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ANOMALOUS DIELECTRIC DISPERSION IN BIMOLECULAR LIPID MEMBRANES

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SUMMARY

The response of lipid membranes to alternating electric fields has been investigated. The electrical measurements yield the equivalent parallel combination of conductance and capacitance seen by the a.c. bridge. An attempt has been made to determine the film parameters through the use of equivalent circuits for the system. Both the film conductance and capacitance showed a dispersion suggestive of the presence of thin water layers adjacent to the membranes where the electrical properties differ from those of bulk water.

INTRODUCTION

In principle a knowledge of the response of lipid membranes to alternating electric fields can furnish useful information relating to film thickness and dielectric constant, and to the organisation both of the molecules in the films and of the ions in the electrical double layers. This awareness has prompted investigations into the behaviour of the impedance, as a function of frequency, of different types of bilayer systems[1-5].

The preponderance of experimental data appears to indicate that the capacitance of these systems is independent of frequency at least up to 20000 cycles/sec and varies from about 0.33–0.38 μF/cm^2 for lecithin in decane films[1,4] to up to 0.7–1.3 μF/cm^2 for bovine brain lipid membranes[2].

The data for membrane impedance as a function of frequency has been customarily analysed in terms of the equivalent parallel combination of film conductance and film capacitance. MUELLER *et al.*[2] have reported that these parameters remain constant over the frequency range 1–20000 cycles/sec for bovine brain lipid membranes. HANAI *et al.*[1] found no significant variations for lecithin in decane membranes between 50 and $5 \cdot 10^6$ cycles/sec. On the other hand SCHWAN *et al.*[5] found that although the capacitance of lecithin–tetradecane–chloroform–methanol membranes was independent of frequency between 20 cycles/sec and 20 kcycles/sec at higher frequencies the impedance of the system became less than that of the bathing solution and electrodes.

The bulk of the work performed to date has been on membranes of small area (about 1 mm^2). It would seem that ideally a rationalisation of effects such as that observed by SCHWAN *et al.*[5] can be achieved more easily with larger membranes. The present report is concerned with impedance data for membranes of area about 40 mm^2. A reduction of impedance at high frequencies below that which resulted when no membrane was present was repeatedly observed. In tracing the origin of this effect, the electrode aqueous-phase impedance characteristics were subtracted from those of the total circuit in an attempt to obtain the capacitance and conductance of the film. The resulting values showed a strong dispersion which is anomalous at least in so far as it has not been reported elsewhere. Subject to the validity of the subtraction procedure the effect has been tentatively attributed to a modification of the electrical properties of the aqueous phases adjacent to the membrane, to a depth of about 40000 Å.

EXPERIMENTAL METHODS

The measurements were made using platinum electrodes (3 cm × 1.5 cm) which had a fresh coating of platinum-black.

In one series of measurements the frequency range 0.3–50 kcycles/sec was investigated using a Wayne-Kerr Model B221 bridge. The a.c. signal was supplied by a standard laboratory signal generator (Advance, type H1). The null was detected with an Advac wide-band (15 cycles/sec–5 Mcycles/sec) amplifier (Advance Inst.) the output of which was displayed on a C.R.O. (see Fig. 1). The measurements yielded the equivalent parallel combination of conductance and capacitance for the system.

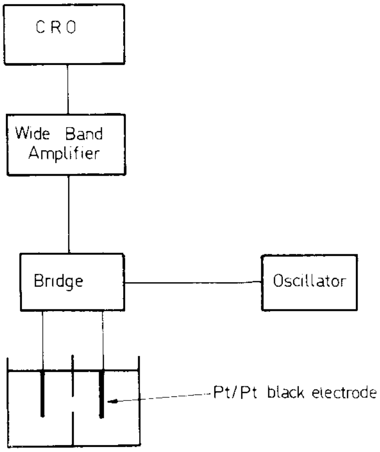


Fig. 1. Schematic of experimental arrangement used in Wayne-Kerr bridge measurements.

A second series of measurements were made using a vector impedance meter (Hewlett Packard 4800A) the analogue outputs of which were displayed on digital voltmetres (Digital Measurements DM2022S). In these measurements the dependence on frequency of the impedance and phase angle, was obtained over a frequency range 10 cycles/sec–500 kcycles/sec. The data was converted to the equivalent parallel combination of conductance and capacitance on an IBM 360/50 computer.

