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Dielectric Saturation of the Aqueous Boundary Layers Adjacent to Charged Bilayer Membranes

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Summary. The surface charge density resulting from the adsorption of hydrophobic anions of dipicrylamine onto dioleyl-lecithin bilayer membranes has been measured directly using a high field pulse method. The surface charge density increases linearly with adsorbate concentration in the water until electrostatic repulsion of impinging hydrophobic ions by those already adsorbed becomes appreciable. Then Gouy-Chapman theory predicts that surface charge density will increase sublinearly, with the power $[z^+/(z^+ + 2)]$ of the adsorbate concentration, where z^+ is the cation valence of the indifferent electrolyte screening the negatively charged membrane surface. The predicted 1/3 and 1/2 power laws for univalent and divalent cations, respectively, have been observed in these experiments using Na^+, Mg^{++}, and Ba^{++} ions. Gouy-Chapman theory predicts further that the change from linear to sublinear dependence takes place at a surface charge density governed by the static dielectric constant of water and the concentration of indifferent electrolyte. Quantitative agreement with experiment is obtained at electrolyte concentrations of 10^{-4} M and 10^{-3} M, but can be maintained at higher concentrations only if the aqueous dielectric constant is decreased. A transition field model is proposed in which the Gouy-Chapman theory is modified to take account of dielectric saturation of water in the intense electric fields adjacent to charged membrane surfaces.

In this paper we present evidence for dielectric saturation of the aqueous boundary layers exposed to the intense electric fields adjacent to charged lipid bilayer membranes. The nonlinear polarization of a dielectric medium by an intense electric field can result from several mechanisms (Böttcher, 1973). A homogeneous polar medium usually displays normal saturation, a sublinear increase of polarization with field appropriately described by higher order terms of the Langevin function. In a theoretical treatment of the case of water, Booth (1951), found that the dielectric constant would decrease from the static value ($\varepsilon \sim 80$) in low applied fields, approaching the optical value, equal to the square of the refractive index, at applied fields in excess of 10^7 V/cm. Such a

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transition reflects an approach to maximal alignment of the permanent dipole moments of the water molecules in the applied field. High field dielectric measurements (Kolodziej, Parry-Jones & Davies, 1975) provide evidence that bulk water does indeed display normal saturation as described above, although experimental difficulties restrict the applied field to 10^5 V/cm at which the observed decrease in dielectric constant is no more than 0.1 %.

Further experimental evidence for dielectric saturation of water in more intense electric fields is necessarily indirect, being provided, for example, by measurement of the differential or integral capacitance of the mercury/aqueous electrolyte interface. The capacitance is attributed to the series combination of a diffuse Gouy-Chapman region and a Stern layer reflecting the finite size of ions impinging upon the interface (Grahame, 1947). At high concentrations of weakly adsorbing electrolyte (e.g., 1.0 M NaF) the Stern layer determines the measured capacitance, the results obtained being consistent with dielectric saturation of the water in the layer. A theoretical treatment of the integral capacitance of charged aqueous interfaces, based upon the assumption of a reduced dielectric constant in the inner layer, has been given by Robinson and Levine (1973), who cite references to earlier work on this problem. Additional evidence of saturation is provided by measurement of the static dielectric permittivity of aqueous electrolyte solutions, found to be lower than that of pure water (Giese, Kaatze & Pottel, 1970). The real part of the dielectric permittivity of such solutions, measured at microwave frequencies, drops markedly with increasing electrolyte concentration (Barthel, Schmithals & Behret, 1970). Such findings are generally attributed to dielectric saturation of water in the vicinity of ions in solution. Earlier references to this extensively studied topic will be found in the papers cited. Finally, dielectric saturation of water can also be inferred from studies of proton transfer reactions (Schwarzenbach, 1970) for which the rate is controlled by electrostatic interactions which in turn are mediated by the effective dielectric constant of the aqueous medium separating the charged reactant species. Additional information on the high field properties of both polar and nonpolar media has been provided in a recent review by Davies (1976).

Further evidence of immediate biophysical significance is given by studies of the hydrogen ion titration curves of proteins (Tanford, 1962) and of ionic equilibria as revealed by spectral titration studies on proteins incorporating azomercurial and naphthalene reporter groups (Klotz, Stellwagen & Stryker, 1964). These studies frequently reveal shifts of the pK_a of ionizable groups which indicate that charge separation takes place less readily in native protein than in low molecular weight analogs involving the same group. Reduced polarity of the microenvironment at the site of reaction – tantamount to a reduction of local dielectric constant – has been offered as a possible explanation of such observations (Cohen, 1970). Additional biophysical implications of high electric fields have been considered by Davies (1971).

The experiments to be described here employ bilayer membranes formed from dioleyl-lecithin, the zwitterionic head groups of which carry no net charge. Such bilayers will, however, acquire a net negative surface charge density due to adsorption of hydrophobic anions of dipicrylamine (DpA^-) when this compound is introduced into the surrounding aqueous phases. Studies of current relaxation accompanying the application of a voltage step to this system (Ketterer, Neumcke & Läuger, 1971) have provided strong evidence that transport of DpA^- across the bilayer takes place in three distinct steps, namely, interfacial adsorption, translocation across the membrane, and desorption. Their observation of initial currents of much greater magnitude than steady-state currents indicates that the membrane translocation step is much faster than the interfacial reactions. Subsequent experiments using higher voltage steps (Bruner, 1975) have shown that it is possible to transfer all adsorbed DpA^- ions to one membrane/solution interface, completely eradicating the initially equal distribution of adsorbed charge between the two interfaces. Since negligible desorption of ions takes place during the time required for this transfer, a graphical integration of the observed current-time transient provides a direct and model-independent measure of the density of charge present on the membrane/solution interfaces prior to application of the voltage step. The same technique has been used by Andersen and Fuchs (1975), to measure the surface charge density resulting from adsorption of tetraphenylborate anions onto phosphatidylethanolamine bilayer membranes.

Availability of the system described above permits a direct experimental investigation of the relationship between the density of adsorbed membrane surface charge and the concentration of adsorbate in the surrounding aqueous phases. This relationship is expected to be linear so long as the surface charge density is sufficiently low as not to perturb the aqueous phase concentration of adsorbate near the interfaces. As the surface charge density increases, however, a point will be reached at which impinging hydrophobic anions will experience electrostatic repulsion by those already adsorbed, leading to a sublinear increase of surface charge density with further increase of adsorbate concentration in the aqueous phases. A quantitative description of this dependence, based upon classical Gouy-Chapman theory, will be given in the next section. This theory has been applied to the interpretation of conductance measurements both on bilayers formed from lipids possessing an intrinsic surface charge (McLaughlin, Szabo, Eisenman & Ciani, 1970), and on bilayers possessing surface charge due to adsorbed hydrophobic ions (McLaughlin, 1972).

