

STUDIES OF ELECTRIC CAPACITANCE OF MEMBRANES

I. A MODEL MEMBRANE COMPOSED OF A FILTER PAPER AND A LIPID ANALOGUE

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ABSTRACT A hydrophobic filter paper of a given pore size containing a synthetic lipid, i.e. dioleoyl phosphate, was interposed between aqueous electrolyte solutions having the same chemical composition and temperature. The electric capacitance and conductance of the membrane immersed in various concentrations of KCl were measured in the frequency range from 20 to 3×10^6 cycle/sec. The observed capacitance and conductance were found to be strongly dependent on the applied frequency. A theory is proposed to account for this dispersion of impedance observed in the present membrane-electrolyte system. The dispersion is attributed to the formation of bilayer membranes of the lipid inside the filter paper. The effects of the salt concentration, the adsorbed quantity of the lipid, and the pore size of the filter paper on the capacitance and conductance of the membrane are discussed in terms of the distribution function of bilayers formed within the filter paper.

INTRODUCTION

The electric capacitance observed in living cells has been the subject of considerable study in the field of neurophysiology, especially in connection with excitable membranes (1). However, little is known about the physicochemical mechanism of the capacitance. This stems mainly from the lack of our knowledge of the detailed structure of the living membrane. Membranes of known physicochemical structure, (e.g. a lipid bilayer membrane and/or an asymmetric ion-exchange membrane composed of cation and anion exchangers) are known to exhibit a frequency-dependent capacitance (2, 3). But the magnitudes of the capacitance and conductance as well as the critical frequency for dispersion of impedance in these membranes are quite dif-

ferent from those observed in living cells. In order to clarify the cause of the capacitance observed in living cells, we have either to determine the chemical structure of living membrane itself, or to make a model system of known chemical structure with an artificial membrane which has an impedance similar to that of a living membrane. In this series of papers we have taken the latter approach. A similar attempt has been discussed in a recent paper by Ilani (4).

In this paper the model system is a filter paper that contains a synthetic lipid, i.e., the dioleoyl phosphate (DOPH). We propose a theoretical model which enables us to calculate the frequency dependence of capacitance and conductance for the Millipore-DOPH system. The observed capacitance and conductance for the Millipore-DOPH system with filter paper of various pore sizes and various concentrations of external KCl solution are in reasonable agreement with the theory. Subsequent papers will be concerned with more complicated natural lipids such as phosphatidyl serine, phosphatidyl ethanolamine, and lecithin, in view of the theory proposed here.

EXPERIMENTAL

Materials, Apparatus, and Cell

The dioleoyl phosphate was kindly furnished by Miss M. Yoshida of the Tokyo Institute of Technology, Tokyo, Japan. A chloroform solution of the calcium salt of DOPH was purified by passing it through a column of Ca silicate. It was converted to the acid form by using an ion exchange column of Dowex 50-8X (Dow Chemical Co., Midland, Mich.). A check by mass-spectroscopic analysis showed that DOPH thus obtained was 98% of the material used; the rest was recognized to be an alkyl chloride. Further purification gave no appreciable change of the dielectric behavior of the membrane. Millipore filter papers (Millipore Filter Corp., Bedford, Mass.) of a given pore size were immersed overnight in a benzene solution of DOPH at various weight concentrations. The immersed filter paper was dried in the air and weighed. The quantity of DOPH adsorbed by the Millipore filter was determined by weighing. The adsorbed quantities, Q , of DOPH on a Millipore filter examined were between 0.1 and 10 mg per cm² of the filter paper. The filter paper containing DOPH was conditioned overnight in an aqueous solution of 1 mM KCl, and used as the sample membrane. As will be shown later, the concentration of KCl solution for the conditioning was immaterial, since the observed capacitance, C_p , and conductance, G_p , varied reversibly with the concentration of the external solution with which the membrane was in contact. The water used as solvent was prepared by treating distilled water with both cation and anion exchangers; the carbon dioxide dissolved in it was not degassed. Before use, KCl was purified by repeated recrystallization.

The impedance was measured by a dielectric loss bridge (Ando Electric Co., Ltd., Tokyo, Japan, type TR-1B). A G-C box (Ando Electric Co., Ltd., type YS-1) and decade resistor and capacitor (Yokogawa Electric Co., Tokyo, Japan, model

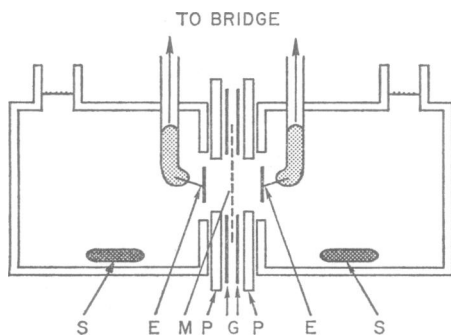


FIGURE 1 Schematic diagram of the cell used. *E*: Pt-Pt electrode ($1 \times 1 \text{ cm}^2$); *P*: spacer of plastic plate having a bore of 1.5 cm in diameter; *G*: silicon rubber gasket; *M*: membrane; *S*: magnetic stirrer tip.

CD-41) were combined with the bridge when necessary. The bridge was designed for measurements with conductive materials, and was suitable for the large variations both in capacitance and conductance with applied frequency that were encountered here. The measurable range for capacitance and conductance of the apparatus used were $1\text{--}10^6 \text{ pF}$, and $0.1\text{--}10^6 \text{ }\mu\text{mho}$, respectively, with reasonable accuracy in the whole range examined. The measurements were carried out between 20 and 3×10^6 cycle/sec in applied frequency.

