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THE SURFACE CHARGE AND DOUBLE LAYERS OF THIN LIPID FILMS FORMED FROM NEUTRAL LIPIDS

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SUMMARY

1. The specific capacitance (C_m) of thin lipid films formed from dispersions of glyceryl-1-monoolein in *n*-decane has been measured as function of NaCl concentration in the presence and absence of 2,4-dinitrophenol in order to examine the double layers and surface charge of films formed from neutral lipids.

2. When 2,4-dinitrophenol is absent, plots of C_m against NaCl concentration (10^{-4}–10^{-1} M) accurately fit curves predicted by double layer theory (Everitt, C. T. and Haydon, D. A. (1968) *J. Theor. Biol.* 18, 371–379) assuming an adsorbed surface charge of $0.769 \cdot 10^{-7}$ C/cm^2 corresponding to $2.08 \cdot 10^4$ Å^2/charge.

3. In the presence of 10^{-4} M 2,4-dinitrophenol, an adequate fit of the data to the theoretical curves is obtained if the surface charge is assumed to be $2.53 \cdot 10^{-7}$ C/cm^2 corresponding to $6.32 \cdot 10^3$ Å^2/charge. This finding supports the hypothesis of S. McLaughlin (*J. Membrane Biol.* 9 (1972) 361–372) that 2,4-dinitrophenol adsorbs to the surface of neutral bilayers and imparts a negative charge.

4. The geometrical capacitance (C_g) of the bilayer in the absence of 2,4-dinitrophenol is 0.4057 ± 0.0067 (S.D.) μF/cm^2 if the NaCl concentration is 10^{-1} M or less. $C_g = 0.4348 \pm 0.0071$ (S.D.) μF/cm^2 in 1 M NaCl. This increase is probably a result of either a real or apparent decrease in hydrocarbon thickness of approx. 3 Å. In the presence of 10^{-4} M 2,4-dinitrophenol, $C_g = 0.3982 \pm 0.0119$ (S.D.) μF/cm^2 which is not statistically different from the value obtained in the absence of 2,4-dinitrophenol.

INTRODUCTION

The measured specific capacitance (C_m) of neutral thin lipid films should depend upon the concentration of the surrounding electrolyte[1,2]. C_m is determined primarily by the dielectric coefficient and thickness of the hydrocarbon layer[3,4], *i.e.* by the so-called specific geometric capacitance (C_g). However, the polarization charge on the geometrical capacitor is in the form of diffuse double layers which have finite thicknesses and specific capacitances (C_d) that depend upon electrolyte concentration. C_g is in series with two double layers so that

$$\frac{1}{C_m} = \frac{1}{C_g} + \frac{2}{C_d} \tag{1}$$

For high electrolyte concentrations and/or significant surface charge[1,2] $(1/C_g) \gg (2/C_d)$ so that $C_m \simeq C_g$. C_m should differ considerably from C_g when $(1/C_g) \approx (2/C_d)$. This situation prevails in the absence of surface charge at low electrolyte concentrations.

The purpose of this paper is to report precise and accurate measurements of the specific capacitance (C_m) of thin lipid films formed from the neutral lipid glyceryl-1-monoolein dispersed in *n*-decane. The results show that C_m as a function of electrolyte concentration is accurately predicted by double layer theory. The addition of the weak acid 2,4-dinitrophenol to the electrolyte causes a significant increase in surface charge. The findings are important because of the great interest in the dependence of bilayer conductance on surface charge[5-8]. It appears to be feasible to measure low and intermediate levels of surface charge using precise measurements[9] of specific capacitance.

THEORY

The geometrical capacitance of a thin lipid film is given by[3]

$$C_g = \frac{\varepsilon_0 \varepsilon_{HC}}{\delta_{HC}} \tag{2}$$

where $\varepsilon_0 = 8.85 \cdot 10^{-14}$ F/cm, ε_{HC} is the dielectric coefficient of the hydrocarbon region, and δ_{HC} is the hydrocarbon thickness. The polar groups generally make no contribution[4] although there are exceptional cases[10].

