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THE MOLECULAR ORGANISATION OF BIMOLECULAR LIPID MEMBRANES

THE DIELECTRIC STRUCTURE OF THE HYDROPHILIC/HYDROPHOBIC INTERFACE

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Summary

Improvements to a previously described very low-frequency impedance-measuring technique have now allowed the characterisation of a third, electrically distinct, type of substructural region in phosphatidylcholine bimolecular lipid membranes. This region was found to have properties intermediate to those of the hydrophobic (hydrocarbon) layer and the regions containing the polar heads of the phosphatidylcholine molecules. Its properties are consistent with it being associated with the oxygen-rich carboxyl ester portions of the phosphatidylcholine molecules which lie at the hydrophilic/hydrophobic interface. We will refer to these regions in the membrane as the acetyl regions.

The individual properties of the three distinct types of regions in the phosphatidylcholine membranes were determined at KCl electrolyte concentrations of 1, 10, 100 and 1000 mM. It was found that with increasing KCl concentration: (a) The capacitance, C_H, of the hydrophobic region increased slightly, indicating a decrease in the thickness of this region. (b) The conductance, G_H, of this hydrophobic region increased by a factor of 20 in going from 1 to 1000 mM KCl electrolyte. (c) The capacitance of the acetyl region was independent of KCl concentration although its conductance increased 5-fold over the range 1—1000 mM KCl. (d) The volume-specific electrical properties of the region containing the polar heads appeared to be essentially independent of KCl con-

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centration. However, a change in thickness of these regions was observed which was consistent with the cholinephosphate dipole being oriented normal to the bilayer surface in 1 mM KCl and parallel to the surface in 1000 mM KCl external solutions.

Introduction

The presence of two electrically distinct regions within bilayer membranes formed from egg phosphatidylcholine was detected (Refs. 1—3) from measurements of the frequency dependence of their impedance (over the range 0.2—90 Hz). The individual capacitance and conductance of these distinct layers (or regions) were consistent with their being the hydrocarbon and polar head regions of the bilayer. Subsequent modifications to our original four terminal digital impedance-measuring technique have improved its resolution and frequency range sufficiently to permit resolution of an additional dispersion in the impedance associated with additional substructure. The measured impedance data were fitted by the method of least squares to that of a membrane model composed of a multilayered dielectric 'sandwich'. The resultant interfacial polarisation dispersion of such a system is identical to that of an equivalent circuit composed of a parallel capacitance-conductance element for each layer connected in series (see Figure 1). Symmetry considerations indicate that this five layer model of the membrane would present as three electrically distinct membrane regions. This analysis indeed revealed the presence of a third region with electrical properties intermediate to those previously ascribed to the polar head and hydrocarbon regions. We present here the results of varying the external KCl concentration on the electrical properties of these three electrically distinct regions in phosphatidylcholine bilayers.

Theoretical considerations

Examination of the molecular structure of phosphatidylcholine reveals three regions of distinctly different chemical character (see Fig. 1) which are presumably also present as substructural regions within bilayers formed from this lipid. These regions are:

Hydrophobic region: subscript H. This region is associated with the acyl chains. Such a region would be expected to exhibit a dielectric constant $\epsilon_H \approx 2.13$, this being a representative value for unbranched, long alkanes [4]. Partitioning of ions into such a medium is energetically extremely unfavourable and so this region would be expected to have a very low conductance, as has been demonstrated by many experimenters. The width of this region will depend upon three factors; the degree of kinking in the acyl chains, the equilibrium amount of alkane solvent 'dissolved' in the bilayer and the limiting surface area per lipid molecule at the bilayer/electrolyte interface.

Polar-head region: subscript P. This region corresponds to the cholinephosphate dipole which possesses rotational freedom and flexibility around the phosphate group. Water penetrates this region and a dielectric constant ϵ_P of 20—50 is likely [1]. It is also likely that significant ion penetration into this

