by Prieto et al. (1998). The following values have been used in the potential calculation according to GC theory: $A = 42$ Å^2, p$K_a = 2.5$, pH$_b = 7$. The inset shows the corresponding change of the degree of dissociation.

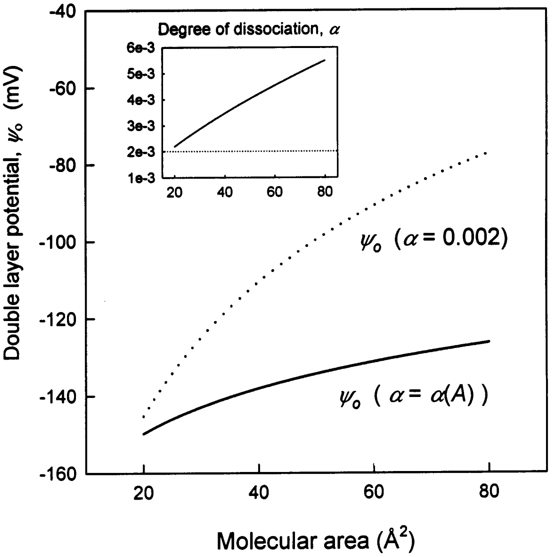

Fig. 2. Theoretical calculation of double layer potential below a condensed stearic acid monolayer assuming constant α (upper curve) and taking into account that α changes with concentration (lower curve). The lower curve exhibits almost identical slope as the experimental data reported by Taylor et al. (1989) under the conditions $A = 20$ Å^2, p$K_a = 5.7$, pH$_b$ = 5.6.

Similarly, some studies where the molecular area is varied do not consider how this variation affects the degree of dissociation. An example is found in the paper by Oliveira et al. (1992) on stearic acid monolayers at the air–water interface. Although the effects are not so clear in this case due to the large number of parameters involved in the surface potential (several effective dielectric constants and dipole moments). These authors estimated that $\alpha = 0.002$ from an empirical fitting to the surface potential measurements (Taylor et al., 1989), and calculated the double layer potential assuming constant α. Such a procedure neglects the fact that α changes with A even when c is kept constant ($c = 2.5$ µM). Fig. 3 shows a plot of the calculated double layer potential assuming $\alpha = 0.002$ (dotted line) and considering the dependence of α with A at constant $c = 0$ and pH = 5.6 (solid line). The pK_a value used is 5.7 (the same as in Fig. 1 of Oliveira et al. 1992). The inset in Fig. 3 shows the variation of α with A, and the dotted line corresponds to the constant value $\alpha = 0.002$. Once again, the differences between the approximated and exact values are very significant.

Nevertheless, the differences obtained in Fig. 3 are not so marked when the total potential drop across the monolayer is computed. Fig. 4 reproduces the theoretical calculation of dipole and double layer contributions to the surface potential–area isotherm of a stearic acid monolayer in pure water. Dipole potential contributions (dashed, dotted and dashed–dotted lines) are calculated in a similar way to Oliveira et al. (1992) following Demchak and Fort (1974), i.e. expressing the surface potential as $\Delta V = (\mu_1/\varepsilon_1 + \mu_2/\varepsilon_2 + \mu_3/\varepsilon_3)/A\varepsilon_o + \psi_o$, where the dipole contributions 1, 2 and 3 correspond to water molecules, hydrophilic headgroups and hydrophobic tails. Thin solid lines show the double layer potential contribution for the cases of constant α (panel b) and α changing with A (panel a). Finally, thick solid lines show the corresponding overall surface potential across the monolayer. The potential ΔV computed in Oliveira et al., 1992 appears to agree reasonably well with their experimental data. The fact is, however, that there are several magnitudes (dipole moments and dielectric constants) whose changes with molecular area can be chosen in such a way that the theoretical results fit to the experimental curve $\Delta V(A)$ in both panels a and b.

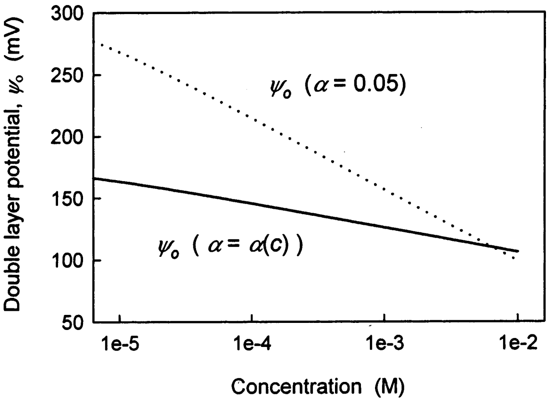

Fig. 3. Calculated double layer potential contribution to the surface potential–area isotherm of a stearic acid (pK_a = 5.7) monolayer in pure water (pH$_b$ = 5.6). Dotted line, assuming constant degree of dissociation α (Taylor et al., 1989; Oliveira et al., 1992); solid line. taking into account the variation of α with the molecular area A. The inset shows the change of α with molecular area and the reference value $\alpha = 0.002$ used by Oliveira et al. (1992)

