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CAPACITANCE, AREA, AND THICKNESS VARIATIONS IN THIN LIPID FILMS

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SUMMARY

1. Thin lipid films are generally assumed to be homogeneous equilibrium structures with a definite chemical stoichiometry. However, occasional reports in the literature suggest that these assumptions may not be valid in all cases because of microlenses of solvent trapped in the films. We have studied in detail the specific capacitance of thin films formed from a chromatographically pure synthetic phospholipid in order to examine the validity of these assumptions.

2. The method of White ((1970) *Biophys. J.* 10, 1127–1148) for measuring the specific capacitance (C_m) of planar lipid bilayers has been improved to allow C_m to be determined with a precision of $\pm 0.3\%$ and accuracy of $\pm 3.0\%$. Bilayer area (A_m) is ascertained from photographs using a weight–area method. It is shown that calculations of A_m based on measurements of film diameter using a microscope reticle are subject to a number of uncertainties which can greatly limit the precision and accuracy of area determinations.

3. The total capacitance (C_T), area, and specific capacitance of thin lipid films formed from 1,2-bisdihydrosterculoyl-3-*sn*-glycerophosphorylcholine in *n*-decane were measured as a function of time and applied voltage (V_A). C_T, A_m, and C_m generally varied with time and were non-reproducible. C_m typically varied by 20% from film to film. A possible cause of these variations is microlenses of solvent trapped in the films and equations are derived which describe their effects on C_m. It is concluded that the bilayer films studied must have a non-reproducible stoichiometry and a non-uniform thickness. The variations with time are probably a result of a disproportionation of *n*-decane (Andrews, D. M. and Haydon, D. A. (1968) *J. Mol. Biol.* 32, 149–150).

4. C_T of films in approximate equilibrium increases in the presence of an applied voltage (V_A) due to an increase in both C_m and A_m. The dependence of C_m on V_A is accurately described by the equation $C_m = C_0 + \beta V_A^2$. A similar, but approximate, equation is derived assuming the bilayer to be an elastic system of constant density which can be deformed by the force generated by the electric field.

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INTRODUCTION

The planar lipid bilayer membrane first described by Mueller *et al.*[1] is an important model system for studying molecular interactions which occur in biological membranes. These thin lipid films have been produced from a variety of lipids (see reviews, refs 2–5). It is generally assumed that the films are homogeneous equilibrium structures with a well defined stoichiometry. There are, however, reports in the literature which suggest that these assumptions may not be valid in all cases. White[6] showed that the specific electrical capacitance of films formed from oxidized cholesterol varies with time. Pagano *et al.*[7] have shown by means of a direct method that the solvent composition of thin films can be highly variable. These observations may be a result of microlenses of solvent trapped in the films[8,9]. Henn and Thompson[9] and Andrews and Haydon[8] suggested that the microlenses would probably have a negligible effect on electrical measurements.

Electric fields cause the total capacitance[10,11] and the specific capacitance[6,12] of bilayer films to increase. The increase in specific capacitance has been reasonably attributed to a decrease in membrane thickness[6,13]. However, some doubt[14] remains about this conclusion because the microlenses might have an effect on measurements of total capacitance and area.

We report here high precision measurements of total capacitance and area as a function of time and applied voltage for thin films of exceptional purity. We will (1) show that bilayer films are not necessarily homogeneous equilibrium structures with a well defined stoichiometry, (2) present a detailed analysis of the effects of microlenses on measurements of specific capacitance, (3) examine in greater detail the question of whether or not microlenses are responsible for voltage dependent changes in specific capacitance, and (4) derive an equation for the relation between specific capacitance and applied voltage.

The primary purpose of this paper is to indicate that the thin lipid film is not as simple and well behaved as generally believed. We have performed our measurements on thin films formed from a chromatographically pure synthetic lipid which is extremely stable chemically. The results obtained using this particular lipid should not be interpreted to mean that all bilayer films are heterogeneous non-equilibrium structures. They do mean, however, that *a priori* assumptions in this regard should not be made and that one must experimentally verify that a given bilayer system is homogeneous and at equilibrium.

PRELIMINARY REMARKS

The planar lipid bilayer membrane may be treated as a parallel plate capacitor with static capacitance (C_T)

$$C_T = \frac{\varepsilon_0 \varepsilon_m}{\delta_m} A_m \tag{1}$$

Where $\varepsilon_0 = 8.854 \cdot 10^{-12}$ F/m^2, ε_m the effective membrane dielectric coefficient, δ_m the effective membrane thickness, and A_m the area of the bilayer portion of the film. The polar regions of the film make little contribution to the measured capacitance because their high capacitance is in series with the low capacitance of the hydrocarbon

interior[6,15]. Thus, to an excellent approximation, $\varepsilon_m = \varepsilon_{HC}$ and $\delta_m = \delta_{HC}$ where HC refers to the hydrocarbon interior of the film. There are, of course, exceptions to this rule[16]. In addition, the surrounding electrolyte must be high enough in concentration to make the double layer capacitance negligible[17].

Molecular properties are independent of bilayer film area and it is necessary to normalize capacitance with respect to area. Therefore, the parameter of real interest is the specific capacitance (C_m) defined from Eqn 1 as

$$C_m \equiv \frac{C_T}{A_m} = \frac{\varepsilon_0 \varepsilon_m}{\delta_m} \tag{2}$$

Both C_T and A_m must be precisely determined if C_m is to be determined with precision. Measurements of these two parameters are subject to a number of uncertainties which must be understood and controlled in order to properly interpret experimental results. These uncertainties may be summarized as follows:

(1) Total membrane capacitance is usually measured using an A.C. bridge[6,15] which contains a decade resistor (R_p) in parallel with a decade capacitor (C_p) in the known arm. Since the bilayer film is immersed in an electrolyte, the bridge measures the parallel equivalent admittance ($A = [1/R_p] + i\omega C_p$) of the membrance–electrolyte system which in many cases can be represented by an equivalent circuit consisting of the total membrance capacitance (C_T) in series with the electrolyte resistance (R_E). At low frequencies (100 Hz to 1 kHz depending upon the value of R_E), $C_p = C_T + C$ to an excellent approximation. C_s is the stray capacitance associated with the unknown arm of the bridge and can be easily accounted for.

