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THE MOLECULAR ORGANISATION OF BIMOLECULAR LIPID MEMBRANES

A STUDY OF THE LOW FREQUENCY MAXWELL–WAGNER IMPEDANCE DISPERSION

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SUMMARY

Theoretical considerations show that the presence of the polar group regions in bimolecular lipid membranes will produce a small (2–3 %) dispersion of the bimolecular lipid membrane capacitance at low frequencies (0.1–100 Hz). A dispersion in conductance will also result. Calculations are given of the resolution of phase angle and impedance amplitude required to detect this dispersion and a new measuring technique is described which can achieve this. From the experimental results presented for lecithin bimolecular lipid membranes a determination was made of the individual capacitances and conductances of both the hydrocarbon and polar group regions. The polar group conductance was found to vary from 700 $\mu\Omega^{-1} \cdot cm^{-2}$ (in 1 mM KCl) to 2000 $\mu\Omega^{-1} \cdot cm^{-2}$ (in 1 M KCl). The polar group capacitances were found to be approx. 30 $\mu F \cdot cm^{-2}$ and not systematically dependent on the concentration of the external electrolyte.

INTRODUCTION

Useful information on the molecular organisation of bimolecular lipid membranes can be obtained from the variation of their impedance with frequency.

Thus, for example, the presence within the membrane of substructural layers with distinctly different conductivities and dielectric constants will give rise to interfacial polarisations which lead to a Maxwell–Wagner dispersion in the membrane impedance. In this dispersion the overall membrane capacitance decreases and the conductance increases with increasing frequency until at sufficiently high frequencies both parameters reach new, frequency independent, values.

The form of this type of dispersion can be readily calculated from the separate equivalent capacitances and conductances of the substructural layers.

Using justifiable estimates for the values of the relevant parameters (e.g. see [1]), the Maxwell–Wagner dispersion resulting from the presence of the two outer polar group regions and the hydrophobic central region in bimolecular lipid membranes should occur in the frequency range 0.1–100 Hz.

Theoretical considerations of this type of dispersion in bimolecular lipid membranes have been given by Hanai et al. [1] although these authors were not able to experimentally detect this dispersion, largely because of its small magnitude and for other reasons which will become apparent. A difference, however, between the a.c. (15 Hz) and d.c. capacitance for lecithin–cerobroside bilayers has been reported [2]. Indeed the resolution required, particularly because of the low frequencies involved, is not available using conventional methods of measuring complex impedances.

In this communication we present results we have obtained for the low frequency dispersion in bimolecular lipid membranes formed from egg lecithin in *n*-tetradecane, using a new, high resolution, digital technique for measuring the conductance and capacitance of cell membranes.

THEORY AND REQUIREMENTS FOR EXPERIMENTAL RESOLUTION AND ACCURACY

The Maxwell–Wagner system

The theoretical and experimental studies of Hanai et al. [1] and Everitt and Haydon [3] have shown that the contribution to the capacitance and conductance of the polar group region in lecithin bimolecular lipid membranes due to diffuse electric double layers is likely to be negligible. Accordingly, as a first approximation, following these authors, we treat these two polar group regions as isotropic dielectrics with a dielectric constant similar to the bulk value of other polar materials. Further justification for this treatment of the polar group regions based on the dispersive properties of double fixed charge membranes [4, 5] is given in the Discussion.

The bimolecular leaflet of lecithin, shown diagrammatically in Fig. 1, can thus be divided into three regions; two polar group regions and a hydrocarbon interior. Each is associated with a capacitance and conductance, to yield the equivalent circuit shown at the top of the figure.

The overall capacitance and conductance of such a system is frequency dependent; the capacitance, C and conductance, G, being given by,

$$C = \frac{\omega^2 2C_P C_H(2C_P+C_H)+C_H 4G_P{}^2+2C_P G_H{}^2}{(2G_P+G_H)^2+\omega^2(2C_P+C_H)^2} \tag{1}$$

$$G = \frac{2G_P G_H(2G_P+G_H)+\omega^2(2G_P C_H{}^2+4C_P{}^2 G_H)}{(2G_P+G_H)^2+\omega^2(2C_P+C_H)^2} \tag{2}$$

where C_P is the capacitance of each polar group region, G_P is the conductance of each polar group region, C_H is the capacitance of the hydrocarbon region, G_H is the conductance of the hydrocarbon region and ω is the angular frequency.

An order of magnitude estimate of the values of the polar group region capacitances and conductances can be obtained as follows. The thickness of the polar group region is somewhere between 0.6–1 nm. With a dielectric constant of approx. 10–80 the capacitance of this region is between 10 and 100 $\mu F \cdot cm^{-2}$.

Similarly the specific conductances of non-aqueous, polar, liquids are typically in the range 10^{-11}–$10^{-8}\ \Omega^{-1} \cdot cm^{-2}$. On this basis the conductance of the polar-group regions would be of the order of 100–100 000 $\mu\Omega^{-1} \cdot cm^{-2}$.