Unless otherwise stated the membranes were formed over a 6.8-mm diameter hole in a polycarbonate septum.

RESULTS

The total system parameters

During the period between deposition of the initial thick film and fracture of the final black membrane the low frequency capacitance C_T of the total system (*i.e.*, film + septum + electrodes) underwent characteristic changes which could be grouped roughly as follows: (1) an increase as the film became black; (2) a further increase, by about 25 % during the first 5 min after black-film formation; (3) long term drifts or oscillations during the succeeding 20-min to 2-h period, the changes usually being less than 5 %; (4) a steady state; (5) a sharp rise a few seconds prior to film rupture.

It seems unlikely that the rise in capacitance during the second phase is associated with a further increase in black film area such as might arise from a shrinkage of the torus. Generally the torus was less than 0.02 mm wide so that torus shrinkage cannot account for area changes in excess of 1 % whereas the film capacitance increased by about 25 %. The effect has been attributed tentatively to either a further thinning of the membrane or else a reorganisation of the molecules within it.

Figs. 2 and 3 show the results which were obtained for G_T and C_T as a function of frequency when the electrolytes were 0.1, 1, 10 and 100 mM NaCl. It is seen that both quantities exhibit a large dispersion, the frequency range of which is dependent on the electrolyte concentration. Generally the low-frequency capacitance values were reproducible to within a few percent while the low-frequency conductance varied by a factor of less than 4. These experiments were carried out at room temperature which was between 20 and 23°. The applied a.c. signal was less than 50 mV and in this range no dependence of G_T and C_T on voltage was detected.

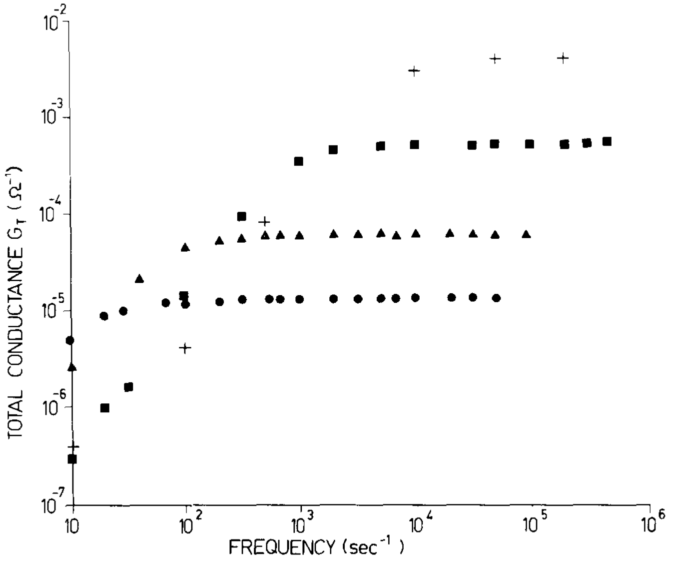


Fig. 2. Results for conductance G_T of total system (*i.e.*, membrane + electrolyte + electrodes) as a function of frequency. ●, 0.1 mM NaCl; ▲, 1 mM NaCl; ■, 10 mM NaCl and +, 0.1 M NaCl.