The exact form of the double layer capacitance depends upon the fixed surface charge (σ_s) and membrane potential (V_m) of the lipid film. If $\sigma_s = 0$, then[1]

$$C_d = \frac{\varepsilon_0 \varepsilon_w \sinh \alpha}{(1/\kappa)\alpha} \tag{3}$$

where ε_w is the dielectric coefficient of water and $(1/\kappa)$ is the Debye–Hückle length given by

$$(1/\kappa) = \left(\frac{\varepsilon_0 \varepsilon_w RT}{c}\right)^{\frac{1}{2}} \tag{4}$$

where c is the concentration of 1:1 electrolyte, R the gas constant, and F the Faraday. α is determined from the transcendental equation

$$\frac{\varepsilon_0 \varepsilon_w}{(1/\kappa) C_g} \sinh \alpha + 2 = \frac{V_m}{2(RT/F)} \tag{5}$$

or alternatively from

$$C_m V_m = \sigma_p = 4cF(1/\kappa) \sinh \alpha \tag{6}$$

where σ_p is the polarization charge on the capacitor. Eqn 3 reduces to

$$C_d = \frac{\varepsilon_0 \varepsilon_w}{(1/\kappa)} \tag{7}$$

if $V_m \ll RT/F$ so that $\sinh\alpha \simeq \alpha$.

If $\sigma_s \neq 0$ so that there is a surface potential ψ_0 related to σ_s by[11]

$$\sigma_s = (8\varepsilon_0\varepsilon_w RTc)^{\frac{1}{2}} \sinh \frac{\psi_0}{2(RT/F)} \tag{8}$$

and if $\psi_0 \gg V_m$ then[2]

$$C_d = \frac{\varepsilon_0\varepsilon_w}{(1/\kappa)} \cosh \frac{\psi_0}{2(RT/F)} \tag{9}$$

METHODS AND MATERIALS

Specific capacitance (C_m) was determined by measuring the total membrane capacitance (C_T) and membrane area (A_m) using the relation

$$C_m = \frac{C_T}{A_m} \tag{10}$$

The techniques and equipment for these measurements have been described in detail elsewhere[9,12]. The Wheatstone bridge was modified, however, to include a General Radio (Concord, Mass.) 1413 decade capacitor. The bridge measures the parallel equivalent capacitance (C_p) and resistance (R_p) of the membrane–electrolyte–electrode system. To an excellent approximation, this system can be considered as the total membrane capacitance in series with the electrolyte resistance (R_E). $C_p \approx C_T$ at low frequencies when R_E is small. When R_E is large $C_p < C_T$ because of the dispersion of the system. The actual C_T was calculated using the transform equation

$$C_T = C_p \frac{1 + \omega^2 R_p{}^2 C_p{}^2}{\omega^2 R_p{}^2 C_p{}^2} \tag{11}$$

where $\omega = 2\pi f$. All measurements were made at a frequency (f) of 100 Hz. The bridge is constructed in such a way that stray capacitances[3] normally associated with such measurements have a negligible effect. The total voltage (V_{uk}) applied to the system was kept constant at either 14 mV (rms) in 10^{-4} M NaCl or 7 mV (rms) in 10^{-3} to 10^0 M NaCl. The efficacy of using the transform Eqn 11 was carefully checked using dummy circuits which included the electrode–electrolyte system. C_m can be determined with a precision of $\pm 0.3\%$ and accuracy of $\pm 1\%$.

Unbuffered electrolyte solutions (10^{-4} to 10^0 M) were prepared from Heico, Inc. (Delaware Water Gap, Pa.) extreme purity (99.95%) NaCl and ultrapure water using certified glassware. The water was prepared by distillation from a Corning (Corning, New York) AG-3ADA still preceded with a LD-2A demineralizer (3508A ultra high purity cartridge) and a Barnstead (West Roxbury, Mass.) D8904 organic removal cartridge. Water from this system was distilled from $KMnO_4$ and then redistilled using a vertical Freidrichs distilling apparatus with a heating collar to prevent "creep" of $KMnO_4$. When 2,4-dinitrophenol was present the solutions were titrated to pH 7. The pH in the absence of 2,4-dinitrophenol was about 5.8.

Glyceryl-1-monoolein was obtained from Sigma Chemical Company (St. Louis, Mo.). It migrated as a single spot on thin-layer chromatography using ether–benzene–ethanol–acetic acid (40:50:2:0.2, by vol.) as a developer[13]. The glyceryl-1-monoolein was lyophilized from benzene and dispersed (20 mg/ml) in 99.5% *n*-decane (La Chat Chemicals, Chicago Heights, Ill.) which had been passed through alumina several times just prior to use.