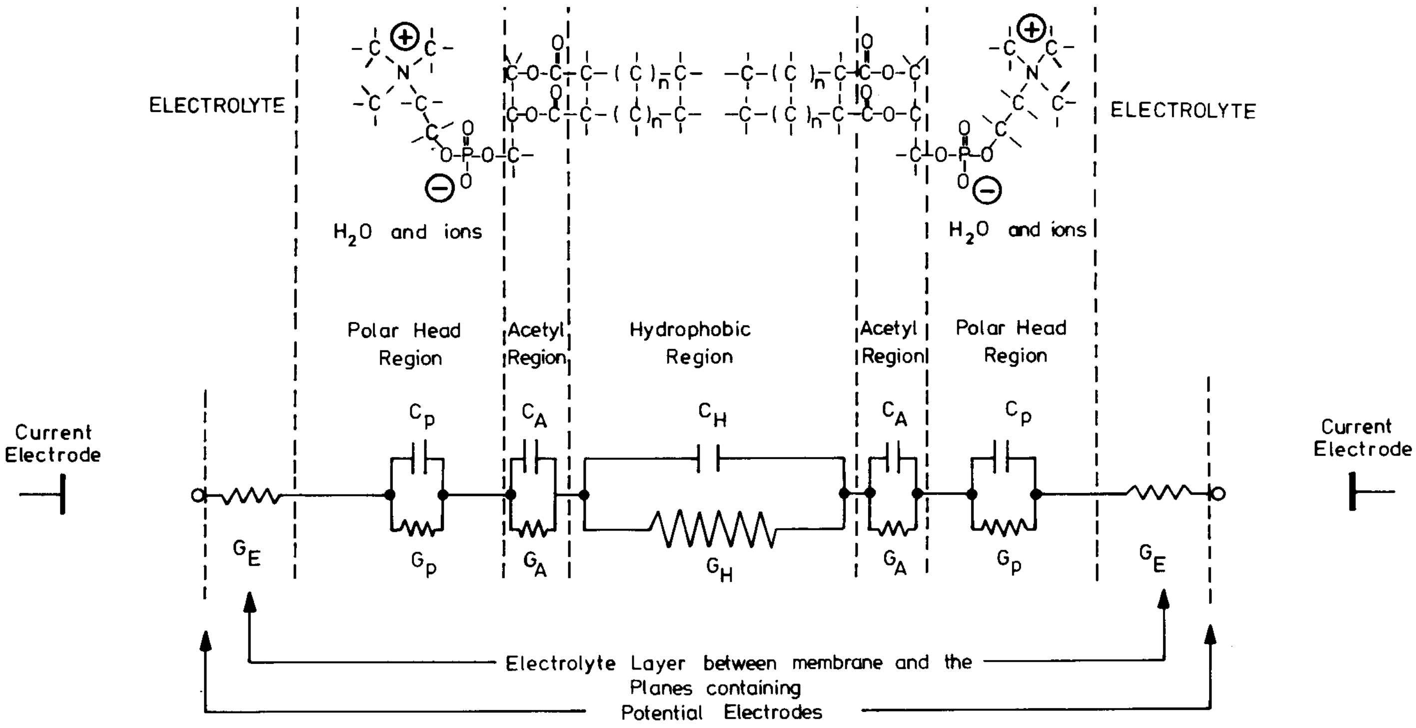


Fig. 1. An equivalent circuit for a bilayer/electrolyte system. Each region of the bilayer is associated with a chemically distinct portion of the phosphatidylcholine molecule, together with any ions and water molecules present in that region. These regions have each been represented by an equivalent parallel capacitance and conductance. The thin slabs of electrolyte between the potential-measuring electrodes and the bilayer/electrolyte interface have been represented as a conductance element alone, the capacitance of these regions being negligible at the frequencies concerned.

region will occur, resulting in a specific volumetric conductivity perhaps 10—100-times that of pure water. The width of this region will depend upon the orientation of the cholinephosphate dipole relative to the bilayer/electrolyte interface.

Acetyl region: subscript A. This region has a high density of oxygen atoms due to the carboxyl ester portions of the phosphatidylcholine molecules. There is evidence that some water can bind to this region [5,6] (see also Discussion) and a dielectric constant ϵ_A of 20—50 may be reasonable. In the bilayer configuration this region is the most rigid part of the molecule [7,8] and generally has the largest cross-sectional area. Thus this acetyl region will be the most tightly