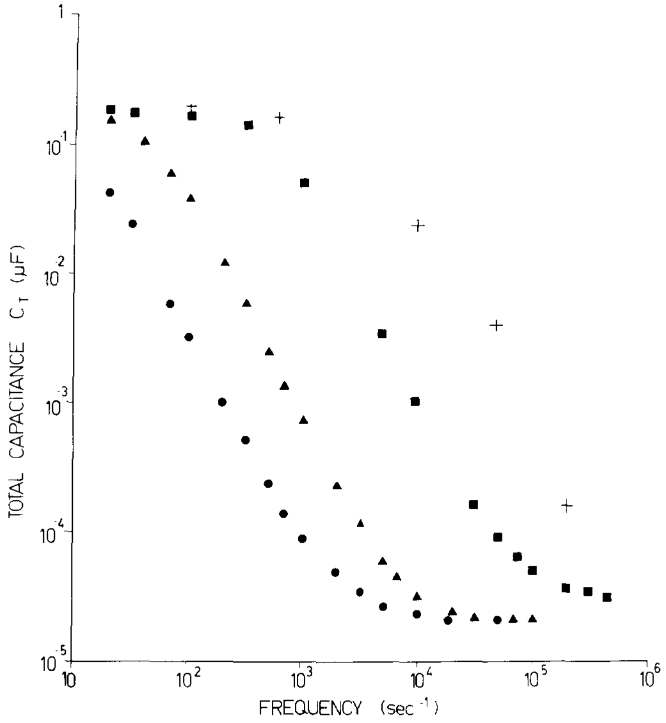


Fig. 3. Results for capacitance C_T of total system (*i.e.*, membrane + electrolyte + electrodes) as a function of frequency. ●, 0.1 mM NaCl; ▲, 1 mM NaCl; ■, 10 mM NaCl and +, 0.1 M NaCl.

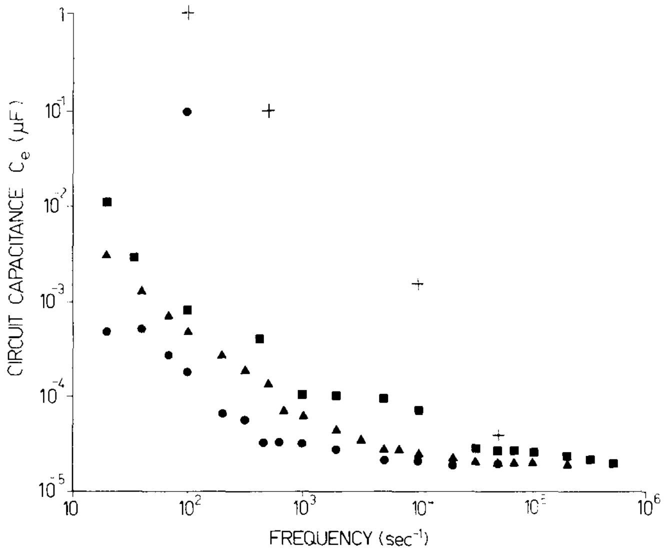
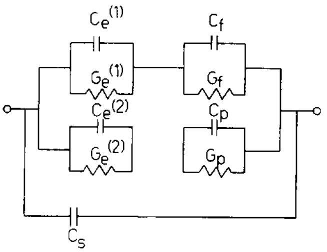


Fig. 4. Results for the frequency dependence of the capacitance C_e of the system after the membrane had fractured. ●, 0.1 mM NaCl; ▲, 1 mM NaCl; ■, 10 mM NaCl and +, 0.1 M NaCl.

Fig. 4 shows the results for the capacitance (C_e) of the 0.1, 1, 10 and 100 mM NaCl systems of Figs. 2 and 3, but after the membrane had fractured. The corresponding conductances (G_e) were almost independent of frequency and had values of $0.51 \cdot 10^{-5}$, $0.60 \cdot 10^{-4}$, $0.53 \cdot 10^{-3}$ and $0.2 \cdot 10^{-2}\,\Omega^{-1}$ in the 0.1, 1, 10 and 100 mM NaCl solutions, respectively. The non-linearity between G_e and electrolyte concentration is largely attributable to different volumes of electrolyte solution used in the different experiments. It can be seen from Fig. 4 that the electrode + aqueous system by itself also displayed dispersive properties and in a frequency region which, to some extent, overlaps with that in which the dispersion occurs when a membrane is present (Figs. 2 and 3).

The results shown in Figs. 2–4 resemble those of HANAI *et al.*[1] in that at sufficiently high frequencies $G_T \rightarrow G_e$, $C_T \rightarrow C_e$ while at sufficiently low frequencies it seems that $G_T \rightarrow G_f$, $C_T \rightarrow C_f$ where G_f and C_f are respectively the low-frequency conductance (about $10^{-7}\,\Omega^{-1} \cdot \mathrm{cm}^{-2}$) and capacitance (about 0.6 $\mu\mathrm{F/cm}^2$) of the films. (They differ, however, from the results of HANAI *et al.* in that in the latter work C_e was a frequency-independent quantity.) In Fig. 5 the equivalent circuit for the total system when a membrane is present, is given following HANAI *et al.*

