

THE FORM BIREFRINGENCE OF LAMELLAR SYSTEMS CONTAINING THREE OR MORE COMPONENTS

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INTRODUCTION

A system of cytoplasmic membranes, referred to as endoplasmic reticulum (1), has been seen in many types of cells with the electron microscope. This structure is of great interest because several lines of evidence identify it as a probable site of protein synthesis (1-4). Such membranes are often arranged in more or less parallel layers and in some types of cells, particularly in plasma cells (5, 6), and in the exocrine cells of the pancreas (7, 8), these layers form an irregular system of concentric lamellae around the nucleus. If such arrangements exist in the living cell then corresponding areas in the cytoplasm should show birefringence resulting from: (a) form component which, according to the Wiener (9) equation for plates, must be negative with respect to an axis perpendicular to the plates, and (b) a possible intrinsic component whose axis has not been deduced from electron micrographs.

Birefringence has been observed in a number of cellular structures such as the myelin sheath of nerve fibers (10), retinal rods (11), and the basal areas in the cytoplasm of a number of glandular cells (12). The optical behavior of these structures in fresh material and during subsequent treatment with fixatives and imbibition fluids has often been interpreted as evidence for the existence of protein lamellae with interspersed layers of perpendicularly oriented lipid molecules. Subsequent studies with the electron microscope and, in some cases, with x-ray diffraction support this interpretation. Recent observations of continuity between myelin lamellae and the plasma membrane of the Schwann cell (13, 14), between the nuclear membrane and endoplasmic reticulum (15), etc. suggest that the classical lipoprotein models may be applicable to cellular membranes generally.

With the large amount of new information that is available, particularly from electron microscopy and from biochemistry of "structural lipides," a thorough review of evidence on the structure of living membranes is desirable. If the classical model is correct, however, it is evident that in many cases the form birefringence of oriented membranes must be interpreted on the basis of three components of differing refractive index, *i.e.*, protein, lipid, and cellular

fluids, rather than on the basis of the two component system treated by the Wiener equations. In this paper the equation for the form birefringence of lamellae is extended to cover systems containing three or more components. The preceding remarks are intended to explain the interest in this problem and to suggest possible applications for the equations which have been derived.

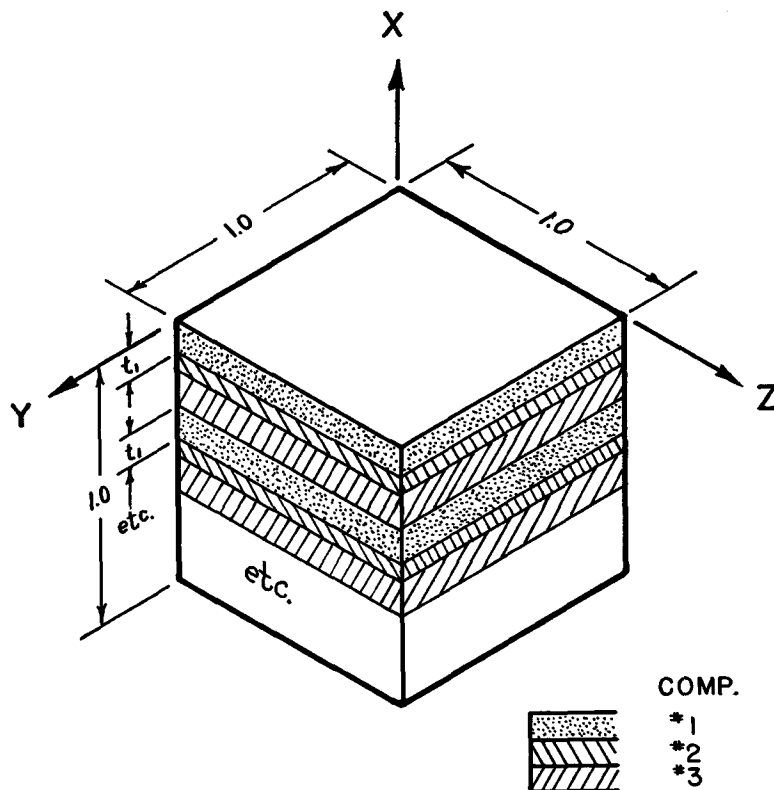


FIG. 1. Diagram of a unit volume composed of parallel plates. The sum of all elements with thickness t_1 and dielectric constant K_1 (stippled) have a partial volume d_1 . The x axis is the optical axis.

DERIVATION

The capacity and, hence, the refractive index of a stack of thin dielectric plates (parallel membranes immersed in a dielectric medium) may be computed on the basis of classical electrostatic theory. In this derivation we shall express the refractive indices of such a system for fields whose electric vector is either perpendicular or parallel to such plates by obtaining sums of the capacities of elements in series and in parallel, respectively. This model has been previously employed by Oster (16) and is formally analogous to the original derivation by Wiener (9).