From the many studies now made on bimolecular lipid membranes the capaci-

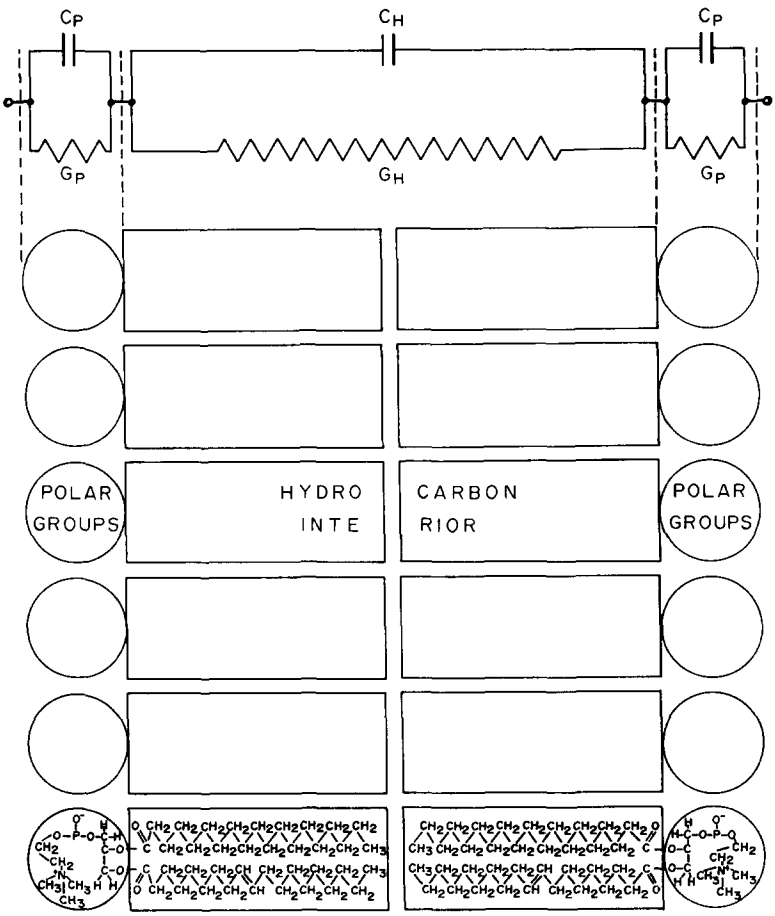

Fig. 1. A schematic diagram of a bimolecular leaflet of lecithin molecules. Each of the hydrocarbon (subscript H) and polar group regions (subscript P) are assigned an equivalent capacitance and conductance to yield the equivalent circuit shown at the top of the figure for the bimolecular lipid membrane as a whole. The total capacitance and conductance of the bimolecular lipid membrane, which are then frequency dependent, are given by Eqns 1 and 2.

tance C_H and conductance G_H of the hydrocarbon region are known to be of the order of 0.5 $\mu F \cdot cm^{-2}$ and 0.1 $\mu\Omega^{-1} \cdot cm^{-2}$, respectively.

Fig. 2 shows plots of Eqns (1) and (2) when the parameters have the following values: $C_P = 30\ \mu F \cdot cm^{-2}$, $G_P = 2000\ \mu\Omega^{-1} \cdot cm^{-2}$, $C_H = 0.5\ \mu F \cdot cm^{-2}$, $G_H = 0.1\ \mu\Omega^{-1} \cdot cm^{-2}$. It is evident that the expected dispersion occurs at very low frequencies (approx. 10 Hz) and that the total variation with frequency of the capacitance is very small (2–3 %). The dispersion in the conductance is much greater, but as will be shown presently, such a variation of conductance with frequency is difficult to detect experimentally.

Theoretical limitations for experimental resolution

The variation with frequency of the phase angle between current and potential difference for the system with the same parameters as before (Fig. 2) is shown in