(2) The bilayer film is in equilibrium with an annulus of bulk lipid solution which might make a contribution to C_T. This problem has been studied in detail[18] and it is concluded that the contribution will be on the order of 0.01% if the bilayer film diameter is large compared to the width of the annulus. The effect can be safely neglected.

(3) The transition region between thin film and annulus is much thicker than the bilayer; but, for a non-negligible distance, it is still smaller than a wavelength of light. It is therefore difficult to define reproducibly the exact boundary of the bilayer film using reflected light. The uncertainty can cause errors of 2–20% in measurements of film diameter[18] corresponding to errors in area of 2.8–28%. The methods of White[6] and Kolarov *et al.*[19] are not subject to this uncertainty. There may be a small systematic error but the boundary is defined by explicit criteria.

(4) The bilayer film must be kept flat at all times. Small asymmetric changes in hydrostatic pressure cause significant bulging and concomitant over-estimates of specific capacitance.

(5) An applied potential causes the interfacial free energy of the bilayer to decrease[6,8]. Theoretical analysis[18] shows that if the volume of the annulus remains constant, the area of the bilayer film will increase when the voltage is applied. Thus, when studying the effects of applied voltages, it is imperative that area (A_m) be determined[6].

(6) Small droplets or lenses of bulk lipid may be present in the bilayer film in significant numbers[8,9]. In such cases the calculated specific capacitance includes contributions from the microlenses as well as the true bilayer. This is a potentially serious problem and is considered in detail in an appendix to this paper.

METHODS

Electrical measurements

Total capacitance (C_T) was measured using a modified version of a bridge described elsewhere[6,20]. The known arm contained a General Radio (West Concord, Mass.) GR1412-BC decade capacitor and the null was detected using a GR1232-AP null detector with preamplifier. The voltage (V_{AC}) from the GR1309-A oscillator appearing across the membrane was maintained constant at 20 mV peak to peak (7 mV r.m.s. (root mean squared)). The frequency was 100 Hz. The precision of the measurement of C_T under these conditions is $\pm 0.02\%$ while the accuracy is $\pm 0.5\%$. D.C. bias voltages (V_{DC}) were applied to the membrane through a low impedance voltage source in series with the unknown arm of the bridge. The total applied instantaneous voltage is $V_{DC} + V_{AC}(\max) \sin\omega t$. The relevant parameter[10,12] is the r.m.s. applied voltage V_A given by

$$V_A = [V_{DC}^2 + \tfrac{1}{2}V_{AC}^2(\max)]^{\frac{1}{2}} \tag{3}$$

Area measurements

Area (A_m) of the bilayer membrane was determined by the weight-area method previously described[6]. The membrane is photographed through a grid reticle in the microscope eyepiece and the weight of the image of the bilayer on the photographic film is compared with that of a known area defined by the grid. The weights were determined using a Mettler H-20T balance. In the present case, the membrane was observed with an American Optical (Buffalo, N.Y.) K-1360B utility microscope oriented normal to the plane of the membrane. Light from a Bausch and Lomb (Rochester, N.Y.) 31-33-40-44 General Purpose Illuminator transilluminated the bilayer. The membrane was photographed on Polaroid Corporation (Cambridge, Mass.) Type 107 film using a Romicron R-700 camera (Paul Rosenthal, Great Neck, N.Y.) mounted on an AO series 23 stereomicroscope stand. An example of a photograph of the bilayer membrane and annulus is shown in Fig. 1. The membrane was observed simultaneously through a Bausch and Lomb 40× macroscope using reflected light so that the membrane could be maintained flat at all times. The precision of the area determination has been checked repeatedly and found to be better than $\pm 0.3\%$. The accuracy of the measurement is limited by the precision with which the grid reticle can be calibrated using a substage micrometer. This accuracy is $\pm 0.8\%$.

Area is measured by most investigators by observing the membrane under reflected light and measuring the diameter of the bilayer portion of the film with a calibrated eyepiece reticle. This method is unreliable for three reasons. First, there is an uncertainty in the location of the boundary of the film (see Preliminary Remarks, item 3). Second, most available reticles under the usual experimental conditions allow the diameter to be determined with a precision of only about $\pm 4\%$. Third, the bilayer region is assumed to be circular or elliptical. However, small irregularities in the boundary shape cause significant deviations from the calculated area. To examine the size of this effect, membrane areas determined from photographs using the diameter method and the weight-area method were compared. The citreria for defining the boundary of the bilayer were the same in both cases. Table I shows the comparison for measurements on 26 membranes. Overall, the diameter method leads to an average

