THEORETICAL CALCULATION OF THE DIELECTRIC CONSTANT OF A BILAYER MEMBRANE

WEI-TZE HUANG, *Department of Physics, University of Idaho, Moscow, Idaho 83843*
DAVID G. LEVITT, *Department of Physiology, University of Minnesota, Minneapolis, Minnesota 55455*

ABSTRACT The dielectric constant (ϵ) and refractive index (n) of a bilayer lipid membrane is determined from the known values of the polarizabilities of the carbon-carbon and carbon-hydrogen bonds. It is assumed that the hydrocarbon chains are hexagonally arranged in an all-*trans* conformation perpendicular to the plane of the membrane. The only variable in the calculation is the average separation between the chains and the theory relates ϵ to this separation. The calculation and results differ significantly from those presented in a 1968 publication by Ohki. It is shown that a thin membrane is not homogeneously polarized by the applied field. This effect is analysed and the dependence of ϵ on the membrane thickness is determined. The theoretical results are in good quantitative agreement with experimental measurements on bulk paraffins and on oriented multilayers of saturated fatty acids. The most important conclusion is that the dielectric constant for an applied field perpendicular to the membrane (which is the appropriate value for capacitance measurements) differs by only a few percent from the value for the macroscopic (bulk) liquid hydrocarbon. Thus the dielectric constant of a bilayer membrane can be approximated by the value for the appropriate bulk hydrocarbon.

INTRODUCTION

One of the easiest and most frequently made measurements of a membrane property is the electrical capacitance (C). An important use of the capacitance is as a measure of the thickness (d) of the hydrocarbon region of a membrane by the use of Eq. 1:

$$C = \epsilon/4\pi d \tag{1}$$

The use of this expression is limited primarily because of the uncertainty about what value should be used for the dielectric constant (ϵ). Although it is possible to measure the dielectric constant of a macroscopic liquid that resembles the hydrocarbon region of the membrane, it is questionable whether this value is representative of a membrane in which the hydrocarbons have a markedly anisotopic orientation and which is only about 30 Å thick. Because of this orientation, the value of $\epsilon_\parallel$ for a field parallel to the hydrocarbon chains (normal to the plane of the membrane) should differ from the $\epsilon_\perp$ for a field perpendicular to the chain (in the plane of the membrane). In Eq. 1, $\epsilon_\parallel$ should be used while measurements on the bulk liquid yield only some average of $\epsilon_\parallel$ and $\epsilon_\perp$. This anisotropy shows up as a birefringence in optical studies of the mem-

brane and it is possible to measure directly the index of refraction ($n_\parallel, n_\perp$) in the two directions. Since the hydrocarbon region is nonpolar, n for this region can be simply related to the ϵ of the hydrocarbon region (for light in the visible range) by Eq. 2:

$$\epsilon = n^2. \tag{2}$$

However, the value of n actually measured represents an average over both the hydrocarbon and polar regions of the membrane and therefore can only be used to provide an estimate of the $\epsilon_\parallel$ needed in Eq. 1.

The absence of a direct experimental approach increases the importance of trying to determine $\epsilon_\parallel$ from theoretical considerations. Ohki (1968, 1969, 1970) has derived theoretical values for $\epsilon_\parallel$ and $\epsilon_\perp$ by a detailed molecular approach in which the dipole interactions in the hydrocarbon region of the membrane were directly calculated. However, Ohki's theoretical results do not agree very well with experimental measurements on built up layers of barium stearate. Recently, Den Engelsen (1976) has argued that the molecular approach of Ohki cannot, in general, be applied to this type of calculation. Den Engelsen has used a different theoretical approach, in which the hydrocarbon chain is replaced by a cylindrical hole and the rest of the membrane is modeled by a continuum approximation. The theoretical values of ϵ obtained by this continuum approach are in good agreement with experimental ellipsometric measurements recently obtained by Den Engelsen and his colleagues. The purpose of this paper is to show that the molecular approach can provide useful information about the values of ϵ in lipid membranes and that the difficulties with Ohki's results are not inherent in this approach but are due to some specific erroneous assumptions.

GENERAL THEORETICAL CALCULATION

The derivation of the relation between the capacitance and ϵ (Eq. 1) is usually based on the assumption that the dielectric is uniformly polarized by the applied field. Since it will be shown that this assumption is not valid for thin membranes, this assumption will not be used in the derivation of the general results in this section. To evaluate the dependence of ϵ on the membrane thickness, the general relations will first be applied to the special case of a thick membrane, in which one can assume uniform polarization. These results will be compared with experimental values of ϵ for bulk hydrocarbons. Then, the calculation for the thin, nonuniformly polarized membrane will be presented and these results will be compared with experimental measurements on lipid bilayers.

The derivation for the case of an applied field parallel to the axis of the hydrocarbon (perpendicular to the membrane surface) is based on an analysis of the Gaussian box drawn in Fig. 1. The hydrocarbon (insulating) region of the membrane is indicated by the two parallel lines. The box is drawn so that the left-hand surface is in the bulk solution far enough from the membrane that the electric field (E) is zero, while the other surface is in the hydrocarbon region of the membrane. [The theory and notation used in this section follows, in general, the treatment presented in a recent monograph by Bottcher et al. (1973).] Because of the applied field, an amount of charge, Q per unit

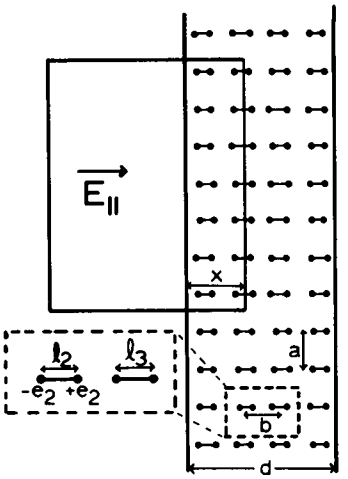

FIGURE 1 Schematic representation of hydrocarbon dipoles induced by a field applied parallel to the hydrocarbon axis.

area, is accumulated external to the hydrocarbon membrane. First, assume that all these external charges are fixed in position and then the hydrocarbons are removed and a vacuum is left. Then, according to Gauss's law, there will be a uniform field in the membrane region given by:

$$E^0 = 4\pi Q. \tag{3}$$

When the hydrocarbons are replaced there will be an additional charge in the box due to the induced dipoles. The E field will then be given by:

$$E(x) = 4\pi[Q + q(x)], \tag{4}$$

where $q(x)$ is the charge in the membrane region of the box as a function of the position of the surface of the box. It will be assumed that the membrane dipole (CH_2) groups are arranged in planes parallel to the surface, and from symmetry considerations, all the dipoles in a given plane have the same dipole strength. Thus, $q(x)$ will be zero unless the surface of the box passes through the line connecting the dipoles in the ith plane (separated by a distance l_i) in which case it will equal $-se_i$ (where e_i is the charge of the dipole in the ith plane and s is the number of dipoles per unit area in a plane). Thus, the average value of E in the membrane is given by:

$$\bar{E} = \frac{1}{d}\int_0^d E(x)\mathrm{d}x = E^0 - \frac{4\pi s}{d}\sum_{i=1}^{N} e_i l_i, \tag{5}$$