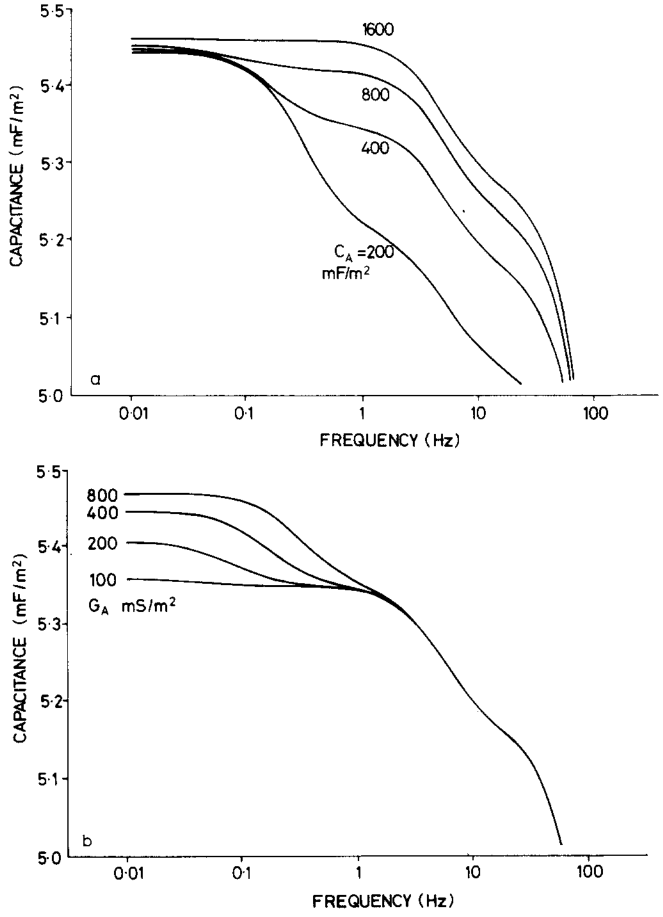


Fig. 2. Theoretical variations in the total capacitance of a bilayer/electrolyte system as a function of frequency for the model shown in Fig. 1. These plots show the effects of including an acetyl region with various parameter values. In both figures $C_H = 5.5$ mF/m², $G_H = 1$ mS/m², $C_P = 300$ mF/m², $G_p = 10\,000$ mS/m² and $G_E = 20\,000$ mS/m². These values are comparable with those found for phosphatidylcholine bilayers formed in 1 mM KCl. (a) The effect of varying the C_A value while the G_A value is held constant at 200 mS/m². It is apparent that an additional dispersion of detectable magnitude is introduced as the time constant τ_A (= C_A/G_A) of this region becomes distinct from τ_H (= C_H/G_H = 5.5 s) (b) The effect of varying G_A value while maintaining C_A at 400 mF/m². The additional dispersion can again be clearly seen. The total conductance as a function of frequency is virtually unaffected by the inclusion of the acetyl region and has not been shown here.

packed in a bilayer. It is unlikely that there will be much salt penetration into this region, and its specific volumetric conductance might be comparable with that of pure water.

Previous measurements [1—3] allowed characterisation of the hydrocarbon and polar head regions. If the time constant of this putative acetyl region is sufficiently distinct, it can manifest itself as an additional dispersion in the membrane impedance. Fig. 2 shows the theoretical variations of the total capacitance, $C(\omega)$, with frequency for the bilayer/electrolyte system shown in Fig. 1 for various plausible values of the acetyl region capacitance and conductance. These figures indicate that the presence of the intermediate acetyl region should be detectable by accurate measurements at frequencies below 1 Hz.

The equivalent circuit shown in Fig. 1 assumes that the equivalent parallel C and G values of each region are themselves each independent of frequency. Essentially this implies that the alternating electric field should be independent of position within each of the regions concerned. Since the width of each region appears to be appreciably smaller than the estimated Debye length for mobile ions in that region (as calculated from the measured conductance [9]), this condition should be satisfied. Thus each region should, in isolation, act as a simple frequency independent parallel capacitance-conductance network.

The equivalent circuit also assumes that the dielectric constant and conductivity change abruptly at the interfaces between adjacent regions. Calculations show that smoothly graded transitions in time constant between the regions have no gross effect on the dispersions of $C(\omega)$ and $G(\omega)$ [9].