In Fig. 5 C_s denotes the stray capacitance of the leads and electrodes. The quantities $G_e^{(1)}$ and $C_e^{(1)}$ denote the equivalent parallel combination of conductance and capacitance for the aqueous phases in series with the film while $G_e^{(2)}$ and $C_e^{(2)}$ are the corresponding parameters in series with the septum, of parameters G_p and C_p. The parameters G_p and C_p may be estimated from the known geometry, resistivity and dielectric constant of the septum. Similarly, rough estimates for $G_e^{(2)}$ and $C_e^{(2)}$ may be obtained by supposing that the values relate primarily to the water layers perpendicular to the septum but not to the hole. In practice all these parameters varied from experiment to experiment, however, some typical values are shown in Table I.



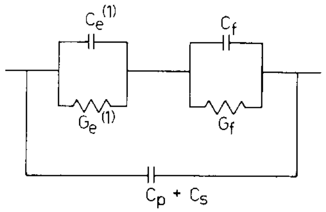


Fig. 5. Equivalent circuit for total system when a membrane is present. See text for details.

Fig. 6. The effective equivalent circuit for the total system.

In Table I are also shown the relaxation frequencies, f_0, for the septum in the different electrolyte solutions. These values were calculated using the relation

$$f_0 = \frac{G_p + G_e^{(2)}}{2\pi(C_p + C_e^{(2)})}$$

It is seen that the relaxation process occurs at frequencies which are much higher than the frequencies at which the dispersions in G_T and C_T were observed. It would seem therefore that the units in Fig. 5, which represent the septum in series with the aqueous phase, may be combined with the stray capacitance. Thus for the frequency range of interest the circuit of Fig. 5 becomes effectively that of Fig. 6. For this circuit the following relations apply[1].

$$C^* = C' - jC'' \tag{1}$$

$$C_e^{(1)*} = C_e^{(1)} + G_e^{(1)}/j\omega \tag{2}$$

$$C_f^* = C_f + G_f/j\omega \tag{3}$$

where the superscript * is used to denote complex capacitance and C^* is the complex capacitance of the whole circuit. Also

$$C' = \frac{C_f C_e^{(1)}}{C_f + C_e^{(1)}} + \frac{\dfrac{C_e^{(1)}G_f^2 - G_e^{(1)2}C_f}{(G_e^{(1)} + G_f)^2} - \dfrac{C_e^{(1)}C_f}{C_e^{(1)} + C_f}}{1 + \omega^2\tau^2} + (C_s + C_p) \tag{4}$$

$$C'' = \omega\tau \frac{\dfrac{C_e^{(1)}G_f^2 + G_e^{(1)2}C_f}{(G_e + G_f)^2} - \dfrac{C_e^{(1)}C_f}{C_e^{(1)} + C_f}}{1 + \omega^2\tau^2} + \frac{G_e^{(1)}G_f}{\omega(G_e^{(1)} + G_f)} \tag{5}$$

$$\tau = \frac{C_e^{(1)} + C_f}{G_e^{(1)} + G_f} = \frac{1}{2\pi f_0} \tag{6}$$

Provided that

$$\frac{G_e^{(1)}G_f}{\omega(G_e^{(1)} + G_f)}$$

is sufficiently small and all quantities are frequency-independent Eqn. 1 gives a semicircular plot on the complex plane.

The plots obtained for 1 and 10 mM NaCl systems are shown in Fig. 7. (At the other concentrations only parts of near-semicircular plots were obtained.) It is seen

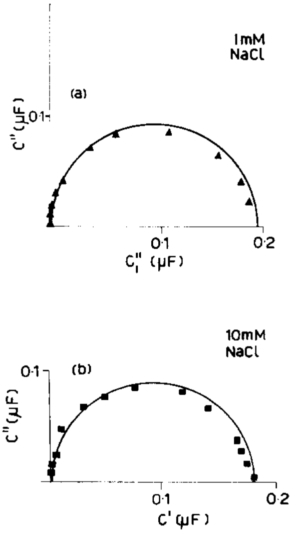


Fig. 7. Plots of the complex capacitance of the total circuit in 1 mM (a) and 10 mM (b) NaCl.