Where d is the thickness of the hydrocarbon region and N is the number of planes. Since $l_i e_i$ is, by definition, the dipole strength (p_i), Eq. 5 can be written in the form:

$$\bar{E} = E^0 - 4\pi\bar{P}, \tag{6}$$

Where $\bar{P}$ is the polarization vector and is defined by:

$$\bar{P} = \rho\bar{p}; \quad \bar{p} = \frac{1}{N}\sum_i p_i; \quad \rho = s/b, \tag{7}$$

where ρ is the dipole density and b is the distance between dipoles in adjacent planes. In general, the dielectric displacement ($\bar{D}$) and the dielectric constant (ϵ) in the membrane are defined by:

$$\bar{D} = \epsilon\bar{E} = \bar{E} + 4\pi\bar{P}. \tag{8}$$

Comparing Eqs. 6 and 8, we can see that for an applied field parallel to the hydrocarbon axis:

$$\bar{D}_\parallel = E^0_\parallel. \tag{9}$$

From these relations, the expression for the capacitance (Eq. 1) can be obtained immediately:

$$C = \frac{Q}{\Delta\phi} = \frac{(E^0/4\pi)}{d\bar{E}} = \frac{\epsilon}{4\pi d}. \tag{10}$$

The purpose of this detailed derivation was to emphasize the point that the value of ϵ (defined operationally by Eq. 1) can be obtained rigorously in terms of the average value of the membrane dipoles ($\bar{p}$), even if the membrane is not uniformly polarized.

The strength of each dipole is obtained from the relation:

$$\mathbf{p}_i = \alpha\mathbf{E}_i, \tag{11}$$

where α is the polarizability and $\mathbf{E}_i$ is the "local" field that acts on the ith dipole. The vector $\mathbf{E}_i$ is the superposition of the field $\mathbf{E}^0$ (defined above) and field due to all the other dipoles (not including p_i) in the hydrocarbon region:

$$\mathbf{E}_i = \mathbf{E}^0 - \sum_j \mathbf{T}_{ij} \cdot \mathbf{p}_j, \tag{12}$$

where $\mathbf{T}$ is the dipole field tensor and is defined by:

$$\mathbf{T} = r^{-3}(\mathbf{I} - 3r^{-2}\,\mathbf{r}\,\mathbf{r}); \quad \mathbf{T}_{ii} = 0, \tag{13}$$

where $\mathbf{r}_{ij}$ is the vector directed from dipole $\mathbf{i}$ to $\mathbf{j}$ and $\mathbf{I}$ is the unit tensor. Since the induced field is parallel to the applied field (in the direction e):

$$\mathbf{E}^0 = E^0 e; \quad \mathbf{E}_i = E_i e; \quad \mathbf{p} = pe, \tag{14}$$

and Eq. 12 can be reduced to a scaler equation:

$$e \cdot \mathbf{E}_i = e \cdot \mathbf{p}_i/\alpha = p_i/\alpha = E^0 - \sum_j T_{ij}p_j;$$

$$T_{ij} = e \cdot \mathbf{T}_{ij} \cdot e. \tag{15}$$

For example, for an applied field parallel to the hydrocarbon (z) axis:

$$T_{ij} = r^{-3}(1 - 3r^{-2}z_{ij}^2). \tag{16}$$

THICK MEMBRANE

For the special case of a thick membrane, the surface effects can be neglected so that all the p's are identical and Eq. 15 can be immediately solved:

$$p = \frac{\alpha E_0}{1 + \alpha T}, \quad T = \sum_j T_{ij}, \tag{17}$$

where i is any dipole that is not near the surface. From Eqs. 7, 8, and 17 the expression for ϵ or χ (susceptibility) is obtained:

$$\chi = \frac{\epsilon - 1}{4\pi} = \frac{\alpha\rho}{1 + \alpha T}\frac{E_0}{\bar{E}}. \tag{18}$$

To complete the derivation, it is necessary to know the relation between E_0 and $\bar{E}$, which depends on the direction of the applied field. This relation is obtained from the boundary condition that D is continuous in the direction normal to the membrane surface, while E is continuous in the direction of the plane of the surface. Thus for an applied field normal to the membrane (see Eq. 9):

$$E_{\parallel}^0 = \bar{D}_{\parallel} = \epsilon \bar{E}_{\parallel}. \tag{19}$$

For a field applied parallel to the membrane:

$$E_{\perp}^0 = \bar{E}_{\perp}. \tag{20}$$

From Eqs. 19 and 20 the final expressions for the dielectric constant for a field parallel ($\epsilon_{\parallel}$) and perpendicular ($\epsilon_{\perp}$) to the hydrocarbon chains are obtained:

$$\frac{\epsilon_{\parallel} - 1}{4\pi} = \frac{\alpha_{\parallel}\rho}{1 + \alpha_{\parallel}(T_{\parallel} - 4\pi\rho)}; \tag{21a}$$

$$\frac{\epsilon_{\perp} - 1}{4\pi} = \frac{\alpha_{\perp}\rho}{1 + \alpha_{\perp}T_{\perp}} \tag{21b}$$

It is important to note the difference in the two expressions that results from the boundary conditions. Ohki (1968) did not take account of this and used Eq. 21*a* for both $\epsilon_{\parallel}$ and $\epsilon_{\perp}$. This should produce a very large error in his calculation of $\epsilon_{\perp}$.