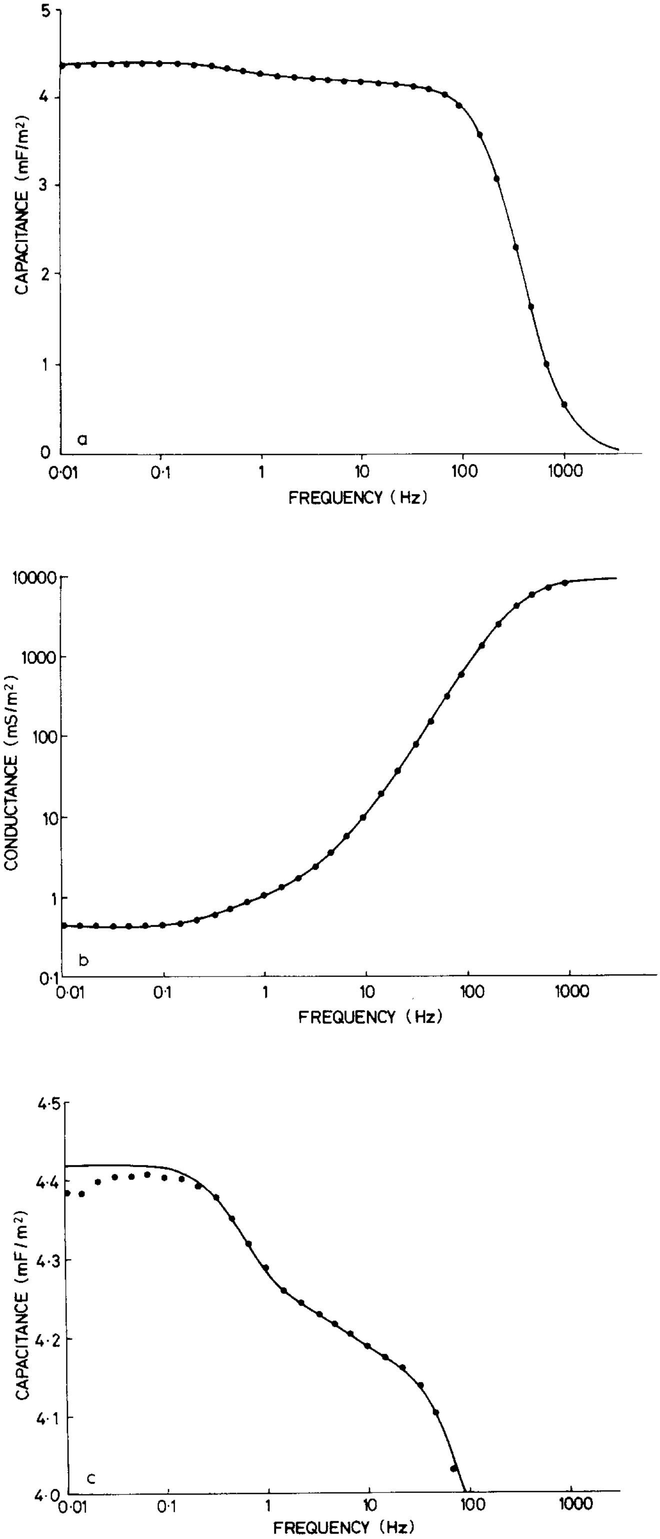
Additionally it appears unlikely that effects due to modulation of space charge associated with the polar head dipoles will influence the value of C_P in the frequency range over which it contributes to the frequency dispersion. This is because such effects predominate only at very low frequencies, whereas the polar head region only contributes significantly to the impedance at high frequencies (see Discussion of Ref. 1).

Materials and Methods

The bilayers were formed from a saturated solution of egg phosphatidylcholine in *n*-tetradecane or *n*-decane solvent. A film was made (by either syringe or brush) over a 1.2 mm diameter hole in a polycarbonate septum dividing a cell containing a 1, 10, 100 or 1000 mM KCl solution.

All measurements were made at 18—22°C. The thinning of each film was monitored by measuring the capacitance at 1 Hz. Once the impedance of the bilayer had settled (variation less than 1%/h), a series of frequency scans, each involving 25—30 measurements of impedance in the frequency range 0.01—220 Hz, were performed until the bilayer broke. Data from the final few scans were then averaged and the S.D. calculated. During each frequency scan the a.c. signals were applied in order of increasing frequency. Because the bilayer capacitance decreases with increasing frequency, any increase with time due to bilayer thinning will tend to reduce the apparent frequency dependence of the capacitance. In order to minimise any such effects all bilayers reported here had been completely black for at least 3 h.

Before each bilayer was formed across the hole in the septum, the impedance


CAPACITANCE (mF/m²)
5
4
3
2
1
0
a
0·01
0·1
1
10
100
1000
FREQUENCY (Hz)
CONDUCTANCE (mS/m²)
10000
1000
100
10
1
0·1
b
0·01
0·1
1
10
100
1000
FREQUENCY (Hz)
CAPACITANCE (mF/m²)
4·5
4·4
4·3
4·2
4·1
4·0
c
0·01
0·1
1
10
100
1000
FREQUENCY (Hz)

of the system was measured. This returned G_E, the conductance of each layer of electrolyte between the potential-measuring electrodes and the bilayer itself. This appears in series with the bilayer when it is present. The same procedure was repeated after each bilayer had broken to recheck the value of G_E.

The four terminal digital impedance technique has been described elsewhere [1,10]. In this method a sinusoidally varying current is passed through the membrane and the resultant potential difference generated across both the bilayer and a known impedance in series are compared using digital techniques to obtain a direct measure of the relative phase and amplitude. The equivalent parallel capacitance and conductance are then calculated. Separate Ag/AgCl electrodes are used for injecting the current and measuring the potential difference. This technique eliminates complications arising from the frequency-dependent complex impedance of the solution/electrode interfaces. Subsequent to the earlier descriptions several modifications have significantly improved the accuracy, reproducibility and range of the impedance measurements [9].

Test of experimental resolution. It is, of course, essential to verify whether the experimental technique is really capable of detecting the very small dispersions expected at low frequencies. This is best achieved by measuring an equivalent circuit composed of resistors and capacitors with values comparable with those expected on theoretical grounds and from hindsight following actual experiments. The results of such a test are shown in Fig. 3. It can be seen that the agreement between the measured total $C(\omega)$ and $G(\omega)$ values and those calculated from the individual component values is good. This demonstrates that our impedance measurements have the necessary accuracy to resolve the three individual electrically distinct types of substructural regions.

