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INTERFACIAL pH AT ELECTRICALLY CHARGED LIPID MONOLAYERS INVESTIGATED BY THE LIPOID pH-INDICATOR METHOD*

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SUMMARY

Monomolecular films of various charge densities are prepared by mixing methylstearate with long chain sulfate and quaternary ammonium ions. A lipoid fluorescence pH indicator is embedded in the charged interface. Interfacial pH changes are detected as a function of the charge density of the monolayer and the NaCl concentration of the subphase. In the case of the positive films the results are in agreement with the Gouy–Chapman theory of the diffuse double layer. In the case of the negative film significant deviations are found.

INTRODUCTION

At an electrically charged interface in contact to an electrolyte solution an electrical potential is built up by the electrical double layer. This interfacial potential affects the concentration of ions at the interface. Accordingly the pH at the charged interface is different from the bulk pH, and consequently an acid–base equilibrium located at the interface is sensitive to the interfacial potential.

Hitherto this effect has been studied with soluble pH indicators adsorbed to charged micelles and liposomes [1–3]. The drawbacks of this method are the unknown location of the dye molecule and the invariable charge density of the colloid surface.

In a previous paper it has been shown, that these difficulties may be overcome by incorporating a lipoid fluorescence pH indicator in lipid monolayers at the air–water interface. In this case the pH-sensitive chromophore is fixed in the plane of the polar headgroups of the lipid film[†]. The titration characteristics of this acid–base-sensitive probe were found to be dependent on the lipid composition of the monolayer, especially on the presence of electrically charged lipids [4].

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[*] Third communication on "Lipoid pH indicators in monolayers", first and second communication, see refs 4 and 5.

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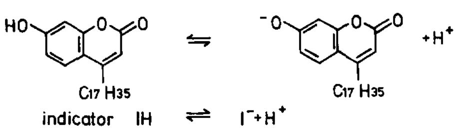
[†] This is suggested by molecule models [4] and can be proved by energy transfer measurements in dye/lipid multilayers [5].

This paper deals with the reaction of the probe chromophore on the variation of the monolayer charge density and of the electrolyte concentration.

MATERIALS AND METHODS

Monolayers with various charge densities are made from mixtures of methylstearate (Merck) and eicosyltrimethylammonium bromide (Schuchardt; twice recrystallized) and eicosylsodium sulfate (Schuchardt), respectively [4]. To these mixtures the lipoid pH indicator 4-heptadecylumbelliferone (Formula I) is added at a ratio of indicator/total lipid of 1 : 400. The monolayers are spread from 5 mM solutions in chloroform (freshly destilled; 20 % dimethylsulfoxide is added to solve the sulfate) on a 10 mM aqueous solution of NaCl (Merck, suprapure) and compressed to 30 dynes/cm (water is distilled twice, once from alkaline permanganate [6]).



Formula I

The fluorescence of the monolayers is measured as a function of the pH of the subphase. Using an excitation wavelength of 366 nm the fluorescence at 450 nm originates exclusively from the dissociated indicator molecules [7, 8]. Thus after dividing the pH-dependent fluorescence intensities by the intensity at high pH (i.e. complete dissociation of the indicator) one obtains the degree of dissociation of the indicator as a function of the bulk pH, as determined by a glass electrode relative to NBS buffer solutions. Details of the experimental procedure are found in ref. 4.

RESULTS

The titration curves of the indicator are shown in Fig. 1 with five monolayer compositions and a 1 mM NaCl subphase. In the eicosyltrimethylammonium/methylstearate films the indicator takes up a proton at a 10^4 times higher bulk proton concentration than in the pure methylstearate film. In the eicosyl sulfate/methyl-

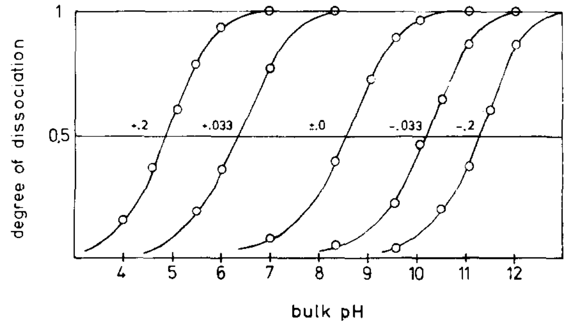


Fig. 1. Degree of dissociation of 4-heptadecylumbelliferone in monolayers of various composition versus pH of the 1 mM NaCl subphase. The molar fraction of eicosyltrimethylammonium (+) and eicosylsulfate (—) in the methylstearate monolayers are indicated in the figure.