One of the major problems in the present membrane-electrolyte system is that the impedance of the membrane phase should be discriminated from that of the solution phase. Also, it is known that the major source of experimental error with conductive media is electropolarization of the electrode in the low frequency region. In order to overcome these difficulties, the cell was designed so that the distance between the two electrodes was variable. Fig. 1 shows a schematic diagram of the cell used. The distance between two Pt-Pt plate electrodes, each 1 cm^2 in area, was changed by inserting a pair of plastic plates, having a bore of 1.5 cm in diameter, in two sides of the membrane; this electrode distance, d , was measured by a travelling microscope. The distance d was varied between 0.2 cm and 2.35 cm. The Cole-Cole plot (5) was used to obtain the low frequency plateau of dispersion curves when necessary.

All measurements were made in an air oven regulated at 25°C .

Results

By way of an example, the observed capacitance C_p and conductance G_p for a given membrane (5μ pore size of Millipore filter with 1.51 mg/cm^2 of adsorbed DOPH) with 2 mM KCl solution are shown in Fig. 2 as functions of frequency, ν , at various electrode distances. The ordinate for C_p and G_p and abscissa for ν are given in logarithmic scale. In Fig. 2, the C_p and G_p for the filter paper of the same pore size with no DOPH are shown (broken lines) for the case where d is fixed at 0.225 cm. It is noted that C_p and G_p do not exhibit any dispersion for the latter case,¹ and that the

¹ The frequency dependence of C_p and G_p shown by broken lines in Fig. 2 is to be due to the electrode polarization (6, 7).

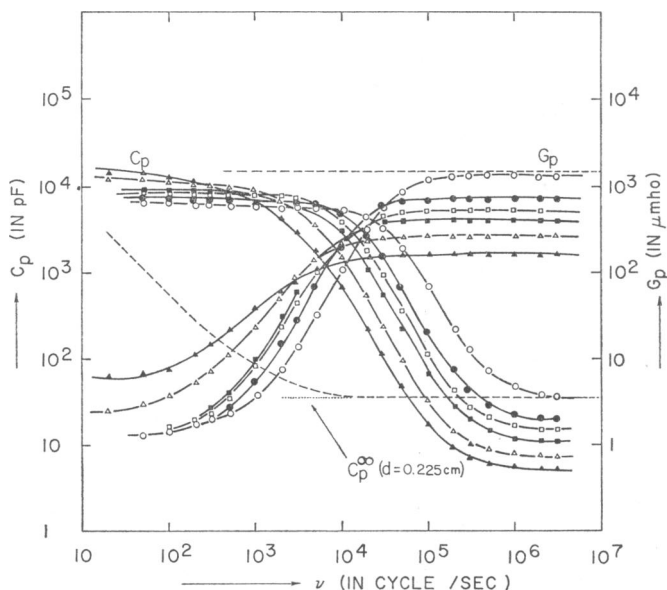


FIGURE 2 Plots of observed capacitance C_p and conductance G_p against frequency ν , with various electrode distance d . The concentration of KCl $c_s = 2$ mM, the pore diameter of the Millipore filter $\phi = 5 \mu$, the adsorbed quantity of DOPH $Q = 1.51$ mg/cm², and temperature = 25°C. \circ : $d = 0.225$ cm; \bullet : $d = 0.435$ cm; \square : $d = 0.656$ cm; \blacksquare : $d = 0.872$ cm; \triangle : $d = 1.47$ cm; \blacktriangle : $d = 2.35$ cm. Broken lines show C_p and G_p for a Millipore filter alone, with $c_s = 2$ mM KCl, $d = 0.225$ cm, $\phi = 5 \mu$, and $Q = 0$ mg/cm².

values of C_p and G_p at high frequencies for the system containing DOPH approach those for Millipore filter alone (see open circles). The limiting values of C_p and G_p at the high frequency, (denoted hereafter C_p^∞ and G_p^∞) are found to be inversely proportional to the electrode distance. Fig. 3 A shows C_p^∞ and G_p^∞ plotted against d^{-1} ; Fig. 3 B shows C_p and G_p at the low frequency limit (denoted C_p^0 and G_p^0) as a function of d . Note that C_p^0 increases with the distance d between two electrodes. This implies that the edge capacitance (6, 7), i.e. the capacitance that comes from nonuniformity of the electric field around the peripheral edges of the plate electrode, contributes to the observed capacitance. In other words, the effective area of the membrane increases with the electrode distance d , since the area of the membrane is larger than that of a pair of electrodes. We can evaluate the membrane capacitance by extrapolating to $d = 0$ of C_p^0 , where only the area of the electrode (1 cm² in the present case) is effective to the membrane. However, as seen in Figs. 2 or 3 B, even when the electrode distance d is finite, the value of C_p^0 does not differ much from C_p^0 at $d = 0$, as long as d is not very large. Therefore, we shall use the observed value of C_p at $d = 0.225$ cm for the subsequent arguments in this paper unless otherwise noted.