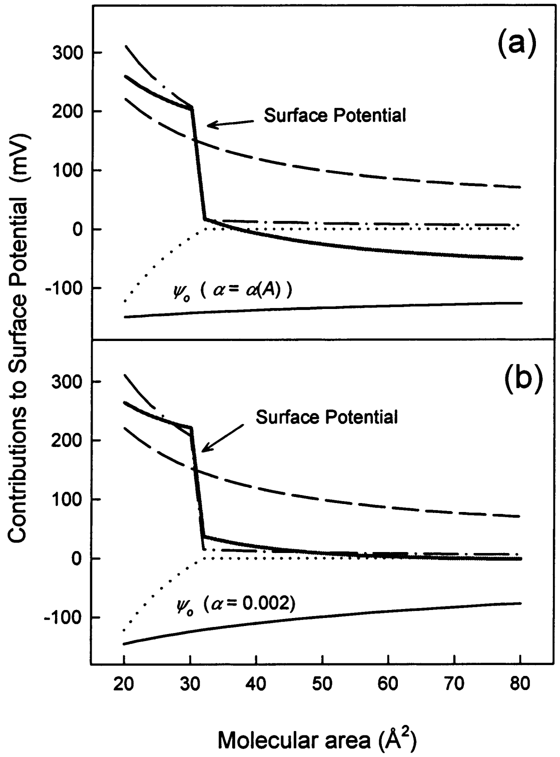

Fig. 4. Calculated dipole and double layer contributions to the surface potential–area isotherm of a stearic acid monolayer in pure water. Dipole potential contributions (dashed, dotted and dashed–dotted lines) have been calculated in a similar way to Oliveira et al. (1992). Thin solid lines show the double layer potential calculated by using either $\alpha(A)$ (panel a) or $\alpha = 0.002$ (panel b). Thick solid lines show the corresponding overall surface potential across the monolayer.

In conclusion, our results show clearly that the description of monolayers with surface charge due to ionizable sites should take into account all the relevant factors (bulk ion concentration, density of charged sites, pH, ion binding) and their mutual influence. Omitting this mutual influence may lead to serious misinterpretation of experimental results (see Figs. 1–3), although in some cases the significant discrepancies obtained here can be masked by the effective values assumed for the relatively high number of free parameters involved in the models (see Fig. 4). This is probably the reason why simplified forms of Eqs. (2) and (3) have usually been employed in the literature. Note finally that an improved description of the monolayer would require more accurate evaluations of both surface and double layer potential, while we have concentrated our analysis only on the latter contribution.

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References

Brockman, H., 1994. Dipole potential of lipid membranes. Chem. Phys. Lipids 73, 57–79.

Cseh, R., Benz, R., 1999. Interaction of phloretin with lipid monolayers: relationship between structural changes and dipole potential change. Biophys. J. 77, 1477–1488.

Demchak, R.J., Fort, T.J., 1974. Surface dipole moments of close-packed unionized monolayers at the air–water interface. J. Colloid Interface Sci. 46, 191–202.

Healy, T.W., White, L.R., 1978. Ionizable surface group models of aqueous interfaces. Adv. Colloid Interface Sci. 9, 303–345.

McLaughlin, S., 1989. The electrostatic properties of membranes. Annu. Rev. Biophys. Biophys. Chem. 18, 113–136.

Moncelli, M.R., Becucci, L., Buoninsegni, F.T., Guidelli, R., 1998. Surface dipole potential at the interface between water and self-assembled monolayers of phosphatidylserine and phosphatidic acid. Biophys. J. 74, 2388–2397.

Morgan, H., Taylor, D.M., Oliveira Jr, O.N., 1988. Two-dimensional proton conduction at a membrane surface: influence of molecular packing and hydrogen bonding. Chem. Phys. Lett. 150, 311–314.

Oliveira Jr, O.N., Taylor, D.M., Morgan, H., 1992. Modeling the surface potential–area dependence of a stark acid monolayer. Thin Solid Films 210/211, 76–78.

Prieto, I., Camacho, L., Martín, M.T., 1998. Ion interactions and electrostatic effects on TMPyP/DMPA monolayers. Langmuir 14, 1853–1860.

Taylor, D.M., Oliveira Jr, O.N., Morgan, H., 1989. The surface potential of monolayers formed on weak acidic electrolytes: implications for lateral conduction. Chem. Phys. Lett. 161, 147–150.