The theoretical calculation of ϵ then requires evaluation of T (Eq. 17) for the assumed molecular structure of the membrane. The summation in Eq. 17 is approximated by an exact sum over the near dipoles and an integral approximation for the far dipoles. This far integration is simplified by using the classical approach of Clausius and Mosotti. This approach involves making a hole in the dielectric around the dipole. The "local" field (E_i) can then be written as the superposition of (*a*) the

sum over the dipoles in the hole, (*b*) the field from the charge on the surface of the hole (E_h), and (*c*) the internal field in the dielectric (E):

$$E_i = \bar{E} + E_h - \sum_{\text{hole}} T_{ij} p_j. \tag{22}$$

This can be rewritten as:

$$E_i = \bar{E} + P\left(\kappa - \frac{T_h}{\rho}\right) = \bar{E}\left[1 + \frac{\epsilon - 1}{4\pi}\left(\kappa - \frac{T_h}{\rho}\right)\right], \tag{23}$$

where Eq. 8 and the following relations have been used.

$$p = \frac{P}{\rho}; \quad E_h = \kappa P, \quad \sum_{\text{hole}} T_{ij} = T_h \tag{24}$$

The value of κ depends on the shape of the hole and the direction of the applied field. From Eqs. 8 and 11:

$$\frac{\epsilon - 1}{4\pi} E = P = \rho\alpha E_i. \tag{25}$$

Finally, solving Eqs. 23 and 25 for ϵ:

$$\frac{\epsilon - 1}{4\pi} = \frac{\rho\alpha}{1 - \rho\alpha[\kappa - (T_h/\rho)]} \tag{26}$$

The form of this solution is independent of the direction of the applied field although the values of α, κ, and T_h depend on this direction. The calculation of ϵ then requires the determination of: (*a*) the polarizabilities of the unit dipoles (α), (*b*) the sum over the interactions from the dipoles in the hole (T_h) and (*c*) the value of κ. These three calculations will be described in the next three sections.

Polarizability of the Unit Dipole

An exact calculation of the polarizability (α) of a molecule requires a complete quantum mechanical solution and is only possible for very simple molecules (Phillips, 1969). However, it has been shown that it is possible to accurately predict the polarizability of hydrocarbons if one assumes that the contribution to the polarizability from each bond is simply additive. This procedure involves an empirical assignment of the polarizability of, for example, the C—C or C—H bond and simply adding them (vectorially) to determine the total polarizability of the molecule (Denbigh, 1940). The approximations involved in this procedure are essentially the same as those that allow the use of the concept of a bond length. That is, to a first approximation one assumes that the length of a C—C bond is independent of the other atoms attached to the carbon and that there is no interaction between the bonds. Similarly, one can define the polarizability of a C—C bond from the assumption that there is no interaction between

the bonds. It is important to note that the polarizability assigned to the C—C bond is not the polarizability of a "bare" C—C bond (which does not exist). It is the empirical polarizability that when assigned to the bond allows one to predict the molecular polarizability on the assumption that the bonds do not interact (are additive). We have emphasized this point because it implies that when the interaction between the dipoles is determined by summing over T_{ij}, it is essential that one does not include the interactions between bonds in the same molecule, since these have already been accounted for by the additivity assumption. This is very important because the nearest C—C bonds are only separated from each other by about 1 Å, and the dipole interaction between these bonds is so large that the susceptibility becomes infinite or negative. This is another point at which we feel Ohki's solution is incorrect. In his calculation of the interaction between dipoles, he summed over all the neighbors, including those on the same molecule. To avoid the problem of the very large interactions between the nearest neighbors, Ohki took as his unit dipole a C_2H_4 group (so that the neighbors were further apart), rather than the CH_2 unit that we used. Further analysis of Ohki's calculations are presented in the appendix. In this section we will calculate the polarizability of the CH_2 unit averaged over the rotation of the hydrocarbon chain about its axis. In the next section, the interaction between this CH_2 unit and all neighboring CH_2 groups not on the same molecule will be determined.

To determine the polarizability of the CH_2 group, one must know how it is oriented in the membrane. We have made the simplifying assumption that the hydrocarbon chains are all in the *trans* conformation, perpendicular to the plane of the membrane

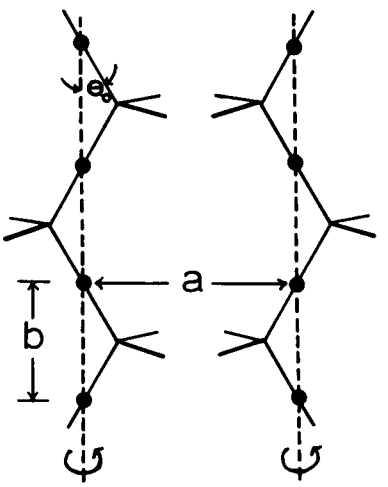

FIGURE 2 Arrangement of the hydrocarbon chains. It is assumed that the chains are in all-*trans* conformation perpendicular to the plane of the membrane and are free to rotate about the chain axis (dotted line). The carbon-hydrogen bonds remain in the membrane plane during this rotation. In the calculation the CH_2 group is replaced by a unit with the same polarizability located at the center of the C—C bond (solid circles). The C—C bond distance projected on the chain axis is b, the separation between the chains is a, and θ_0 is the angle between the C—C bond and the chain axis.

(Fig. 2) and are arranged in a regular hexagonal array (Fig. 3). This should be a good approximation to the membrane in the low temperature "crystalline" state but is only a rough approximation to the "liquid" phase. In addition, we are neglecting the effect of any ethylenic double bonds and the CH_3 terminal group. We will use the polarizabilities assigned by Denbigh (1940) to the C—C and C—H bonds:

$$\alpha_{C-C}^{\parallel} = 1.88\ \text{Å}^3 \quad (E \parallel \text{C—C bond})$$
$$\alpha_{C-C}^{\perp} = 0.02\ \text{Å}^3 \quad (E \perp \text{C—C bond})$$
$$\alpha_{C-H}^{\parallel} = 0.79\ \text{Å}^3 \quad (E \parallel \text{C—H bond})$$
$$\alpha_{C-H}^{\perp} = 0.58\ \text{Å}^3 \quad (E \perp \text{C—H bond}).$$

$E \parallel$ Hydrocarbon Axis. As is shown in Fig. 2, the C—H bonds all lie in the plane of the membrane or perpendicular to E:

$$\alpha_{C-H}^{E \parallel \text{axis}} = \alpha_{C-H}^{\perp} = 0.58\ \text{Å}^3, \tag{27}$$

where $\alpha_{C-H}^{\perp}$ means $\perp$ to the C—H bond. In the hexagonal array the hydrocarbon chains are free to undergo rapid rotation about the chain axis. Thus, in the calculation of the interaction between neighboring chains (there is no interaction between groups on the same chain), the polarizability should be averaged over this rotation. As is shown in Fig. 2, the C—C bond rotates on the surface of a cone at an angle $\theta_0 = \cos^{-1}\sqrt{2/3}$ to the chain axis as the chain rotates about its axis. Since:

$$\alpha(\theta) = \alpha^{\parallel}\cos^2\theta + \alpha^{\perp}\sin^2\theta, \tag{28}$$