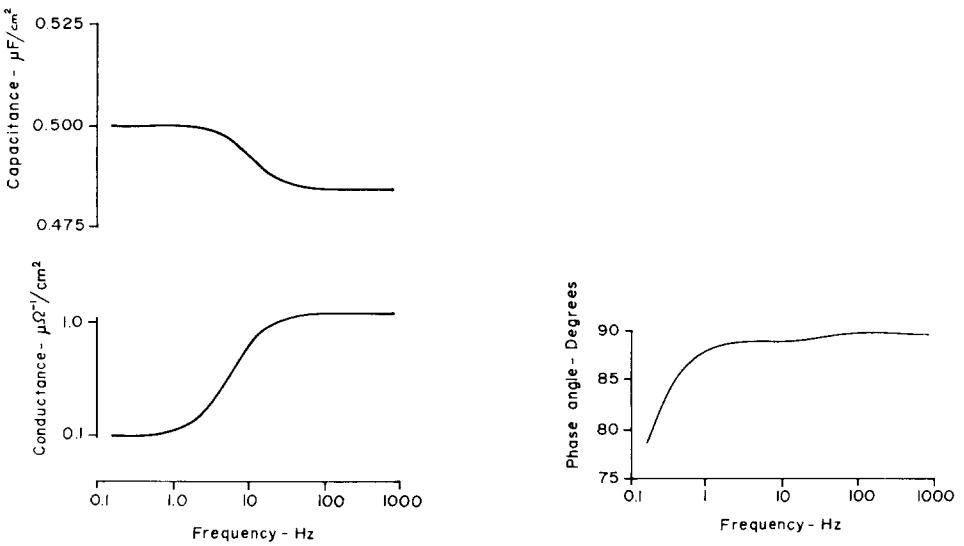

Fig. 2. The theoretical variation of the capacitance (upper curve) and conductance (lower curve) with frequency for the bimolecular lipid membrane shown in Fig. 1. In the calculations the following parameters were used (see also text). Hydrocarbon region: capacitance; $C_H = 0.5\,\mu F \cdot cm^{-2}$; conductance, $G_H = 0.1\,\mu\Omega^{-1} \cdot cm^{-2}$. Polar group region: capacitance; $C_P = 30\,\mu F \cdot cm^{-2}$; conductance, $G_P = 2000\,\mu\Omega^{-1} \cdot cm^{-2}$.

Fig. 3. The variation of phase angle with frequency for the system specified in Fig. 2.

Fig. 3. As expected the phase angle is large (approx. 80°) even at frequencies as low as 0.2 Hz and increases rapidly to approx. 90° with increasing frequency. It is hence to be expected that small errors in the measurement* of the phase angle, ϕ, will lead to very large errors in the conductance deduced for the membrane since,

$$G = \cos\phi/Z, \tag{3}$$

where Z is the magnitude of the membrane impedance.

Similarly the measured capacitance,

$$C = \sin\phi/\omega Z, \tag{4}$$

will be more sensitive to errors in the measurement of the impedance since $\sin\phi$ is approx. 1 and is therefore not very sensitive to ϕ.

The degree of resolution and accuracy required in the measurement of the phase angle and magnitude of the impedance of the membrane in order to detect the changes in C and G with frequency can be calculated from Eqns 1–4.

Results for the calculated effect of such errors on the overall membrane capacitance and conductance, for a system with the same parameters used in the calculations for Fig. 3, are shown in Figs 4a and 4b. It is immediately apparent that in order to detect the variation of C and G with frequency due to the presence of the polar group

* The measurement of the real and imaginary parts of the impedance or admittance using an a.c. bridge or similar instrument is formally equivalent to the measurement of the phase angle ϕ which is respectively given by $\cos\phi = R/Z$ or $\cos\phi = GZ$.

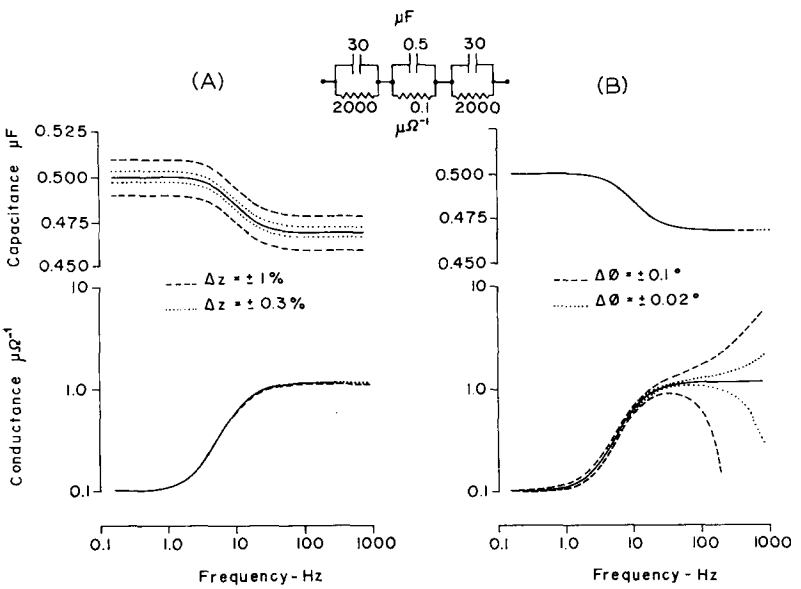

Fig. 4. (A) and (B) The effect of errors in the determination of phase angle and impedance magnitude, on the uncertainty of the calculated capacitance and conductance as a function of frequency. The curves shown were calculated for the system shown in the inset at the top of the figures, with the parameter values indicated. The full curves are direct plots of Eqns 1 and 2. The regions between either the dashed or dotted curves indicates the range of uncertainty in determining C and G. Note that the errors in ΔZ (A) lead to an uncertainty in the capacitance but not the conductance. This is so because the phase angle is very close to 90°. Errors in phase angle (B) do not lead to appreciable uncertainties in capacitance. However, the uncertainty in the conductance is very sensitive to errors in phase angle. It is apparent that to resolve the dispersion an accuracy of $\Delta\phi = \pm 0.02°$ or better needs to be achieved.

regions it is necessary to achieve an accuracy of 0.3 % in measuring the magnitude of the impedance and a phase angle resolution of 0.02° or better. These figures refer to measurement of parameters of the membrane alone.