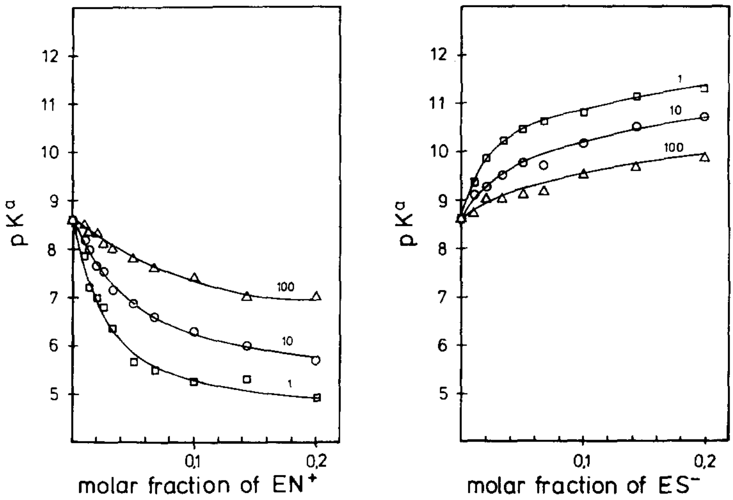


Fig. 2. Apparent pK_a values of 4-heptadecylumbelliferone versus the molar fraction of the charged lipid ions eicosyltrimethylammonium (EN^+) (Fig. 2a) and eicosylsulfate (ES^-) (Fig. 2b) in the methylstearate films. The NaCl concentrations (mM) of the subphase are indicated in the figure.

stearate film the indicator accepts a proton at already a 10^3 times lower bulk proton concentration than in the methylstearate monolayer.

To characterize the titration of the reaction $IH = I^- + H^+$ (cf. Formula I) under the various reaction conditions, an apparent equilibrium constant K_a is introduced. Denoting the bulk pH as pH_b and the interfacial density of dissociated and undissociated indicator as d_{I^-} and d_{IH}, the pK_a is defined by Eqn 1:

$$pK_a = pH_b - \log(d_{I^-}/d_{IH}) \qquad (1)$$

The values of pK_a are determined by the value of pH_b for $d_{I^-} = d_{IH}$ i.e. degree of dissociation 0.5.

The pK_a values taken from titrations in monolayers and subphases of various compositions are comprised in Fig. 2*.

The apparent equilibrium constant is lowered by eicosyltrimethylammonium bromide and rised by eicosylsodium sulfate, indicating stabilization and destabilization of the dissociated indicator. Increase of the NaCl concentration reduces the stabilization and the destabilization effect. By mixing equal molar fractions of both charged lipids (e.g. 10 %) they compensate each other, such that the same pK_a is found as in pure methylstearate.

DISCUSSION

The observation that the same chromophore may take up a proton at different

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* The pK_a in methylstearate (8.6) differs from the value given in ref. 4 for methylpalmitate (9.5) because of the high indicator concentration (1 : 50) used there. In that case the negative charges of the indicator molecules itselves contribute to the charge density of the monolayer appreciably, shifting the pK_a to higher values.

proton concentrations suggests that the measured bulk pH values do not reflect in all cases the actual pH at the interface affecting the chromophore.

One may assume that for the neutral methylstearate film (mst) the interfacial $\mathrm{pH_i}$ and the bulk $\mathrm{pH_b}$ coincide (Eqn 2).

$$\mathrm{pH_i(mst)} = \mathrm{pH_b} \tag{2}$$

This implies that the apparent $\mathrm{p}K_\mathrm{a}$ measured with the methylstearate film $\mathrm{p}K_\mathrm{a}(\mathrm{mst})$ is assumed to be the "real" $\mathrm{p}K$ of the indicator in the interface. For the other monolayers the difference of the apparent $\mathrm{p}K_\mathrm{a}$ values and the $\mathrm{p}K_\mathrm{a}(\mathrm{mst})$ may be attributed the difference of the bulk and interfacial pH (Eqn 3)*.