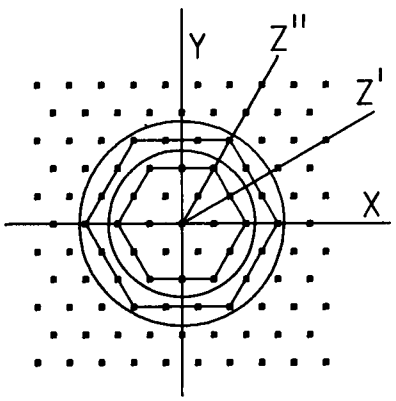

FIGURE 3 Hexagonal arrangement of hydrocarbon chains. The calculation of ϵ involves a direct summation over dipoles in a cylindrical hole and a continuum assumption for the dipoles that lie outside that hole. The calculation was carried out for cylinders with the two different radii, indicated by the circles. The hexagonal region indicates the dipoles included in the direct sum. The x and y axes are for the case of an applied field perpendicular to the membrane, and the z' and z'' axes indicate the two orientations of the field in the membrane plane that were considered.

we obtain:

$$\alpha_{C-C}^{E\parallel \text{axis}} = \alpha_{C-C}^{\parallel}\left(\frac{2}{3}\right) + \alpha_{C-C}^{\perp}\left(\frac{1}{3}\right) = 1.88\left(\frac{2}{3}\right) + 0.02\left(\frac{1}{3}\right) = 1.26\ \text{Å}^3. \tag{29}$$

E ⊥ to Hydrocarbon Axis. As the chain rotates about its axis, the C—H bonds rotate in a plane perpendicular to the axis. Then $\alpha_{C-H}^{E\perp}$ can be obtained by averaging over the angle between E and the direction of the bond (ϕ):

$$\alpha_{C-H}^{E\perp \text{axis}} = \frac{1}{2\pi}\int_0^{2\pi} d\phi(\alpha_{C-H}^{\parallel}\cos^2\phi + \alpha_{C-H}^{\perp}\sin^2\phi)$$

$$= \frac{1}{2}[\alpha_{C-H}^{\parallel} + \alpha_{C-H}^{\perp}] = 0.685\ \text{Å}^3. \tag{30}$$

The angle Θ between the C—C bond and E is given by the relation:

$$\cos\Theta = \sin\Theta_0\cos\phi, \tag{31}$$

where Θ_0 is the angle between the C—C bond and the chain axis and ϕ is the angle of rotation of the chain about its axis. Since $\cos\Theta_0 = \sqrt{2/3}$:

$$\cos\Theta = \sqrt{1/3}\cos\phi, \quad \sin\Theta = \sqrt{1 - 1/3\cos^2\phi}.$$

Then, averaging over ϕ:

$$\alpha_{C-C}^{E\perp \text{axis}} = \frac{1}{2\pi}\int_0^{2\pi} d\phi(\alpha_{C-C}^{\parallel}\cos^2\Theta + \alpha_{C-C}^{\perp}\sin^2\Theta)$$

$$= \left(\frac{1}{6}\right)\alpha_{C-C}^{\parallel} + \left(\frac{5}{6}\right)\alpha_{C-C}^{\perp} = 0.33\ \text{Å}^3. \tag{32}$$

Finally, the polarizability of the CH_2 unit is obtained simply by adding the contributions from one C—C bond and two C—H bonds:

$$\alpha_{\parallel} = \alpha_{C-C}^{E\parallel} + 2\alpha_{C-H}^{E\parallel} = 2.42\ \text{Å}^3,$$
$$\alpha_{\perp} = \alpha_{C-C}^{\perp} + 2\alpha_{C-H}^{E\perp} = 1.70\ \text{Å}^3. \tag{33}$$

where $\alpha_{\parallel}$ and $\alpha_{\perp}$ are the polarizabilities of the CH_2 unit parallel and perpendicular to the molecular chain axis, respectively.

Summation over Near Interactions

In the low-temperature crystalline phase the hydrocarbon chains are in a close-packed hexagonal structure with a separation of about 4.8 Å. In the liquid phase this packing becomes looser and less orderly and the average interchain distance is increased to about 5.0 Å. This interchain distance ("a") is the only adjustable parameter that enters into the calculation of ϵ.

A diagram of the assumed lattice structure is shown in Figs. 2 and 3. Each CH_2 group is taken as a unit dipole placed at the center of the C—C bond. In the plane of

the membrane the dipoles form a triangular lattice at a separation of a and a unit cell area of $A = a^2\sqrt{3/2}$. The C—C bond distance is 1.54 Å and its projection on the molecular axis is $1.54 \cos\Theta_0 = 1.257$ Å, which is now the distance between two neighboring CH_2 groups on the same chain and will be denoted by b. The number density of the dipole units is then:

$$\rho_{CH_2} = (Ab)^{-1} = (\sqrt{3}/2\ a^2 b)^{-1}. \tag{34}$$

The continuum approximation used for the region outside the hole should become exact as the hole becomes larger and the calculated value of ϵ should become independent of the hole size if the hole is big enough. To test this approximation, the calculation was carried out for two different values for the radius (R) of the hole and different values for the hole length (h). The two circles in Fig. 3 indicate the two values of the radius used and the solid line outlines the hexagonal region of dipoles included in the summation. The radius of the circle (R) was chosen so that the continuum number of dipoles in the hole was equal to the number of discrete dipoles in the summation:

$$\pi R^2 = N\sqrt{3}a^2/2, \tag{35}$$

where N is the number of dipoles in a single plane and the dipole density (Eq. 34) has been used.

$E \parallel$ to Hydrocarbon Axis. For this case E and the z axis is directed normal to the plane of the paper and the x and y axis are oriented as shown in Fig. 3. The expression for T_h (Eqs. 16 and 24) can then be written in the form:

$$T_h^{\parallel} = \sum_{[j]} r_j^{-5}(\rho_j^2 - 2z_j^2); \qquad \rho^2 = x^2 + y^2, \tag{36}$$

where the subscript j indicates the distance between the dipole j and the dipole at the origin and the brackets around j indicates that dipoles that belong to the same hydrocarbon chain as the dipole at the origin are not included in the summation. For the larger region in Fig. 3 the summation in Eq. 24 is over dipoles that have z coordinates of $0, \pm b, \ldots \pm nb$ and (x,y) coordinates of $(\pm a, 0)$, $(\pm 2a, 0)$, $(\pm 3a, 0)$, $(\pm 1/2a, \pm\sqrt{3}/2a)$, $(\pm 3/2a, \pm\sqrt{3}/2a)$, $(\pm 5/2a, \pm\sqrt{3}/2a)$, $(0, \pm\sqrt{3}a)$, $(\pm a, \pm\sqrt{3}a)$, $(\pm 2a, \pm\sqrt{3}a)$, $(\pm 1/2a, \pm 3\sqrt{3}/2a)$, $(\pm 3/2a, \pm 3\sqrt{3}/2a)$. The dipoles that belong to the same hydrocarbon as the dipole at the origin ($x = y = 0$, $z = 0, \pm b, \ldots \pm nb$) are not included.