TABLE I

VALUES OF SOME OF THE VARIABLES INVOLVED IN THE a.c. MEASUREMENTS

Variable	*0.1 mM NaCl*	*10 mM NaCl*
$G_e^{(1)}$	$0.6 \cdot 10^{-6}\,\Omega^{-1}$	$0.6 \cdot 10^{-4}\,\Omega^{-1}$
$C_e^{(1)}$	0.5 pF	0.5 pF
$G_e^{(2)}$	$0.15 \cdot 10^{-4}\,\Omega^{-1}$	$0.15 \cdot 10^{-2}\,\Omega^{-1}$
$C_e^{(2)}$	13.5 pF	13.5 pF
G_p	$< 10^{-14}\,\Omega^{-1}$	$< 10^{-14}\,\Omega^{-1}$
C_p	13.4 pF	13.4 pF
C_s	3 pF	3 pF
f_0	0.2 Mcycles/sec	20 Mcycles/sec

that there are small though possibly significant deviations from semicircles. It seems unlikely that these can be attributed to the dispersion found for C_e since except at very high frequencies (*i.e.*, $f \gg f_0$), C' and C'' turn out to be almost independent of C_e. A possible mechanism for these deviations is discussed in the next section.

The overall film parameters

If the equivalent circuit of Fig. 6 is valid and provided that the intrinsic conductance and capacitance of the rest of the circuit does not alter after the membrane has broken, the parameters G_f and C_f may be calculated from the data of Figs. 2–4 using the relations

$$G_f = \frac{G_e^{(1)}G_T^{(1)}(G_e^{(1)} - G_T^{(1)}) + \omega^2(C_e^{(1)2}G_T^{(1)} - C_T^{(1)2}G_e^{(1)})}{(G_e^{(1)} - G_T^{(1)})^2 + \omega^2(C_e^{(1)} - C_T^{(1)})^2} \qquad (7)$$

$$C_f = \frac{G_e^{(1)2}C_T^{(1)} - G_T^{(1)2}C_e^{(1)} + \omega^2 C_T^{(1)}C_e^{(1)}(C_e^{(1)} - C_T^{(1)})}{(G_e^{(1)} - G_T^{(1)})^2 + \omega^2(C_e^{(1)} - C_T^{(1)})^2} \qquad (8)$$

where $C_T^{(1)} = C_T - (C_s + C_p)$; $C_e^{(1)} = C_e - (C_s + C_p)$; $G_T^{(1)} = G_T$; and $G_e^{(1)} = G_e$. Results obtained in this way are shown in Figs. 8 and 9. At low frequencies (about 10 cycles/sec) these were generally as reproducible as the corresponding values for G_T and C_T. The steady levels attained by G_f at high frequencies were fairly reproducible, the variability factor being about 2. However, at sufficiently high frequencies, where there was only a small difference between the measured values of G_T and G_e and of C_T and C_e, the calculated values for G_f and C_f were frequently unreliable. As may be deduced from Eqns. 7 and 8 small percentage errors in the measured parameters can give rise to large fractional changes in the calculated values for G_f and C_f in this latter frequency region.

It is seen that both G_f and C_f exhibit a strong dispersion over frequencies which are about 3 times as large as those for the total system parameters (see Figs. 2 and 3). This result cannot be attributed in an obvious way to inaccuracies in the experimental measurements since at many frequencies errors in excess of 15 % would be implied whereas the instruments appeared to be reliable to about 1 %. The low-frequency capacitances are independent of the concentration of electrolyte. However, it is uncertain whether the same applies to the high-frequency capacitances. The latter appear to level off at values less than $0.3 \cdot 10^{-8}$ F at least in the case of the 0.1, 1 and 10 mM solutions.