Fig. 4 shows the dependence of C_p and G_p on the amount of adsorbed DOPH, Q , in the Millipore filter of a given pore size. The nominal pore diameter, ϕ , is 5μ ,

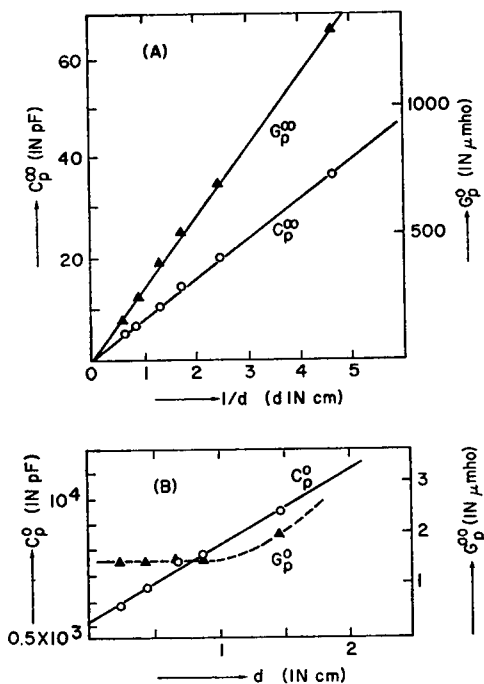


FIGURE 3 (A) C_p^∞ and G_p^∞ as a function of the inverse of electrode distance, $1/d$. (B) C_p^0 and G_p^0 as a function of d . The system is the same as in Fig. 2.

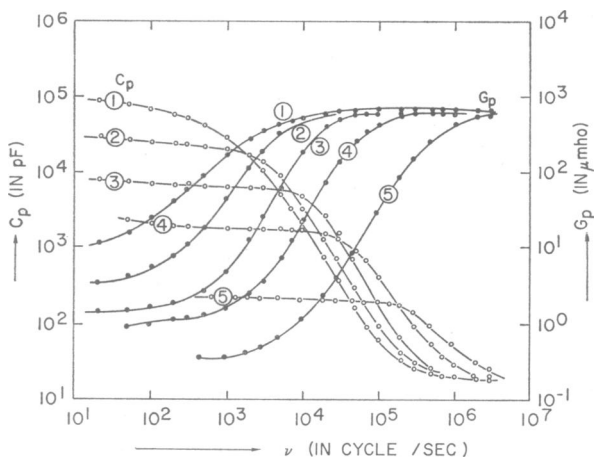


FIGURE 4 C_p and G_p as a function of frequency ν , with various absorbed quantity of DOPH, Q . Pore size of the Millipore filter is 5μ ; the electrode distance d is fixed at 0.225 cm . Curve 1: $Q = 0.17 \text{ mg/cm}^2$; curve 2: $Q = 0.42 \text{ mg/cm}^2$; curve 3: $Q = 1.31 \text{ mg/cm}^2$, curve 4: $Q = 2.69 \text{ mg/cm}^2$; curve 5: $Q = 9.69 \text{ mg/cm}^2$.

the concentration of the external solution is 2 mM KCl , and d is 0.2 cm . It is observed that the value C_p^0 decreases with increase of Q , and that both C_p^∞ and G_p^∞ are almost independent of Q in the range studied. The capacitance C_p in the low frequency region increases with decrease of frequency when Q is small (see curves 1 and 2 in Fig. 4).

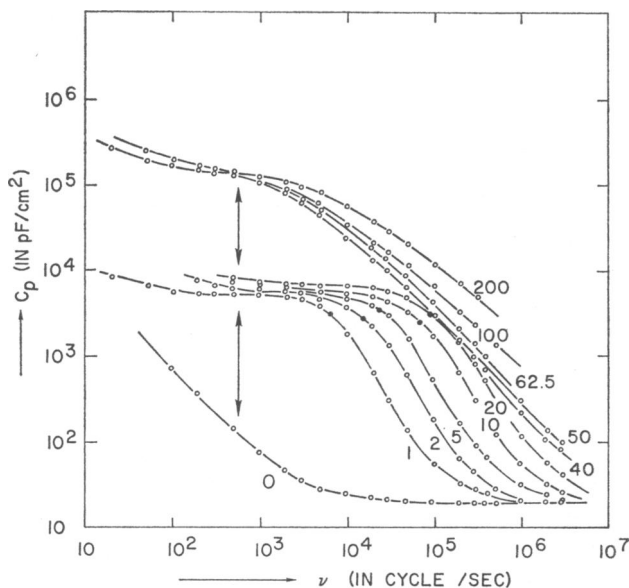


FIGURE 5 Relations between C_p and ν with various concentration of KCl. The pore size of Millipore filter $\phi = 5 \mu$, electrode distance $d = 0.225$ cm, and absorbed quantity of DOPH $Q = 2.20$ mg/cm². Number on each line shows the concentration of KCl in mM. Darkened circles show characteristic frequency of C_p dispersion for a given KCl concentration.

Fig. 5 relates $\log C_p$ to $\log \nu$ at various concentrations of the external KCl solution for a membrane with $\phi = 5 \mu$ and $Q = 2.20$ mg/cm². As seen in the figure, the value of C_p^0 does not change very much when the concentration of the external solution is changed from 1 to 20 mM KCl. However, if the concentration of KCl is raised to 40 mM, C_p in the low frequency region rises rather discontinuously more than 10 times, and remains at the same value with further increase of the external KCl concentration. On the other hand, when the external salt solution is replaced by a distilled water, the capacitance becomes almost the same as that of Millipore filter alone (see broken line in Fig. 2). A similar abrupt change in the dispersion curve of C_p can be observed when a divalent cation species such as Ca^{++} is added to the external salt solution. The effects of Ca^{++} , pH value, salt species, as well as salt concentration of the external solution, are quite different from one lipid to the other. The behaviors are reversible as long as the system stands more than 2 hr after the external solution is replaced by a new solution of a given composition.