$E \perp$ to Hydrocarbon Axis. The direction of E (and z) now lies in the plane of the paper in Fig. 3. We carried out the summation for the two different orientations of E(z' and z'') indicated by the two lines in Fig. 3. The results for these two orientations, which are at the two extremes, differed by only 0.1% and in the paper the average of these two values was used.

Calculation of Field Due to Surface Charge on the Hole (κ)

E Parallel to Molecular Chain Axis. The hole is a cylinder of radius R and

length h with its axis parallel to the hydrocarbon chains and centered on the dipole of interest (Fig. 3). For E parallel to the axis, the surface charge is induced only on the ends of the cylinder. For a polarization P the charge induced on a ring of radius r and thickness dr at the end of the cylinder is given by:

$$de = -2\pi r P dr. \tag{37}$$

From symmetry considerations the field on the dipole at the center of the cylinder must be in the axial direction and is given by:

$$dE = -\frac{de}{\rho^2}\cos\Theta. \tag{38}$$

The total field due to the surface charge is then given by:

$$E_{cyl} = 4\pi Ph \int_h^{\sqrt{R^2+h^2}} \rho^{-2}\, d\rho = 4\pi P[1 - h(R^2 + h^2)^{-1/2}], \tag{39}$$

where the following relations have been used:

$$r^2 + h^2 = \rho^2; \qquad r\mathrm{d}r = \rho\mathrm{d}\rho; \qquad \cos\Theta = h/\rho. \tag{40}$$

From the definition of κ (Eq. 24):

$$\kappa_\parallel = 4\pi[1 - h(R^2 + h^2)^{-1/2}]. \tag{41}$$

E Perpendicular to Molecular Chain Axis. In this case the charge is induced on the cylinder walls. The surface charge (de) in a region located at an axial distance x and polar angle ϕ is given by:

$$de = -PR\cos\phi\, \mathrm{d}x\, \mathrm{d}\phi. \tag{42}$$

The field exerted in the direction parallel to E is given by:

$$\mathrm{d}E = -\frac{\mathrm{d}e}{r^2}\sin\Theta\cos\phi; \qquad \sin\Theta = \frac{R}{r}, \qquad r^2 = x^2 + R^2. \tag{43}$$

Finally, integrating over the entire surface:

$$E_{cyl}^{\perp} = 8PR^2 \int_0^{\pi/2} \cos^2\phi \mathrm{d}\phi \int_0^h (x^2 + R^2)^{-3/2}\, \mathrm{d}x = 2\pi Ph(h^2 + R^2)^{-1/2}. \tag{44}$$

And

$$\kappa_\perp = 2\pi h(h^2 + R^2)^{-1/2}. \tag{45}$$

Calculation of the Dielectric Constant (ϵ)

The values of $\epsilon_\parallel$ and $\epsilon_\perp$ are then determined by substituting the above expressions for $\alpha_\parallel$, $\alpha_\perp$, $T_h^\parallel$, $T_h^\perp$, $\kappa_\parallel$, and $\kappa_\perp$ into Eq. 36. The values of $\epsilon_\parallel$ and $\epsilon_\perp$ as a function of the radius and length of the hole (for $a = 5.0$ Å) are shown in Table I. It can be seen

TABLE I

EFFECT OF HOLE SIZE ON ϵ FOR A THICK MEMBRANE (a = 5.0 Å b = 1.257 Å)

	Hole radius	Hole length						
		17 b	21 b	25 b	29 b	33 b	37 b	41 b
$\epsilon_\parallel$	2.29 a	2.160	2.146	2.138	2.134	2.131	2.128	
	3.19 a	2.155	2.141	2.134	2.130	2.127	2.125	2.124
$\epsilon_\perp$	2.29 a	2.283	2.290	2.293	2.296	2.297	2.298	
	3.19 a	2.268	2.277	2.281	2.284	2.286	2.287	2.288

that ϵ changes by less than 1% in going from the smaller to the larger radius and that ϵ has approached to within 1% of its limiting values for h greater than 21 b (26 Å). The results for the largest hole (R = 3.19a, h = 41b) are tabulated in Table II and plotted in Fig. 4 as a function of the interchain distance.

Comparison with Bulk Dielectric Constant of Liquid Paraffins

X-ray diffraction analysis (Warren, 1933; Pierce, 1935) has indicated that the *n*-paraffins in the liquid phase have a local hexagonal ordering similar to that assumed in the theoretical calculation for the membrane, and therefore the dielectric constant (ϵ_b) of the bulk liquid is approximately equivalent to an average over all possible orientations of the membrane:

$$\epsilon_b = \tfrac{1}{3}(\epsilon_x + \epsilon_y + \epsilon_z) = \tfrac{1}{3}\epsilon_\parallel + \tfrac{2}{3}\epsilon_\perp. \tag{46}$$

The density and refractive index (and therefore the dielectric constant from Eq. 2) of a large number of liquid hydrocarbons have been tabulated by Egloff (1939). The value of ϵ_b is relatively independent of chain length and has a value of about 2.06 at the melting point. To compare this experimental value with our theoretical calculation, it is necessary to know the value of the average interchain distance (a). It has usually been assumed that this distance is about 5.0 Å for the liquid paraffins. For this value

TABLE II

THEORETICAL VALUES OF $\epsilon_\parallel$ AND $\epsilon_\perp$ FOR A THICK MEMBRANE

a	ρ	$\rho\alpha_\parallel$	$\rho\alpha_\perp$	$\kappa_\parallel$	$\kappa_\perp$	$T_h^\parallel$	$T_h^\perp$	$\epsilon_\parallel$	$\epsilon_\perp$
Å	Å^{-3}								
4.75	0.0407	0.0985	0.0692	1.7372	5.4146	0.06847	−0.03423	2.245	2.534
4.80	0.0399	0.0965	0.0678	1.7666	5.3999	0.06817	−0.03409	2.219	2.479
4.85	0.0391	0.0945	0.0664	1.7960	5.3852	0.06788	−0.03394	2.194	2.427
4.90	0.0383	0.0926	0.0650	1.8255	5.3704	0.06759	−0.03379	2.170	2.378
4.95	0.0375	0.0907	0.0637	1.8551	5.3557	0.06729	−0.03365	2.146	2.332
5.00	0.0367	0.0889	0.0625	1.8847	5.3408	0.06700	−0.03350	2.124	2.288
5.05	0.0360	0.0872	0.0612	1.9143	5.3260	0.06670	−0.03335	2.101	2.247
5.10	0.0353	0.0855	0.0600	1.9440	5.3112	0.06641	−0.03320	2.080	2.208
5.15	0.0346	0.0838	0.0589	1.9738	5.2963	0.06611	−0.03306	2.059	2.171
5.20	0.0340	0.0822	0.0578	2.0036	5.2814	0.06582	−0.03291	2.039	2.136
5.25	0.0333	0.0807	0.0567	2.0334	5.2665	0.06552	−0.03276	2.019	2.102