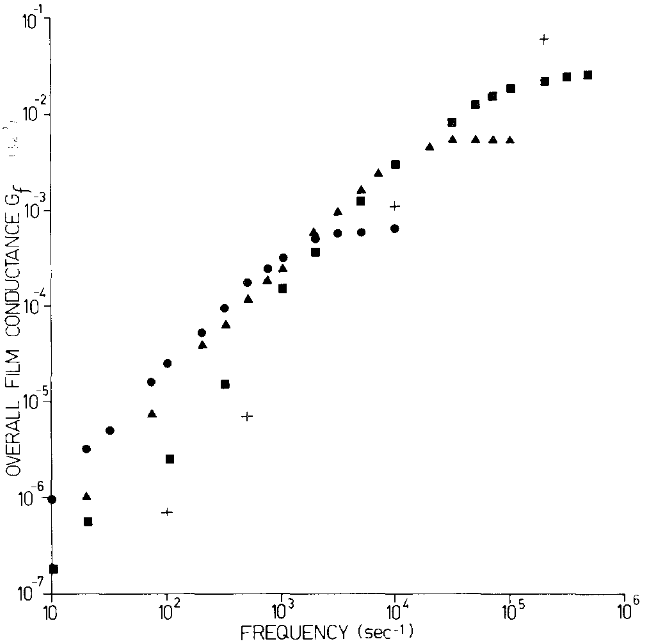
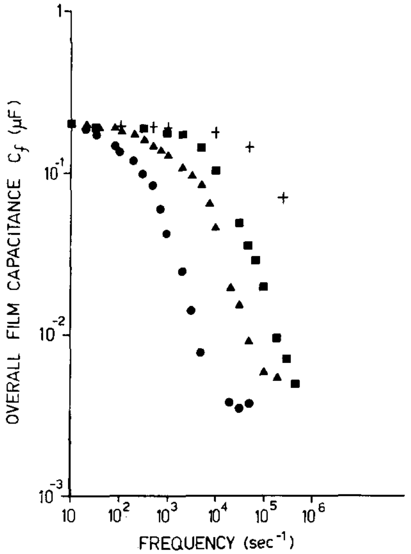


Fig. 8. Results for the overall film conductance G_f as a function of frequency. ●, 0.1 mM NaCl; ▲, 1 mM NaCl; ■, 10 mM NaCl and +, 0.1 M NaCl.

A capacitance value of $0.3 \cdot 10^{-8}$ F for a 40-mm^2 area of dielectric corresponds to a thickness of about 3000 Å if the dielectric constant ε is 3 and 80000 Å if ε is 80. The dielectric constant of the membrane is roughly 3 (ref. 6). However, its thickness as determined by optical techniques is only 50 Å (ref. 7). It seems necessary therefore, either to regard the high-frequency capacitance value as an anomaly which results from the subtraction procedure being invalid or else to attribute it to water layers (ε about 80) adjacent to the membrane with electrical properties different from those of the bulk aqueous phases. In the case of 0.1 mM NaCl (C_∞ about $0.3 \cdot 10^{-8}$ F) each layer would have a thickness of about 40000 Å while in 1 mM and 10 mM NaCl solutions ($C_\infty \leqslant 0.3 \cdot 10^{-8}$ F) the thickness might be greater.

If there are indeed disturbed aqueous layers adjacent to the film then the high-frequency conductance data of Fig. 7 might be expected to agree, at least roughly, with the conductances of such layers as calculated from bulk solution resistivity data. The conductance of a centimetre cube of NaCl solution of concentration 0.1 mM is approx. $124 \cdot 10^{-7}\,\Omega^{-1}$ (American Inst. of Physics Handbook). Thus the conductance of two layers of 0.1 mM NaCl solutions each of cross sectional area 40 mm^2, in series and of combined thickness of 80000 Å would be about $6 \cdot 10^{-3}\,\Omega^{-1}$. By comparison the value, found for the high-frequency conductance G_f in 0.1 mM NaCl solutions was about $10^{-2}\,\Omega^{-1}$. The figures appear in reasonable agreement when allowance is made for the variability in G_f and the uncertainty as to what extent the dielectric constant and resistivity of thin water layers adjacent to an interface might reflect the properties of bulk solutions. It would seem therefore that both the capacitance and conductance data are consistent with the water-layer hypothesis.



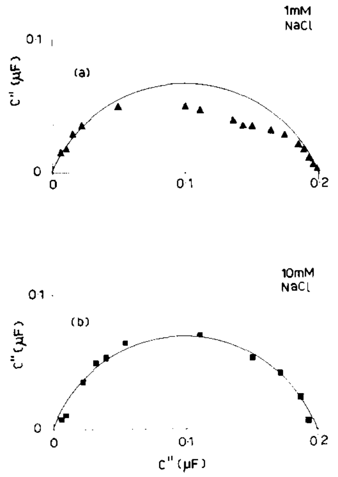


Fig. 9. Results for the overall film capacitance C_f as a function of frequency. ●, 0.1 mM NaCl; ▲, 1 mM NaCl; ■, 10 mM NaCl and +, 0.1 M NaCl.