$$\mathrm{pH_i} = \mathrm{pH_b} - (\mathrm{p}K_\mathrm{a} - \mathrm{p}K_\mathrm{a}(\mathrm{mst})) \tag{3}$$

The difference in osmotic energy between the interface and the bulk subphase must be compensated by an electrostatic energy difference. With k as Boltzmann's constant, T as absolute temperature and e as electronic charge an electrical potential ψ_i is defined by Eqn 4.

$$\psi_\mathrm{i} = (2.3\ kT/e)(\mathrm{pH_i} - \mathrm{pH_b}) \tag{4}$$

The potentials calculated from the experimental $\mathrm{p}K_\mathrm{a}$ values (Fig. 2) according to Eqns 3 and 4 are shown in Fig. 3 as a function of the charge density. Each eicosyltrimethylammonium ion and each eicosylsulfate ion is assumed to contribute one positive and one negative electronic charge, respectively.

This representation of the experiments may be compared to interfacial potentials obtained from theories of the electrical double layer near charged interfaces.

The Gouy–Chapman theory of the diffuse double layer considers the electrostatic interaction of point charge ions with a homogeneously charged, impenetrable interface in a medium of constant permittivity [9, 10]. With ε as relative permittivity, ε_0 as permittivity of the vacuum, Eqn 5 represents the relation of the interfacial potential ψ^{GCh} and the interfacial density of electronic charges σ and the concentration c of a 1-1 electrolyte.

$$\psi^{\mathrm{GCh}} = (2kT/e)ar\sinh(\sigma^2 e^2/8\varepsilon\varepsilon_0 ckT)^{\frac{1}{2}} \tag{5}$$

Inserting the values of the constants and $T = 293$ °K and $\varepsilon = 80$ (of bulk water) into Eqn 5 one obtains the theoretical curves shown in Fig. 3.

The general appearance of the relation of the experimental potential ψ_i, the charge density and the electrolyte concentration is reflected by the theoretical

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* Actually the apparent $\mathrm{p}K_\mathrm{a}(\mathrm{mst})$ (= 8.6) differs from the $\mathrm{p}K$ of the unsubstituted indicator in bulk solution $\mathrm{p}K_\mathrm{b}$ (= 7.75 [3]). (This may be caused by a lower dielectric constant in the immediate surroundings of the indicator. It may be excluded that this shift is caused by adsorbed $\mathrm{Cl^-}$, since the $\mathrm{p}K_\mathrm{a}(\mathrm{mst})$ is independent of the NaCl concentration). In fact Eqn 2 should be considered as a convention. It defines the conditions of the methylstearate film as a thermodynamical standard state for the indicator reaction in the various monolayers. The "interfacial $\mathrm{p}K$" $\mathrm{p}K_\mathrm{a}(\mathrm{mst})$ includes the effects of the surroundings of the indicator in the pure methylstearate film. The influence of the charged lipids, is then included in the "interfacial pH". This partitioning of the total shift of $\mathrm{p}K_\mathrm{a}$ into a $\mathrm{p}K$ and a pH shift is convenient, but obviously somewhat arbitrary. However terms as "interfacial $\mathrm{p}K$" and "interfacial pH" are not essential, since the experimental results ($\mathrm{p}K_\mathrm{a} - \mathrm{p}K_\mathrm{a}$ (mst) or $\mathrm{p}K_\mathrm{a} - \mathrm{p}K_\mathrm{b}$) may be related directly to a potential difference, comparable to theoretical double layers potentials (Fromherz, P., unpublished).

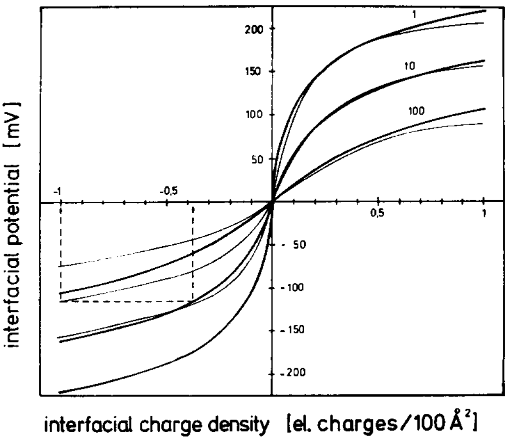

Fig. 3. Interfacial potential versus interfacial charge density. Heavy lines: theoretical ψ^{GCh} according the Gouy–Chapman theory (Eqn 5). Light lines: experimental ψ_i (Fig. 2 and Eqns 3 and 4). The concentrations of 1-1 electrolyte (mM NaCl) in the subphase is indicated in the figure. The charge densities indicated on the abscissa are the reciprocal monolayer areas of the mixed monolayers per charge lipid at 30 dynes cm. The broken arrows indicate the relation of this intrinsic charge density with the effective charge density calculated from the experimental potentials using the Gouy–Chapman theory (cf. text).