Gouy-Chapman Theory of Self-Limited Adsorption

Classical Gouy-Chapman theory (Aveyard & Haydon, 1973) states that the electrostatic potential, ψ_s, at a plane carrying a surface charge density, σ_s, and adjoining a symmetric electrolyte solution will be given by,

$$\frac{\sigma_s}{2\sigma_o} = \sinh\left(\frac{e z \psi_s}{2kT}\right) \tag{1}$$

where

$$\sigma_o = \sqrt{2\varepsilon\varepsilon_o kT c_o} \tag{2}$$

and where ψ_s is measured relative to $\psi = 0$ in the bulk solution. Here e is the magnitude of the electron charge, $z = z^+ = -z^-$ where z^+ and z^- are the cation and anion valence, respectively, of the dissolved electrolyte, k is the Boltzmann constant, and T is the absolute temperature. The parameter σ_o is a characteristic surface charge density involving as additional parameters, ε, the relative permittivity or static dielectric constant of the solvent, ε_o, the permittivity of free space, and c_o, the concentration of indifferent electrolyte in the bulk solution. Equation (1) is valid for both signs of surface charge density, the sign of ψ_s being identical to that of σ_s. For the experiments to be described it will always be true that the concentration of dipicrylamine in the bulk solutions, $c_{\mathrm{aq}}(\mathrm{DpA}^-)$, will be much less than that of the indifferent electrolyte, and hence will not enter into the determination of σ_o by Eq. (2).

We relate the magnitude of the surface charge density due to absorbed DpA^- to its concentration in the bulk aqueous phases by the expression,

$$|\sigma_s| = \beta c_{\mathrm{aq}}(\mathrm{DpA}^-) \exp\left[-\frac{e|\psi_s|}{kT}\right] \tag{3}$$

where β is a partition coefficient having the dimension of length (Ketterer *et al.*, 1971). The Boltzmann factor introduced here incorporates the absolute magnitude of the negative surface potential, but does not involve z since the DpA$^-$ ion is monovalent. At low surface potentials Eq. (3) reduces to,

$$|\sigma_s| \cong \beta c_{aq}(\mathrm{DpA}^-); \qquad \left(|\psi_s| \ll \frac{kT}{e}\right). \tag{4}$$

When the magnitude of the surface potential is large, Eq. (1) may be written,

$$\frac{|\sigma_s|}{\sigma_o} \cong \exp\left[\frac{ez|\psi_s|}{2kT}\right]; \qquad \left(|\psi_s| \gg \frac{kT}{e}\right). \tag{5}$$

Eqs. (3) and (5) may then be combined to eliminate the factor $(e|\psi_s|/kT)$, yielding,

$$|\sigma_s| \cong \sigma_o \left[\frac{\beta c_{aq}(\mathrm{DpA}^-)}{\sigma_o}\right]^{\frac{z}{z+2}} \tag{6}$$

when $(e|\psi_s|/kT) \gg 1$.

Equations (4) and (6) indicate that a log-log plot of $|\sigma_s|$ vs. $c_{aq}(\mathrm{DpA}^-)$ which covers a sufficient range should show a linear segment of unit slope at low concentrations of DpA$^-$, followed by a smooth transition to another linear segment of lower slope $[z/(z+2)]$ at higher concentrations of DpA$^-$. The transition takes place when $|\psi_s| \approx (kT/e)$, or when $|\sigma_s| \approx \sigma_o$. In fact, by eliminating $\beta c_{aq}(\mathrm{DpA}^-)$ between Eqs. (4) and (6), it may be shown that the linear segments will intersect at a surface charge density, $|\bar{\sigma}|_s = \sigma_o$. Since both $|\sigma_s|$ and $c_{aq}(\mathrm{DpA}^-)$ are determined by the experiments described below, it follows that verification of the predicted log-linear dependences will permit an experimental determination of $|\bar{\sigma}_s|$. Comparison with σ_o as given by Eq. (2), with c_o also experimentally determined, then permits an assessment of the adequacy of the static dielectric constant for the characterization of the state of polarization of the aqueous solvent adjacent to the charged membrane/solution interface.

The description of self-limited adsorption in terms of the power law of Eq. (6) reflects the increase of screening capacity with increasing valence of the indifferent electrolyte (McLaughlin, Szabo & Eisenman, 1971; Muller & Finkelstein, 1972). As z increases, the sublinearity decreases; Eq. (6) approaches coincidence with Eq. (4) as z becomes indefinitely large. As a final point, though the analysis has been given for the case of symmetric indifferent electrolyte, we would expect it to apply equally well to the asymmetric case by simply replacing z by z^+ in Eqs. (5) and (6). The indifferent cation will govern the space charge distribution adjacent to membranes which are negatively charged by adsorption of DpA^-.

Materials and Methods

Bilayer membranes were formed by the brush technique from dioleyl phosphatidylcholine (Analabs) dissolved in *n*-decane. The conductance cell used was of Teflon and quartz construction, the membranes being formed on a 2-mm diameter aperture in a Teflon septum. The cell was water-jacketed, with temperature control at 25 °C provided by a Lauda Model K-2 circulating bath. Dipicrylamine (2, 2′, 4, 4′, 6, 6′ hexanitrodiphenylamine from Aldrich Chemical Co.) was carefully dried in small quantities, with weighed amounts then being dissolved in dimethyl sulfoxide, (DMSO), to form stock solutions with concentrations ranging from 10^{-2} to 10^{-5} M. Measured aliquots of these were then added to the aqueous electrolyte solutions to secure the desired concentration of DpA^-; the resulting DMSO concentrations never exceeded 0.2% by volume. Aqueous solutions were prepared using reagent grade salts and deionized distilled water. The solutions were unbuffered, having a pH of about 6, sufficiently high to insure that dipicrylamine with a pK_a of 2.66 (Gaboriaud, 1966) would be fully ionized.

Voltage pulses were applied to the membrane through Ag/AgCl electrodes of 1 cm^2 area, using a pulse generator built in our laboratory. Current transients were recorded by monitoring the voltage drop across an external series resistor, with care being taken to minimize the membrane charging time (Ketterer *et al.*, 1971). Applied voltage pulses and resulting current transients were recorded on a Tektronix Model 7313 storage oscilloscope equipped with a Model 7B50 time base and two Model 7A22 differential amplifiers. The records were photographed; charge transport was measured by subsequent integration of the current transients using a planimeter as described previously (Bruner, 1975).

