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THE THICKNESS OF BIMOLECULAR LIPID MEMBRANES

R. SIMONS

University of New South Wales, School of Physics, Kensington (Australia)

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SUMMARY

The DRUDE theory for the reflection of light by transition layers has been employed to obtain expressions for the reflectivity of optically inhomogeneous thin films. The range of validity of the different expressions has been examined by comparing the values predicted for a number of symmetrical trilayers with those calculated using multilayer theory. Application of the treatment to lipid bilayer films reveals a large uncertainty in the thickness estimates obtained by the single layer model.

INTRODUCTION

In recent years several groups of workers[1-4] have investigated the optical properties of lipid membranes. Their interest has evolved from the realization that a knowledge of the light reflectance and refractive index of the films can, for specific membrane models, lead to estimates of film thickness. The two membrane models which have received attention are the single layer and the triple layer models.

(*i*) *The single layer model.* This was originally proposed by HUANG AND THOMPSON[1], and is the simplest possible membraneous model. In it the membrane and adjacent aqueous phases are regarded as being isotropic dielectrics characterized by refractive indices that are uniform up to the membrane–water interfaces (Fig. 1).

(*ii*) *The triple layer model.* This has been proposed by TIEN[3]. It attempts to take account of the heterogeneity in membrane structure which arises from the presence of both polar and non-polar groups in the membraneous molecules. The polar groups are regarded as being located at the interfaces of the membrane and water phases where they form layers of a refractive index that is different from that of the membrane interior. The membrane is thus considered to be a symmetrical trilayer with a refractive index profile similar to that shown in Fig. 1.

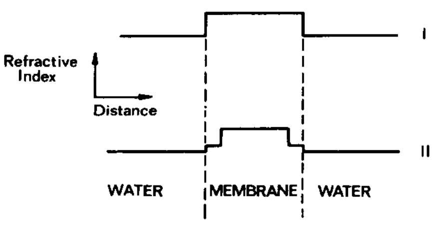


Fig. 1. Scheme of refractive index profile: (I) single layer model; (II) triple layer model.

In both the above models the dielectric constant is assumed to change discontinuously at an interface. Now it has been pointed out by DRUDE[5,6], and confirmed by VASICEK[7], that at any interface there exists a thin transition layer within which the refractive index varies continuously between that of the two adjacent media. One effect of this transition layer is to cause the slight elliptical polarization often observed in light reflected at the Brewster angle.

The thickness of a transition layer depends on the particular system involved. At the air–glass interface it can be around 100 Å (see refs. 5–7). However, the degree of elliptical polarization present in light reflected at the Brewster angle at a liquid–air interface is smaller than that for glass[6], and it seems that in these examples the transition layer may be of a smaller thickness.

An approximate theory for the reflection and refraction of light by transition layers was developed by DRUDE[5,6]. It has been verified both theoretically and experimentally by VASICEK[7]. The theory applies not only to transition layers *per se* but also to any region where the refractive index varies in a single direction over a distance l which is small compared with the wavelength of the incident light.

It seems likely that lipid bilayers contain such regions. TIEN[3] has drawn attention to the presence of both polar and non-polar groups in the membraneous molecules as one factor which contributes to a variation in refractive index. The presence of transition layers between the different membraneous regions constitutes another factor. Additional variation would result from factors such as interdigitation of the hydrocarbon chains[2] or a spatial distribution in the amount of hydrocarbon solvent or water trapped within the membrane.

From the knowledge derived from electrical[8–10], electron microscopic[11] and optical[1,2,4] techniques that lipid bilayer membranes have thicknesses around 100 Å it appears that the DRUDE transition layer theory can be applied to these systems to obtain formulae for the intensity of light which is reflected or refracted by the bilayers.

This is done here for light at near-normal incidence on the membranes. The formula for the reflected light intensity turns out to be a rather general expression that is not limited to a single membrane model. It is a relatively simple function of parameters such as refractive index, wavelength *etc.* which can be measured. However, it also involves the spatial dependence of the dielectric constant which is currently unknown.

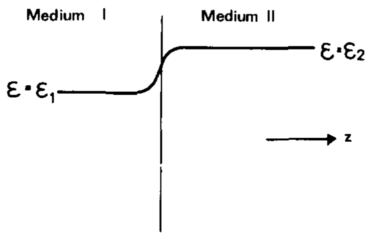
THEORY

Complex amplitude and phase relations for light incident on an inhomogeneous region

Let us consider the case where the boundary region between two dielectrics is inhomogeneous with respect to the dielectric constant. Inside the inhomogeneous region the dielectric constant changes continuously from a value of ε_1, which equals that of the bulk medium I, to a value of ε_2 equal to that of medium II. The variation in ε is restricted to the z direction only as shown in Fig. 2.