Fig. 10. Plots of the apparent complex capacitance for films in 1 mM (a) and 10 mM (b) NaCl.

The plots for the complex capacitance (C_f^*) are shown in Fig. 10. These results are fairly typical of the two types of plot obtained. In the 10 mM NaCl case the points lie very close to a circle with a depressed centre suggesting a distribution of relaxation times[5]. In the 1 mM NaCl case, however, the existence of a single circle with depressed centre cannot be construed from the data.

DISCUSSION

In the previous section it was pointed out that subject to the validity of the subtraction procedure (Eqns. 7 and 8) the available evidence supports the conclusion that there are water layers of thickness of 40000 Å adjacent to the membrane in which the electrical properties differ from those of the bulk aqueous phases. Supporting this conclusion were the dispersion in the quantities G_f and C_f obtained by subtracting the electrode and bulk aqueous phase parameters G_e and C_e from those of the total system G_T and C_T and the correspondence between the thickness of such layers as determined from the high-frequency capacitances (C_f) and the high-frequency conductances (G_f).

Evidence which might support the subtraction procedure lies in the low-frequency capacitance data of Figs. 3 and 9. It is seen that whereas the low-frequency capacitance (C_T) for the total system in 0.1 mM NaCl is only about one half of that for the other systems the low-frequency values for C_f are all about the same. It seems that this agrees with the data of HANAI *et al.*[1] and also with the prediction of EVERITT AND HAYDON[8] that the capacitance of the membrane should be independent of the concentration of the adjacent electrolyte solutions at least in so far as electrical double-layer effects are concerned.

To the authors knowledge there have been no previous reports confirming the existence of the water layers, discussed above, for membranes which separate identical solutions of electrolyte. It might be considered on these grounds, that the present results are suspect. However, it might be note worthy that measurements of the mechanical properties of water (and other liquids) adjacent to quartz and glass surfaces, indicate the presence of boundary layers of thickness of about 1000 Å (ref. 9). Furthermore the thermodynamic and mechanical properties of water in capillaries of small radius (up to 22 μ) appear to be different from those of normal water[9]. In view of the current lack of understanding of these effects, it seems conceivable, that the properties of the aqueous regions adjacent to the membrane may have been modified to a depth of 40000 Å and that the a.c. technique provides a very sensitive method for their detection.

On the other hand there remains the possibility that the subtraction procedure is invalid. This would be the case if the equivalent circuit of Fig. 6 is invalid. It could also be the case if the electrical characteristics of the electrodes were sensitive to the change in current or local voltage when a film is formed. However, the largest changes occur at low frequencies where, as pointed out above, the subtraction procedure seems more likely to be valid.

Invalidation of the subtraction procedure could also result from variations, of significant magnitude, in the direction of the local current lines, brought about by the presence of the film. Such variations would be expected in the region near the film's torus where the impedance is presumably very large. However, they might also arise if the bulk of the current passes through a few "active patches" in the membrane or else through a few microleaks at the boundary between the film and the septum.

The remarks of the preceding paragraph suggest the possible relevence of performing measurements on films of different areas. This was done for membranes formed in 1 mM NaCl and of areas of 2.7 and 7.0 mm². The calculated values for C_f and G_f were in the same range as those for the 40-mm² membranes for frequencies ω such that $C_f(\omega) < C_f(\omega = 0)/2$. However, the independence of these parameters on membrane area was uncertain at higher frequencies, there being large errors involved in the calculation because of the diminution in the difference between G_T and G_e and between C_T and C_e.

The finding that the low-frequency capacitance is independent of the electrolyte concentration agrees with the results of HANAI *et al.*[1].

As pointed out by these authors this result appears to imply that the capacitance is not of the MAURO type[10] so that a rough idea for the dielectric constant of the film may be obtained by treating it as a parallel plate capacitor. The membrane areas were about 40 mm² so that the capacitance per unit area is 0.57 μF/cm². Using the thickness estimate of 50 Å and treating the membrane as a homogeneous dielectric the value 3.2, arrived at for the low-frequency dielectric constant is in fair agreement with theoretical predictions[6].

ACKNOWLEDGEMENTS

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