If anything but a four terminal method is used for such measurements the accuracy required will need to be significantly better than this (approx. 2 orders of magnitude). Furthermore, since the membrane capacitance tends to change with ageing* of the film, the complete set of measurements covering the frequency range 0.1–100 Hz must be made rapidly in comparison with the ageing process. Under these conditions when a conductance of the order of 0.1 $\mu\Omega^{-1}$ is involved at frequencies below 100 Hz the accuracies quoted above are very difficult, and below 10 Hz virtually impossible, to attain using conventional techniques.

Using a modification of a computer-based digital technique developed for the study of impedance dispersion in cell membranes [5] we have been able to achieve the accuracy required (i.e. $\pm\Delta\phi < 0.02°$, $\pm\Delta Z < 0.3\,\%$) to detect and characterise the dispersion due to the presence of the polar group regions.

* This could be due to internal changes such as the removal of solvent in the hydrocarbon region [8].

METHODS

Cell and formation of the membrane

The technique for generating the membranes was essentially that described by Hanai et al. [7], except that in the present work the membranes were much larger in area (4.45 mm^2). They were formed from a solution of egg phosphatidylcholine in *n*-tetradecane over a 2.4-mm diameter hole in a polycarbonate septum. Except for a small torus the entire film covering the hole became black. The septum divided a "Perspex" cell filled with, 1, 10, 100 mM or 1 M KCl solutions, into two compartments. The cell was held in a clamp mounted on a 150 kg concrete block supported on damped springs.

Electrical apparatus

A four terminal method was used for the impedance measurements. This avoids the large errors which will be otherwise introduced when the frequency-dependent conductance and capacitance of the electrode-solution system has to be subtracted from the total parameters (e.g. see [9]).

In the present set-up sinusoidal current was injected via two AgCl-coated silver electrodes in each compartment of the cell. The potential developed across the membrane was measured with two miniature calomel $\frac{1}{2}$ cells.

The capacitance and conductance of the membranes were determined by the direct measurement of the relative phase and amplitude of the membrane current and potential difference. This was achieved, to a very high degree of accuracy, using the set-up shown in Fig. 5 which is described below.

A voltage-controlled oscillator generated a pulse train whose frequency was controlled by one channel of a four channel digital-to-analogue converter interfaced with a small computer (Digital Equipment Corpn. PDP 11/20). The frequency range of the voltage-controlled oscillator was selected, via magnetic reed relays, by the output of a digital, general device, interface on the computer. The computer controlled sinewave signal was synthesised digitally by using the pulse train from the voltage controlled oscillator to scan a read-only memory which had been programmed with a sine function. With the aid of a digital-to-analogue converter the sinusoidal signal was then obtained.

The frequency of the sinewave signal injected into the membrane chamber was monitored by a frequency counter, the digital output of which was connected to the input register of the general device interface and was hence measured directly by the computer.

The current was measured by the potential developed across a known resistance in the current circuit.

Both the current and membrane potential were measured using high input impedance ($10^{14}\ \Omega$) electrometer amplifiers (Keithley Inst. No. 604). The outputs of these electrometers were sampled, in turn, by two channels of a 11 bit analogue-to-digital converter interfaced with the computer. Optimum selection of the analogue-to-digital converter gain (4 ranges) was automatically selected by software which entailed sampling of one cycle of the two signals. The clocking pulses to initiate the sampling were derived from the pulse train of the voltage-controlled oscillator, after a computer controlled frequency division. Since the pulse train from the voltage-

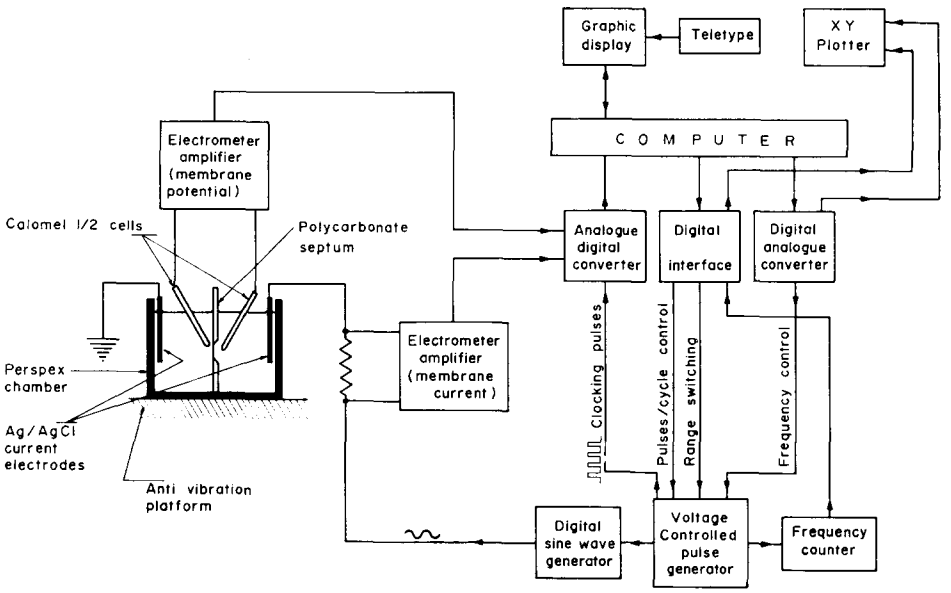

Fig. 5. A schematic diagram of the experimental apparatus. The membrane was formed over a 2.4-mm diameter hole in the polycarbonate septum. The a.c. potential appearing across the membrane was measured with the aid of two miniature calomel $\frac{1}{2}$ cells and a high input impedance ($10^{14}\,\Omega$) electrometer amplifier. AgCl-coated silver wires in each compartment served as the current electrodes. This four terminal measuring technique yields the impedance between the two planes in the solution where the tips of the potential measuring electrodes are located, irrespective of the impedance of the current electrodes. The computer-controlled measuring technique is described in the text.

