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SURFACE CHARGE, SURFACE DIPOLES AND MEMBRANE CONDUCTANCE

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SUMMARY

The conductance of lipid membranes in the presence of nonactin is changed by the adsorption of small amounts of ionic and zwitterionic surfactants. The conductance changes are, in many instances, not accounted for by the variation in surface charge or diffuse double layer potential as calculated from Gouy–Chapman theory. The changes are, however, all accurately accounted for by the variation in total potential across the membrane interface. This potential includes contributions from surface dipoles and specific adsorption, as well as any diffuse double layer effects not included in the Gouy–Chapman theory.

The total potential changes were inferred from Volta or compensation potential changes at bulk oil (and monolayer)/aqueous solution interfaces. Surface charge densities were found by standard thermodynamic methods involving the use of the Gibbs equation. Electrokinetic potentials for the appropriate surfaces were also measured and, in general, agreed well with the diffuse double layer potentials calculated from the Gouy–Chapman theory.

INTRODUCTION

The influence of the surface charge of a thin membrane on its ion permeability has attracted the attention of several groups of workers during the past few years. Chandler *et al.*[1] and Gilbert and Ehrenstein[2] have shown that the presence of a surface charge could account for the shifts along the voltage axis of the sodium and potassium channel inactivation curves for perfused squid giant axons. More recently, attempts to demonstrate quantitative relationships between surface charge and membrane conductance have been made by means of experiments with black lipid bilayer membranes[3–6]. This work has been discussed elsewhere[7]. The results showed that the membrane conductance was readily influenced by the ionization of the lipid, and by the electrolyte in the aqueous solution. It also seemed likely that the conductance changes could to some extent, at least, be accounted for by the variations in the diffuse layer potential. The actual values of the surface charge density of the membranes were not known with any precision, however, nor was it certain that the

Abbreviations: PTFE, polytetrafluoroethylene; DTAB, dodecyl trimethylammonium bromide.

surface charge density was independent of electrolyte concentration. It is also possible that, in these experiments, the surface dipole moments may have varied and affected the results[7]. Indeed, surface charge and surface dipole contributions to interfacial potentials are very difficult to separate in any satisfactory way[8].

The chief purpose of the present paper is to show that bilayer membrane conductance changes may be accurately predicted from estimations of the change in total potential across a membrane interface and that there is, in general, a contribution to this change not accounted for by the Gouy–Chapman equation.

The conductance in the limit of zero applied potential, $G(0)$, of a bilayer membrane in the presence of lipid-soluble ions may be written

$$G(0) \propto \bar{u} c_s \tag{1}$$

where $\bar{u}$ is the mean ionic mobility in the membrane and c_s is the concentration of ions at a specified point within the hydrocarbon of the membrane. It is assumed that the principal barrier to ion transfer lies in the non-polar part of the bilayer and that, although such parameters as the viscosity and image potential energy in the hydrocarbon region are important in the rate-determining processes, they remain constant in the present systems. Under these conditions, $G(0)$ should depend only on c_s, and c_s should be related to the concentration c_b of the ions in the bulk aqueous phase by an expression of the form

$$c_s \propto c_b \exp\left[-\frac{zF\Delta\varphi}{RT}\right] \tag{2}$$

where Δ_φ is the difference in the electrostatic potential between a point in the aqueous phase far from the membrane and the point in the hydrocarbon of the membrane at which the concentration is c_s. Insomuch as $\Delta\varphi$ is a potential between two bulk phases, one of which is extremely non-polar, changes in $\Delta\varphi$ may be equated to the changes in the Volta potential of the system, and these, at least in principle, are accurately measurable[8]. In order to achieve some physical understanding of the system, $\Delta\varphi$ may be regarded as having several components, *e.g.*

$$\Delta\varphi = \Delta\varphi\,(\text{diffuse double layer}) + \Delta\varphi\,(\text{Stern layer}) + \Delta\varphi\,(\text{dipole layer}) + \ldots \tag{3}$$

Such a separation is possible, however, only in terms of theoretical models and there are no completely unambiguous means of measuring the various contributions. In general, any attempt to vary one component will also change the others. Nevertheless, one possible approach to the separation of the diffuse double layer from the dipole potentials was mentioned in an earlier paper, where it was pointed out that if ions crossed the membrane through aqueous pores they would not necessarily be subject to a dipole potential, at least in the sense discussed above[7]. A preliminary examination of this idea has been carried out using gramicidin A as a source of pores[7]. The addition of $5 \cdot 10^{-5}$ M sodium dodecyl sulphate, however, produced specific conductance increases (sometimes approx. $\times 10^3$) out of all proportion to those expected and which were very irreproducible. These anomalous results were presumed to be a consequence of the breaking up by the surfactant of aggregates of the polypeptide in the membrane, so liberating fresh potentially conducting material. This approach was not, therefore, pursued.