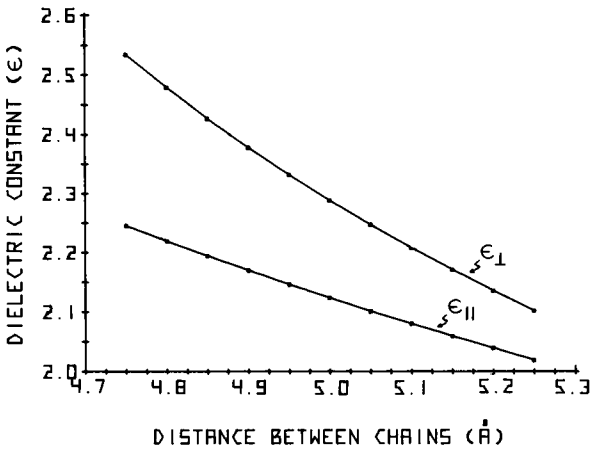

FIGURE 4 Theoretical value of the dielectric constant of a thick membrane for a field applied parallel ($\epsilon_\parallel$) and perpendicular ($\epsilon_\perp$) to the membrane plane as a function of the average separation (a) between the hydrocarbon chains.

of a (Table II): $\epsilon_\parallel = 2.124$; $\epsilon_\perp = 2.288$; and from Eq. 46: $\epsilon_b = 2.23$, about 8% larger than the experimental value of 2.06. Although this agreement between theory and experiment is satisfactory, the following arguments show that the agreement may actually be much better than this.

The value of 5.0 Å for the interchain separations was obtained from a calculation of the volume per molecule in the liquid and with the assumption of a hexagonal packing arrangement. This calculation is based on the assumption (Müller, 1932) that the separation between the CH_3 groups at the chain ends has the same values as in the crystalline phase (determined from X-ray diffraction). However, it is possible that when the hydrocarbon melts and the average interchain separation increases, the CH_3 groups could slightly interdigitate and the average length per chain might be less than in the crystal state. Thus, as an alternative approach, we have determined the interchain separation by an approach that does not require any assumption about the length per chain. We assume that the molecular volume (V_n) for a paraffin that contains n carbon atoms is described by the following equation:

$$V_n = [a^2\sqrt{3}/2][(n - 1)b + 2d], \tag{47}$$

where the first set of brackets is the cross-sectional area for hexagonal packing (Eq. 34) and the second set is the chain length where b is the length associated with each C—C bond (1.257 Å) and d is the additional length associated with each CH_3 group. A plot of V_n versus n for a series of paraffins should be a straight line whose slope should provide an estimate of the interchain distance (a). Such a plot is shown in Fig. 5 for saturated straight chain paraffins containing an even number of carbon atoms (n = 18–34) with the hydrocarbon density at the melting point (Egloff, 1939). The straight line in Fig. 5 is a least squares fit with a slope of 29.63 and an intercept of 11.06, corresponding to an a of 5.22 Å and d of 0.86 Å. This value of d is approximately equal to length of the C—H bond (1.09 Å) and is significantly smaller than the

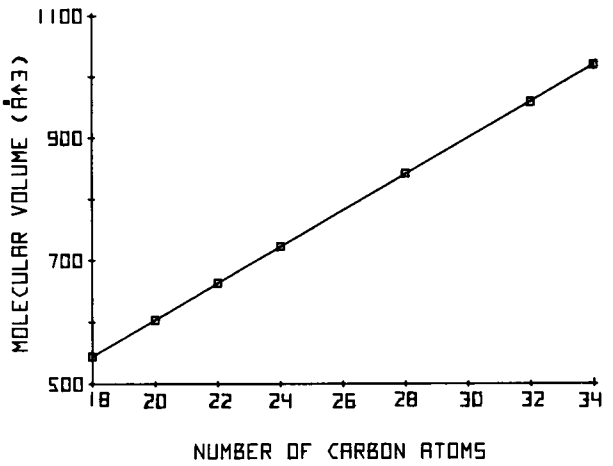

FIGURE 5 Plot of the molecular volume of straight chain liquid *n*-paraffins at their melting point as a function of the number of carbon atoms. The straight line is a least square fit to the data.

value determined for the crystalline phase. Using a value of a of 5.22 Å in Fig. 4 (and Eq. 46) yields a theoretical value for ϵ_b of 2.09, very close to the experimental value of 2.06. Although the X-ray diffraction pattern for the liquid hydrocarbons is consistent with an interchain separation of 5.0 Å, these data are probably not good enough to distinguish between a value of 5.0 and 5.2 Å.

THIN MEMBRANES

It has been established by electron diffraction that the hydrocarbon chains in films built up from repeated bilayers of unsaturated fatty acids (e.g., barium stearate) are hexagonally arranged with their axis perpendicular to the plane of the membrane (Germer and Storks, 1938). Thus these films have a structure similar to that assumed in our theoretical calculations and should provide a good experimental test. Blodgett and Langmuir (1937) first measured the values of the refractive index parallel ($n_\parallel$) and perpendicular ($n_\perp$) to the membrane plane. More recently, Den Engelsen and his colleagues (for review, see Den Engelsen, 1976) and Tomor and Srivastavo (1973) have determined n by ellipsometry. As mentioned in the Introduction, n for the hydrocarbon region can be related to ϵ by the use of Eq. 2. The value of n that is actually measured has contributions from both the polar head groups and the hydrocarbon regions, so that there is some uncertainty in the value for n for the hydrocarbon region. However, for the long-chain fatty acids, the relative contribution from the polar head groups should be small. Den Engelsen (1971) obtained values of $n_\parallel = 1.549$ and $n_\perp = 1.518$ for a mixture of cadmium arachidate and arachidic acid ($C_{20}H_{40}O_2$). For a chain separation (a) of 4.85 Å (determined from electron diffraction), our predicted theoretical results (Table II) for a thick film are (by using Eq. 2) $n_\parallel = 1.48$ and $n_\perp = 1.56$, which are within 5% of the experimental values. However, it is disturbing that we predict the wrong sign for the optical birefringence ($n_\parallel - n_\perp$). This dis-

crepancy might be due to the application of a thick membrane model to a bilayer membrane, and in this section the effect of membrane thickness will be examined.