Characterisation of substructural parameters. The values of the substructural parameters were derived by using the method of least squares to minimise the deviation between the equivalent circuit of Fig. 1 and the measured frequency dependence of both the amplitude ratio Z_R and phase difference ϕ_D, although in principle the substructural parameters could be extracted from the data for either $Z_R(\omega)$ or $\phi_D(\omega)$ alone. These directly measured parameters were used because both their magnitudes and errors depend less upon frequency than those of the calculated parameters $C(\omega)$ and $G(\omega)$ for the membrane itself (for example see Fig. 4 of Ref. 1). The normal equations were used to adjust parameter values of the model until the reduced χ^2 in Z_R and ϕ_D had both reached a value less than one. Further improvements were then selected solely for improving the variance of the fit in Z_R.

Fig. 3. Results of measuring the total capacitance (a) and conductance (b) as a function of frequency for an equivalent circuit of the bilayer/solution system (as in Fig. 1). For this test the network was composed of resistors and capacitors with values of the order that might prevail for a bilayer. Values of the individual components are given in Table I. Each of the two polar head, acetyl and electrolyte regions were lumped together and each represented by a single parallel capacitance and conductance. (The actual component values have been multiplied by the same area factor as a bilayer to make the test realistic.) (c) The same data as (a), but replotted on a scale magnified 10-fold. One each figure the continuous lines are the theoretical curves generated from the equivalent circuit by substituting the individual component values. The agreement is good, except at frequencies below 0.1 Hz, where the capacitance is underestimated by up to 0.5%. It is apparent, however, that the experimental accuracy should be adequate to resolve these three distinct types of region in bilayers.

TABLE I

COMPARISON OF PARAMETERS

Comparison of results obtained by curve fitting the frequency dependence of the measured amplitude ratio Z_R and phase difference ϕ_D for a network of discrete components arranged as in equivalent circuit of Fig. 1. Individual component values were measured on a Wayne-Kerr B224 bridge. The fitting routine returned $\chi^2 = 0.02$ for Z_R and $\chi^2 = 0.09$ for ϕ_D. The variance of the fit to Z_R was less than 10^{-6}.

Method of determination	Parameters						
	Capacitance (mF/m^2)			Conductance (mS/m^2)			
	C_H	C_A	C_P	G_H	G_A	G_P	G_E
Measurement of individual components by bridge	4.48	202	614	0.460	804	24 200	9950
Frequency dependence of complete network	4.43	195	520	0.453	768	23 800	9820

To check the performance of this procedure on experimental data, the measurements shown in Fig. 3 on an equivalent circuit of resistors and capacitors were fitted. The results shown in Table I indicate that individual values of the substructural parameters can be reliably extracted from measurements of the frequency dependence of the total impedance, and also give some idea of the accuracy that might be expected.

With experimental bilayer data it was found that the fitting routine produced a much improved fit when three (as opposed to two) distinct types of substructural region were used in the bilayer equivalent circuit. A significant decrease in χ^2 (always to less than 1) then occurred. The introduction of this third type of region also produced new features in the theoretical dependence of the capacitance on frequency (see Fig. 2) which matched those observed experimentally. The addition, however, of a fourth type of bilayer substructural region produced only a marginal improvement in the fit variance. It thus appears justifiable to extract the parameters for three distinct types of substructural regions from the present bilayer experimental data.

Results

The measured impedance ratio Z_R and phase difference ϕ_D for a bilayer/electrolyte system (with respect to the known reference impedance) are shown in Fig. 4 for a phosphatidylcholine/tetradecane bilayer formed in 1 mM KCl. Fig. 5a shows the same data plotted in terms of an equivalent parallel capacitance and conductance. In Fig. 5b the capacitance of the bilayer alone is shown plotted on an expanded scale for clarity (the measured conductance G_E of the electrolyte in series having been vectorially subtracted). The two dispersions due to the three electrically distinct types of regions can be clearly seen.

Similar results were found for bilayers formed in electrolytes of increasing salt concentration, the main effects being: (a) a large increase in the low-frequency conductance; (b) a small increase in the low-frequency capacitance; and (c) a reduction of the magnitude of the frequency dispersions in the capacitance.