In the experiments described below the carrier molecule nonactin was used to produce conducting membranes. Changes in the $\Delta\varphi$ potentials at the membrane surfaces were brought about by the adsorption of water-soluble surfactants and were estimated from Volta (strictly speaking, compensation) potential measurements for oil–water interfaces and for lipid monolayers at air–water interfaces. The diffuse double layer potential has been calculated from the Gouy–Chapman equation which, for uni-univalent electrolytes, is[9]

$$\sigma = \frac{N_0}{F}\left(\frac{2\varepsilon RTc}{\pi}\right)^{\frac{1}{2}} \sinh\frac{F\Delta\varphi_{\text{Gouy}}}{2RT} \tag{4}$$

where σ is the surface ion density (ions/cm^2), c is the electrolyte concentration in mole/cm^3, ε is the dielectric constant, and N_0 is the Avogadro number. The surface ion density σ was obtained from the measurement of the adsorption of an ionic surfactant. The diffuse double layer potential has also been estimated by the measurement of the electrokinetic potential. No attempts have been made to calculate precisely the potential arising from the surface dipoles, although some indications of the sign and magnitude of this effect have been gleaned from monolayer data.

MATERIALS AND METHODS

Membrane conductance measurement

The conductance cell consisted of a rectangular perspex vessel divided symmetrically by a sheet of polytetrafluoroethylene (PTFE). Satisfactory sealing of the partition into the perspex was facilitated by moistening with *n*-decane. Very small quantities were used and no significant amount of the decane was exposed to the aqueous solutions. The black lipid films were formed across a hole (approx. diameter 1 mm) in the PTFE partition by the pipette method[10], and were viewed under reflected light by means of a binocular microscope. The two sides of the cell could be linked through Ag–AgCl electrodes to either a.c. capacitance or d.c. conductance measuring circuits[11]. Both compartments in the cell could be stirred by means of magnetic stirrers. The experiments were commenced by the rinsing and equilibration of the compartments of the cell with the aqueous solutions to be used initially. It was then checked that, with a thick lipid film in the hole, the electrical insulation between the compartments was adequate. With a black film formed and in a steady state the first measurement of the specific conductance was made. The surfactants used to modify the membrane were then added simultaneously to the aqueous phases on each side of the membrane so as to give equal concentrations in the two compartments. This was achieved by the use of coupled glass syringes. The new specific conductance of the membrane was then measured, special attention being given to the changes in area sometimes caused by the addition of the surfactant. Stirring was continuous except during the measurement of the specific conductance.

Volta (compensation) potential measurements

These measurements were accomplished by means of a vibrating-plate potentiometer somewhat similar to that described by Kinloch and McMullen[12]. The main differences were in the replacement of the old vacuum tube electronics by transistorised units. The plate was vibrated at approx. 70 Hz. (Prior to the construction

of this apparatus, trial experiments were conducted at Unilever, Port Sunlight, by kind permission of Drs Pethica and Mingins.)

The vibrating gold plate was suspended at less than 1 mm from the oil– or air–water interface. A steady (d.c.) potential was applied between the bulk aqueous phase (through a calomel electrode) and the gold plate so as to reduce the a.c. signal generated by the vibration to zero. The value of the steady potential was noted. The plate was then raised slightly, the lipid or surfactant added to the aqueous phase (which was then stirred thoroughly by means of a magnetic stirrer) and the plate returned to a position the same distance from the interface. The new steady potential was recorded, the difference from the initial value being the quantity required. Some further technical matters will be discussed under Results.

Electrophoretic mobility measurement

The electrokinetic measurements were carried out on the droplets of an emulsion of the membrane-forming lipid solution suspended in the appropriate aqueous phase (approx. 0.05 ml lipid in 15 ml aqueous solution). A microelectrophoretic method, employing a closed cylindrical cell immersed in a water thermostat, was used[13,14]. The usual precautions were taken to focus at the stationary level, as calculated from the equation of Henry[15], and the accuracy of the setting was checked by observing the fresh human erythrocyte in 0.145 M NaCl at pH 7.2[14]. As the radii of the emulsion droplets selected for measurement were always large compared with the Debye–Hückel parameter ($1/\kappa$), the electrokinetic or zeta potentials were calculated from the equation[16]

$$\zeta = \frac{4\pi\eta}{\varepsilon X} \cdot U, \tag{5}$$

where η and ε are respectively the viscosity and dielectric constant of the solution in the electrical double layer adjacent to the surface, U is the mobility and X is the applied field strength. In the present systems the surface potentials and electrolyte concentrations were relatively small and η and ε were taken as equal to their bulk values[17,18].

Interfacial tension measurements

Interfacial tensions were measured for the film-forming lipid solution in contact with aqueous solutions containing a range of concentrations of the ionic or zwitterionic surfactant. The drop-volume technique, using the Harkins and Brown correction curve, was employed[19]. As the tensions were all very low, stainless steel tips of small radius were required, and the usual calibration methods had to be supplemented by an intermediate standardization using solutions of pure glyceryl mono-oleate in decane against 0.1 M NaCl. The tensions of these systems had previously been determined[20].

The electrokinetic and interfacial tension measurements were carried out at 20 ± 0.1 °C and the conductance and compensation potential measurements at 20 ± 1 °C.