Our ability to equate measured charge transport per unit area of membrane to the surface charge density of adsorbed DpA^- ions is based upon three characteristics of the observed current transients. First, that portion of the transient resulting from charging of the membrane capacitance is of very short duration (typically ~10 μsec) in comparison with the slower current decay (0.5 to 10 msec) associated with translocation of DpA^- ions across the membrane interior. Thus, extrapolation of the slow transient to zero time, i.e., to the time at which the voltage pulse is applied, may be accomplished with negligible error. Secondly, the slow current transient decays to the prepulse baseline with no appreciable "tail" due to diffusion limited flow of hydrophobic ions through the membrane/solution interfaces (Ketterer *et al.*, 1971). Illustration of these characteristics may be obtained by reference to Fig. 1 of the paper by Bruner (1975) where typical current transients are displayed. Under extreme conditions of aqueous electrolyte and DpA^- ion concentration, as detailed in the following section, these characteristics no longer obtain and accurate data on surface charge density cannot be secured. Finally, the surface density of transported charge, measured as a function of applied voltage pulse amplitude, increases sublinearly to a limiting value which corresponds to complete depletion of adsorbed DpA^- ions from the lower potential (negative) membrane/solution interface. Thus, this limiting value of transported charge density may be equated to the

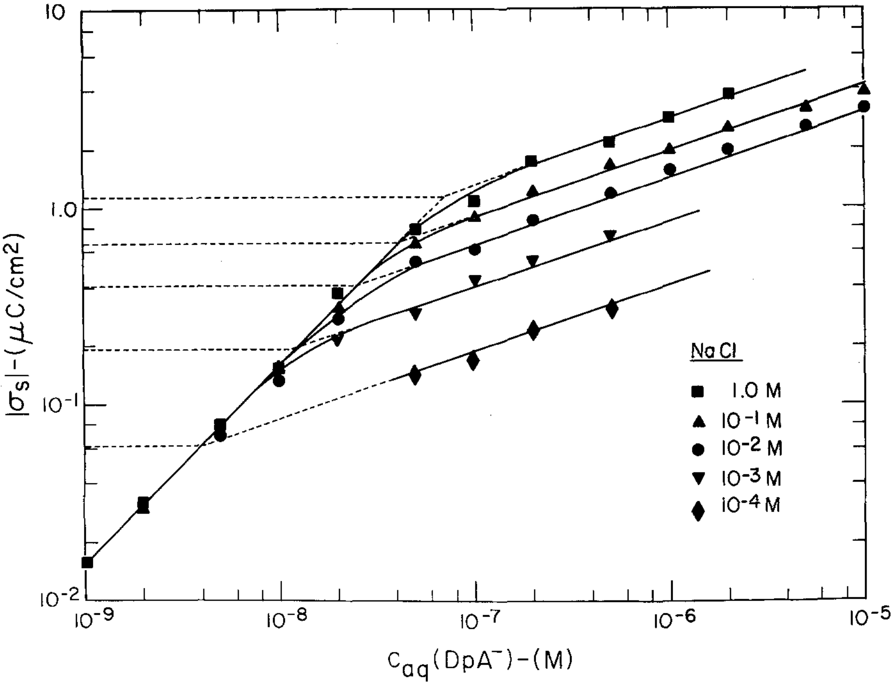

Fig. 1. Log-log plot of the magnitude of the density of adsorbed membrane surface charge, $|\sigma_s|$, vs. $c_{aq}(DpA^-)$, the concentration of dipicrylamine in the aqueous solutions. Data are shown for five fixed concentrations of NaCl as indifferent electrolyte

surface density of adsorbed DpA^- ions. Representative plots illustrating the sublinear dependence described above are shown in Fig. 2 of Bruner (1975). A similar plot for the voltage dependent transport of tetraphenylborate anions has been given by Andersen and Fuchs (1975), as Fig. 5 of their paper. In the present work voltage pulse amplitudes were increased in 50 mV steps to at least 300 mV to insure determination of the density of adsorbed surface charge to an accuracy of ±10%. Since the pulse durations required for the recording of complete current transients were relatively short, typically 10 msec or less, the membranes were usually able to withstand applied voltages of this amplitude.

Results

All data presented graphically in this section are in the form of log-log plots of the magnitude of the density of adsorbed surface charge, $|\sigma_s|$, measured vs. $c_{aq}(DpA^-)$, the concentration of DpA^- in the bulk aqueous phases. Figure 1 illustrates the results obtained when NaCl is employed as a symmetric univalent indifferent electrolyte, at five concentrations ranging in decade steps from 10^{-4} M to 1.0 M. The data for all salt concentrations converge to a common line of unit slope at low concentrations of DpA^-. At higher DpA^- concentrations the data points for each salt concentration are well fit by lines of slope 1/3 as illustrated, these lines being displaced upward with increasing salt concentration. The intersection of each of these lines with the common line of unit slope is illustrated, with the projection of the intersection point onto the $|\sigma_s|$-axis also being shown. The surface density, $|\bar{\sigma}_s|$, determined by this projection, is given in Table 1 for each salt concentration. There a comparison is made with σ_o, the value of $|\bar{\sigma}_s|$ predicted by the Gouy-Chapman theory of self-limited adsorption as given above.

The range over which data can be taken is limited by several factors. At high (1.0 M) salt concentration and high ($\geqq 5 \times 10^{-6}$ M) DpA$^-$ concentrations, membrane thinning characteristics are poor, with persistent unthinned areas being evident. At lower salt concentrations ($\leqq 10^{-3}$ M) and high DpA$^-$ concentrations ($\geqq 10^{-6}$ M), the current transients generated by high field pulses show large residual currents thought to arise by desorption of DpA$^-$ from the membrane/solution interface. In the ranges cited these desorption currents precluded accurate measurement of charge transport by displacement of DpA$^-$ from one interface to the other. When both salt and DpA$^-$ concentrations are low (typically $\leqq 10^{-3}$ M and $\leqq 10^{-8}$ M, respectively) increased solution resistance lengthens the membrane charging time, while charge transport by displacement of DpA$^-$ across the membrane is reduced. These effects combine to prevent accurate measurement of surface charge density. Limits of error in the determination of $|\sigma_s|$, for the data given, are estimated to be $\pm 10\%$. The remarks of this paragraph apply to all data presented (Figs. 1–3).