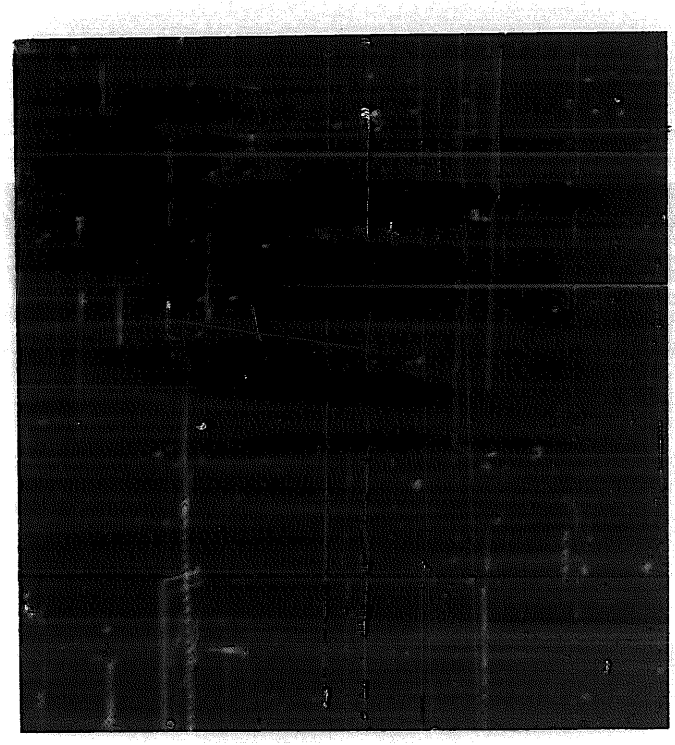

Fig. 1. An example of a photograph of a planar lipid bilayer membrane from which bilayer area (A_m) can be determined using the weight–area method (see text). The photograph was made using transmitted light. The outer light circle is a countersink made in the aperture to aid film stability. The broad dark circle is the annulus whose outer edge corresponds to the aperture diameter. The inner narrow white circle is the beginning of the transition between annulus and bilayer film. The inside edge of this inner circle is taken as the line of demarcation between bilayer and annulus. Magnification × 33.

TABLE I

COMPARISON OF THE WEIGHT–AREA AND DIAMETER METHODS FOR DETERMINING BILAYER AREA

$A_m{}^D$ is the area determined by the diameter method. $A_m{}^W$ is the area determined by the weight–area method. Both methods were used to determine area from photographs similar to that shown in Fig. 1. The criteria for defining the edge of the bilayer were the same in both cases.

Number of films	*Average value (%) of* $\frac{A_m{}^D - A_m{}^W}{A_m{}^W} \times 100$	*Range*
16	+3.4	+1.2 to +10.7%
4	0.0	—
6	−1.6	−1.2 to − 1.7%
26	+1.7	−1.2 to +10.7%

over-estimate of area of +1.7%. The probability of an over-estimate rather than an under-estimate or correct estimate is 16÷26 or 0.615 and in such cases the over-estimate will average +3.4%. It appears that the diameter method is unlikely to have a precision much better than ±4% and the accuracy could easily be only ±10%. The

inaccuracies favor over-estimation of area and therefore under-estimation of specific capacitance.

Specific capacitance

C_m was obtained from Eqn (2) using the simultaneously measured values of C_T and A_m described above. The total precision of the specific capacitance is estimated to be $\pm 0.3\%$ and the accuracy is better than $\pm 3\%$. In terms of detecting changes in specific capacitance it is the precision which is important. Accuracy is only important in comparing absolute measurements made in different laboratories.

Chamber

The chamber is an improved version of the one described earlier[20] and consists of two water-jacketed compartments (Plexiglass) clamped on either side of a partition with a 1/16-inch hole carefully drilled through it. The partition was constructed from chlorotrifluoroethylene polymer (CTFE). The aperture was designed using criteria established by White[18] and had an aspect ratio of 1.0. The partition was cleaned by prolonged soaking in conc. H_2SO_4:HNO_3 (4:1, v/v), exhaustively rinsing in tap water and distilled water, dehydrating with absolute ethanol, and soaking in glass-distilled light petroleum. A water-tight seal between the partition and chamber halves was achieved using gaskets made from 0.020 inch Silastic rubber (Dow Corning Corp., Midland, Mich.). Silastic contains no antioxidants or fillers which might contaminate the lipid. However, trace amounts of dichlorobenzene (a polymerization side-product) were detected in aqueous washings of the material using a G. K. Turner Associates (Palo Alto, Calif.) Model 210 spectrofluorometer. They could be removed by boiling the gaskets for 24 h in distilled water. The temperature of the chamber was maintained constant to within ± 0.1 °C using a Lauda K-2/R circulator (Brinkman Instruments, Westbury, N.Y.). The temperature at the aperture was measured using a small thermistor probe of negligible thermal mass connected to a DC bridge constructed in the laboratory.

Membrane formation

The membranes were "painted" using the pipet technique of Szabo *et al.*[24] except the lipid solution (approx. 1 μl) was applied with halflengths of 1-mm diameter melting point capillaries attached to a rubber bulb made from a short length of rubber tubing. The tips of the capillaries were flamed in a small bunsen burner to remove any organic contaminants. Each capillary was used once and discarded.

Materials

Films were formed from solutions consisting of 1.33–5.00 mg/ml of 1,2-bisdihydrosterculoyl-3-*sn*-glycerophosphorylcholine (Fig. 2) in *n*-decane. This lecithin was a gift kindly provided by Dr J. A. Weisbach of Smith, Kline and French Laboratories, Philadelphia, Pa. It was further purified by silicic acid column chromatography using glass distilled solvents as eluants The final product migrated as single spot on thin-layer chromatography. The *n*-decane was 99.5% pure as obtained from LaChat Chemicals (Chicago Heights, Ill.) and was further purified by passage through alumina. The lecithin was stored in a freezer in glass-distilled benzene under argon. To prepare the solutions, the lecithin was lyophilized at solid CO_2 temperatures, allowed to