Materials

Inorganic electrolytes were of A.R. grade and the NaCl and KCl were roasted at 700 °C to remove organic impurities. The *n*-alkanes were from Koch-Light Ltd.

and were of puriss grade. They were further purified by passage through columns of chromatographic alumina and were used shortly afterwards. The glyceryl mono-oleate was obtained from Nu-Chek Prep. A specimen of nonactin was kindly provided by Dr Barbara Stearns of Squibb. The di-*n*-octyl glyceryl phosphatidylcholine was a gift from Dr N. J. M. Birdsall of the National Institute for Medical Research, Mill Hill, and was more than 98% pure. The sodium dodecyl sulphate was a pure specimen synthesized by Dr Gillian Rich (University of East Anglia) and gave a critical micelle concentration in water of 8.0 mM. It showed no minimum in the vicinity of the critical micelle concentration and no ageing. The dodecyl trimethyl-ammonium bromide was also a pure specimen, supplied by I.C.I. (Dyestuffs) Ltd, and described previously[21]. The water was twice distilled, the second time from a pyrex still. Its pH was approximately 5.6.

RESULTS

Membrane conductance

Black films were formed from solutions of glyceryl mono-oleate (8 mM) in *n*-decane[20,24]. Early results of the effect of the ionic surfactants on the conductance due to nonactin were irreproducible. This appeared from electrokinetic measurements to be a consequence of the sensitivity, especially at low electrolyte concentrations, of the surface potential of the glyceryl mono-oleate membranes to pH. Indeed, except at about pH 7 the glyceryl mono-oleate–hydrocarbon/water interface carried a significant charge. This varied from one system to another but was of the order of 1000 Å^2 per (positive) ion at pH 5.5. All systems except those in 0.001 M electrolyte were therefore buffered by the addition of sodium acetate–acetic acid solutions so as to yield final concentrations of 0.01 M acetate in 0.1 M electrolytes (pH approx. 6.9) and 0.001 M acetate in 0.01 M electrolytes (pH approx. 6.8). In these solutions the electrophoretic mobilities (as described below) were zero and the specific conductance changes became more readily reproducible. In the 0.001 M electrolytes, adequate buffering was not attainable by this means and, in order to obtain well-defined data, some ionic surfactant was added to the systems initially, the conductance measured and then further surfactant added. Owing, probably, to the very low initial concentrations of the surfactant ($5 \cdot 10^{-6}$ M) the reproducibility was still poorer than in the buffered systems.

It was checked that the various surfactants did not significantly affect the membrane capacitance (0.39 μF$\cdot$cm^{-2}) and conductance (approx. $10^{-9}\Omega^{-1}\cdot$cm^{-2}) in the absence of nonactin. The nonactin, as a concentrated solution in ethanol, was added to the membrane-forming lipid. The final nonactin concentration was controlled so as to keep the membrane specific conductance both well above the background value in absence of nonactin and well below that which would involve limitation of the fluxes through boundary layer diffusion[29]. The substantial reductions in the specific conductance produced by the dodecyl trimethylammonium ions necessitated the use of the relatively highly conducting KCl systems. Owing to the possibility of specific ion effects with KCl, however, NaCl was used with the sodium dodecyl sulphate.

On the addition of the surfactants the new steady conductance was reached within approximately 30 s and remained at that level until the membrane broke

(approx. 5–10 min). The conductances were measured with an applied potential of 10 mV. Higher surfactant concentrations were not used owing to the instability that these induced in the black films. Each system was measured at least twice and, in some instances, three or more times. The mean values of the conductance ratios, together with the spread, are given in Table I.

TABLE I

THE SPECIFIC CONDUCTANCE $G_2(0)$ FOR GLYCERYL MONO-OLEATE–DECANE–NONACTIN BILAYER MEMBRANES IN SURFACTANT SOLUTIONS OF CONCENTRATION c (mole·l^{-1} × 10^5) RELATIVE TO THEIR CONDUCTANCE $G_1(0)$ IN THE INITIAL AQUEOUS SOLUTION

Initial aqueous solution	$G_2(0)/G_1(0)$			
	$c=2$	$c=2.5$	$c=4$	$c=5$
Sodium dodecyl sulphate				
0.1 M NaCl	9.3 ± 1	12.7 ± 1	20.4 ± 1	24.9 ± 1
0.01 M NaCl	9.3 ± 1	—	18.2 ± 1	23.0 ± 1
0.001 M NaCl + 5·10^{-6} M sodium dodecyl sulphate	—	—	—	5.5 ± 1
	$c=2$	$c=4$		$c=5.4$
Dodecyl trimethylammonium bromide (DTAB)				
0.1 M KCl	0.37 ± 0.02	0.24 ± 0.01		0.195 ± 0.01
0.01 M KCl	0.24 ± 0.02	0.16 ± 0.01		0.126 ± 0.01
0.001 M KCl + 5·10^{-6} M DTAB	—	—		0.187 ± 0.02
	$c=0.807$	$c=1.61$	$c=2.4$	$c=3.2$
Dioctyl lecithin				
0.1 M KCl	0.45 ± 0.02	0.23 ± 0.01	—	—
0.01 M KCl	0.55 ± 0.02	0.37 ± 0.02	0.27 ± 0.02	0.168 ± 0.01