If it is assumed that the membrane is uniformly polarized ($p_i = p_j$), then one can derive Eq. 26 and the only possible effect of membrane thickness is that it limits the size of the hole that can be used. However, as is shown in Table I, the value of ϵ for a hole 21 carbon atoms long (a very thin membrane) differs from the limiting value for a thick membrane by only about 1%. Thus, this effect of membrane thickness is negligible. However, a thin membrane is not uniformly polarized and this effect will be examined in this section. From symmetry the value of p should be the same for all atoms in the same plane parallel to the membrane surface. Then, summing T_{ij} over all the dipoles in a given plane, one can write Eq. 15 as:

$$p_i = \alpha\left[E^0 - \sum_{j=1}^{N} S_{ij}p_j\right], \tag{48}$$

where p_i is the dipole strength in the ith plane, and S_{ij} is the interaction of all the dipoles in jth plane with a dipole in the ith plane, and N is the number of planes (carbon atoms) across the membrane.

Eq. 48 can be written in matrix form in terms of the dimensionless variable p_i':

$$[\mathbf{I} + \alpha\mathrm{S}]p' = I; \qquad p_i' = p_i/\alpha E^0. \tag{49}$$

Eq. 49 can then be solved for p_i' by inverting the $N \times N$ matrix. The expression for S_{ij} is determined by the following procedure. The interaction is divided into an exact summation over the near dipoles (S_{ij}^n) plus an integration over the far dipoles (S_{ij}^f). For example, for an applied field parallel to the axis, T_{ij} is given by Eq. 30, and the interaction between the near dipoles in plane j (by using the smaller circle in Fig. 3) with a dipole in plane 1 is given by:

$$S_{1j}^n = 6\left\{\frac{a^2 - 2(jb)^2}{[a^2 + (jb)^2]^{5/2}} + \frac{(2a)^2 - 2(jb)^2}{[(2a)^2 + (jb)^2]^{5/2}} + \frac{(\sqrt{3}a)^2 - 2(jb)^2}{[(\sqrt{3}a)^2 + (jb)^2]^{5/2}}\right\}. \tag{50}$$

As discussed above, there is no interaction between dipoles on the same hydrocarbon chain. The far interaction component of S_{1j} is given by:

$$\mathrm{S}_{1j}^f = 2\pi s \int_R^\infty \frac{[r^2 - 2(jb)^2]}{[r^2 + (jb)^2]} r\mathrm{d}r = \frac{4\pi R^2}{\sqrt{3}a^2[R^2 + (jb)^2]^{3/2}} \tag{51}$$

where R is determined from Eq. 35 and s is the number of dipoles in a plane per unit area (Eqs. 7 and 44). With S_{ij} known, Eq. 49 can be solved for the p_i'. Table III gives an example of one such calculation for a membrane 37 atoms thick. It can be seen that p' varies from a value of 0.6330 at the membrane surface to 0.4736 at the center.

From the definition of ϵ for a nonuniformly polarized membrane (eq. 8):

$$\epsilon = 1 + 4\pi\bar{P}/\bar{E} = 1 + 4\pi\alpha\rho\bar{p}'(E^0/\bar{E}). \tag{52}$$

TABLE III

VARIATION OF p' FOR A MEMBRANE 37 CARBON ATOMS IN THICKNESS (a = 5.0 Å)

Position	1 (edge)	2	3	5	7	9	12	15	19 (middle)
p'	0.6330	0.5472	0.5026	0.4798	0.4764	0.4751	0.4742	0.4737	0.4736

Finally, $\bar{E}$ can be related to E^0 by Eqs. 19 and 20 and Eq. 52 solved for ϵ:

$$\epsilon_{\parallel} = (1 - 4\pi\alpha\rho\bar{p}')^{-1}; \qquad \epsilon_{\perp} = 1 + 4\pi\alpha\rho\bar{p}' \tag{53}$$

The values of $\epsilon_{\parallel}$ and $\epsilon_{\perp}$ as a function of membrane thickness (N) are listed in Table IV and compared with the corresponding value determined as described above for a thick membrane (Eq. 26). (The slight difference, about 1%, between the bulk values in Tables II and IV is due to the smaller hole used in Table IV.) It can be seen that in going from a very thin membrane (N = 19) to a thick membrane, $\epsilon_{\parallel}$ decreased by about 8% and $\epsilon_{\perp}$ increased by about 4%. Although these changes are small, their direction improves the agreement between our theoretical results and experiments on built-up films of fatty acids. For example, the experimental values of $n_{\parallel}$ = 1.549 and $n_{\perp}$ = 1.518 for arachidic acid are now within 2% of our values (from Eq. 2) for a = 4.85 Å (the value determined from electron diffraction) and N = 37. However, we still predicted the wrong sign for the optical birefringence. Possible reasons for this uiscrepancy are: (*a*) Errors in our calculation: in particular we have neglected correlations in the polarization of neighboring dipoles; (*b*) Error in interpreting the optical measurements as representing just the hydrocarbon region of the membrane, since the observed birefringence could result from an anisotropic orientation of the polar head groups; (*c*) Although the experimental value of ϵ is given exactly in terms of the average polarization (Eq. 53), the interpretation of n for a nonuniformly polarized membrane is more complicated since each plane will tend to scatter light separately. Cherry and Chapman (1969*b*) have shown that if the variation in n for the different layers is small, then the experimental value of n is equivalent to the average value for the membrane, which is the assumption that we made in the above comparison. However, this is only an approximation and could account for the discrepancy.

TABLE IV

DEPENDENCE OF ϵ ON MEMBRANE THICKNESS (N)

N	a = 4.85 Å		a = 4.95 Å		a = 5.05 Å		a = 5.15 Å	
	$\epsilon_{\parallel}$	$\epsilon_{\perp}$	$\epsilon_{\parallel}$	$\epsilon_{\perp}$	$\epsilon_{\parallel}$	$\epsilon_{\perp}$	$\epsilon_{\parallel}$	$\epsilon_{\perp}$
19	2.3885	2.3347	2.3275	2.2494	2.2684	2.1729	2.2152	2.1040
25	2.3366	2.3568	2.2793	2.2691	2.2256	2.1906	2.1753	2.1200
31	2.3074	2.3706	2.2521	2.2815	2.1998	2.2018	2.1511	2.1301
37	2.2871	2.3801	2.2331	2.2900	2.1825	2.2094	2.1350	2.1370
43	2.2730	2.3871	2.2201	2.2962	2.1702	2.2150	2.1234	2.1421
49	2.2623	2.3924	2.2100	2.3010	2.1609	2.2193	2.1147	2.1459
55	2.2540	2.3965	—	2.3047	2.1536	2.2226	2.1079	2.1490
Thick ($N = \infty$)	2.1879	2.4311	2.1404	2.3356	2.0957	2.2505	2.0536	2.1742

The theoretical values can also be compared with experimental measurements of $n_\parallel$ and $n_\perp$ on black lipid membranes (Cherry and Chapman, 1969*a*; Den Engelsen and de Koning, 1975). This comparison is a much weaker test of our calculations because: (*a*) the structure and composition of these membranes are uncertain; (*b*) the contribution of the polar head groups of lipids such as lecithin will be greater than for the fatty acids; and (*c*) the measurement of $n_\parallel$ and $n_\perp$ on these membranes is less direct and requires additional assumptions. In any case, the experimental values of $n_\parallel = 1.47$ and $n_\perp = 1.45$ for a lecithin-decane membrane are in good agreement with our result of 1.48 for both $n_\parallel$ and $n_\perp$ for $N = 37$ and $a = 5.05$ Å (a reasonable value since these membranes are in the fluid phase).