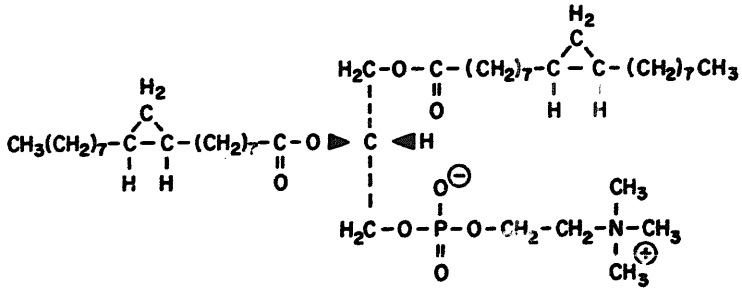

Fig. 2. The structural formula of 1, 2-bisdihydrosterculoyl-3-*sn*-glycerophosphorylcholine. Mol. wt 815.198.

absorb moisture from the air for about 2 min, and taken up in the *n*-decane. Reagent-grade NaCl roasted to remove organic contaminants was used to prepare the electrolyte solutions. The solutions were unbuffered and had a pH of between 5.6 and 5.8. Water was thrice distilled with the last distillation being from a $KMnO_4$ solution.

RESULTS

Capacitance and area variations in the absence of applied d.c. voltages

A given bilayer system must be in an equilibrium state and have reproducible physical parameters if it is to be a useful model system. Fig. 3 shows that neither of these conditions necessarily holds for 1,2-bisdihydrosterculoyl-3-*sn*-glycerophosphorylcholine in *n*-decane. The specific capacitance (C_m) typically varies by 20% from film to film and the value of C_m for a particular film is not necessarily stable in time. In the series of experiments from which these data are taken, only about 10% of the films were in approximate equilibrium (*e.g.* Curve D, Fig. 3). Such films had values of C_m ranging from 0.375 to 0.500 μF/cm^2. The total capacitances (C_T) and areas (A_m) as a function of time for two of the films of Fig. 3 are shown in Fig. 4. It is clear that one cannot assume *a priori* that bilayer area or total capacitance will remain steady in time; careful measurements of both A_m and C_T are necessary if

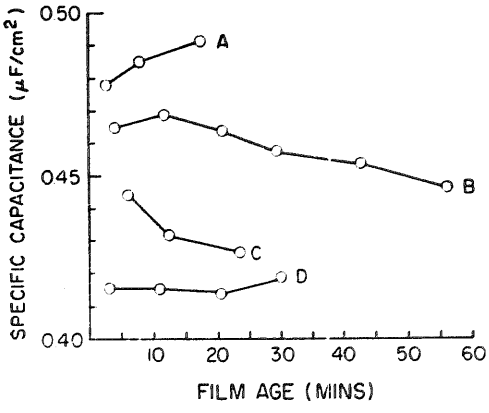

Fig. 3. The specific capacitance (C_m) of four different bisdihydrosterculoyl lecithin–*n*-decane membranes as a function of film age. Film age = 0 at the instant the membrane is fully thinned. Membranes A and B: 1.33 mg/ml lecithin in *n*-decane, $1.0 \cdot 10^{-1}$ M NaCl, pH 5.70, temp. 20.0 °C. Membranes C and D: 5.00 mg/ml. $1.0 \cdot 10^{-1}$ M NaCl, pH 5.76, temp. 20.0 °C. The diameters of the data points indicate the approximate precision ($\pm 0.3\%$) of the measurement of C_m.

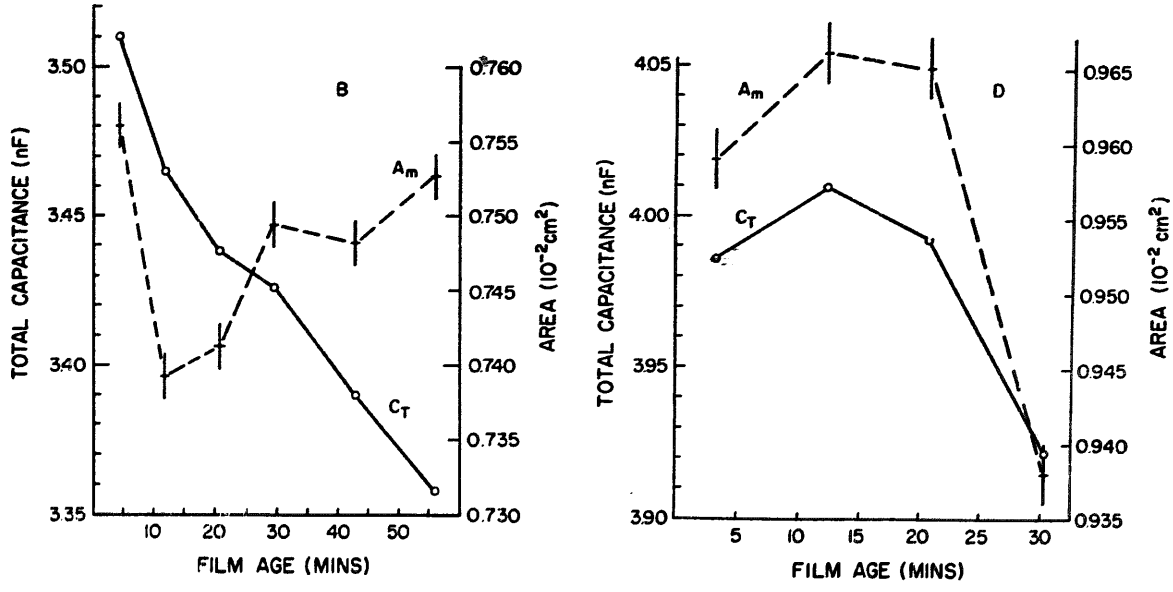

Fig. 4. Variations of total capacitance (C_T) and membrane area (A_m) with film age for two of the membranes (B and D) of Fig. 3. The diameters of the data points and lengths of the data bars indicate the approximate precision of the measurement of C_T ($\pm$ 0.02%) and A_m ($\pm$0.3%), respectively.

the specific capacitance (C_m) is to be accurately described. Careful observations of the films under reflected light did not reveal the changes in membrane area. No lenses of the type described by Andrews and Haydon[8] or other surface irregularities were observed. However, lenses were easily observed in glycerol monooleate–*n*-decane films.