The temperature of the membranes was maintained at 20.0 ± 0.5 °C.

RESULTS

The specific capacitance of the films as a function of NaCl concentration was determined by making single measurements of C_m on 7–10 films at each concentration. The films must be in a state of approximate equilibrium[12] if the data are to be

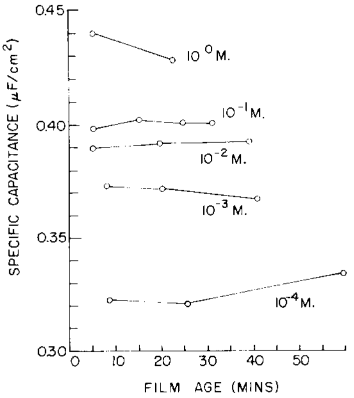

Fig. 1. Specific capacitance (C_m) of glyceryl-1-monoolein in *n*-decane films as a function of film age with NaCl concentration as a parameter. The diameters of the data points represent the approximate experimental precision of ±0.3%. C_m is reasonably steady with time usually not varying more than about ±1%. It is concluded that the films are in approximate equilibrium. The variations observed are probably due to the presence of microlenses in the thin films. Temp., 20.0 ± 0.5 °C.

TABLE I

THE SPECIFIC CAPACITANCE (C_m) OF GLYCERYL-1-MONOOLEIN IN *n*-DECANE (20 mg/ml) FILMS IN VARIOUS NaCl SOLUTIONS

The values of C_m have been corrected for the dispersion caused by the electrolyte resistance (R_E) acting in series with the total membrane capacitance (C_T) using Eqn 11. C_m decreases as the electrolyte concentration decreases because the double layer capacitance (C_d) decreases and becomes comparable to the geometric capacitance (C_g) (see Eqn 1). Temp. 20.0 ± 0.5 °C.

[NaCl] (M)	*C_m(μF/cm²) ± S.D.*	*Number of films*
10^{-4}	0.3142 ± 0.00395	10
10^{-3}	0.3631 ± 0.00536	8
10^{-2}	0.3964 ± 0.00387	8
10^{-1}	0.3968 ± 0.00531	8
10^{0}	0.4348 ± 0.00710	8

meaningful. Fig. 1 shows examples of measurements of C_m as a function of film age from which it is concluded that the films are in approximate equilibrium. All data reported below are obtained from films 2–6 min of age. Film age equals zero at the instant a film becomes full "black".

Table I summarizes the measurements of C_m as a function of NaCl concentration when no 2,4-dinitrophenol was present. C_m decreases with decreasing electrolyte concentration. The specific capacitance of certain kinds of thin lipid films can be highly non-reproducible varying as much as 20% from film to film[12]. The standard deviations in Table I are about 1% indicating that non-reproducibility is not a significant problem with glyceryl-1-monoolein–decane films.

Glyceryl-1-monoolein is a neutral lipid and the data of Table I should represent an example of the variation of C_m with electrolyte concentration when the surface charge is near zero. The measurements of McLaughlin[16] suggest that 2,4-dinitrophenol should adsorb to neutral bilayers and thereby impart a negative surface charge ($pH \gg pK$). Therefore, a series of measurements was made after 2,4-dinitrophenol (10^{-4} M) was added to the NaCl solutions. These measurements are summarized in Table II. C_m is again seen to decrease with decreasing electrolyte concentration but not as much as when 2,4-dinitrophenol is absent.

TABLE II

THE SPECIFIC CAPACITANCE (C_m) OF GLYCERYL-1-MONOOLEIN IN *n*-DECANE (20 mg/ml) FILMS IN VARIOUS NaCl SOLUTIONS CONTAINING 2,4-DINITROPHENOL (10^{-4} M)

The pH was adjusted to approximately 7. The values of C_m have been corrected for the dispersion caused by the electrolyte resistance (R_E) acting in series with the total membrane capacitance (C_T) using Eqn 11. C_m decreases as the electrolyte concentration decreases but not as much as when 2,4-dinitrophenol is absent (see Table I). Temp., 20.0 ± 0.5 °C.