controlled oscillator was also used to digitally synthesise the sinewave signal, the clocking pulses were phase locked to the signal.

At each of a programmed series of frequencies, a programmed number of cycles of current were injected into the membrane cell and the results at each frequency separately accumulated in the computer memory. In this fashion a good signal-to-noise ratio could be obtained. In the final analysis, which was only begun after the data for all the programmed frequencies had been collected, the method of least squares was employed to fit sinusoidal waveforms to the stored data points for current and potential. In this way the relative phase and amplitude of the current and potential difference waveforms were calculated.

The total time to acquire the data for a complete set of frequencies (usually about 25) was simply equal to the sum, over the frequencies selected, of the periods of each sinewave times the number of cycles at each frequency sampled (usually 5–30). Typically the data for a complete dispersion curve was collected in <90 s. At <100 Hz the system was capable of resolving phase angles of 0.01–0.02° and amplitude ratios of 0.2 %. The a.c. potential across the membrane never exceeded 7 mV rms.

The present system yields the capacitance and conductance between the two points where the tips of the potential measuring electrodes are located. The impedances of the electrode–solution system do not enter into the measurements. The values derived are therefore those of the membrane in series with the thin slices of external solution between the potential electrodes and the surface of the membrane. The

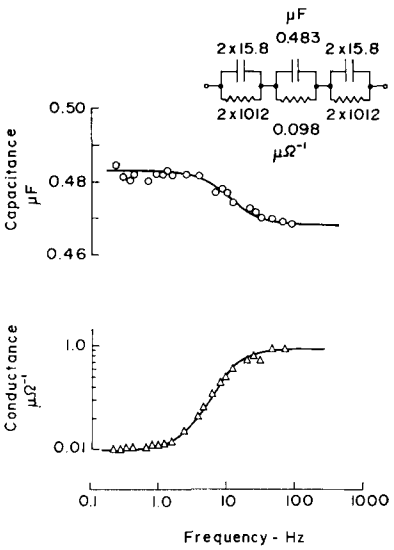

Fig. 6. The results of a test run, using the digital measuring technique described, with the circuit shown in the inset at the top of the figure which was constructed from elements with the values indicated. Since the outer two segments of the 3 segment circuit were identical they were lumped together in the actual circuit constructed. The values of the components used in the test circuit were determined, before assembly, using a Wayne Kerr Model B224 bridge. After assembly this bridge was not able to resolve the values of the individual components (not even the value of the 0.098 $\mu\Omega^{-1}$ conductance shunting the 0.483 μF capacitor). The points plotted are the experimental values obtained while the curves are plots of the theoretical values of C and G predicted by Eqns 1 and 2 with values of C_H, G_H, C_P and G_P equal to the values of the corresponding circuit elements. It is seen that the system described was able to accurately resolve the theoretical dispersion predicted in the total C and G. The values chosen for the test circuit elements are close to those expected for a bimolecular lipid membrane (see text) and hence the system should be capable of detecting such a dispersion in these membranes.

TABLE I

Errors represent scatter between different membranes.

	External solution concn (KCl)	1 mM	10 mM	100 mM	1 M
Hydrocarbon region					
Capacitance, C_H ($\mu F \cdot cm^{-2}$)		0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1
Conductance, G_H ($\mu\Omega^{-1} \cdot cm^{-2}$)		0.024	0.17	0.9	1.6
Thickness from C_H (assuming $\varepsilon = 2.13$ [6]) (nm)		approx 3.9	approx 3.9	approx 3.9	approx 3.9
Polar group region					
Capacitance, C_P, ($\mu F \cdot cm^{-2}$)		30±6	30±6	30±6	30±6
Thickness (assuming $\varepsilon = 20$) (nm)		approx. 0.61	approx. 0.61	approx. 0.61	approx. 0.61
Conductance, G_P, ($\mu\Omega^{-1} \cdot cm^{-2}$)		700±200	1500±200	2000±200	2000±200

conductances of the latter are very high compared to the membrane but nevertheless were allowed for (vectorially) in each case in the determination of C and G of the membrane.

In order to test the apparatus, the capacitance and conductance of a circuit containing resistors and low loss capacitors, with values similar to those used in the calculations for Figs 2–4, were measured. The actual values of the components used in the circuit were measured before assembly* using a Wayne-Kerr Model B224 bridge. The results so obtained, together with the theoretical values deduced from the component values using Eqns 1 and 2, are shown in Fig. 6, as a function of frequency. The impedance measuring system is seen to be accurate and capable of resolving the predicted changes in both C and G with frequency.