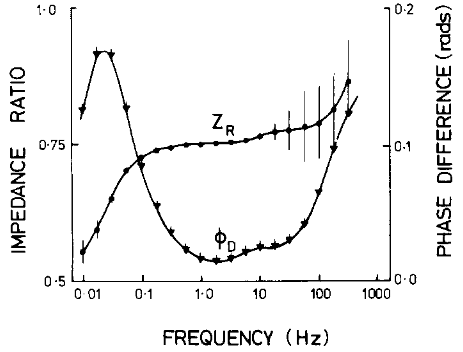


Fig. 4. The measured impedance ratio Z_R and phase difference ϕ_D (with respect to the reference impedance) for a phosphatidylcholine/tetradecane bilayer in 1 mM KCl. The data points represent the average of five successive frequency scans. The vertical bars indicate S.E. The full curve is the curve of best fit to these data found by the procedure described in the text. The enhanced errors in the impedance ratio at high frequencies are a consequence of a limitation on the maximum sampling rate of the analogue-to-digital converter used. The fitting routine returned $\chi^2 = 0.077$ for Z_R and $\chi^2 = 0.086$ for ϕ_D. The variance of the fit to Z_R was $5.6 \cdot 10^{-9}$. The fit parameters returned were $C_H = 5.34$ mF/m², $C_A = 445$ mF/m², $C_P = 305$ mF/m², $G_H = 0.923$ mS/m², $G_A = 530$ mS/m², $G_P = 17\,200$ mS/m², $G_E = 2074$ mS/m². The reference impedance in this instance was composed of a resistor (928 MΩ) and capacitor (14.67 nF) connected in parallel with another resistor (46.38 kΩ) in series.

The results of fitting the multilayer membrane model to frequency scans for membranes formed in 1, 10, 100 and 1000 mM KCl are given in Table II. The age of the bilayers reported were in the range 180—450 min after turning com-

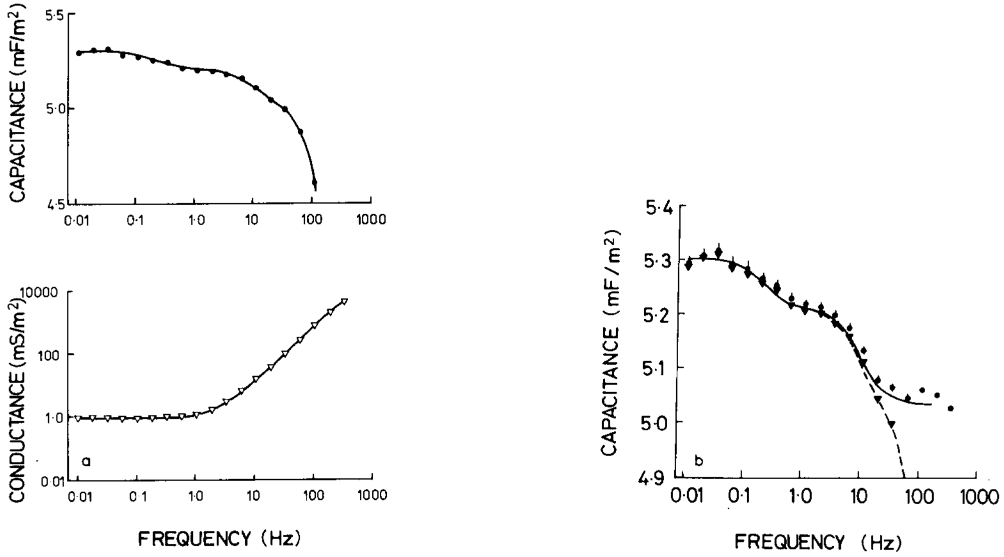


Fig. 5. The same data as Fig. 4, but now expressed in terms of an equivalent parallel capacitance and conductance as a function of frequency. The continuous line is again the theoretical plot given by the parameter values that gave the best fit to Z_R and ϕ_D. It is now possible to see the dispersion in the frequency dependence of the capacitance caused by the presence of the electrically distinct types of substructural region. (b) The capacitance of the bilayer/electrolyte system (▲. . . . . .▲)) and the bilayer alone (●————●) on an expanded scale. The impedance of the electrolyte was subtracted using the value of G_E measured in the absence of the bilayer.

TABLE II

SUBSTRUCTURAL PARAMETERS FOR PHOSPHATIDYLCHOLINE/TETRADECANE BILAYERS

Data are mean ± S.D. BLM, bilayer membrane. For bilayers in 1000 mM KCl it was found to be very difficult to obtain suitable frequency scans for fitting to the multilayer model because of technical difficulties associated with the very small frequency dispersions and problems due to spontaneous jumps in the bilayer conductance. Only on two occasions was it possible to get frequency scans of sufficient quality to permit resolution of the three substructural layers. From the fitting of these it was found that the capacitance at 1 Hz is very similar to the C_H value. Such data for an additional six bilayers were thus included in the statistics for C_H and G_H values in 1000 mM KCl.