Volta (compensation) potential changes

At first, these potentials were examined at the interface between the appropriate aqueous solution and the membrane-forming lipid (glyceryl mono-oleate in *n*-decane). In these experiments the lipid solution layer was sufficiently thick for the gold vibrating plate to be immersed in the oil. While this approach seemed satisfactory in principle, in practice it was extremely tedious owing to the frequent occurrence of long time effects, and the reproducibility of the results was poor. Such problems are well-known for systems in which the vibrating plate is immersed in the oil, and arise probably from the long periods needed for the equilibration of water and polar solutes between the two phases. Although some apparently reliable data were obtained, all the systems were re-examined using, instead of the bulk oil phase, spread monolayers of the film-forming lipids in equilibrium with small lenses of the bulk lipid. This method of estimating the potential changes across the black film interfaces was employed recently by MacDonald and Bangham[22] for phospholipid systems. Its validity is important and will be discussed in more detail.

When small amounts of the film-forming lipid solution are placed on the surface of the aqueous solution the material spreads and the compensation potential changes. Changes, or fluctuations, in the potential continue until the amount of lipid

added corresponds roughly to a continuous monolayer in which the gmo occupies approx. 40 Å^2 per molecule and, thereafter, remains constant and independent of further additions of lipid. The lipid added in excess of the monolayer forms lenses on the water surface.

It has been pointed out in earlier papers[23,20,24] that the composition of the two monolayers in a black film differ only minutely from those at the interfaces of the thick lipid solution with which it is in equilibrium. The present approach, in which excess lipid solution is spread at an air–water interface, differs only from the former one in that the bulk oil phase above the monolayer is replaced by air. Thus, one non-polar medium has been replaced by another slightly more so. As the relative interactions of the lipid and hydrocarbon chains with the non-polar medium above the monolayer are much smaller than the relative interactions of the hydrocarbon chains and lipid polar groups with the water on the other side of the monolayer, it would not be expected that the replacement of hydrocarbon by air would greatly affect the monolayer structure. There are consequently theoretical reasons for supposing that the monolayer of lipid and hydrocarbon at the air–water interface approximates closely to that in the black bilayer membrane. Experimental evidence that this is so has been given by MacDonald and Bangham and, in the present work, no obvious difference could be found in the changes in compensation potential for the bulk lipid and monolayer systems. Whereas in the black film and bulk oil–water interface experiments, decane was used as the solvent hydrocarbon, it was found (in common with MacDonald and Bangham) that this substance was too volatile for accurate results to be obtained by the monolayer approach. Instead, hexadecane was used. In earlier work[20] the adsorption of glyceryl mono-oleate was concluded to be similar for decane and hexadecane, and such data as could be obtained for the present systems indicated that the surface potentials were also comparable.

The compensation potentials both before and after the addition of the sur-

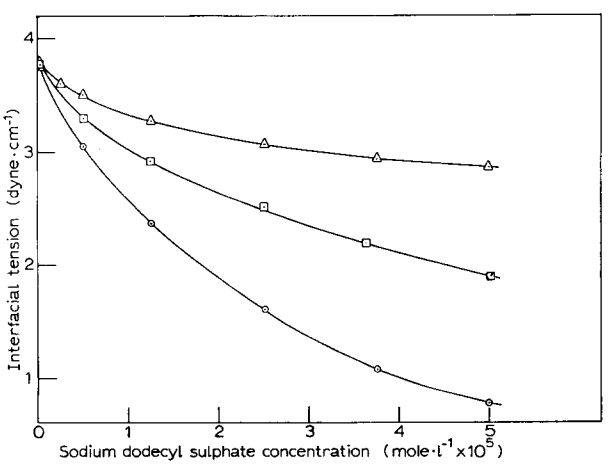


Fig. 1. Interfacial tensions for glyceryl mono-oleate–decane/aqueous solution interfaces as a function of sodium dodecyl sulphate concentration. ⊙, 0.1 M NaCl, pH 6.9; ⊡, 0.01 M NaCl, pH 6.8; △, 0.001 M NaCl (not buffered).

factant became steady within two or three minutes of setting up the system. Each experiment was repeated at least three times. The spread of the data was approximately 5%. The results are plotted in Figs 3–6.