RESULTS

After formation, the coloured films thinned until a small patch of "black" membrane formed, usually at the lower end of the circular aperture. The area of black membrane then expanded until it occupied the entire area of the aperture (4.45 mm^2), except for a small torus (approx. 1 % of the area of the hole) around the edge. The area could be measured to within 1 %. However, the specific capacitance varied from membrane to membrane by 10–20 %.

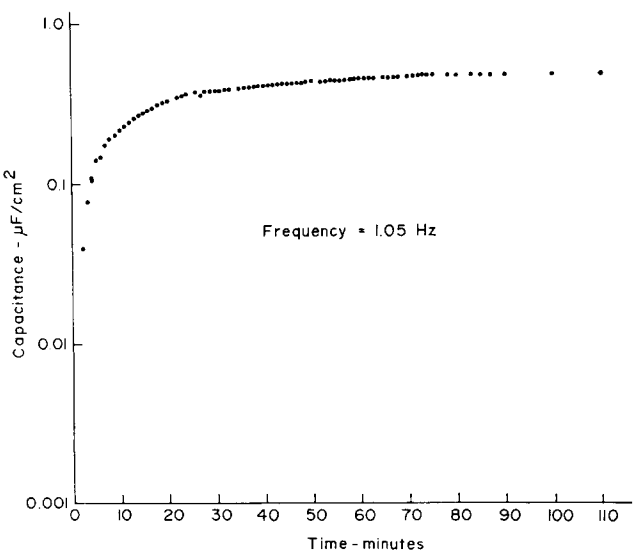

Fig. 7. A typical time course of the capacitance, per cm^2 of a film, at 1.05 Hz. At this frequency the measured capacitance of a bimolecular lipid membrane is entirely that due to the hydrocarbon interior. The initial increase in capacitance is due to an increase in the area of "black" membrane. After approx. 15 min the entire film had become black but the membrane capacitance continued to increase, although at longer times the rate of increase of C was very small. This particular bimolecular lipid membrane was formed in 10 mM KCl.

* It was necessary to measure the components separately using this bridge since only then was it able to give accurate values for the individual components in the test circuit. It was in error by up to 10 000 % in deducing the values of these parameters in the complete circuit.

The membranes were often quite stable, a lifetime of 2–4 h not being uncommon, although in some cases the membrane would last only a few minutes.

While the films were thinning the computer-controlled measuring system was set to continuously monitor the membrane capacitance and conductance at one frequency. Usually a low frequency was selected (approx. 1 Hz) since, with reference to Fig. 2, this would be expected to reflect the hydrocarbon region capacitance. A typical time course of the membrane capacitance is shown in Fig. 7. It was found that the membrane capacitance continued to increase even after the entire membrane was black and continued to do so, albeit more slowly, for up to 2 h after formation.

The variation of membrane capacitance and conductance with frequency is shown in Fig. 8a; in this particular example the external solution was 10 mM KCl. In this figure and Fig. 8b, which shows the same data, plots are also shown of the theoretical variation of C and G expected from Eqns 1 and 2 for various values of the parameters C_P and G_P of the polar group regions. The choice of the values C_H and G_H is determined by the very low frequency experimental values obtained.

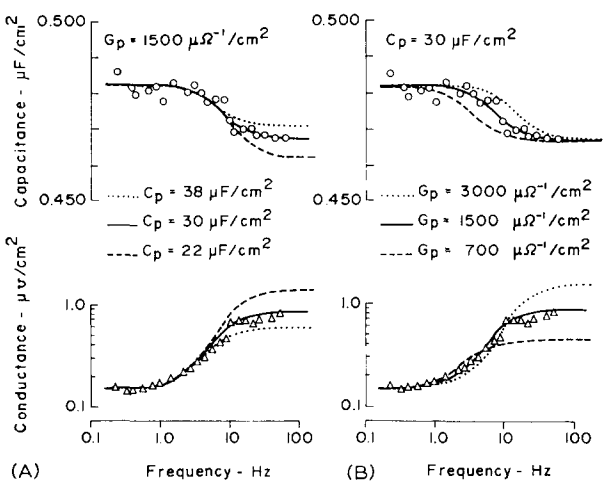

Fig. 8. (A) and (B) The capacitance and conductance as a function of frequency for a lecithin bimolecular lipid membrane made in 10 mM KCl. The points plotted are the experimental values obtained (points in (B) are a replot of those in (A)). The curves are the theoretical values expected from Eqns 1 and 2 for various combinations of the values of C_P and G_P. In both figures C_H (= 0.482 μF · cm^{-2}) and G_H (= 0.165 $\mu\Omega^{-1}$ · cm^{-2}) were fixed by the very low frequency experimental values obtained. (A) This figure shows theoretical plots with G_P = 1500 $\mu\Omega^{-1}$ · cm^{-2} for C_P = 22, 30 and 38 μF · cm^{-2}. It is clear that the value of 30 μF · cm^{-2} gives a good fit to the data and the choice of the value of C_P is very restricted. Note that the value of C_P also affects the high frequency conductance. (B) This figure shows theoretical plots with C_P = 30 μF · cm^{-2} and G_P = 700, 1500 and 3000 $\mu\Omega^{-1}$ · cm^{-2}. The value of G_P determines not only the high frequency conductance of the bimolecular lipid membrane but also the frequency range in which the dispersion occurs. The latter is most evident from the dispersion curves for capacitance in (B). The value of G_P, however, does not affect the high frequency capacitance. The final choice of G_P is also very restricted.