THEORY

Membrane Model and Assumptions

To make the problem under study clear, the experimental facts described above and some relevant observations are summarized as follows: (a) all lipids and DOPH

that were used have the characteristic of forming a bilayer membrane under appropriate conditions (reference 2, and T. Hanai, S. Morita, and M. Yoshida, private communication); (b) at low frequency region the observed capacitance approaches an extraordinarily large value (10^3 – 10^6 pF/cm²); (c) this large value of C_p^0 is, in general, observed when the value of pH in the external solution is close to the isoelectric point of the lipid used; (d) variations in both C_p^0 and G_p^0 with environmental conditions such as salt concentration, pH value, and the presence of Ca^{++} ions, etc., are of the order of magnitude of 10^4 – 10^5 ; (e) a simple charged membrane, e.g. an ion exchange membrane (with various fixed charge densities), never shows a large capacitance increase with frequency change (M. Yuasa and Y. Kobatake, unpublished data); and (f) the emulsions of the lipids used give no large capacitance dispersion at low frequencies. Considering all of these, we may infer that the observed frequency-dependent capacitance for the system can be attributed to the formation of foamlike bilayer membranes in the network skeleton of cellulose constituting the Millipore filter paper. The foams formed in the filter paper by lipid bilayers must enclose the electrolyte solution. Since the DC conductance of a lipid bilayer membrane is extremely small compared with that of an electrolyte solution (2), the electrolyte solution enclosed in a bilayer foam does not contribute to the DC conductance of the over-all membrane system, but participates in its AC conductance.

To make the theoretical analysis possible for the membrane model described above, we choose a capillary model for the membrane. The membrane is supposed to consist of two portions: a solid portion and an array of uniform cylindrical capillaries having equal cross-sectional areas. The length of capillary is denoted by L . Ions and water molecules can penetrate the membrane only through the capillary portions when the membrane contains no lipid. The lipid adsorbed in the filter paper forms bilayer membranes in some of the capillaries as schematically shown in Fig. 6. The fraction of capillaries having no bilayer membrane is denoted by f_0 , the fraction of capillaries having only one bilayer membrane is f_1 , that having i bilayer membranes

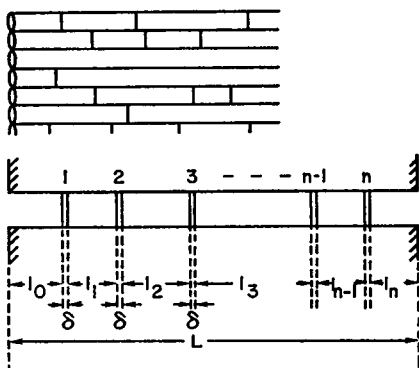


FIGURE 6 Schematic diagram of the membrane model used, including the symbols used in subsequent analysis.

is f_i , and so on. Then we have

$$\sum_{i=0}^{\infty} f_i = 1. \quad (1)$$

The electrical resistance (DC resistance) of a DOPH bilayer membrane is known to be $1 \times 10^8 \Omega/\text{cm}^2$, while the capacitance of a bilayer membrane has been determined to be $0.35 \pm 0.03 \mu\text{F}/\text{cm}^2$ irrespective of the concentration of the electrolyte solution with which the bilayer membrane is in contact (Hanai et al., private communication). Therefore, the impedance Z for the system in which a bilayer membrane of DOPH is in contact with a salt solution having the electrical resistance r ($\Omega \cdot \text{cm}^2$) can be represented by the following equation for unit area (see Appendix I):

$$Z = r - j/\omega C, \quad (2)$$

where C is the capacitance of unit area of DOPH bilayer membrane, ω is the applied angular frequency ($\omega = 2\pi\nu$), and j is $(-1)^{1/2}$. Equation 2 implies that the conductance of the bilayer membrane is negligibly small compared to that of the external electrolyte solution. Then the impedance of a capillary containing n bilayer membranes is represented by the equation

$$Z_n = \sum_{i=0}^n Z_i = \sum_{i=0}^n (r_i - j/\omega C_i), \quad (3)$$

where r_i is the electrical resistance of the solution layer confined between i th and $(i + 1)$ th bilayer membranes in the capillary (see Fig. 6, bottom), and C_i is the electric capacitance of i th bilayer membrane in the capillary containing n bilayers. According to the model described above, the capillaries having n bilayers are connected in parallel with the fraction f_n , we have the following equation for the total impedance for the membrane in question

$$1/Z_p = \sum_{n=0}^{\infty} f_n/Z_n = \sum_{n=0}^{\infty} \left(\frac{f_n \omega^2 C_n^2 r_n}{1 + \omega^2 C_n^2 r_n^2} \right) + j\omega \sum_{n=0}^{\infty} \left(\frac{f_n C_n}{1 + \omega^2 C_n^2 r_n^2} \right), \quad (4)$$

where C_n and r_n are defined by the equations

$$1/C_n = \sum_{i=1}^n (1/C_i),$$

(5)

and

$$r_n = \sum_{i=0}^n r_i.$$

As noted above, the electric capacitance of a bilayer membrane is known to be constant irrespective of the concentration of the external electrolyte solution, and given

by (reference 2):

$$C = \epsilon/4\pi\delta. \quad (6)$$

Here δ is the thickness of the bilayer membrane, and ϵ is the dielectric constant of the lipid used. Thus the capacitance of a capillary containing n bilayer membranes, C_n , can be simplified to give