Capacitance and area changes in the presence of applied D.C. voltages:

The effect of applied voltages on the specific capacitance (C_m) of several films is shown in Fig. 5. These films were in approximate equilibrium during the course

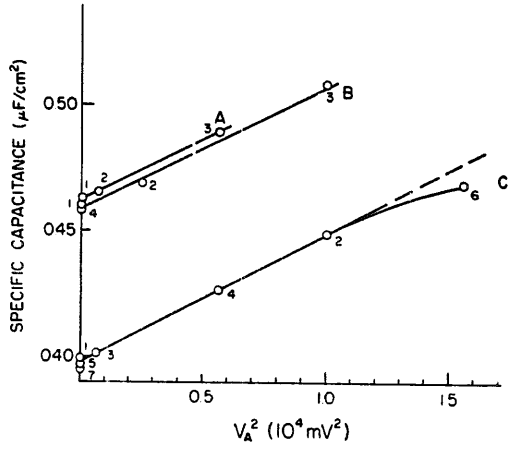

Fig. 5. Variation of specific capacitance (C_m) as a function of the square of the applied voltage (V_A^2) for three membranes. The diameters of the data points indicate the approximate precision of the measurement of C_m ($\pm$0.3%). The numbers next to the data points indicate the sequence in which the measurements were made. Membranes A and B: 1.33 mg/ml bisdihydrosterculoyl lecithin in *n*-decane, $1.0 \cdot 10^{-1}$M NaCl, pH 5.60, temp. 20.5 °C. These films were in an approximate state of equilibrium.

of the measurements which took from 10–30 min depending upon the number of points measured. The values of C_m have been plotted against V_A^2 and it will be noted that the plots are linear for applied voltages of 100 mV or less. A similar observation has been made on the specific capacitance of oxidized cholesterol films[12]. Other investigarors[10,11] have observed that the total capacitance (C_T) of other types of films varies as the square of the applied voltage. Bilayer area (A_m) in these latter experiments was not measured precisely enough to determine whether the variation was due to changes in area or specific capacitance.

C_T and A_m of Films B and C (Fig. 5) are plotted against V_A^2 in Fig. 6. The important finding from these data is that both C_T and A_m vary in an approximately linear way with V_A^2. The changes in area which occur with the application of a voltage have been predicted from theoretical studies[18]. The data of Figs 5 and 6 demonstrate conclusively that specific capacitance is affected by electric fields and that variations in total capacitance cannot be explained by area variations alone.

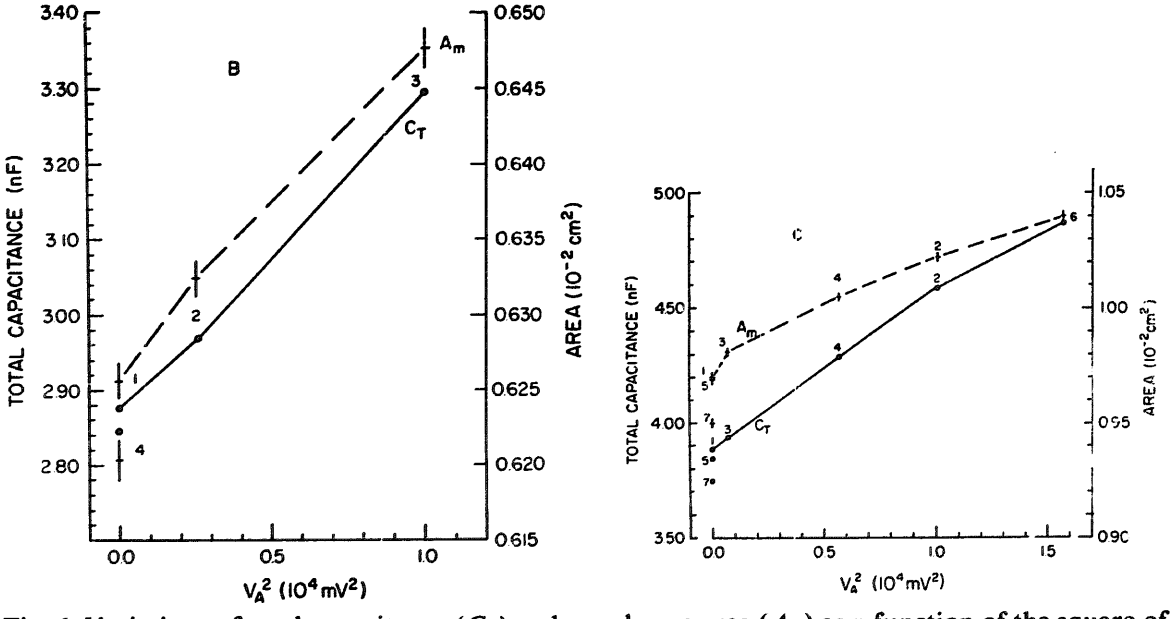

Fig. 6. Variations of total capacitance (C_T) and membrane area (A_m) as a function of the square of applied voltage (V_A^2) for Membranes B and C of Fig. 5. The diameters of the data points and lengths of the data bars indicate the approximate precision of the measurement of C_T ($\pm 0.02\%$) and A_m ($\pm 0.3\%$), respectively.