Log-log plots of $|\sigma_s|$ vs. $c_{aq}(\text{DpA}^-)$ are presented in Fig. 2 for three concentrations of the symmetric divalent indifferent electrolyte, $MgSO_4$, ranging in decade steps from 10^{-2} M to 1.0 M. Convergence to a common line of unit slope is again observed at low concentrations of DpA$^-$. Linear segments of slope 1/2 are observed at higher concentrations of DpA$^-$; upward displacement of these lines with increasing salt concentration is again observed. Projections for the determination of $|\bar{\sigma}_s|$ are also illustrated. In this case it has been found that, in the range of self-limited adsorption, as the salt concentration is reduced below 10^{-2} M at fixed concentration of DpA$^-$, the measured value of $|\sigma_s|$ actually increases slightly rather than decreasing as expected. This finding has been tentatively attributed to specific adsorption of Mg^{++} ions at the membrane/solution interface. Partial neutralization of the negative membrane surface charge density by such adsorption would promote adsorption of additional DpA$^-$ ions.

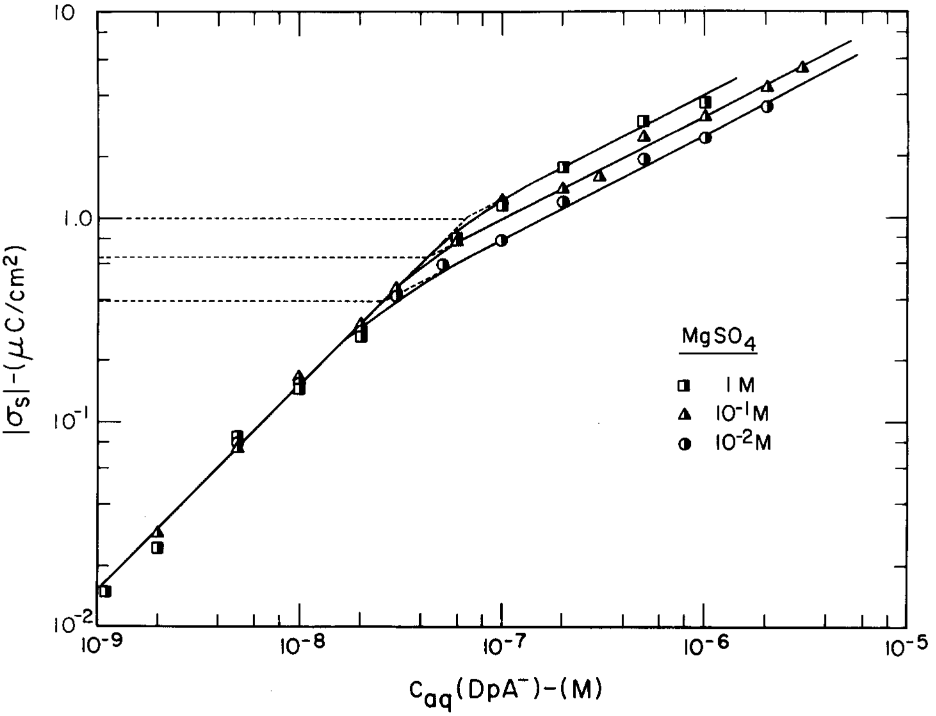

Fig. 2. Log-log plot of $|\sigma_s|$ vs. c_{aq}(DpA$^-$), illustrating results for three fixed concentrations of $MgSO_4$ as indifferent electrolyte

In view of the complications associated with $MgSO_4$, additional measurements have been made using $BaCl_2$ as indifferent electrolyte. The data, shown in Fig. 3, cover the same range of salt concentration employed for NaCl. The data closely resemble those of Fig. 1, the most striking difference being that the slope of the lines fitting the data in the range of self-limited adsorption is 1/2 for $BaCl_2$ and 1/3 for NaCl. These differences in slope are most clearly illustrated in Fig. 4, where data for all three salts, each at a fixed concentration of 10^{-1} M, are replotted. These data confirm our expectation, stated in the preceding theoretical discussion, that the cation valence of the indifferent electrolyte will determine the observed power law in the range of self-limited adsorption of a negatively charged ion species.

In Table 1 we list, for each concentration of each of the three salts used, the value of $|\bar{\sigma}_s|$ determined as described above. The ratio, $(|\bar{\sigma}_s|/\sigma_o)$, is also given in each case, with σ_o being calculated from Eq. (2), using $\varepsilon = 78.3$ for the static dielectric constant of water at 25 °C (Malmberg &

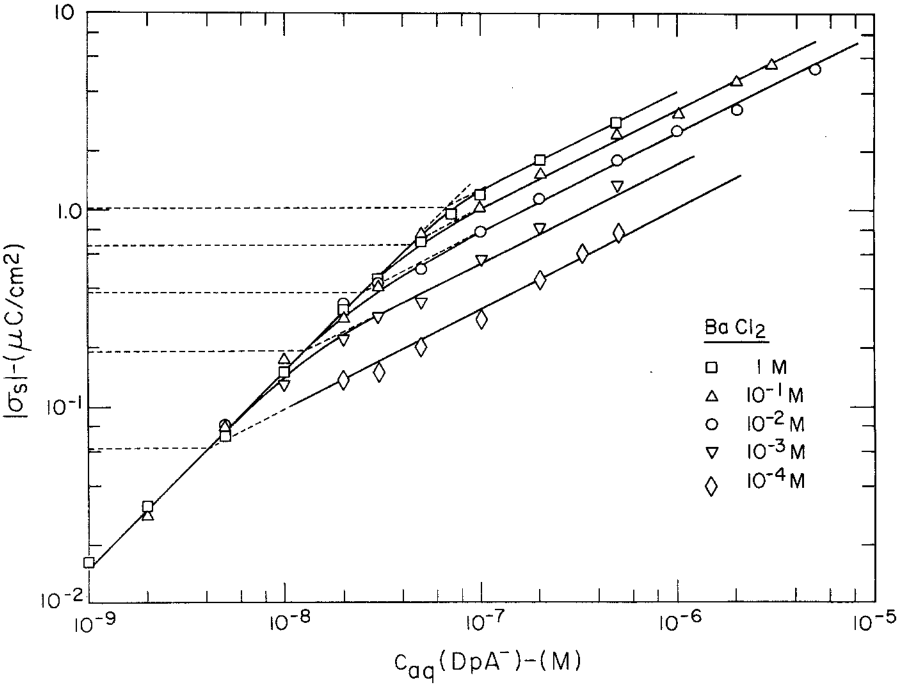

Fig. 3. Log-log plot of $|\sigma_s|$ vs. $c_{aq}(\mathrm{DpA}^-)$, illustrating results for five fixed concentrations of $\mathrm{BaCl_2}$ as indifferent electrolyte

Maryott, 1956). The preceding theoretical discussion leads to the expectation that the tabulated ratio of surface charge densities should equal unity at all concentrations of indifferent electrolyte. While this expectation is realized at 10^{-4} M and 10^{-3} M salt, to within experimental error, the ratio decreases sharply as the electrolyte concentration is increased to 1.0 M.