$$C_n = C/n. \quad (7)$$

Denoting the length of the solution layer confined between i th and $(i + 1)$ th bilayers by l_i , we have the relation

$$L = \sum_{i=0}^n (l_i + \delta) = \sum_{i=0}^n l_i + n\delta.$$

Since the thickness, δ , of the lipid bilayer is known to be about 50 Å (2), while the length of the capillary (which is equivalent to the effective thickness of the Millipore filter) L is about 10^{-2} cm, $n\delta$ is less than a few per cent of L even when the number of bilayer membrane, n , is as large as 10^3 . Then to a first approximation, we have $\sum_{i=0}^n l_i = L$. Denoting the equivalent conductivity of the electrolyte solution by Λ in mho·cm²/mole, and the concentration of the electrolyte component by c_s in moles/10³·cm³, we have the following expression for the electrical resistance for the salt solution confined between i th and $(i + 1)$ th bilayer membranes:

$$r_i = (l_i/\Lambda c_s) \times 10^3 \text{ in mho} \cdot \text{cm}^2.$$

The AC resistance of a capillary having n bilayers, r_n , is then represented by the equation

$$r_n = \sum_{i=0}^n r_i = \sum_{i=0}^n l_i \times 10^3/\Lambda c_s = L \times 10^3/\Lambda c_s = R_0(\Omega \cdot \text{cm}^2). \quad (8)$$

Here, R_0 denotes the electrical resistance of a capillary having no bilayer membrane. Equation 8 implies that the capillary containing lipid bilayer membranes has the same AC resistance as that of a capillary having no bilayer membrane as far as the condition that $L \gg n\delta$ is fulfilled. It is important to note that the capillary containing at least one bilayer membrane does not contribute to the DC conductance, since the DC resistance of a DOPH bilayer membrane is several orders of magnitude higher than that of the bathing media. Furthermore, it is noted that no difference may be observed in AC conductance between the case where discrete bilayers are formed in a capillary as depicted in Fig. 6, and the case in which globular micelles plug up a capillary (8), or even in the case where an oil droplet plugs up a capillary, so far as the condition $L \gg n\delta$ is satisfied.

Equations for Capacitance and Conductance of the Membrane

The reciprocal of the observed impedance Z_p is expressed in terms of capacitance C_p and conductance G_p as

$$1/Z_p = G_p + j\omega C_p. \quad (9)$$

Introducing equations 7 and 8 into equation 4, and equating the resulting equation with equation 9, we obtain the following expression for C_p and G_p :

$$C_p = C_0^0 + \sum_{n=0}^{\infty} \frac{f_n(C/n)}{[1 + \omega^2(C/n)^2 R_0^2]}, \quad (10)$$

$$G_p = \sum_{n=0}^{\infty} \frac{f_n \omega^2 (C/n)^2 R_0^2}{[1 + \omega^2 (C/n)^2 R_0^2] R_0}. \quad (11)$$

Here, C_0^0 has been introduced for convenience, because we may observe a capacitance C_0^0 at the high frequency region even when there is no membrane in the system. Detailed discussion on C_0^0 is given later (see also Appendix II).

With the critical relaxation time τ for a bilayer membrane with a given concentration of the electrolyte solution defined by

$$\tau = CR_0 = CL \times 10^3 / \Lambda c_s, \quad (12)$$

equations 10 and 11 are simplified to give

$$C_p = C_0^0 + \sum_{n=0}^{\infty} \frac{nf_n C}{[n^2 + (\omega\tau)^2]}, \quad (13)$$

$$G_p = \sum_{n=0}^{\infty} \frac{f_n (\omega\tau)^2}{[n^2 + (\omega\tau)^2] R_0}. \quad (14)$$

The relaxation time τ given by equation 12 is considered to be constant for a given lipid bilayer membrane and an electrolyte solution of a fixed concentration. Thus the present problem has been reduced to that of knowing the distribution function f_n of a system for a given pair of membrane and salt solution. The distribution function f_n may vary with the pore size, ϕ , of the filter paper used, the quantity of adsorbed lipid, Q , as well as the composition of the external electrolyte solution. However, f_n is considered to be independent of the applied frequency ν . Then we may obtain information about f_n for a given system by changing the frequency ν . For example, C_p and G_p at low frequency limit, i.e. C_p^0 and G_p^0 , are represented by the equations

$$C_p^0 = C_0^0 + \sum_{n=1}^{\infty} (f_n/n) C, \quad (15)$$

$$G_p^0 = f_0/R_0.$$

On the other hand, the observed capacitance and conductance at the high frequency limit, C_p^∞ and G_p^∞ , are given by

$$\begin{aligned} C_p^\infty &= C_0^0, \\ G_p^\infty &= (\sum_{n=0}^{\infty} f_n)/R_0 = 1/R_0. \end{aligned} \quad (16)$$

The agreement between G_p^∞ and $1/R_0$ (with no adsorbed lipid) can be used as a partial check of the assumptions used above. Combining relations given equations 15 and 16, we obtain

$$\begin{aligned} (C_p^0 - C_p^\infty)/C &= \sum_{n=1}^{\infty} (f_n/n), \\ (G_p^\infty - G_p^0)/G_p^\infty &= \sum_{n=1}^{\infty} f_n = 1 - f_0, \end{aligned} \quad (17)$$

where equation 1 has been introduced. The above two equations are combined to give

$$\left(\frac{C_p^0 - C_p^\infty}{G_p^\infty - G_p^0} \right) \frac{G_p^\infty}{C} = \sum_{n=1}^{\infty} (f_n/n) / \sum_{n=1}^{\infty} f_n = \overline{(1/n)}. \quad (18)$$

Here $\overline{(1/n)}$ represents the average of the reciprocal number of bilayer membranes formed in the filter paper.