Curve C of Fig. 5 shows that for applied voltages in excess of 100 mV the relation between C_m and V_A^2 becomes non-linear. Note that the slopes in the linear regions of Curves B and C are approximately the same even though the values of the zero voltage capacitances are different.

DISCUSSION

Phospholipids from natural sources are inherently heterogeneous in alkyl chain composition and a synthetic lecithin was used in the present study to eliminate this variable. In addition, the cyclopropane ring gives the lipid an alkyl chain fluidity similar to that imparted by a double bond but is less subject to autooxidation. It was surprising to find that the specific capacitance of these films was not reproducible

and that most of the films were non-equilibrium structures. However, for those films which were in approximate equilibrium, the specific capacitance was accurately a linear function of applied voltage squared.

Critical to the interpretation of these data are the effects of microlenses[8,9] on the specific capacitance of the bilayer film. The lenses, if present, would occupy area attributed to true bilayer and would have capacitances somewhat lower. They could be responsible for the non-reproducibility and the time and voltage dependence of the specific capacitance. Lenses 1 μm or greater in diameter should have been detected with the optical system used in this study. Since no lenses were seen, they must have been smaller than 1 μm if they were present. Equations describing the effects of microlenses are derived and discussed in the Appendix. The conclusion is, as expected, that the effect of the lenses on specific capacitance depends upon the volume of solvent trapped in the film and the average diameter of the lenses. It is concluded in Appendix that there is at most about $50 \cdot 10^{-8}$ cm^3 of solvent trapped per cm^2 of film. If the lenses are slightly smaller than 10^{-4} cm (1 μm) they probably occupy no more than 10% of the film area. The maximum observed variation in specific capacitance from film to film was greater than 30%.

The area of film occupied by lenses is only one factor which might affect the specific capacitance. The analysis in Appendix and the findings of Fettiplace *et al.*[13] suggest that the volume fraction (f) of true bilayer occupied by the alkyl chains of the lecithin also plays a major role. The thickness of the bisdihydrosterculoyl lecithin bilayer will be 35 Å if no solvent is trapped in the film assuming the dihydrosterculoyl alkyl chain has a molecular volume equal to that of octadecane and that the area per lecithin molecules is about 61 Å^2 (ref. 13). If $50 \cdot 10^{-8}$ cm^3 of decane were present per cm^2 of film and distributed uniformly, the thickness would be 85 Å. This range of thicknesses corresponds to specific capacitances of about 0.52–0.22 μF/cm^2. The observed variation was from approx. 0.50 to approx. 0.38 μF/cm^2.

The data presented in this paper are consistent with the following hypotheses: (1) variable amounts of solvent can be trapped in the film during formation. (2) There is no reproducible stoichiometry between solvent and lecithin in the true bilayer portions of the films. (3) Solvent is disproportioned[8] as a function of time between bilayer and lenses which are generally smaller than 1 μm. The exact time dependence of the specific capacitance depends upon the combined effects of solvent disproportionation and diffusion of microlenses into the annulus of the film. (4) The exact value of specific capacitance depends upon the volume fraction of true bilayer occupied by the lecithin alkyl chains[13] and the number and dimensions of the lenses formed. The characteristics of the lenses depend in turn upon the amount of solvent trapped and the amount disproportioned.

The lenses are apparently smaller than 1 μm. The conclusions of the Appendix suggest that in this case the film can probably be visualized as simply having a non-uniform thickness which is on the average close to a bilayer thickness. This would be consistent with the electron microscopic findings of Henn and Thompson[9] who reported variations in thickness of phosphatidylethanolamine–decane films of 37.5–116 Å. They also observed that formal large lenses occurred only rarely. Thus, it appears that to a good approximation the lenses do not need to be considered separately from the bilayer. The film behaves as though it has a non-uniform thickness and non-reproducible stoichiometry.

The variation of specific capacitance with applied voltage might be a result of changes in the submicroscopic lenses.[14] The application of a 100-mV potential causes the relative surface free energy of the bilayer to decrease by a factor of approx. 4 (ref. 6). In such a case, the contact angle between the lens and bilayer would increase causing the area occupied by the lenses to decrease. The resulting increase in area of true bilayer, if unaccounted for in the determination of film area, would be interpreted as an increase in specific capacitance. The lens contact angle is likely to be on the order of 2° (ref. 21). A 100-mV potential would cause the angle to increase to 4°. This change would cause at most a 4% apparent increase in specific capacitance. The change actually observed is greater than 10% (Fig. 5). In addition, if the zero voltage value of capacitance depended upon the percentage of area occupied by lenses, the fractional change in capacitance with applied potential would decrease as the area occupied by lenses decreased. This does not occur in the present films (Fig. 5). Area changes of lenses may have some effect but they clearly are not dominant. The voltage effect must be primarily a result of changes in the average thickness of the bilayer.

The results of this study confirm earlier findings[12,20] that the specific capacitance (C_m) of bilayer films is a linear function of V_A^2 ($V_A \leq 100$ mV) and can be described by the equation

$$C_m = C_0 + \beta V_A^2 \tag{4}$$

This dependence on V_A^2 may be attributed[6,13] to thickness changes in the bilayer brought about by the force generated across the film due to the electric field. The force on a unit area of membrane is given by

$$F_E = \frac{\varepsilon_0 \varepsilon_m}{2\delta_m^2} V_A^2 \tag{5}$$

The implicit assumption is that the film is either elastic[22] (density constant) or compressible[11] (density not constant). Rosen and Sutton[11] have pointed out that the bulk modulus (B) of most hydrocarbons is such that the force generated by the electric field should have a negligible effect on thickness if the assumption of compressibility is made. If $B = 10^{+4}$ atm, then the thickness would change by only about 1 part in 10^5 for a 100-mV potential. The changes observed here are about 10%. The reasonable conclusion is that the film is elastic and the deformation involves a decrease in thickness accompanied by an increase in area such that the specific volume of bilayer remains constant. This is consistent with the observation that area increases with increased potential.