ψ^{GCh} functions. This indicates that the shifts of the acid–base equilibrium observed may be considered to be due essentially to the interfacial potential, i.e. the interfacial pH shift, caused by the electrostatic interaction of the charged lipids in the monolayer with the electrolyte in the subphase.

pK_a shifts caused by pure electrostatic interaction of the interface with the electrolyte should be antisymmetrical with respect to the sign of the charge density. The asymmetry observed (Fig. 3) may have two reasons: (a) Different chemical nature of the positively and negatively charged groups in the interface and of the cations and anions of the electrolyte. (b) Negative charge of the dissociated indicator.

Specific adsorption of counterions to the charged groups may reduce the effective charge density. Within the framework of the Gouy-Chapman theory the effective charge density is obtained by inserting the experimental potential ψ_i as ψ^{GCh} in Eqn 5 (a graphical procedure is indicated in Fig. 3). The considerable reduction of the effective charge density in the case of the negatively charged film would indicate a strong interaction of Na^+ with the sulfate groups*. The fraction of the neutralized sulfate groups may be correlated to an adsorption energy by applying Stern's equation [12]. However, this adsorption energy is found not to be constant for the various conditions of charge density and NaCl concentration.**

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* Although purest reagents and carefully distilled water have been used, it is rather difficult to rule out the presence of traces of multivalent cations, which would shift the pK_a in the sulfate films to lower values [4] by adsorption to the sulfate groups (cf. ref. 11).

** It should be considered that $(d\psi_i/d \log c)_\sigma$ i.e. the slope of the potential as a function of the electrolyte concentration at constant surface charge density, in the negative film at high potentials ($>$ 100 mV) is 40.5 mV which does not agree with the theoretical value of 58 mV predicted by the Gouy–Chapman and the Stern equation [13]. For the positive monolayer this slope is found to be 58 mV.

A more detailed discussion is postponed at the moment, since it cannot be decided whether this inconsistency is due to the shortcomings of the Gouy–Chapman or Stern theory with respect to the monolayers considered, or to the presence of the indicator anion itself. A more extensive treatment of the lipoid pH indicator method with respect to its implications as measuring device for interfacial potentials as well as with respect to the interpretation in terms of various double layer theories, will be possible only when the pK_a measurements of various lipoid pH indicators of different chemical nature as well as measurements of the surface potential may be compared (Fromherz, P., unpublished).

REFERENCES

1 Hartley, G. S. and Roe, J. W. (1940) Trans. Faraday Soc. 36, 101–109
2 Mukerjee, P. and Banerjee, K. (1964) J. Phys. Chem. 68, 3567–3574
3 Montal, M. and Gitler, C. (1973) Bioenergetics 4, 363–382
4 Fromherz, P. (1973) Biochim. Biophys. Acta 323, 326–334
5 Fromherz, P. (1974) Chem. Phys. Lett. 26, 221–224
6 Pfau, J. and Sondermann, J. (1972) G.I.T., Fachz. Lab. 1149–1152
7 Chen, R. F. (1968) Anal. Lett. 1, 423–428
8 Shank, C. V., Dienes, A., Trozzolo, A. M. and Myer, J. A. (1970) Appl. Phys. Lett. 16, 405–407
9 Verwey, E. J. W. and Overbeek, J. Th. G. (1948) Theory of the Stability of Lyophobic Colloids, p. 22 ff, Elsevier, New York
10 Davies, J.-T. and Rideal, E. K. (1963) Interfacial Phenomena, p. 79, Academic Press, New York
11 Mingins, J. and Pethica, B. A. (1963) Trans. Faraday Soc. 59, 1892–1905
12 Stern, O. (1924) Z. Elektrochem. 30, 508–516
13 Davies, J.-T. and Rideal, E. K. (1963) Interfacial Phenomena, p. 76, Academic Press, New York