	KCl (mM)			
	1 (4BLM, 7 scans)	10 (6BLM, 12 scans)	100 (5BLM, 13 scans)	1000 (8BLM, 2 scans)
Capacitance (mF/m^2)				
C_H	5.35 ± 0.05	5.43 ± 0.06	5.5 ± 0.1	5.8 ± 0.1
C_A	390 ± 50	450 ± 25	370 ± 50	480 ± 80
C_P	275 ± 100	425 ± 40	500 ± 100	850 ± 100
Conductance (mS/m^2)				
G_H	0.75 ± 0.35	8.9 ± 0.1	17 ± 10	15 ± 10
G_A	470 ± 100	360 ± 40	2 500 ± 500	1 500 ± 300
G_P	16 500 ± 650	20 300 ± 1700	27 500 ± 5000	41 000 ± 4000
Time constant (s)				
τ_H	15 ± 7	0.63 ± 0.03	4.7 ± 4.2	5 ± 4
τ_A	0.90 ± 0.13	1.35 ± 0.18	0.26 ± 0.15	0.33 ± 0.1
τ_P	0.018 ± 0.007	0.023 ± 0.004	0.020 ± 0.005	0.020 ± 0.04

pletely 'black' and their capacitance had reached a steady value, indicating that any alkane 'dissolved' in the bilayer had reached its equilibrium value.

Bilayers formed with n-decane

The following differences were found for bilayers formed with *n*-decane solvent.

(a) Their total capacitance at low frequencies was significantly lower (e.g. in 1 mM KCl the capacitance at 1 Hz was only approx. 4.1 mF/m^2 after 6 h compared to approx. 5.3 mF/m^2 for tetradecane). This is consistent, as has been often reported previously, with the presence in the bilayer of comparatively large amounts of this shorter chain-length alkane solvent.

(b) The bilayers continued to thin slowly throughout their lifetime, unlike bilayers formed with tetradecane which reach a stable thickness some 1—3 h after they become completely 'black'. Such an effect of decane has been reported previously for bilayers formed from phosphatidylcholine [11] and phosphatidylcholine cholesterol [12]. Generally, however, most reports do not indicate either the age of the bilayers or whether they had reached equilibrium. It was found that the capacitance of bilayers formed with decane still increased at a rate sufficient to badly distort the measured frequency dispersion even 6 h after becoming completely black and so resolution of substructural parameters for these bilayers was not possible.

Discussion

The hydrophobic region

The hydrophobic capacitance C_H is seen to increase with increasing external KCl concentration. Assuming $\epsilon_H = 2.13$ [4] the thickness δ_H of the hydrophobic region in different external KCl varies as follows:

KCl (mM)	1	10	100	1000
δ_H (nm)	3.52	3.47	3.42	3.25

The fully extended acyl chain length for egg phosphatidylcholine is 4.2 nm, and hence some effective shortening of the projected chain length must occur. X-ray diffraction data for multilayers appears to rule out any interdigitation. Our measured values for δ_H could possibly arise from:

(a) Chain kinking. An estimate of an average of 3.5 kinks/chain would reduce δ_H by approx. 0.4 nm [13].

(b) The two acyl chains on each phosphatidylcholine may penetrate different amounts into the hydrophobic region. If the chains are out of step in the two opposing monolayers this could effectively reduce the width by up to 0.2 nm [13].

(c) There is some evidence that water may penetrate the bilayer beyond the carboxyl groups of the fatty acids, perhaps as far as the β carbon of the acyl chains. Thus the hydrophobic region of low dielectric constant may not necessarily include all of the acyl chains.

Ionic double layers

A possibility that must be considered is that the measured bilayer capacitance may be underestimated because of the presence of ionic double layers in the external electrolyte. These arise because the charge associated with potential differences across the bilayer is not completely confined to the bilayer/electrolyte interface, but extends into the external electrolyte to a distance governed by the Debye length for mobile ions. This gives rise to an additional capacitance at each electrolyte interface which will appear in series with the membrane and possesses a magnitude equal to that of a parallel plate capacitor with plates separated by a Debye length [14]. Such an effect has been observed for bilayers formed from glyceryl 1-monoolein in *n*-decane [15]. If there is no surface charge on the bilayer and the electrolyte concentration remains unaltered right up to the bilayer interface, then the corrected values of C_H would be

KCl	(mM)	1	10	100	1000
Corrected C_H	(mF/m^2)	6.26	5.70	5.59	5.80

It is apparent that in 1 mM KCl the membrane capacitance could be severely underestimated. Such effects will be reduced, however, if the mobile ion concentrations at the bilayer/electrolyte interface are higher than in the bulk electrolyte. The specific volume conductivity of the polar head region, however, was found to be lower than that of the external electrolyte (by a factor of approx. 10^{-5} in 1 mM KCl), which would suggest that an enhancement of ion concentration in that region probably does not occur. Alternatively the presence of a net surface charge on the bilayer would produce a concentration increase at the interface and it has been suggested [16] that the surface charge is usually high enough for the effects of double layers to be neglected.