Further Approximation of Theoretical Equations

As noted above, detailed dielectric behavior of the system in question can be described in terms of the distribution function f_n of the membrane. To a first approximation, however, we may take a simple δ -function approximation for describing the general behavior of the system, where only two f_n 's at $n = 0$ and $n = k$ have finite values, and the other f_n assumed to be zero. Then we have

$$f_0 + f_k = 1,$$

and equations 13 and 14 are simplified to give

$$C_p = C_0^0 + \frac{k f_k C}{[k^2 + (\omega\tau)^2]}, \quad (19)$$

$$G_p = \frac{f_0}{R_0} + \frac{f_k (\omega\tau)^2}{[k^2 + (\omega\tau)^2] R_0}. \quad (20)$$

Equations 19 and 20 are rearranged to give the relations for a single dielectric dis-

persion given by Debye (9), with the following critical frequency

$$\nu_c = \tau/2\pi k = CR_0/2\pi k. \quad (21)$$

The values of f_0 and f_k as well as k ($= \bar{n}$) are evaluated from the experimental data of C_p^0 , G_p^0 , C_p^∞ , and G_p^∞ . Using these characteristic constants for the system of a given membrane and electrolyte, we can compare the theory with experiments at an arbitrary frequency ν . It must be remembered that the agreement of equations 19 and 20 with experiments does not always support the correctness of the underlying δ -function approximation.

DISCUSSION

Comparison between Theory and Experiment

With the assumption introduced above, i.e. $G_p^\infty = 1/R_0$, together with equation 8, G_p^∞ can be approximated by the equation

$$G_p^\infty = \Lambda c_s \times 10^{-3} (A/d) \quad \text{in mho,}$$

where A is the area of the electrode in cm^2 , and d is the distance of two electrodes in cm. On the other hand, C_0^0 , i.e. C_p^∞ , may be approximated by the equation

$$C_p^\infty = (\epsilon/4\pi\epsilon_0) (A/d) \quad \text{in pF,}$$

where ϵ is the dielectric constant of the solvent, 80 for the present case at 25°C , ϵ_0 is the absolute dielectric constant of free space. Then we have

$$C_p^\infty = 7.083 (A/d) \quad \text{in pF.}$$

The equations given above indicate that both C_p^∞ and G_p^∞ are inversely proportional to the electrode distance d . These relations were already shown in Fig. 3 A. Elimination of (A/d) from C_p^∞ and G_p^∞ leads to

$$G_p^\infty/C_p^\infty = 24.3$$

when the concentration of KCl solution is 2 mM as in Fig. 2. The experimental value of G_p^∞/C_p^∞ gives 28.6 ± 0.8 for the whole range of d examined. This discrepancy between theory and experiment can be attributed to the effective area of the Millipore filter paper (84% for the present system).

Taking one pair of curves of C_p and G_p from the data given in Fig. 4, and using equations 17 and 18 with the δ -function approximation, we obtain f_0 , f_k as well as the value of k for a given system. With the use of these experimentally determined values, equations 19 and 20 are compared to experiments at an arbitrary frequency. The comparison is made in Fig. 7 for several systems, where the data are the same as

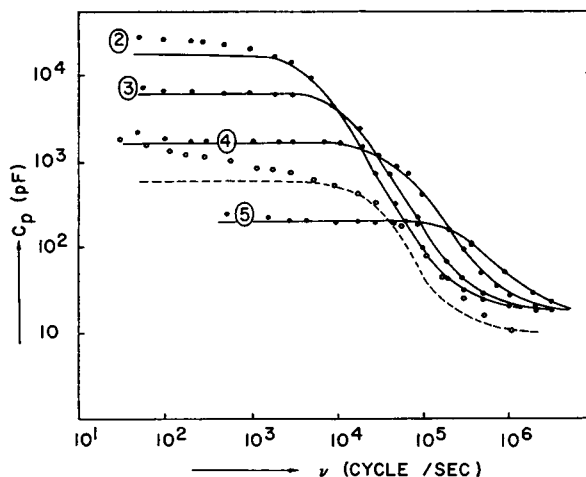


FIGURE 7 Comparison between theory and experiments for C_p as a function of frequency ν , for the systems of $\phi = 5 \mu$, and $c_s = 2 \text{ mM KCl}$. The data and the notations are the same as in Fig. 4. Darkened circles are experimental data, and solid lines show theoretical curves calculated from Equation 19. Open circles show the experimental C_p for the system of $\phi = 10 \text{ m}\mu$, $Q = 2.0 \text{ mg/cm}^2$, and $c_s = 2 \text{ mM KCl}$. Corresponding theoretical values are given by a broken line.

in Fig. 4. The agreement between theory and experiments seems satisfactory, although perhaps fortuitous. As an example of disagreement, Fig. 7 also contains the case where the pore size $\phi = 10 \text{ m}\mu$ with 2 mM KCl aqueous solution and $Q = 2.0 \text{ mg/cm}^2$. Here the observed C_p exceeds that expected by theory in both low and high frequency regions (refer to broken line in Fig. 7). In this case we have to take a broad distribution function f_n rather than δ -function into account. Detailed discussion on the distribution function f_n will be given in a subsequent paper. In general, the δ -function approximation can be used only when the pore size of the Millipore filter is relatively large, and when the adsorbed quantity Q of DOPH is high. This is also seen in Fig. 4, where we observe that the capacitance C_p in the low frequency region increases with decrease of frequency when Q is small (see curves 1 and 2 in Fig. 4). The fact that the observed capacitance in the low frequency region, C_p^0 , decreases with increase of Q as observed in Fig. 4, is attributed to the increase of the average number of bilayer membranes formed in the filter paper (see equation 15).