Assume, then, that the bilayer is an elastic system with a Young's modulus Y_{BL} and that the electric field causes the thickness to decrease by $\Delta\delta$ from δ_0 to $\delta_0 - \Delta\delta$ so that $\delta_m = \delta_0 - \Delta\delta$. From Eqn (2)

$$C_m = \frac{\frac{\varepsilon_0 \varepsilon_m}{\delta_0}}{\left(1 - \frac{\Delta\delta}{\delta_0}\right)} \tag{6}$$

If $(\Delta\delta/\delta_0) \ll 1$, then

$$C_m \simeq \frac{\varepsilon_0 \varepsilon_m}{\delta_0}\left(1 + \frac{\Delta\delta}{\delta_0}\right) \tag{7}$$

Since the bilayer film is assumed to be elastic,

$$\frac{\Delta\delta}{\delta_0} = \frac{F_E}{Y_{BL}} \tag{8}$$

F_E (Eqn 5) depends upon thickness but as a first approximation write

$$\frac{\Delta\delta}{\delta_0} \simeq \frac{1}{Y_{BL}}\left(\frac{\varepsilon_0 \varepsilon_m}{2\delta_0^2} V_A^2\right) \tag{9}$$

Substitution of Eqn 9 into Eqn 7 yields

$$C_m \simeq \frac{\varepsilon_0 \varepsilon_m}{\delta_0} + \frac{1}{Y_{BL}}\left(\frac{\varepsilon_0^2 \varepsilon_m^2 V_A^2}{2\delta_0^3}\right) \tag{10}$$

Comparison with Eqn 4 suggests that

$$C_0 = \frac{\varepsilon_0 \varepsilon_m}{\delta_0} \tag{11}$$

$$\beta = \frac{1}{Y_{BL}}\left(\frac{\varepsilon_0^2 \varepsilon_m^2}{2\delta_0^3}\right) = \frac{1}{Y_{BL}}\left(\frac{C_0^2}{2\delta_0}\right) \tag{12}$$

Even though Eqn 10 is similar to Eqn 4, it suffers from several problems. First, Eqn 12 implies that β should be a strong function of C_0. Fig. 5 is not consistent with this. However, Y_{BL} may vary with δ_0 in such a way as to keep β approximately constant from film to film. Y_{BL} is a partial measure of the repulsive potential energy[6] of the bilayer which should depend strongly on film thickness. Second, Eqn 8 assumes a force independent of thickness which F_E is not. In addition, the thickness-dependent London–van der Waals force[6] between the separated aqueous phases has been neglected. Finally, the non-linearity of Curve C Fig. 5 is not properly accounted for. This curve indicates that β decreases with decreased thickness. Eqn 12 has the opposite change unless Y_{BL} also changes which would not be unimaginable.

The idea that the bilayer is an elastic system may at first seem inconsistent with the finding[23] that bilayer interfacial tension (γ_{BL}) is independent of area. However, measurements of γ_{BL} are made by bulging the bilayer using a hydrostatic pressure difference. In this case new film is being generated from the annulus and the bilayer is not in fact under stress at equilibrium. Elasticity can be revealed only by stressing the membrane in some way[22].

If the film is elastic as assumed and if no new film is generated from the annulus during the application of the voltage, the volume of film must remain constant and $(C_0/C_m)=(A_0/A_m)$ where C_0 and C_m are the values of specific capacitance in the absence and presence of the voltage and ε_m is assumed to remain constant. From Curve B of Fig. 5 $(C_0/C_m)=0.903$ ($V_A=100$ mV). From Fig. 6, $A_0/A_m \simeq 0.95$ depending upon how one draws the curve through the points. Letting C_{T0} represent the total

capacitance in the absence of a voltage and C_{Tm} the total capacitance in the presence of a voltage, then $(C_{T0}/C_{Tm})=(C_0^2/C_m^2)$ making the same assumptions as above. Using data for the membrane of Curve B Fig. 5 the ratios are approx. 0.86 and 0.815, respectively. The agreement in these two cases is not particularly good but the trends are consistent. It is quite likely that the volume of the annulus is changing during these experiments which means that film is being lost to or gained from the annulus. The data of Fig. 4 are consistent with this idea. Thus it is difficult to accurately compare the area and total capacitance ratios as done above.

APPENDIX

The specific capacitance of a thin lipid film containing microlenses of solvent

THEORY

Consider a bilayer film with an area of 1 cm^2 containing n microlenses of average radius a. The capacitance of the film, C_m, will be given by

$$C_m = \frac{\varepsilon_0 \varepsilon_B}{\bar{\delta}_E}(1-nA_L) + \frac{n\varepsilon_0\varepsilon_S A_L}{\bar{\delta}_L} \tag{1A}$$

where ε_B is the dielectric coefficient of the bilayer portion of the film which has an average thickness $\bar{\delta}_E$, A_L the area occupied by a lens, $\bar{\delta}_L$ the average thickness of the film in the region of a lens, ε_s the dielectric coefficient of the solvent, and $\varepsilon_0=8.854\cdot 10^{-8}$ μF/cm. The use of ε_s as the dielectric coefficient of the lens is permissable as long as the volume of the lens is much greater than an equivalent layer of bilayer and if the surfactant is insoluble in the solvent.