Thus the experimental evidence fully supports the power law dependence of self-limited adsorption predicted by Eq. (6), at all concentrations of indifferent electrolyte. At low salt concentrations there is also good agreement with theory on the magnitude of surface charge density at which self-limited adsorption begins. With increasing salt concentration this agreement is lost, the observed surface charge density at onset of self-limited adsorption increasing much less rapidly than predicted by the above theory. This is equivalent to stating that the magnitude of the membrane surface potential increases more rapidly with surface charge density than the simple theory [Eqs. (1) and (2) above] would predict. Since the electric field in the adjacent aqueous boundary layers must increase in magnitude with the density of adsorbed

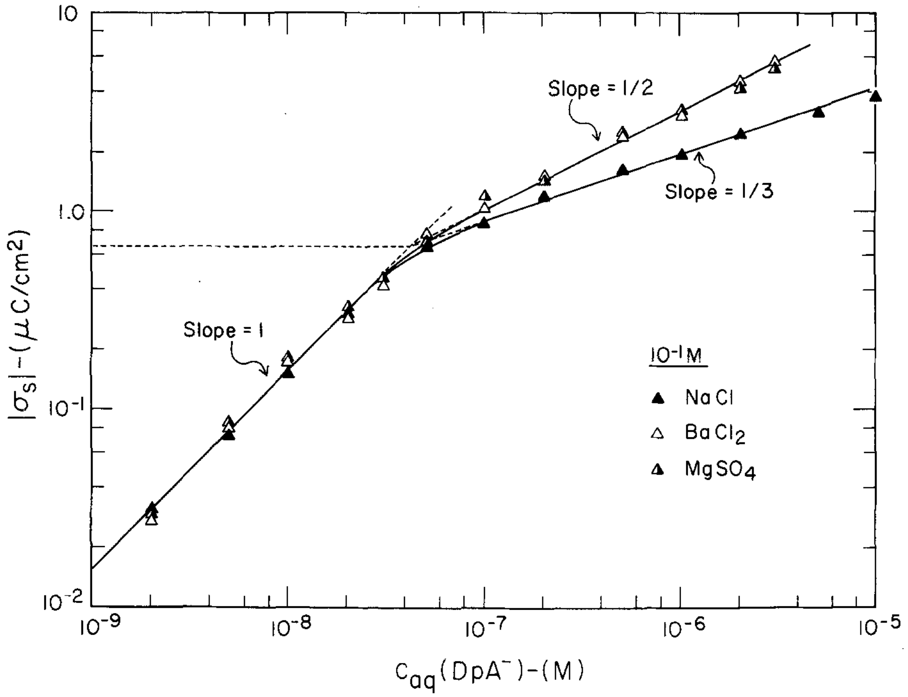

Fig. 4. A comparison of log-log plots of $|\sigma_s|$ vs. $c_{aq}(DpA^-)$ illustrating the characteristic power laws describing self-limited adsorption in the presence of univalent and divalent cations

Table 1. A comparison between theoretical and observed surface charge densities at onset of self-limited adsorption

All salts		NaCl		$MgSO_4$		$BaCl_2$	
(Theory)		(Obs.)		(Obs.)		(Obs.)	
c_o (M)	σ_o (μC/cm^2)	$\lvert\bar{\sigma}_s\rvert$ (μC/cm^2)	$(\lvert\bar{\sigma}_s\rvert/\sigma_o)$	$\lvert\bar{\sigma}_s\rvert$ (μC/cm^2)	$(\lvert\bar{\sigma}_s\rvert/\sigma_o)$	$\lvert\bar{\sigma}_s\rvert$ (μC/cm^2)	$(\lvert\bar{\sigma}_s\rvert/\sigma_o)$
10^{-4}	5.86×10^{-2}	6.2×10^{-2}	1.06	–	–	6.2×10^{-2}	1.06
10^{-3}	1.85×10^{-1}	1.9×10^{-1}	1.03	–	–	1.9×10^{-1}	1.03
10^{-2}	5.86×10^{-1}	4.0×10^{-1}	0.68	3.9×10^{-1}	0.67	3.8×10^{-1}	0.65
10^{-1}	1.85	6.8×10^{-1}	0.37	6.4×10^{-1}	0.35	6.6×10^{-1}	0.36
1.0	5.86	1.1	0.19	1.0	0.17	1.0	0.17

surface charge, account should be taken of the possibility that the field becomes sufficiently intense to cause dielectric saturation of the water in the diffuse charge layers. Such saturation, causing a decrease of effective dielectric constant of the aqueous boundary layers, could account for a more rapid than expected increase of $|\psi_s|$ with $|\sigma_s|$. Data on membrane surface charge density as a function of aqueous phase concentration of DpA^-, reported previously by Bruner (1975), have been recently reexamined in the light of possible dielectric saturation of the adjacent water (Bruner, 1977).

The Transition Field Model

Theoretical investigations of dielectric saturation in aqueous boundary layers have been concerned with the compact layer (Robinson & Levine, 1973), as mentioned in the introduction, and with the diffuse layer as well (Grahame, 1950; Conway, Bockris & Ammar, 1951). The approach taken has been to establish a semi-empirical relationship between electric field and dielectric constant, then to proceed with a numerical integration of the Poisson-Boltzmann equation. The end result is a modified relation between membrane surface potential and surface charge density, presented in graphical or numerical form, which takes account of dielectric saturation of the adjacent water.

To obtain results in analytical form, we will assume the simplified characterization of dielectric saturation depicted in Fig. 5. The macroscopic polarization, P, of water is assumed to increase initially with E, the macroscopic average electric field in the dielectric, with a slope characterized by the static dielectric constant. This constant, ε, takes account of polarization from all sources, namely, orientation of permanent molecular dipoles, atomic polarization involving relative displacement of atomic nucleii within a molecule, and electronic polarization or displacement of electrons relative to nucleii within a molecule. This total polarization increases with field until a transition field, E_t, is reached, at which polarization due to the reorientation of permanent dipoles reaches a limiting value, P_o. This contribution remains constant at all higher fields. Further increase of P with E is attributed solely to atomic and electronic polarization, the slope being characterized by an optical dielectric constant equal to the square of the refractive index of water. We will take $\varepsilon' = n^2 = 1.80$, based upon data of Querry, Curnutte and Williams (1969). A critical discussion of the problem of separating out the permanent dipole contribution to the measured dielectric constant of water has been given by Hasted (1973).