Exact solutions of the Nernst-Planck-Poisson equations for a bilayer/electrolyte system without substructure indicate that the presence of ionic double layers does not introduce any additional frequency dispersion [9], and thus they cannot be characterised with our impedance measurements. The possibility that the observed increase in capacitance with increasing KCl concentration is to some degree a consequence of the presence of external ionic double layers thus cannot be completely dismissed.

How much solvent is present?

The bilayers reported here were formed from a solution of egg phosphatidylcholine in alkane solvent, and an equilibrium amount of alkane will remain 'dissolved' in the bilayer when it reaches its steady-state thickness. For *n*-decane solvent, however, it was found that phosphatidylcholine bilayers did not reach equilibrium even after several hours. The dielectric properties of the alkane and the acyl chains are nearly identical, and hence the presence of the alkane does not alter intrinsic properties of the hydrophobic region, although it may change its thickness.

For *n*-tetradecane solvent in 100 mM KCl electrolyte we found $C_H = 5.5 \pm 0.1\ mF/m^2$, a value slightly higher than the $5.15 \pm 0.1\ mF/m^2$ reported elsewhere [4] for the total capacitance of this system. Comparison of our results with those reported [4] for egg phosphatidylcholine/hexadecane systems ($C = 6.03 \pm 0.1\ mF/m^2$), where the alkane solvent concentration is thought to be very low, suggests that in well-drained tetradecane bilayers the equilibrium concentration of solvent is not very different from hexadecane bilayers.

It is thought that essentially 'solvent-free' bilayers can be formed by various monolayer apposition techniques, although some solvent is still necessary [17]. The capacitance of bilayers formed by such techniques is 7.6 ± 0.4 mF/m^2 for egg phosphatidylcholine [18] and 7.2 ± 0.2 mF/m^2 for dioleoylphosphatidylcholine [11,19]. This suggests that the equilibrium amount of solvent in phosphatidylcholine bilayers formed from lipid solutions in tetradecane or hexadecane are similar in comparison with 'solvent-free' bilayers.

The polar head region

Inspection of Table II shows that both G_P and C_P values increase systematically with increasing external KCl concentration. The time constant $\tau_P(=C_P/G_P)$ of this region, however, does not systematically depend upon the external salt concentration. This suggests that the dielectric and conduction properties of this region are not salt-dependent and the observed variations in C_P and G_P values are mainly consequences of a change in thickness of this region. If we assume, for example, that $\epsilon_P = 25$, the width δ_P of the polar head region $(= \epsilon_0\epsilon_P/C_P)$ varies in the following fashion with external KCl concentration:

KCl (mM)	1	10	100	1000
δ_p (nm)	0.8	0.52	0.44	0.25

These figures are consistent with the cholinephosphate dipole having an orientation normal to the membrane/solution interface in 1 mM KCl and changing to parallel configuration in 1000 mM KCl. In contrast studies on multilayers [6,20,21] show that in such systems (where intermembrane interactions maintain the long-range laminar order), the dipole configuration is parallel to the bilayer surface. The relative independence of G_P on the external KCl concentration indicates that the neutralisation of the cholinephosphate dipole is essentially complete [1].

The specific conductivity of this region is very low in comparison with that of the external electrolyte, being smaller by a factor of 10^{-5} in 1 mM KCl and 10^{-9} in 1000 mM KCl. This suggests that ion partitionining into this region is quite unfavorable.

The acetyl region

The putative acetyl region exhibits a large capacitance that is essentially independent of the external KCl concentration (see Table II). This high value implies a reasonably high dielectric constant for this region, otherwise our values of capacitance would imply an unrealistically * small thickness. This region thus appears to be quite distinct in character from the hydrophobic region. With a dielectric constant of $\epsilon_A = 30$, $\delta_A \approx 0.35$—0.4 nm; a width not inconsistent with the measured separation of the β carbon atoms on the acyl chains from the near-end of the cholinephosphate dipole [6]. The conductance of this region was also found to be intermediate to those of the hydrophobic and polar head regions. The evidence thus strongly suggests that this region be

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* e.g. if the dielectric constant was similar to that of the hydrophobic region (approx. 2) then $\delta_A \approx 0.05$ nm.

ascribed to the layer containing the carboxyl ester portions of the phosphatidylcholine molecules. This region therefore forms an interface between the hydrophobic acyl chains and the hydrophilic regions containing the polar heads. ESR studies on phosphatidylcholine vesicles [5] suggest that water may penetrate into the bilayer as far as the α and β carbons of the acyl chains. It is possible, however, that this is in some degree a consequence of the presence of the bulky nitroxide reporter group used in these studies (impedance measurements on bilayers indicate that such reporter groups can perturb substructure at quite low concentrations [12]). Our results are consistent with the notion that the hydrophobic region does not extend beyond the β carbons of the acyl chains.

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