We wish to point out that $\sum_{n=0}^{\infty} n f_n$ is considered to be a function of total quantity, Q , of DOPH adsorbed in the filter paper. For the system in which δ -function approximation is applicable for f_n , $(k f_k)$ is related to Q . Fig. 8 shows this relation for the case in which the pore size of the Millipore filter used is 5μ , where $\log(k f_k)$'s are plotted against $\log Q$. The slope of the straight line may depend upon the pore size of Millipore filter, but $\sum_{n=0}^{\infty} n f_n$ stays at a constant value irrespective of the salt concentration of the external solution. To proceed with a quantitative discussion along the line mentioned above, the difference between G_p^∞ and R_0^{-1} also has to be

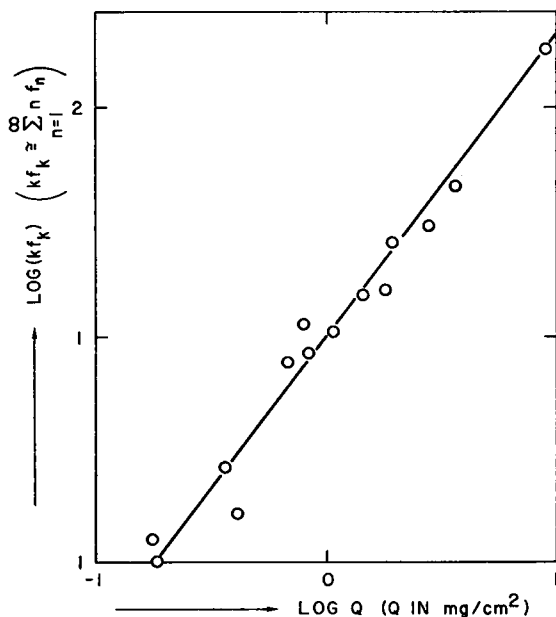


FIGURE 8 $\text{Log } kf_k$ ($kf_k \cong \sum_{n=1}^{\infty} n f_n$) against $\text{log } Q$ relation for the system of $\phi = 5 \mu$, $c_s = 2 \text{ mM KCl}$, at 25°C .

taken into consideration. G_p^∞ and R_0^{-1} do agree with each other within $\pm 5\%$ for all systems studied, although we have not discussed this in detail in the present article in order to make the arguments simple.

The Cole-Cole plot of the observed impedance, i.e. the relationship between $(G_p - G_p^0)/\omega$ and C_p for a given membrane and electrolyte solution, shows a semicircle with the center on the C_p axis as shown in Fig. 9. The data of this figure are taken from Fig. 5. This semicircle implies again the applicability of the δ -function approximation. As seen in Fig. 9, however, the semicircle is gradually depressed with increase of KCl concentration. When the concentration of KCl is brought to 40 mM or more, the impedance locus can never give a semicircle (see open circles in Fig. 9). This implies that the measured capacitance and conductance cannot be represented by a single dispersion given by equations 19 and 20.

The variation of C_p^0 with KCl concentration is discontinuous when the KCl concentration reaches a critical value. This value of concentration depends both upon the adsorbed quantity Q , and upon the pore size ϕ . Fig. 10 shows the values of C_p^0 as a function of KCl concentration, c_s , for $\phi = 5 \mu$ and $Q = 2.20 \text{ mg/cm}^2$. From this figure, we may infer that a change in the configuration of bilayers in the filter paper must be involved at the critical concentration, and that the change should be reflected in the distribution function f_n introduced in this article. In reality, the distribution function f_n in the concentrated KCl solution has been recognized to be a broad

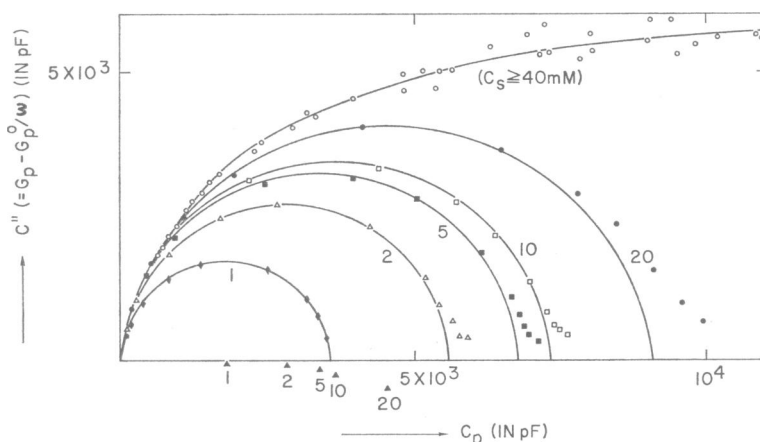


FIGURE 9 $(G_p - G_p^0)/\omega$ against C_p relations with various concentrations of KCl for the system of $\phi = 5 \mu$, $Q = 2.20 \text{ mg/cm}^2$ DOPH. Numbers on semicircles show the concentration of KCl solution in mM, and \blacktriangle -mark with number indicates the center of corresponding semicircle.