A microlens is essentially a double convex lens of solvent embedded in the bilayer. To simplify the derivation, the film is visualized as a uniform bilayer upon which are placed pairs of planoconvex lenses of pure solvent oriented back to back with the uniform bilayer sandwiched between. The total volume of solvent contained in 1 cm^2 of film will be V_S and the total volume of the alkyl chains of the surfactant will be V_{AC}. This latter volume is attributed entirely to the uniform bilayer upon which the plano-convex lenses of pure solvent are placed. Let the total hydrocarbon volume (alkyl chain volume+solvent volume) of the uniform bilayer be V_B. The volume fraction (f) of the uniform bilayer occupied by the alkyl chains of the surfactant will be

$$f = \frac{V_{AC}}{V_B} \tag{2A}$$

The solvent will be distributed between uniform bilayer and microlenses. The volume of solvent, V_{SL}, contained in the lenses will be

$$V_{SL} = V_S - \frac{(1-f)}{f} V_{AC} \tag{3A}$$

since the volume of solvent in the uniform bilayer is $(1-f)V_B$.

A lens of pure solvent will occupy an area of film

$$A_L = \pi a^2 \tag{4A}$$

and have a volume V_L. The volume V_L may be easily derived from geometrical considerations since a single plano-conves lens is a segment of a sphere[21]. If the contact angle between the lens and bilayer is θ and if θ is small, then to an excellent approximation

$$V_L = \frac{\pi\theta a^3}{2} \tag{5A}$$

The average thickness $\bar{\delta}$ of two plano-convex lenses back to back will be

$$\bar{\delta} = \frac{V_L}{A_L} = \frac{a\theta}{2} \tag{6A}$$

so that in the region of a lens the film will have an average thickness $\bar{\delta}_L$ given by

$$\bar{\delta}_L = \bar{\delta}_B + \frac{\theta a}{2} \tag{7A}$$

Since $V_{SL} = nV_L$, one may write from Eqns 3A and 5A

$$n = 2\frac{\left[V_S - \frac{(1-f)}{f} V_{AC}\right]}{\pi\theta a^3} \tag{8A}$$

Using Eqns 1A, 4A, 7A, and 8A, the expression for C_m becomes

$$C_m = \frac{\varepsilon_0\varepsilon_B}{\bar{\delta}_B}\left(1 - \frac{2\left[V_S - \frac{(1-f)}{f} V_{AC}\right]}{\theta a}\right) + \frac{2\varepsilon_0\varepsilon_S\left[V_S - \frac{(1-f)}{f} V_{AC}\right]}{\theta a\left(\bar{\delta}_B + \frac{\theta a}{2}\right)} \tag{9A}$$

ε_B and $\bar{\delta}_B$ are given by Fettiplace *et al.*[13]

$$\bar{\delta}_B = \frac{V_{AC}}{f} \tag{10A}$$

$$\varepsilon_B = f\varepsilon_{AC} + (1-f)\varepsilon_S \tag{11A}$$

where ε_{AC} is the dielectric coefficient of the alkyl chains of the surfactant. Eqn 10A follows from $\bar{\delta}_B \cdot A = V_B$ using Eqn 2A since $A = 1$ cm^2. Eqn 9A shows that C_m depends upon the amount of solvent trapped in the film, the lens radius, and the contact angle. As V_S approaches $(1-f/f)\ V_{AC}$, the microlenses will have a negligible effect on C_m because the solvent is distributed only in the bilayer in that case.

DISCUSSION

The data of Henn and Thompson[9] for phosphatidylethanolamine–decane bilayers indicated that there were 11.8 ± 3.4 decane molecules per phosphatidyl-

ethanolamine molecule. Assuming that each phospholipid molecule occupies an area of 61 Å^2 (ref. 13) and that the alkyl chains are equivalent to heptadecane in molecular volume, $V_{AC}=33\cdot10^{-8}$ cm^3 per cm^2 of film. The volume (V_S) of solvent would be $125\cdot10^{-8}$ cm^3. These numbers may be used to estimate the effects of microlenses on the properties of the film. θ may be taken as 2° (ref. 21). The average size of the microlenses is critical. If a is approx. 10^{-4} cm, then $2[V_S-(1-f)\,V_{AC}]$ is approx. 0.5 θa for a wide range of f and the lenses will have a large effect on C_m. If the lenses are large ($>10^{-3}$ cm), they will have a negligible effect. As the size of the lenses decreases beyond 10^{-4} cm, $(1-nA_L)$ (Eqn 1A) approaches zero, *i.e.* the entire area of film is occupied by lenses. The solvent could be visualized in this case as being uniformly distributed throughout the film and the average film thickness would be $(V_{AC}+V_S)\div 1$ cm^2. It thus appears that the critical lens size is about 10^{-4} cm. If the lenses are much larger or much smaller than this, their effect can be ignored.

The lenses observed by Henn and Thompson[9] should have occupied roughly 50% of the film area if the lens radius was approx. 10^{-4} cm. This means that lenses should have been a common event in the electron micrographs. In fact, lenses were rarely observed. This means that either $a\ll10^{-4}$ cm or that the amount of solvent was over-estimated. A third possibility is that $a\gg10^{-3}$ cm. However, the data presented did not indicate that lenses of this size were ever observed. Suppose that $a\ll10^{-4}$ so that the solvent could be assumed to be uniformly distributed. In this case $\bar{\delta}_B$ is approx. 158 Å. The electron micrographic data indicate that $\bar{\delta}_B$ is approx. 70 Å. Thus, it appears likely that the amount of solvent may have been over-estimated. The amount of solvent actually trapped may be closer to about $50\cdot10^{-8}$ cm^3 if the assumptions made here are correct. This would correspond to about 4 decane molecules per phosphatidylethanolamine molecule. Henn and Thompson[9] suggested that the fixation and embedding procedures they used might have altered the composition of the bilayer. The above calculations indicate that this may in fact be true.

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