CONCLUSION

The primary purpose of this analysis was to determine the value of ϵ that should be used in Eq. 1 in order to relate capacitance measurements to membrane thickness. As discussed above, $\epsilon_\parallel$ should be used in Eq. 1. Most investigators have assumed that the dielectric constant for some appropriate bulk hydrocarbon would be a reasonable approximation to $\epsilon_\parallel$. The most important result of our analysis is that it provides a theoretical justification for this assumption and an estimate of the error involved in using it. For example, for $a = 5.0$ Å, the value of $\epsilon_\parallel$ for a membrane bilayer ($N = 37$) differs from the bulk value of ϵ (from Eq. 46, with the results for a thick membrane) by about 2%. This general conclusion, that $\epsilon_\parallel$ is well approximated by the bulk value, should be relatively independent of the specific structure and composition of the membrane. The value of ϵ has a strong dependence on the distance between the hydrocarbon chains (*a*), as can be seen from our theoretical calculations (Fig. 4) or from comparison of the experimental value of $n = 1.43$ for the bulk (liquid) hydrocarbon with the value of $n_\parallel$ of 1.55 for films of arachidic acid in the solid (crystalline) phase. Thus, a significant error would be introduced into the determination of membrane thickness from Eq. 1 if bulk values of ϵ for the liquid hydrocarbon were used for membranes thought to be in the solid state.

APPENDIX

Further Comparison with Calculations of S. Ohki

In addition to the points discussed in the text, there are several other aspects of Ohki's analysis that we believe are in error. First, it appears that he made an error in the calculation of the polarizability of the C_2H_4 unit that he used. In his Eq. 21, Ohki has given the general expression for the polarizability of a hydrocarbon unit:

$$\alpha = \bar{\alpha}\mathbf{1} + \frac{1}{3}(\gamma_{C-C} - 2\gamma_{C-H}) \sum_{C-C} \mathbf{F}_j \qquad (1A)$$

where the sum is over all the carbon-carbon (C—C) bonds in the unit. The tensor $\mathbf{F}$ is diagonal ($F_\parallel$) if the C—C bond is parallel to the x axis (perpendicular to the membrane surface). In the trans conformation, the C—C bonds are not parallel to the x axis, F is

not diagonal, and Ohki transformed F in order to diagonalize it. However, an error was made in this transformation because his final result (Eq. 32) can be rewritten as:

$$\alpha_{C2H4} = \alpha_{C2H4} \mathbf{I} + \tfrac{2}{3}(\gamma_{C-C} - 2\gamma_{C-H}) F_{\parallel} \qquad (2A)$$

where $F_{\parallel}$ is the form of the tensor for a C—C bond parallel to the x-axis. Comparing Eqs. 1 A and 2 A, it can be seen that the polarizability that Ohki used for the C_2H_4 unit is equivalent to assuming that both the C—C bonds in the C_2H_4 unit are parallel to the x axis, which is incorrect.

We have not been able to reproduce the calculation of $T_{\parallel \text{sum}}$ in the appendix of Ohki's paper. He obtained a value of 0.0699 for a = 5.0 Å and we obtained a value of −0.13 when we tried to reproduce it. Using our value would introduce a very large error into Ohki's calculation of the dielectric constant. Finally, a relatively minor point, Ohki used a square lattice in the membrane plane while we used a hexagonal lattice.

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REFERENCES

BLODGETT, K. A., and I. LANGMUIR. 1937. Built-up films of barium stearate and their optical properties. *Phys. Rev.* **51**:964.

BOTTCHER, C. J. F., O. C. VAN BELLE, P. BORDEWIJK, and A. RIP. 1973. The Theory of Electric Polarization. Elsevier Scientific Publishing Company, Amsterdam.

CHERRY, R. J., and D. CHAPMAN. 1969*a*. Optical Properties of black lecithin films. *J. Mol. Biol.* **40**:19–32.

CHERRY, R. J., and D. CHAPMAN. 1969*b*. Optical determination of the thickness of thin lipid films. *J. Theor. Biol.* **24**:137–146.

DENBIGH, K. G. 1940. The polarisabilities of bonds. I. *Faraday Soc. Trans.* **36**:936.

DEN ENGELSON, D. 1971. Ellipsometry of anisotropic films. *J. Opt. Soc. Am.* **61**:1460–1466.

DEN ENGELSON, D., and B. DE KONING. 1975. Ellipsometry of black lipid membranes of egg lecithin and chloroplast extracts. *Photochem. Photobiol.* **21**:77–80.

DEN ENGELSON, D. 1976. Optical anisotropy in ordered systems of lipids. *Surf. Sci.* **56**:272–280.

EGLOFF, G. 1939. Physical constants of hydrocarbons. Vol. I. Reinhold Publishing Corp., New York.

GERMER, L. H., and K. H. STORKS. 1938. Arrangement of molecules in a single layer and in multiple layers. *J. Chem. Phys.* **6**:280–293.

MÜLLER, A. 1932. An x-ray investigation of normal paraffins near their melting points. *Proc. R. Soc. Lond. A. (Math. Phys. Sci.).* **138**:514.

OHKI, S. 1968. Dielectric constant and refractive index of lipid bilayers. *J. Theor. Biol.* **19**:97.

OHKI, S. 1969. Properties of lipid bilayer membranes. Determination of membrane thickness. *J. Theor. Biol.* **23**:158.

OHKI, S. 1970. Properties of lipid bilayer membranes. Membrane thickness. *J. Theor. Biol.* **26**:277.

PHILLIPS, J. C. 1969. Covalent Bonding in Crystals, Molecules and Polymers. University of Chicago Press, Chicago, Ill.

PIERCE, W. C. 1935. Scattering of x-rays by polyatomic liquids, n-heptane. *J. Chem. Phys.* **3**:252.

TOMOR, R. S., and V. K. SRIVASTAVO. 1973. Anisotropic effects in the ellipsometry of "built-up" films and determination of their optical constants. *Thin Solid Films.* **15**:207–215.

WARREN, B. E. 1933. X-ray diffraction in long chain liquids. *Phys. Rev.* **44**:969.