The values for C_P and G_P not only affect the high frequency (approx. 100 Hz) values of the membrane capacitance and conductance but also the dispersion frequency range.

The choice of the C_P is quite restricted since this parameter completely determines the high frequency capacitance, once C_H is fixed, as is evident from Fig. 8a. It also affects the high frequency conductance.

The effect of G_P on the theoretical curves is shown in Fig. 8b. The value of C_P used in the calculations for the curves in this figure was that required to fix the high frequency capacitance (see Fig. 8a). It can be seen that G_P does not affect the high frequency capacitance. It does, however, affect the dispersion frequency, which is particularly evident from the curve of capacitance versus frequency. G_P also affects the high frequency conductance. These factors make the choice of G_P quite small.

Although the precise values for capacitance and conductance varied from membrane to membrane, very similar dispersion curves were obtained each time.

The results obtained for membranes when the external solution was 1, 10, 100 mM and 1 M KCl are shown in Figs 9a and 9b. To avoid confusion the results for capacitance are shown with arbitrary displacements from the origin since the experimental points overlap. The displacements are indicated by the numbers at the left of each set.

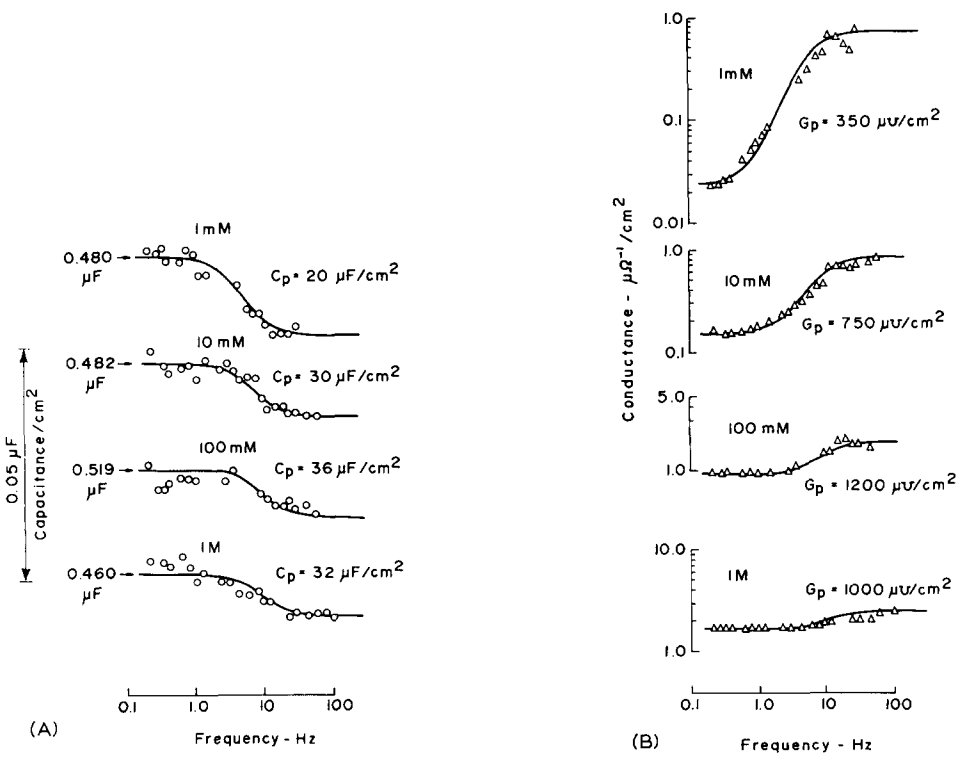

Fig. 9. (A) and (B) The variation with frequency of the capacitance (A) and conductance (B) for lecithin bimolecular lipid membranes made in 1 mM, 10 mM, 100 mM and 1 M KCl solutions. In each case the points plotted are the experimental values obtained. The full curves are theoretical plots derived from Eqns 1 and 2 with the various values of C_P and G_P indicated on the diagram. The values used were the optimum values required to fit the data in each case and were determined in the manner outlined with reference to the results shown in Fig. 8. The values of C_H and G_H in each case were fixed, as indicated before, by the very low frequency values of C and G for the bimolecular lipid membrane. Their values can therefore be read directly from the curves.

Plots of the theoretical dispersion with frequency predicted by Eqns 1 and 2 using appropriate values of the parameters, which were determined as described before in reference to Fig. 8, are also shown. The actual values of these parameters needed are indicated on each set of results.