The relation between electric displacement D, field E, and polarization P is

$$D = \varepsilon_o E + P. \tag{7}$$

At the membrane boundary $D = |\sigma_s|$ by Gauss's Law. Thus it follows that there will be a particular value of surface charge density, $|\sigma_t|$, for which

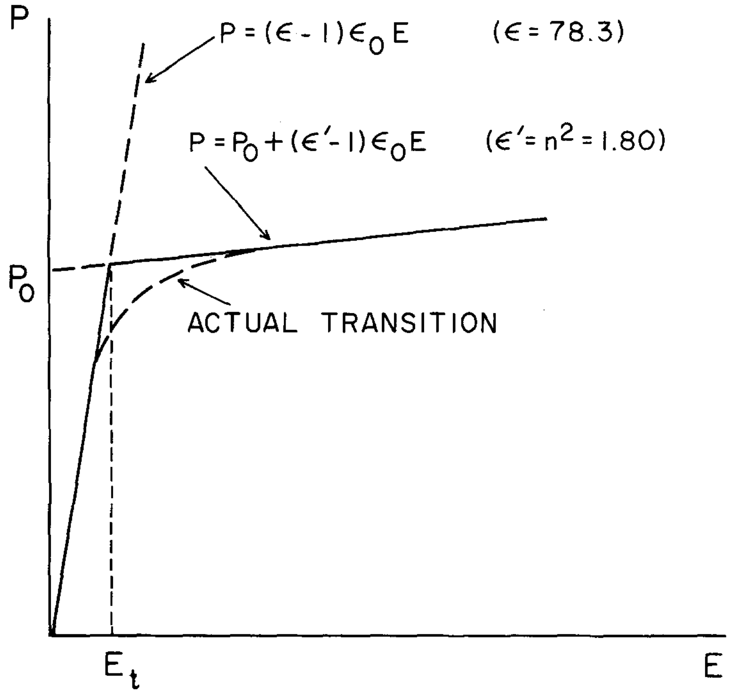

Fig. 5. The dependence of electric polarization, P, of water upon the macroscopic average electric field, E, according to the transition field model. An abrupt change of slope at the transition field, E_t, is assumed by the model. The actual transition from low to high field polarization is illustrated qualitatively by the dashed line curve smoothly connecting the linear segments. The extrapolated polarization, P_0, represents the maximum dipolar contribution to the total polarization

the electric field at the boundary will be E_t and the corresponding membrane surface potential will be $|\psi_t|$. For all $|\sigma_s|<|\sigma_t|$ the relation between $|\psi_s|$ and $|\sigma_s|$ will be as given in Eqs. (1) and (2), and as illustrated by the solid line portion of curve *I* of Fig. 6. When $|\sigma_s|>|\sigma_t|$, then we will have $E>E_t$ over some fraction of the boundary layer, which we designate the high field fraction. An integration of the Poisson-Boltzmann equation over this fraction of the layer would, by our approximation, involve the fixed dielectric constant ε'. If the entire layer could be characterized by ε', then the relation between $|\sigma_s|$ and $|\psi_s|$ would be as illustrated by dashed line curve *II* of Fig. 6. This curve represents Eqs. (1) and (2), modified as follows:

$$\frac{\sigma_s}{2\sigma_o'}=\sinh\left(\frac{ez\psi_s}{2kT}\right) \tag{8}$$

$$\sigma_o'=\sqrt{2\varepsilon'\varepsilon_o kTc_o}. \tag{9}$$

Actually we use curve *II* only to relate the surface charge difference, $|\sigma_s| - |\sigma_t|$, to the potential *difference* existing across the high field fraction of the boundary layer. When the field in the boundary layer decays to E_t, the corresponding potential must be $|\psi_t|$, with further decay of field and potential being described by the Poisson-Boltzmann equation with dielectric constant ε. To insure continuity of the relation between $|\sigma_s|$ and $|\psi_s|$, we transpose the upper segment of curve *II* to the left as illustrated in Fig. 6, obtaining the composite relation depicted by the full solid line curve.

Using the composite relation we show in the Appendix that, while Eq. (4) is unaltered, Eq. (6) becomes

$$|\sigma_s| \cong \bar{\sigma}_s \left[\frac{\beta c_{\mathrm{aq}}(\mathrm{DpA}^-)}{\bar{\sigma}_s}\right]^{\frac{z}{z+2}} \tag{10}$$

when $(e|\psi_s|/kT) \gg 1$, where

$$\bar{\sigma}_s = \sigma_o \left[\frac{1 + \sqrt{1 + \frac{\varepsilon'}{\varepsilon}(2\sigma_o/\sigma_t)^2}}{1 + \sqrt{1 + (2\sigma_o/\sigma_t)^2}}\right]. \tag{11}$$

The transition field model thus leads to the remarkable conclusion that the power law dependence of $|\sigma_s|$ upon $c_{\mathrm{aq}}(\mathrm{DpA}^-)$, in the range of self-limited adsorption, remains identical to that of the unmodified Gouy-Chapman theory at all concentrations of indifferent electrolyte. The surface charge density marking the onset of self-limited adsorption, as determined by the projection method described above, is now given by $\bar{\sigma}_s$ of Eq. (11), however, rather than by σ_o. If the transition field model is valid, we expect the measured projection to be $|\bar{\sigma}_s| \approx \sigma_o$ when $\sigma_o \ll \sigma_t$, but to be $|\bar{\sigma}_s| \approx (\sqrt{\varepsilon'/\varepsilon})\,\sigma_o$ in the opposite limit, $\sigma_o \gg \sigma_t$. For the values of static and optical dielectric constant cited above, $\sqrt{\varepsilon'/\varepsilon} = 0.15$.

In Fig. 7 we compare measured values of $|\bar{\sigma}_s|$, taken from Table 1, with Eq. (11) given by the transition field model. The single adjustable parameter of the model, the transition surface charge density σ_t, has been set equal to 0.7 μC/cm^2 to give the solid line curve of Fig. 7. Agreement between theory and experiment is within experimental error ($\pm 10\%$) in the determination of $|\bar{\sigma}_s|$ over the full range of indifferent electrolyte concentration.