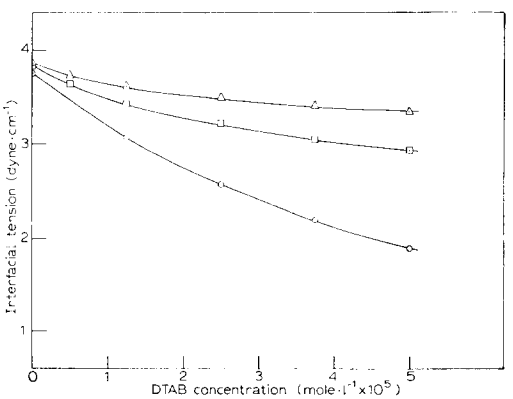
The adsorption of the ionic surfactants

The interfacial tensions of the membrane-forming lipids in contact with various concentrations of the ionic and zwitter-ionic surfactants are shown in Figs 1–3. The adsorption of the surfactant was calculated by the application of the Gibbs equation[8]

$$-\mathrm{d}\gamma = \Gamma RT \mathrm{d} \ln a_{\pm} \tag{6}$$

where γ is the interfacial tension, Γ is the surface excess and $a_{\pm}$ the activity (or mean activity for the sodium dodecyl sulphate and DTAB), of the surfactant in the aqueous phase. This simple form of the Gibbs equation is valid for the present multi-component two condensed phase system partly because the components of each phase are almost insoluble in the other phase and partly because the variation in the concentration of the surfactant occurs at the very low level of approx.10^{-5} M[25]. As a consequence of the low surfactant concentration it has been assumed that in all systems the activity could be replaced by the concentration. Finally, as the surfactant is strongly adsorbed relative to the water, the surface excess, Γ, does not differ significantly from the surface concentration, and the area per molecule, A, of the surfactant may be calculated from the expression

$$A = 10^{16}/\Gamma N_0$$



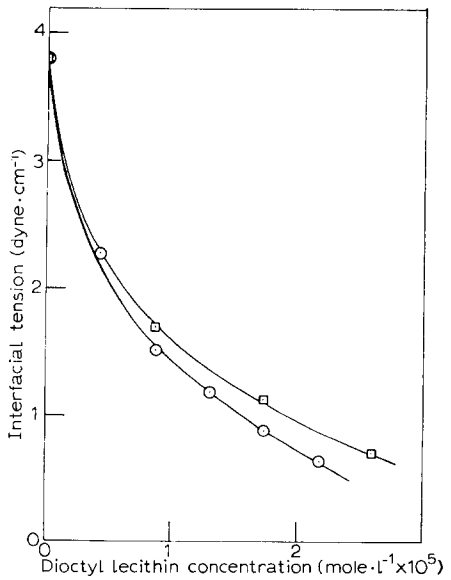


Fig. 2. Interfacial tensions for glyceryl mono-oleate–decane/aqueous solution interfaces as a function of dodecyl trimethylammonium bromide concentration. ⊙, 0.1 M KCl, pH 6.9; ⊡, 0.01 M KCl, pH 6.8; △, 0,0001 M KCl (not buffered).

Fig. 3. Interfacial tensions for glyceryl mono-oleate–decane/aqueous solution interfaces as a function of dioctyl lecithin concentration. ⊙, 0.1 M KCl, pH 6.9; ⊡, 0.01 M KCl, pH 6.8.

where N_0 is the Avogadro number, and A is in Å^2. Values of A are given in Table II. As for the other types of measurement, the 0.1 M and 0.01 M systems were buffered with acetate–acetic acid to pH 6.8–6.9.

Also shown in Table II are the diffuse double layer potentials $\Delta\varphi_{Gouy}$, calculated from Eqn 4, which correspond to the various areas per molecule for the ionic surfactants.

TABLE II

AREAS PER MOLECULE, A, (Å^2) FOR THE VARIOUS SURFACTANTS AT CONCENTRATION, c (mole·l$^{-1}\times 10^5$) ADSORBED ON TO GLYCERYL MONO-OLEATE-DECANE BILAYER MEMBRANES, AND THE CORRESPONDING GOUY-CHAPMAN POTENTIALS $\Delta\varphi_{Gouy}$ (mV) FOR THE IONIC SURFACTANTS

Aqueous phase	*c=0.5*		*c=1*		*c=3*		*c=5*	
	A	$\Delta\varphi_{Gouy}$	*A*	$\Delta\varphi_{Gouy}$	*A*	$\Delta\varphi_{Gouy}$	*A*	$\Delta\varphi_{Gouy}$
Sodium dodecyl sulphate								
0.1 M NaCl	—	—	775	−25	387	−46	310	−55
0.01 M NaCl	—	—	1220	−45	740	−65	585	−76
0.001 M NaCl	2980	−58	2020	−75	1400	−92	1235	−98
							c=5.4	
Dodecyl trimethylammonium bromide								
0.1 M KCl	—	—	1475	14	621	31	455	40
0.01 M KCl	—	—	2400	25	1345	42	1068	51
0.001 M KCl	5468	36	3210	55	2220	70	1800	79
	c=0.87		*c=1.74*		*c=2.61*			
Dioctyl lecithin								
0.1 M KCl	496		363		—			
0.01 M KCl	505		418		352			

Electrokinetic potentials

When an emulsion is formed by dispersing an oil phase, such as the membrane-forming lipid in an aqueous solution, for droplets appreciably greater than molecular dimensions (*e.g.* 1–10 μm) the adsorption at the droplet interfaces is the same as that at a comparable plane interface[18]. The electrokinetic potential calculated from the observation of the movement of the droplet is thus that of the surface of the corresponding planar bulk lipid–aqueous solution interface and, for reasons given elsewhere[23,20,24], is also that for a black lipid bilayer membrane formed from this solution.