It is evident from the results and the theoretical curves fitted to these, that each polar group region capacitance had a value of approx. 30 $\mu F \cdot cm^{-2}$ and that no strong dependence of the capacitance on the concentration of external electrolyte was detected.

The polar group region conductances showed a weak dependence on the concentration of the KCl solutions in which the membranes were formed; varying from 700 $\mu\Omega^{-1} \cdot cm^{-2}$ in 1 mM KCl to approx. 2000 $\mu\Omega^{-1} \cdot cm^{-2}$ in 1 M KCl.

The capacitance of the membrane, which is largely (within 2–3 %) determined by the hydrocarbon regions, varies 10–20 % from membrane to membrane. Outside this, no systematic dependence on concentration of the external solution was observed. The conductance of the hydrocarbon regions was dependent on the solution concentration; varying from 0.024 $\mu\Omega^{-1} \cdot cm^{-2}$ in 1 mM KCl to 1.6 $\mu\Omega^{-1} \cdot cm^{-2}$ in 1 M KCl.

DISCUSSION

The results of our experiments allow us to determine some of the separate properties of the polar group and hydrocarbon regions of the lipid bilayer.

In the polar group regions, persistent separation and orientation of the charges in the electric dipoles of the phospholipids would essentially give rise in each of these two regions to a juxtaposition of two sheets of fixed charge, of opposite sign. The resulting profiles of mobile ion concentrations would lead to a space charge region at the junction of these fixed charge sheets in each polar group region which leads to an associated capacitance [10]. This capacitance would be dependent on the concentration of ions in the external solution.

Due to diffusion polarisation effects, however, this capacitance (and conductance) would also be frequency dependent [4]. The dependence on frequency is so strong that beyond 10–100 Hz the dispersion is complete and the contribution of the diffuse double layer capacitance is negligible; the limiting high frequency capacitance being simply the dielectric capacitance of the complete double fixed charge region which is determined by its thickness and dielectric constant [4, 5].

Experimentally, therefore, any contribution of the bipolar concentration profiles to the polar group capacitance can only be detected at very low frequencies (0.1–$<$10 Hz). But at these frequencies the capacitance and conductance of the complete bimolecular lipid membrane is almost exclusively determined by the hydrocarbon region. These considerations justify our original notion of treating the polar group regions as homogeneous dielectric sheets.

In view of the above, the present experimental findings concerning the dependence of the polar group capacitance on concentration do not necessarily provide support for the notion that the trimethylammonium and phosphate groups are both in a plane parallel to the leaflet [1] or else randomly oriented. On the other hand they are also not inconsistent with this notion.

Treating the polar group regions as isotropic dielectrics with a dielectric

constant of approx. 20 which is similar to other non-aqueous polar liquids such as ethanol ($\varepsilon = 24$) or acetaldehyde ($\varepsilon = 21$), the present values deduced for the capacitance yield a value of approx. 0.61 nm for the thickness of each of these regions. This is close to the value expected from the chemical structure of the polar head of the lecithin molecule. The conductance of these regions is also similar to some non polar solvents, assuming that some contribution from the admixture of the external ionic aqueous solutions is present. However, it was found that the conductance of these regions were only weakly dependent on the concentration of ions in the external solution; a 3-fold increase in G_P was observed for a 1000-fold increase in the KCl solution concentration. This would indicate that the concentration of mobile ions in the polar group regions is relatively constant. Notwithstanding the deductions made from the independence of C_P on the solution concentrations, this finding suggests that neutralisation of the dipole charges by mobile ions is more or less complete.

While at high frequencies (>1 kHz) the capacitance of the plasma membrane of living cells is substantially frequency independent, at low frequencies (<200 Hz) a very strong dispersion, whereby the capacitance increased with decreasing frequency has been reported [11, 5]. Four terminal, intracellular electrode, measurements [5] on cells of the algae *Chara corallina* show that the membrane capacitance increases from its value of 0.8 $\mu F \cdot cm^{-2}$ at >100 Hz to 2–3 $\mu F \cdot cm^{-2}$ at <1 Hz. Such an increase in capacitance cannot be simply explained in terms of a Maxwell–Wagner-type dispersion, as reported here for lecithin bimolecular lipid membranes, even if the cell membrane consists of a lipid bilayer with protein coatings on its surface. Certainly if the latter is correct a much stronger dispersion would be expected than for a naked lipid bilayer, but the initial value at very low frequencies would still be determined by the hydrocarbon interior of the bimolecular lipid membrane. It would appear more than likely that at very low frequencies the capacitance of cell membranes reflects contributions from diffusion polarisation effects in particular ionic channels [4].

CONCLUSIONS

From our experiments on the frequency dependence, in the range 0.2–100 Hz, of the capacitance and conductance of bimolecular lipid membranes formed from a solution of lecithin in *n*-tetradecane we can deduce the information shown in table I concerning the membrane substructure. The accuracies indicated in this table represent the variations from membrane to membrane. The accuracy in the determination of the capacitance and conductance for individual membranes was better than 0.3 %, as can be seen from the results shown in Fig. 6 (cf. also Figs 8 and 9).

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