Assuming water to maintain its static dielectric properties at all fields up to E_t, as per Fig. 5, and applying Gauss's Law, we find for the transition field,

$$E_t = \frac{\sigma_t}{\varepsilon \varepsilon_o} = 1.0 \times 10^5\ \mathrm{V/cm} \tag{12}$$

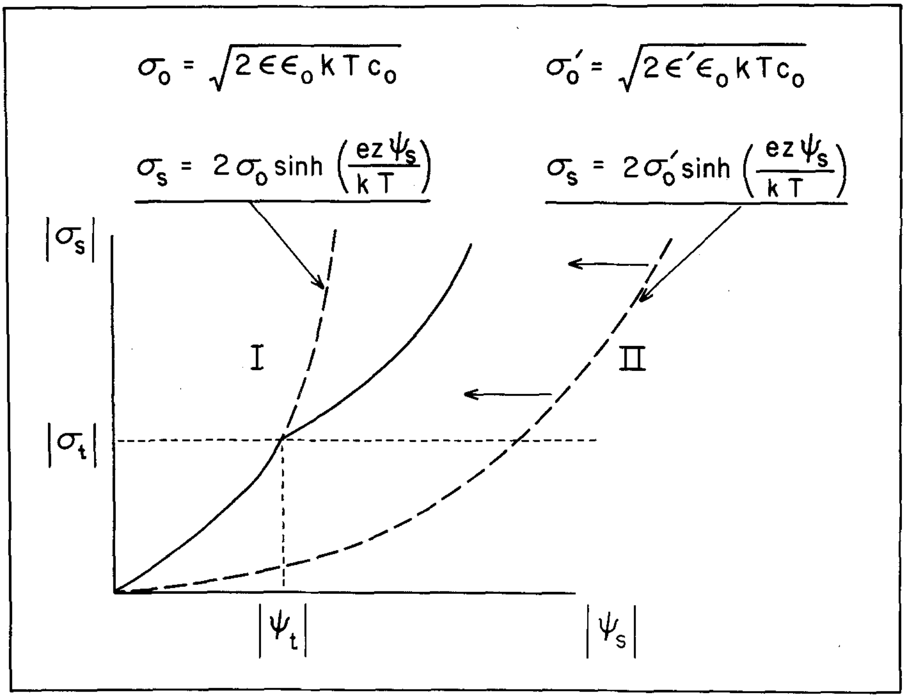

Fig. 6. The solid line curve illustrates the dependence of membrane surface charge density upon surface potential, as assumed by the transition field model

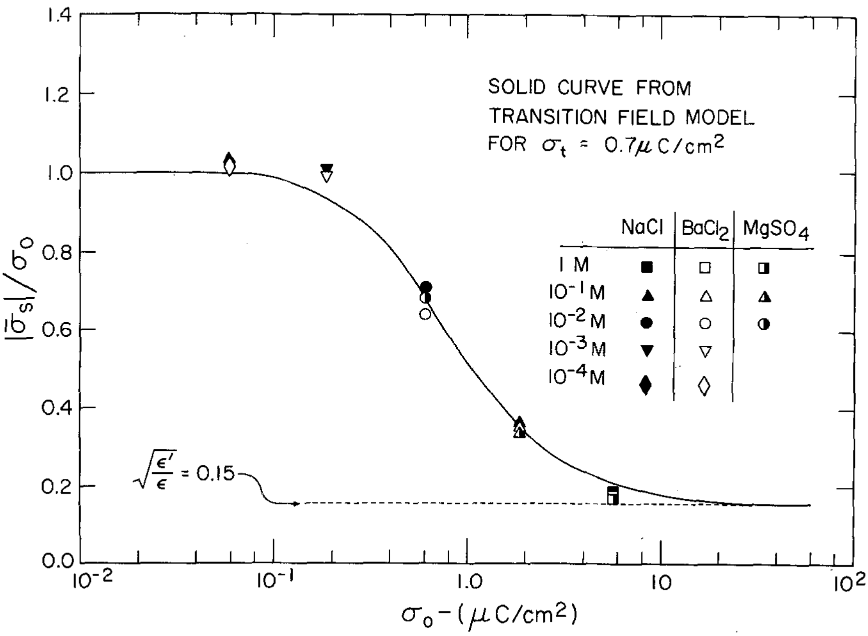

Fig. 7. Experimental values of ($|\bar\sigma_s|/\sigma_o$), taken from Table 1, are plotted vs. $\log\sigma_o$. The quantity $|\bar\sigma_s|$ is the surface charge density marking the onset of self-limited adsorption of DpA$^-$. The parameter σ_o is defined by Eq. (2), with experimental values given in Table 1. The solid line curve is derived from the transition field model, Eq. (11), with the transition surface charge density, σ_t, set equal to 0.7 μC/cm^2

If a uniform field of this magnitude were impressed upon a macroscopic aqueous dielectric medium we could, after Onsager (1936), equate the local orienting field acting upon an individual water molecule to the cavity field, namely,

$$(E_t)'_{\mathrm{loc}} = \left(\frac{3\,\varepsilon}{2\,\varepsilon+1}\right) E_t = 1.5\times 10^5\,\mathrm{V/cm}. \tag{13}$$

The prime signifies an estimated lower limit for the orienting field, as will be discussed below. A comparison with thermal energy of the energy of interaction between this field and a water dipole of moment p_w yields,

$$\frac{p_w (E_T)'_{\mathrm{loc}}}{kT} = 0.02 \tag{14}$$

where for p_w we have taken the value 6.2×10^{-28} C-cm or 1.85 D (McClellan, 1974). The comparison suggests that the local field is not of sufficient strength to cause dielectric saturation. Equation (13) undoubtedly represents a lower limit for the orienting field since, for the case of interest here, the water dipoles are immediately adjacent to a membrane medium of low dielectric constant. In the presence of this inhomogeneity, the reaction field of each water dipole would also contribute to the torque acting to align it perpendicular to the membrane/solution interface. An equivalent point of view, simplified for the purpose of obtaining an upper limit for the orienting field, is to assume that the water molecules immediately adjacent to the charged interface experience an average field perpendicular to the plane which is not attenuated at all by dielectric screening. In this case,

$$(E_t)''_{\mathrm{loc}} = \frac{\sigma_t}{\varepsilon_o} = 8\times 10^6\,\mathrm{V/cm}. \tag{15}$$

The corresponding comparison of electrostatic with thermal energy yields,

$$\frac{p_w (E_t)''_{\mathrm{loc}}}{kT} = 1.2 \tag{16}$$

which is consistent with the onset of dielectric saturation. Furthermore, this discussion takes no account of correlation effects (Kirkwood, 1939) which would be expected to further increase the strength of interaction between water dipoles and the local field.

Discussion

The interpretation of our adsorption data in terms of dielectric saturation rests upon the assumption that the average potential of adsorbed DpA^- ions is primarily determined by the diffuse space charge distribution in the adjacent aqueous phase. In particular, it is asserted that this potential is not significantly augmented by size effect or any other discrete molecular or geometric effect which would serve to separate the primary charge plane of the adsorbed DpA^- ions from the plane of closest approach of the diffuse counterion atmosphere. Such separation, with interposition of a charge-free lamina between these planes, would be equivalent to the formation of a Stern or compact layer. The charge separation resulting from such a layer would augment the surface potential of DpA^- ions by introduction of a uniform electric field in the charge-free lamina. Justification of our exclusion of the compact layer from consideration is provided by experimental confirmation of the power law of self-limited adsorption, with the predicted dependence of the exponent upon cation valence being observed at all concentrations of indifferent electrolyte. Within the framework of the transition field model, the augmentation of potential of adsorbed ions which is indirectly observed can be fully accounted for in terms of dielectric saturation of the aqueous boundary layers.