The electrokinetic potentials relative to their values for the initial aqueous solutions listed in Table I are shown in Table III. In all the initial solutions except the two of 0.001 M containing $5\cdot10^{-6}$ M ionic surfactant, the electrokinetic potential was zero. For $5\cdot10^{-6}$ M sodium dodecyl sulphate the potential was −40 mV and for $5\cdot10^{-6}$ M dodecyl trimethylammonium bromide it was 51 mV. The reproducibility of the data was approx. 10%.

TABLE III

A COMPARISON OF ELECTROKINETIC (ζ) AND DIFFUSE DOUBLE LAYER (GOUY-CHAPMAN) ($\Delta\varphi_{Gouy}$) POTENTIAL CHANGES FOR THE GLYCERYL MONO-OLEATE-DECANE-SURFACTANT SOLUTION INTERFACES. THE VALUES GIVEN ARE RELATIVE TO THOSE FOR THE INITIAL AQUEOUS SOLUTIONS LISTED IN TABLE I

		$\Delta(\Delta\phi_{Gouy})$ (mV)	$\Delta\zeta$ (mV)
Sodium dodecyl sulphate ($5\cdot10^{-5}$ M)	0.1 M NaCl	40	28
	0.01 M NaCl	51	55
	0.001 M NaCl	43	38
Dodecyl trimethylammonium bromide ($5.4\cdot10^{-5}$ M)	0.1 M KCl	−55	−55
	0.01 M KCl	−76	−73
	0.001 M KCl	−40	−37
Dioctyl lecithin ($1.74\cdot10^{-5}$ M)	0.1 M KCl	0	1
	0.01 M KCl	0	1

DISCUSSION

Any change in the surface ionization or dipole composition of a membrane will, in general, be accompanied by changes in the non-polar part of the membrane. In the present systems the perturbation of the non-polar region resulted primarily from the insertion of the hydrocarbon chains of the surfactants. As a consequence, both the thickness and fluidity of the non-polar region may have changed and the specific conductance of the membrane may, for this reason alone, have been affected. There are, however, several reasons for supposing that this effect can, for present purposes, be neglected. Firstly, the surfactants did not affect the membrane capacitance and therefore the thickness did not apparently change. Secondly, there are no aspects of the results which suggest that fluidity changes might be important. In particular, as McLaughlin *et al.*[4] have already observed, membranes which have been charged comparably in positive and negative directions exhibit conductance changes also in opposite directions, whereas any effect on the fluidity of the hydrocarbon would tend to produce changes in the same direction regardless of the sign of the charge. While it is in no way conclusive, this argument does show that fluidity changes are not the dominant influence. It may also be significant that the dioctyl phosphatidylcholine reduced the membrane conductance, whereas the indications so far are that the insertion of small molecules into membranes tends to increase fluidity[26]. Thirdly, the adsorption of the surfactants was apparently far too low to seriously affect the fluidity of the interior of the membrane. The maximum adsorption in any system corresponded to 322 Å^2 per molecule or only 5% by volume of the bilayer hydrocarbon while in most of the systems the adsorption was considerably less than this.

A further complication in the interpretation of the results arises from the fact that on adsorption of the surfactant, the nature of the membrane surface may be changed in such a way that the adsorption of the nonactin is affected. As for the fluidity change just discussed, this effect is likely to be very small since the surfactant

molecules occupy no more than 1 to 10% of the area of the surface. Only a strong specific interaction between the nonactin and the surfactant could have significantly affected the results and, had this occurred, it should have shown up as inconsistencies between the effects of the different surfactants.

In order to use Eqn 1 it is necessary that the conductance should be a linear function of the ion or ion–complex concentration. Rough upper limits to the cation concentrations adjacent to the membrane calculated from the diffuse double layer or electrokinetic potentials given in Tables II or III, lie in the region of 0.9 M. According to the results of Szabo *et al.*[10] the proportionality between conductance and bulk concentration extends at least to 1.0 M for phospholipid membranes and this is also true for glyceryl mono-oleate membranes (Hladky, S.B., private communication). There is, thus, little likelihood of serious error in the present systems.

By combining Eqns 1 and 2 it can be seen that the ratio of the conductance before and after a change in $\Delta\varphi$ is given by

$$\frac{G_2(0)}{G_1(0)} = \exp\left[-\frac{zF}{RT}(\Delta\varphi_2 - \Delta\varphi_1)\right]$$

$$= \exp\left[-\frac{zF}{RT}\Delta(\Delta\varphi)\right] \tag{7}$$

The values of $\Delta(\Delta\varphi)$ calculated from Eqn 7 are plotted in Figs 4–6. Also plotted in these figures are the changes in compensation potential. For every system the agreement between the two sets of data is very good and it appears that the theoretical expectations are entirely borne out. The changes in the diffuse double layer potentials vary considerably in their agreement with the other data. For dodecyl trimethylammonium bromide (Fig. 4) the diffuse double layer potentials, according to the