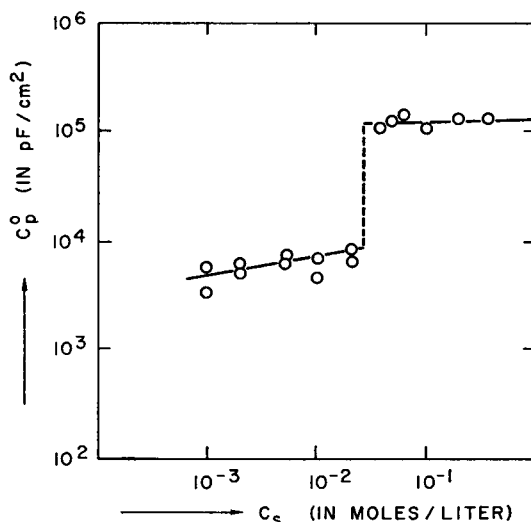


FIGURE 10 C_p^0 as a function of KCl concentration c_s , for the system of $\phi = 5 \mu$, and $Q = 2.20 \text{ mg/cm}^2$ DOPH.

and box type f_n having small average number of bilayers, rather than a δ -function with large k . Note that the variation of C_p and G_p with the external salt concentration is reversible.

Ilani's Observation

In a recent paper, Ilani (4) has demonstrated that a Millipore filter of $10 \text{ m}\mu$ pore size saturated by bromobenzene immersed in an electrolyte solution manifests a

frequency-dependent capacitance. In his observation, the similar but less remarkable frequency-dependent C_p and G_p were reported, and the capacitance in the low frequency region, C_p^0 , rose only to a few hundred pF/cm², to be compared with the values 10⁴–10⁵ pF/cm² in the system reported here. A behavior similar to that in Ilani's system was observed in the case where we use oleic acid with a 10 mμ Millipore filter instead of DOPH or natural lipids. Ilani attributed the observed capacitance to polarization due to displacement of the electric charges of the surfaces between the charged membrane phase and the electrolyte solution. This interpretation seems contrary to our experiments in that we have never observed a dispersion of C_p as was Ilani's observation in a system where a simple charged membrane with various fixed charge densities separates two electrolyte solutions (Yuasa and Kobatake, unpublished data). We wish to point out that the analysis developed here does not exclude the dielectric behavior reported by Ilani, or a Millipore-oleic acid system. In these cases, the assumption that the adsorbed liquid forms a number of bilayer membranes inside the filter paper is questionable; instead the adsorbed oil may be stuck as oil droplets inside the filter paper skeleton. Even in this case, however, the capacitance and conductance can be treated in a similar way as described above by assuming a broad f_n , as will be reported elsewhere.

Concluding Remarks

Even if we confine ourselves to a given pore size of Millipore filter with a fixed adsorbed quantity of DOPH, the theory developed above may be criticized in some respects. The capillary model is one of the possible models for the membrane. One may consider it to be far from realistic to replace a complex matrix of zigzag channels in actual porous membrane by an array of straight, cylindrical capillaries parallel to the membrane thickness. This kind of approximation is, however, rather unavoidable for a quantitative theory to be developed.

The use of δ -function approximation for the distribution function of bilayer membranes may be questioned. At best, it would be approximate even when a single dispersion is observed for frequency dependence of C_p and G_p as shown in Fig. 9. Actually, the δ -function can be replaced by a gaussian distribution function with no appreciable change in C_p and G_p , provided that the center of the gaussian distribution is placed at k , and that the half-width of it is not too broad. One should not forget however, that the δ -function approximation enables us to carry through an analytical treatment of the problem as discussed above.

We would not argue that the mechanism discussed above is the sole cause of the electric capacitance observed in membrane-electrolyte systems. It must be one of many that may account equally well for experimental facts. However, it is our belief that the formation of bilayer membranes inside the filter paper skeletons play a role in the observed dielectric behavior of the system studied here, especially for the appearance of the remarkably large order of magnitude of C_p^0 .

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APPENDIX I

A system which consists of a membrane and an electrolyte solution in series can be represented as a series of two admittances, i.e., the admittance for membrane phase and that for solution phase connected in series (2). Therefore, the i th layer in a capillary containing n bilayer leaflets, which is composed of the i th bilayer leaflet and a solution phase confined between i th and $(i + 1)$ th bilayers (see Fig. 6) may be well expressed by a series of admittances. However, as noted in the text, the capacitance and conductance of a bilayer leaflet of DOPH is 0.35×10^6 pF/cm² and 1×10^{-8} mho/cm², while the order of magnitudes of capacitance and conductance of the solution phase are 10 pF/cm², and 10^{-1} – 10^{-4} mho/cm², respectively, for the system studied. In this case, the series connection of admittances is reduced to the series of a capacitance, C , and a conductance, r^{-1} , with a good approximation, which leads to Equation 2.

APPENDIX II

If we use the series of admittances for representing a membrane-electrolyte system instead of using the approximation described in Appendix I, C_0^0 term would come out automatically. However, as noted in the text, C_0^0 is observed even in the case in which no membrane exists in the system. The interpretation of C_0^0 term is not the purpose of this paper, and the difference in order of magnitude between C_p^0 which stems from the membrane, and C_0^0 exceeds 10^4 – 10^6 as seen in the experimental data shown in Figs. 2, 4, and 5.

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