[*NaCl*] (*M*)	C_m($\mu F/cm^2$) ± *S.D.*	*Number of films*
10^{-4}	0.3516 ± 0.00516	8
10^{-3}	0.3654 ± 0.00413	8
10^{-2}	0.3770 ± 0.00479	8
10^{-1}	0.4028 ± 0.01075	7

DISCUSSION

The data of Table I are compared with theory in Fig. 2 using Eqns 1 and 7. C_g was obtained by applying Eqns 1 and 7 to the values of C_m obtained in 10^{-2} and 10^{-1} M NaCl. It has the value 0.4057 ± 0.00668 (S.D.) μF/cm^2. The use of Eqn 7 is justified by calculations using Eqn 6 which show that $\sinh\alpha \approx \alpha$ for the experimental conditions used. Note that the values of C_m for 10^{-4} and 10^0 M NaCl do not fall on the theoretical curve (solid line, Fig. 2). A better fit is obtained using Eqns 1 and 9 assuming $\psi_0 \approx 31$ mV in 10^{-4} M NaCl. The resulting curve is shown by the lower dashed line in Fig. 2. The surface charge (σ_s) calculated from Eqn 8 is $0.769 \cdot 10^{-7}$ C/cm^2 corresponding to $2.08 \cdot 10^4$ A^2/charge. This low charge density is due either to impurities adsorbed from the electrolyte or to charged impurities in the glyceryl-1-monoolein. In the latter case the contamination would amount to only 1 part in 10^3. The results reported here are entirely consistent with the theories of Everitt and Haydon[2] and Läuger *et al.*[1] and show that it is permissible to assume[5] that the surface charge of films made from neutral lipids is relatively small.

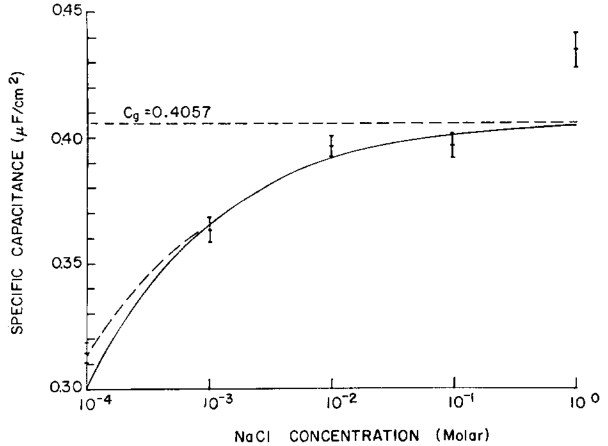

Fig. 2. Specific capacitance (C_m) of glyceryl-1-monoolein in *n*-decane films as a function of NaCl concentration. Each point represents the average of measurements on 8–10 films 2–6-min old. The error bars indicate the standard deviation which is typically ±1%. The geometric capacitance (C_g) is taken as 0.4057 μF/cm^2 (upper dashed curve). The solid curve represents the theoretically expected curve in the absence of surface charge using Eqns 1 and 7. The dashed curve passing through the data points is drawn assuming a surface charge of $2.08 \cdot 10^4$ Å^2/charge calculated using Eqns 1 and 9. The data point for 10° M is higher than expected and suggests that the film is thinner at this concentration.

The value of C_m for 10^0 M NaCl is much greater than expected and cannot be explained by double layer effects. The deviation must be associated with the geometric capacitance. Either the thickness has decreased or the dielectric coefficient has increased. The first possibility seems more likely. The decrease could be due to a real decrease in δ_{HC} or an apparent decrease due to electrolyte penetration into the hydrocarbon region. The difference between 0.4057 and 0.4348 μF/cm^2 corresponds to a thickness decrease from about 46 Å to 43 Å assuming $\varepsilon_{HC} \approx 2.1$. A third possibility is that the polar group capacitance is involved but this appears to be unlikely[14].