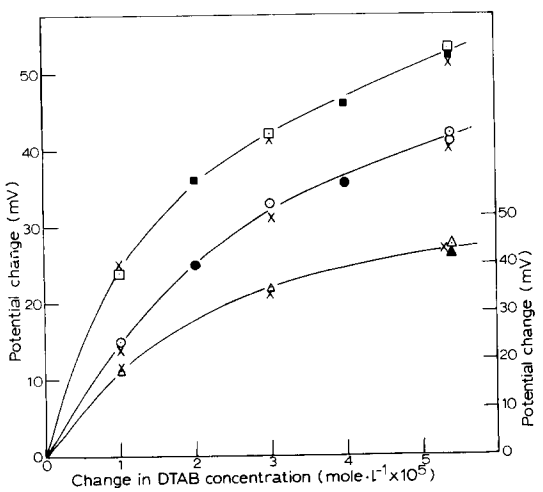


Fig. 4. Compensation potentials (open symbols), Gouy–Chapman diffuse double layer potentials (×—×) and potentials corresponding to conductance changes (from Eqn 7) (closed symbols) for glyceryl mono-oleate–decane membranes in dodecyl trimethylammonium bromide solutions. ⊙, ●, 0.1 M KCl, pH 6.9; ⊡, ■, 0.01 M KCl, pH 6.8; △, ▲, 0.001 M KCl, not buffered (right hand axis).

Gouy–Chapman theory, do not differ appreciably from the compensation potentials and the conductance potentials. For sodium dodecyl sulphate (Fig. 5) the agreement is good for the 0.001 M, questionable for the 0.01 M and very poor for the 0.1 M systems. For dioctyl lecithin the diffuse double layer potential, both according to the Gouy–Chapman theory and from electrokinetic measurements, is effectively zero and the discrepancy with the other potentials is very large.

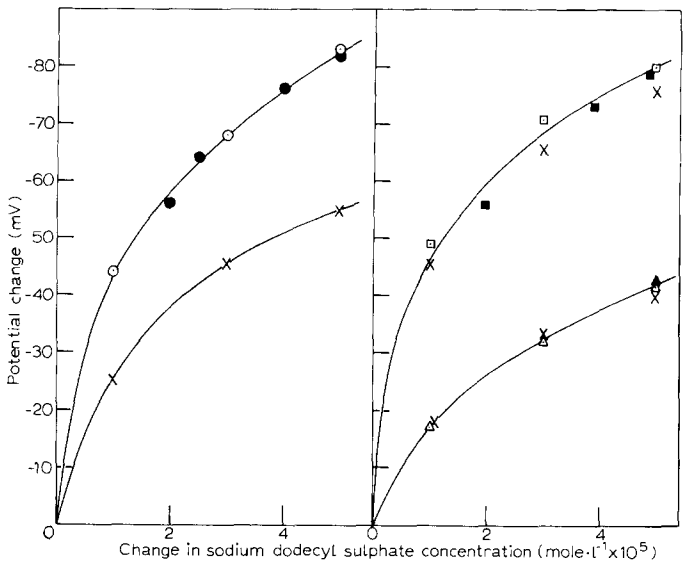


Fig. 5. Compensation potentials (open symbols), Gouy–Chapman diffuse double layer potentials (× — ×) and potentials corresponding to conductance changes (from Eqn 7) (closed symbols) for glyceryl mono-oleate–decane membranes in sodium dodecyl sulphate solutions. ⊙, ●, 0.1 M NaCl, pH 6.9; ⊡, ■, 0.01 M NaCl, pH 6.8; △, ▲, 0.001 M NaCl, not buffered.

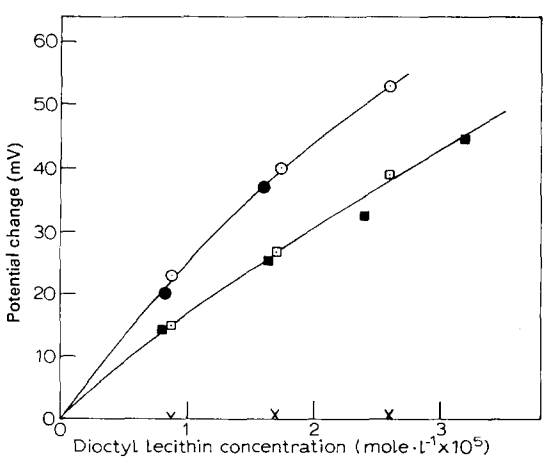


Fig. 6. Compensation potentials (open symbols), electrokinetic potentials (× — ×) (the Gouy–Chapman diffuse double layer potentials are necessarily zero) and potentials corresponding to conductance changes (from Eqn 7) (closed symbols) for glyceryl mono-oleate–decane membranes in dioctyl lecithin solutions. ⊙, ●, 0.1 M KCl, pH 6.9; ⊡, ■, 0.01 M KCl, pH 6.8.