While additional assumptions will be necessary in treating systems which contain elements of other shapes, such as rods, the model is adequate for the restricted case of plates and no new insight into the problem will be provided by a reconsideration of appropriate boundary conditions to Maxwell's equations. The total birefringence of a sample will generally contain intrinsic birefringence as well as form birefringence. When the magnitude and axis of the intrinsic term is known, however, it may be treated in the usual manner. Only the form birefringence is considered below. In order to simplify the notation, the equations below will relate to the properties of the cube of unit dimensions depicted in Fig. 1.

The capacitance of a parallel-plate condenser is equal to $KA/4\pi t$ in which K is the dielectric constant, A is the area, and t the thickness. The total capacitance of a single component taken in the x direction is equal to $K/4\pi d$ in which the area is equal to unity and in which d is the sum of the thicknesses of lamellae contained in a unit of volume and, hence, is equal to the partial volume of that component. The total capacitance of a single component taken in the y or z direction is $Kd/4\pi$ in which the thickness is equal to unity and the area is numerically equal to the partial volume. For the components, numbered 1, 2, and 3, with partial volumes d_1 , d_2 , d_3 , and dielectric constants K_1 , K_2 , and K_3 , the capacitance of a unit cube in the x direction is expressed as the sum of the capacitances of condensers in series:

$$\frac{1}{C_x} = \frac{4\pi d_1}{K_1} + \frac{4\pi d_2}{K_2} + \frac{4\pi d_3}{K_3} \quad (1)$$

$$4\pi C_x = \frac{K_1 K_2 K_3}{d_1 K_2 K_3 + d_2 K_1 K_3 + d_3 K_1 K_2} = K_x$$

Similarly the capacitance of a unit cube in the y or z direction is expressed as the sum of the capacitances of condensers in parallel:

$$4\pi C_y = 4\pi C_z = d_1 K_1 + d_2 K_2 + d_3 K_3 = K_y = K_z \quad (2)$$

While in Fig. 1 the three components are shown in periodic sequence, they may occur in any arbitrary sequence so long as the thickness of any one element or the thickness of a region of random fluctuation in refractive index due to the ordering of elements is small in relation to the resolving power of the instrument used. By setting $C_y = C_z$ the development is restricted to the uniaxial case.

By convention, uniaxial birefringence is defined as the difference in refractive indices, $n_a - n_o$, in which n_a is measured parallel with the optical axis (the x direction in Fig. 1) and n_o is measured perpendicular to the optical axis (such as in the y and z directions). Since the total capacitances of a unit cube with respect to each of the coordinates, C_x , C_y , and C_z , are proportional to the dielectric constants of the composite system, K_x , K_y , and K_z , and since these in

turn can be equated to the squares of the refractive indices, n_x^2 , n_y^2 , and n_z^2 , we may solve for the birefringence, BR , as follows:

$$\begin{aligned} 4\pi C_x &= K_x = n_x^2 = n_a^2 \\ 4\pi C_y &= 4\pi C_z = K_y = K_z = n_y^2 = n_z^2 = n_o^2 \\ n_a^2 - n_o^2 &= K_x - K_y \\ (n_a - n_o)(n_a + n_o) &= K_x - K_y \\ BR &= \frac{1}{n_a + n_o} (K_x - K_y) \end{aligned} \quad (3)$$

Substituting values for K_x and K_y from equations (1) and (2) into equation (3):

$$BR = \frac{+1}{n_a + n_o} \left[\left(\frac{K_1 K_2 K_3}{d_1 K_2 K_3 + d_2 K_1 K_3 + d_3 K_1 K_2} \right) - (d_1 K_1 + d_2 K_2 + d_3 K_3) \right] \quad (4)$$

The expression in brackets may be reduced to a convenient form by algebraic manipulations in which the fact that $1 = d_1 + d_2 + d_3$ is used in two separate steps. Substituting for the K 's the corresponding squares of the refractive indices:

$$BR = \frac{-1}{n_a + n_o} \left[\frac{d_1 d_2 n_3^2 (n_2^2 - n_1^2)^2 + d_2 d_3 n_1^2 (n_3^2 - n_2^2)^2 + d_1 d_3 n_2^2 (n_1^2 - n_3^2)^2}{d_1 n_2^2 n_3^2 + d_2 n_1^2 n_3^2 + d_3 n_1^2 n_2^2} \right] \quad (5)$$

The N component system:

The birefringence of systems containing any number of components, N , is obtained by writing an equation in the same form as (4). Placing the K_y terms over a common denominator produces N^2 terms, N of which may be factored to give a term containing one minus the sum of the squares of the partial volumes. The remaining terms may be collected into $N(N - 1)/2$ pairs each of which contains a coefficient times the sum of two K -squared terms. By completing the squares of the K -squared terms and repeating the steps above, the following expression is obtained:

$$BR = \frac{-1}{n_a - n_o} \left[\frac{\frac{1}{2} \sum_{i,j} \frac{d_i d_j}{n_i^2 n_j^2} (n_i^2 - n_j^2)^2}{\sum_i \frac{d_i}{n_i^2}} \right] \quad (6)$$

DISCUSSION

Several comments on these equations are of interest:

1. If all but two of the d 's become zero or if all but two of the K 's become

equal to one another, both equations (5) and (6) reduce to the form in which the Wiener equation for plates is usually quoted¹:

$$BR = \frac{-1}{n_a + n_o} \left(\frac{d_1 d_2 (n_1^2 - n_2^2)^2}{d_1 n_2^2 + d_2 n_1^2} \right) \quad (7)$$

2. Since both the d 's and the square of differences of the refractive index terms will always be positive, the form birefringence of any system containing two or more lamellar components will always be negative.

3. If the refractive index of a single component is varied, as will be the case when it is diluted or replaced by an imbibition fluid, the form birefringence will also vary. Such effect may be summarized by saying that (a) as a component of higher or lower refractive index approaches an average² refractive index of other components in the system the form birefringence will be decreased, and (b) as a component with an intermediate refractive index attains higher or lower values the form birefringence will increase. This may be illustrated by taking a simple example. Let $d_1 = d_2 = d_3 = \frac{1}{3}$ and $n_1 = 1.3$, $n_2 = 1.4$, and $n_3 = 1.5$. (Such an example may apply to certain systems in which lipoprotein lamellae are suspended in cellular fluids, the refractive indices selected being approximately those of water, lipide, and protein.) The birefringence predicted by equation (5) is 6.8×10^{-3} ; if the refractive index of the first component becomes equal to the second (as might be the case if part of the cellular fluids were replaced with glycerol), the predicted birefringence is 3.0×10^{-3} or approximately one-half of its original value; if the refractive index of the second component becomes equal to the first or third (as might be the case if lipide molecules were replaced by solvents), the birefringence becomes 1.3×10^{-2} , approximately twice its original value.

4. Since it is frequently desirable to obtain an estimate of a partial volume or a refractive index from a measured birefringence, an explicit solution for these terms would be of value. In practice such solutions are quite tedious and it is usually more convenient, even for the two-component systems, to obtain a solution by successive approximation.

5. Equations (5) and (6) contain the expression $1/(n_a + n_o)$. Since its value varies slowly with changes in birefringence, it is common practice to replace it with $\frac{1}{2}n$ where n is the average refractive index of the specimen. Situations

¹ Quotations of this equation in several important reviews on the polarizing microscope, Ambronn and Frey (20), Bennett (19), Oster (16), and Pfeiffer (18), contain a typographical error in which subscripts in the denominator have been reversed. The denominator should read " $d_1 n_2^2 + d_2 n_1^2$ " and not " $d_1 n_1^2 + d_2 n_2^2$."

² In three component systems the term "intermediate" may be substituted for the term "average." With a greater number of components ambiguities may arise since the average will depend upon the way in which the partial volumes are distributed and a detailed calculation may be required in order to predict the direction of the change.

may arise where independent measurement of n_a and n_o with such instruments as the interference microscope are desirable.

6. While transparent isotropic dielectrics have been assumed, the above equations describing systems which contain absorbing components may also be derived by extending Wiener's (17) original treatment of this problem. The optical behavior of absorbing systems, however, is not particularly obvious and in each case it will be desirable to consider the predicted behavior of pertinent alternative distributions of partial volumes, etc. Dramatic changes in birefringence and dichroism are to be expected when lamellar systems are stained with dyes or fixed with heavy metals. The construction of an interesting series of polarizing filters from thin layers of colored materials is suggested.

7. The thicknesses of the plates were assumed to be small in comparison with the dimension of the unit cube, an assumption which assures the approximate linearity of the electric field (across the condenser). The thicknesses must also be small with respect to the wave length of the radiation used, a condition which is often met in biological systems where the thickness of layers is of the order of 100 Å. As the thickness of any plate approaches the wave length, however, elements of the system act more as "discrete scattering centers." In such cases light from the specimen tends to be "depolarized," or with high aperture optical systems, fine structure is "resolved."

8. In the derivation, homogenous plates with sharp boundaries were assumed. If the refractive index varies within a plate (as might be the case for protein membranes in aqueous media), equation (6) will become an integral equation which contains a suitable fitting function for the variation in refractive index.

SUMMARY

Equations are derived which predict the form birefringence of lamellar systems containing three or more components. These equations may be found useful in interpreting the birefringence of biological specimens and in testing certain theories about the structure of natural membranes.

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