As an alternative illustration of the point made above, we can apply to our data a model recently proposed by Andersen, Feldberg, Nakadomari, Levy and McLaughlin (1977), to explain their observations of self-limited adsorption of tetraphenylborate anions onto phosphatidylethanolamine bilayer membranes. They assume the existence of a charge-free lamina separating the plane of adsorbed charge from the diffuse charge region. They determine the specific capacitance which the lamina must have to account for the observed nonlinearity of the adsorption isotherm, obtaining the value of $50\,\mu F/cm^2$. We can obtain an approximate fit to our own data for 0.1 M NaCl (Fig. 4) by using the same specific capacitance value. A similar fit to our data for 0.1 M $MgSO_4$ or $BaCl_2$, on the other hand, can be obtained only if the assumed specific capacitance is increased to $100\,\mu F/cm^2$. This sensitivity of capacitance to the composition of indifferent electrolyte cannot readily be explained on the basis of the compact layer model. It can be explained, however, if the relevant capacitance is that of the diffuse layer, with an effective thickness of the order of the Debye length. The Debye length for the divalent salt will be half that for the univalent salt. For the example cited the high field dielectric constant, ε', should be appropriate to both cases. It is to be emphasized, however, that the remarks of the preceding two paragraphs are not intended to exclude the possibility that charge-free laminae or equivalent dipole sheets may contribute to the overall membrane potential profile. We do conclude that, for the system studied here, any such laminae or sheets must be situated on the membrane side of the charge plane occupied by DpA^- ions.

At the transition surface charge density evaluated above ($\sigma_t = 0.7\ \mu C/cm^2$) we calculate a mean distance between adsorbed DpA^- ions of about 50 Å. At the highest surface charge densities reached in our experiments this mean separation is still of the order of 20 Å. The shortest Debye length encountered here, for 1.0 M divalent salt with an effective solvent dielectric constant of 1.8, is 0.33 Å. Though this is an extreme value, it nevertheless raises a question as to the validity of the one-dimensional Gouy-Chapman theory, for which all physical quantities are averaged in planes parallel to the membrane. The answer must be sought by reference to experiment; in this study verification of the predicted power law of self-limited adsorption provides strong support for the validity of the theory. Other experimental evidence bearing on the point does not appear to be conclusive. Thus Haydon and Myers (1973), using several different systems, compared electrokinetic (ζ) potentials with diffuse double layer potentials, with surface charge densities for calculation of the latter being inferred from interfacial tension measurements by application of the Gibbs adsorption isotherm. Additional comparisons were also made with surface potentials determined by compensation potential measurements on monolayers and by conductance measurements on bilayers. For the positively charged dodecyl trimethylammonium ion adsorbed onto the glycerol-mono-oleate-decane/aqueous solution interface the comparisons showed good agreement at all salt concentrations employed. For negatively charged dodecyl sulfate ions adsorbed onto the same interface, agreement was again good at low salt concentrations. At higher salt concentrations the calculated Gouy-Chapman potentials were significantly lower in magnitude than those determined by compensation potential and conductance measurements. In subsequent studies using a homologous series of dodecyl trialkylammonium salts Carroll and Haydon (1975), reported maxima in surface potentials measured as a function of interfacial surfactant density. Surface potentials were deduced from compensation potential and ζ potential measurements. Such results are clearly inconsistent with diffuse double layer theory; Carroll and Haydon suggested that their results might be explicable in terms of the discrete ion effect (Levine, Mingins & Bell, 1967). If such effects are important, then the activity corrections employed in the use of the Gibbs adsorption isotherm may well be larger and less certain than previously thought. The location of the plane of shear in ζ potential measurements introduces additional uncertainty. At this point it appears most prudent to assess the applicability of diffuse double layer theory on an *ad hoc* basis.

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Appendix

We derive here Eqs. (10) and (11) of the text, which summarize for the transition field model the expected relationship between surface charge density and aqueous phase adsorbate concentration in the range of self-limited adsorption. Equation (3) of the text, expressing the influence of the Boltzmann factor on $|\sigma_s|$, will continue to apply, as will Eq. (4) which follows from it when $|\psi_s| \ll (kT/e)$. The asymptotic form of the self-limited adsorption relation will always be achieved under the condition $|\sigma_s| > \sigma_t$. We will take $\sigma_t > 0$. From Eq. (8) it follows that the potential drop across the high field fraction of the boundary layer, $|\Delta\psi|$, will be given by,

$$\frac{ez|\Delta\psi|}{2kT} = \sinh^{-1}\left(\frac{|\sigma_s|}{2\sigma_0'}\right) - \sinh^{-1}\left(\frac{\sigma_t}{2\sigma_0'}\right). \qquad \text{(A-1)}$$

The potential drop across the remainder of the layer, $|\psi_t|$, will be given by Eq. (1), namely,

$$\frac{ez|\psi_t|}{2kT} = \sinh^{-1}\left(\frac{\sigma_t}{2\sigma_0}\right). \qquad \text{(A-2)}$$

The magnitude of the surface potential at the boundary is then,

$$|\psi_s| = |\psi_t| + |\Delta\psi|. \qquad \text{(A-3)}$$

In what follows we will make use of the identity,

$$\sinh^{-1} x = \ln\left[x + \sqrt{x^2+1}\right]. \qquad \text{(A-4)}$$

Rewrite Eq. (3) in the form

$$\left\{\exp\left[\frac{e|\psi_s|}{kT}\right]\right\}^{\frac{z}{2}}=\left[\frac{\beta c_{\mathrm{aq}}(\mathrm{DpA}^-)}{|\sigma_s|}\right]^{\frac{z}{2}}. \tag{A-5}$$

Then sum Eqs. (A-1) and (A-2), substitute from (A-3), and exponentiate both sides. Then use Eq. (A-4) to obtain in the limit $|\sigma_s| \gg \sigma_o'$,

$$\left\{\exp\left[\frac{e|\psi_s|}{kT}\right]\right\}^{\frac{z}{2}}\cong\frac{|\sigma_s|}{\bar{\sigma}_s} \tag{A-6}$$

where $\bar{\sigma}_s$ is given by Eq. (11) of the text. Use is also made here of the relation

$$\left(\frac{\sigma_o'}{\sigma_o}\right)^2=\frac{\varepsilon'}{\varepsilon} \tag{A-7}$$

obtained from Eqs. (2) and (9). Finally, $(e|\psi_s|/kT)$ may be eliminated from Eqs. (A-5) and (A-6) to obtain Eq. (10) of the text.

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