Obviously some factor other than the Gouy–Chapman potential is required to account for many of the conductance and compensation potential changes. Second order corrections to the Gouy–Chapman theory may be responsible in some instances, but neither the probable order of magnitude of these corrections, nor the general pattern of the discrepancies suggest that they are significant. As pointed out already in this paper and elsewhere[7,27], the dipole potential is almost certainly important. If only diffuse layer and dipole potentials are considered, the compensation potentials may be interpreted according to the equation[8]

$$\Delta(\Delta\varphi) = 4\pi\Delta\mu\Delta\left(\frac{10^{16}}{A}\right) + \Delta(\Delta\varphi_{\text{Gouy}}) \tag{8}$$

where $\Delta\mu$ is the component, normal to the interface, of the change in the surface dipole moment which occurs on the adsorption of each surfactant molecule. The values of $\Delta\mu$ calculated from Eqn 8 are shown in Table IV. They appear to depend on electrolyte concentration and on the magnitude of the surfactant adsorption, and only the range of values for each system is given. Much of this variation may, however, be illusory as in some instances the difference between $\Delta(\Delta\varphi)$ and $\Delta(\Delta\varphi_{\text{Gouy}})$ is comparable to the uncertainties in these quantities.

Independent estimates of $\Delta\mu$ may in principle be made from Volta or compensation potential measurements for monolayers of each of the relevant lipids and surfactants. Thus, on the adsorption of each surfactant molecule at the glyceryl mono-oleate–decane/aqueous solution interface, glyceryl mono-oleate molecules are to some extent displaced. If the extent of the displacement per surfactant molecule and the dipoles of the two molecules were known the nett dipole change might be estimated. In fact the extent of the displacement is not known with any precision and, moreover, there is no reason to suppose that the surface dipoles found from monolayers of the pure components are additive in mixtures of the components. The exercise is nevertheless still of interest at a qualitative level. The changes in the molecular surface dipole $\Delta\mu$ for the pure materials at hydrocarbon–NaCl or –KCl solution interfaces were estimated from Eqn 8 to be: glyceryl mono-oleate–decane, approx. 300 mD; sodium dodecyl sulphate, approx. −100 mD (from the data of ref. 30); dodecyl trimethylammonium bromide, apprlx. 400 mD (also from ref. 30)

TABLE IV

CHANGES IN THE NORMAL COMPONENT OF THE SURFACE DIPOLE ON THE ADSORPTION OF SURFACTANTS AT GLYCERYL MONO-OLEATE–DECANE/BUFFERED ELECTROLYTE SOLUTION INTERFACES

	Surface dipole change $\Delta\mu$ (mD) from Eqn 8:	
	Calculated directly from the data of Figs 4–6	*Estimated from monolayers of pure components (see text)*
Dodecyl trimethylammonium bromide	12 to 57	≈100
Sodium dodecyl sulphate	−62 to −390	≈−400
Dioctyl lecithin	201 to 385	≈460

and lecithin, approx. 760 mD. If the very crude assumption is made that each surfactant molecule displaces one glyceryl mono-oleate molecule, the dipole changes in the various systems should be as shown in the right hand column of Table IV. The signs of the two sets of dipole moments are obviously in agreement and there is sufficient quantitative agreement to give further confidence in the present interpretation. The prospects for obtaining more precise estimates than those given is rather poor as the re-orientation of water molecules evidently contributes strongly to the dipole change[30] and co-operative effects seem likely to occur. This is especially obvious in the case of the dodecyl trimethylammonium ion which has a negligible intrinsic dipole and yet yields a $\Delta\mu$ at the oil–water interface of approx. 400 mD.

As the sodium dodecyl sulphate produces a relatively large dipole change, it is not surprising that in these systems the Gouy–Chapman diffuse layer potentials account less well for the conductance changes than in the dodecyl trimethylammonium systems. In fact it is only for low electrolyte concentrations, and low charge densities, where the second term on the right hand side of Eqn 8 becomes dominant, that the diffuse layer potential provides an adequate explanation for the results. For the dodecyl trimethylammonium systems, the dipole term is evidently too small in each case to be significant. For the dioctyl lecithin on the other hand, it appears that the dipole term alone accounts entirely for the conductance changes.

Although inadequacies in the Gouy–Chapman theory have been dismissed as of secondary importance it is, nevertheless, interesting that there is no indication that discrete ion effects are significant. This may be inferred from the fact that the compensation potentials, which are average values for approx. 1 cm^2 of interface, account satisfactorily for the conductance changes.

The electrokinetic potentials shown in Table III agree well, in many instances, with the diffuse double layer potentials. While it has become accepted that, in general these two quantities will differ, for small adsorbed ions at low surface charge densities and in low electrolyte concentrations it is likely that the discrepancies will be small[28]. These conditions are to a large extent satisfied in the present experiments, and the data are not obviously inconsistent with the findings of earlier investigations[18].

NOTE ADDED IN PROOF (Received April 6th, 1973)

The surface potential measurements from which these dipole moments were calculated showed that the interiors of both phosphatidylcholine and glyceryl mono-oleate membranes were positive with respect to the aqueous phases, and that the phospholipid was some 200 mV more positive than the monoglyceride. If electrostatic effects alone are considered it would therefore be expected that the permeability to lipid-soluble cations or cation–carrier complexes would be some thousand times greater in the monoglyceride than in the phospholipid membranes.

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