The presence of a significant surface charge should cause the dependence of C_m on electrolyte concentration to be decreased[2]. That is, as σ_s increases, C_m should approach C_g at low electrolyte concentrations. McLaughlin's[16] data suggests that 2,4-dinitrophenol should adsorb to neutral bilayers and impart a negative surface charge. The results presented in Table II show that in 10^{-4} M NaCl containing 10^{-4} M 2,4-dinitrophenol $C_m = 0.3516$ μF/cm^2. In the absence of 2,4-dinitrophenol one would expect C_m in a $2 \cdot 10^{-4}$ M electrolyte to be 0.3335 μF/cm^2 (from lower dashed curve, Fig. 2). The data in Table II are compared with theory in Fig. 3. The value of C_g in this case is 0.3982 ± 0.0119 (S.D.) μF/cm^2 obtained as outlined earlier. The solid curve was calculated using Eqns 1 and 7 assuming the surface charge to be zero. The deviation of the data from theory is clearly shown. The lower dashed curve was calculated using Eqns 1 and 9 assuming the surface charge was $2.53 \cdot 10^{-7}$ C/cm^2 corresponding to $6.32 \cdot 10^3$ A^2/charge in 10^{-4} M NaCl. This surface charge is 3.3 times greater than found in the absence of 2,4-dinitrophenol. The data fit this curve reasonably well but the scatter is greater than for the data in Fig. 2. For example, the standard deviation of C_g is almost twice that found in the absence of 2,4-dinitrophenol. The 2,4-dinitrophenol seems to enhance the inherent non-reproducibility. It should be noted, however, that the values of C_g obtained in the presence and absence of 2,4-dinitrophenol are not significantly different statistically. The results are entirely consistent with the theory of Everitt and Haydon[2] and support McLaughlin's[16] hypothesis that 2,4-dinitrophenol adsorbs to neutral bilayers. McLaughlin reports the surface potential of phosphatidylethanolamine bilayers bathed in 10^{-1} M KCl containing 2,4-dinitrophenol (10^{-4} M) to be about -21 mV (ref. 16, Fig. 1). This corresponds to a charge density of about 10^3 A^2/charge or 6 times the value reported here. The discrepancy could be due to differences in lipid structure and/or screening effects[17]. The negative charge of 2,4-dinitrophenol may be screened at higher electrolyte concentrations allowing more 2,4-dinitrophenol to be adsorbed.

Finally, a value of $C_g = 0.383 \pm 0.008\ \mu$F/cm^2 has been measured in D. A. Haydon's laboratory[14,18] for glyceryl-1-monoolein–decane films. This value is somewhat smaller than the value of $0.4057 \pm 0.0068\ \mu$F/cm^2 reported here. The difference may be a result of the visual method used in Haydon's laboratory to measure membrane area. The precision and accuracy of measurements of C_m have been discussed in detail elsewhere[12]. The agreement is probably satisfactory if the absolute accuracies of the methods of the two laboratories are taken into consideration. The standard

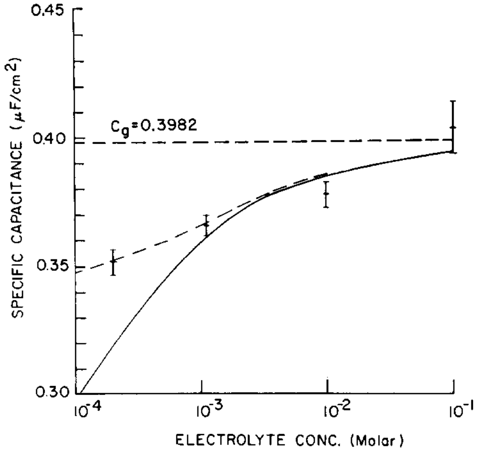

Fig. 3. Specific capacitance (C_m) of glyceryl-1-monoolein in *n*-decane films as a function of NaCl concentration in the presence of 2,4-dinitrophenol (10^{-4} M). Each experimental point represents the average of measurements made on 7–8 films 2–6-min old. The error bars indicate the standard deviation which is typically $\pm 1\%$. The geometric capacitance (C_g) is taken as $0.3982\ \mu$F/cm^2 (upper dashed curve). The solid curve represents the theoretically predicted curve in the absence of surface charge using Eqns 1 and 7. The lower dashed curve is calculated using Eqns 1 and 9 assuming a surface charge of $6.32 \cdot 10^3$ A^2/charge. The results are consistent with the hypothesis that 2,4-dinitrophenol adsorbs to the surface of neutral bilayers[16].

deviation of the value of C_g reported here is about ±1.5% which is much greater than the estimated precision of ±0.3%. The variation is likely due to microlenses trapped in the films[12,15,19]. In addition, there appears to be a slight dependence of membrane specific capacitance on membrane area which cannot be accounted for at the present time by systematic experimental error (White, S. H., unpublished).

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