Suppose now a plane wave is incident from medium I at an angle θ at the boundary of the inhomogeneous region. Let us regard the incident ray as being in the XZ plane and the boundary as being in the XY plane. The ray that emerges from the opposite boundary of the inhomogeneous region will be referred to as the refracted ray and the angle of refraction denoted by χ (Fig. 3).

Let E, R and D denote the electric field amplitudes in the incident, reflected and refracted rays, respectively; E_p, R_p and D_p denote the components of E, R and D in the plane of incidence; and E_s, R_s and D_s denote the components of E, R and D perpendicular to the plane of incidence.



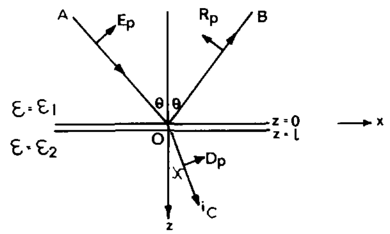


Fig. 2. A possible profile for the dielectric constant at the boundary of two media.

Fig. 3. Plane wave AO incident at the boundary ($z = 0$) of an optically inhomogeneous region of thickness l which is located at the interface of two media. OB and OC are the reflected and refracted rays, respectively. θ and χ are the respective angles of incidence and refraction.

The inhomogeneous region introduces phase shifts δ and δ' in the reflected and refracted rays with respect to the incident ray. It is therefore convenient to introduce complex amplitudes $\vec{R}_s$, $\vec{D}_s$ *etc.* defined by the relations

$$\vec{R}_s = R_s\, e^{j\delta_s} \qquad \vec{R}_p = R_p\, e^{j\delta_p}$$
$$\vec{D}_s = D_s\, e^{j\delta_{s'}} \qquad \vec{D}_p = D_p\, e^{j\delta_{p'}} \tag{1}$$

where, as previously, the subscripts p and s refer to quantities in the plane of incidence and perpendicular to the plane of incidence, respectively.

If the width l of the inhomogeneous region is much smaller than the wavelength λ of the incident light the following relations apply[5–7]

$$(E_p - \vec{R}_p)\cos\theta = \vec{D}_p\left[\cos\chi + j\,\frac{2\pi}{\lambda}\,(l - q\varepsilon_2\sin^2\chi)\sqrt{\varepsilon_1\varepsilon_2}\right]$$
$$E_s - \vec{R}_s = \vec{D}_s\left(1 + j\,\frac{2\pi}{\lambda}\,l\sqrt{\varepsilon_2}\cos\chi\right) \tag{2}$$
$$(E_s - \vec{R}_s)\sqrt{\varepsilon_1}\cos\theta = \vec{D}_s\left[\sqrt{\varepsilon_2}\cos\chi + j\,\frac{2\pi}{\lambda}\,(p - l\varepsilon_2\sin^2\chi)\right]$$
$$(E_p + \vec{R}_p)\sqrt{\varepsilon_1} = \vec{D}_p\left(\sqrt{\varepsilon_2} + j\,\frac{2\pi}{\lambda}\,p\cos\chi\right)$$

where

$$l = \int_1^2 dz \qquad p = \int_1^2 \varepsilon dz \qquad q = \int_1^2 \frac{dz}{\varepsilon} \tag{2a}$$

the integration being performed over the inhomogeneous region from the medium containing the incident ray to the medium containing the refracted ray.

Eqns. 2 were first derived by DRUDE[5,6]. Their approximate validity has been verified both theoretically and experimentally by VASICEK[7]. In his theoretical treatment VASICEK[7] showed that the equations obtained for the reflection of light by a very thin homogeneous film when the normal boundary conditions in the Maxwell theory are assumed, are almost identical with those obtained from DRUDE's theory when the transition layer is assumed to have a uniform refractive index throughout.

Eqns. 2 yield the complex amplitude ratios

$$\frac{\vec{R}_p}{E_p} = \frac{\cos\theta\sqrt{\varepsilon_2} - \cos\chi\sqrt{\varepsilon_1} + j\dfrac{2\pi}{\lambda}[p\cos\theta\cos\chi - (l - q\varepsilon_2\sin^2\chi)\sqrt{\varepsilon_1\varepsilon_2}]}{\cos\theta\sqrt{\varepsilon_2} + \cos\chi\sqrt{\varepsilon_1} + j\dfrac{2\pi}{\lambda}[p\cos\theta\cos\chi + (l - q\varepsilon_2\sin^2\chi)\sqrt{\varepsilon_1\varepsilon_2}]}$$

$$\frac{\vec{R}_s}{E_s} = \frac{\cos\theta\sqrt{\varepsilon_1} - \cos\chi\sqrt{\varepsilon_2} + j\dfrac{2\pi}{\lambda}(l\cos\theta\cos\chi\sqrt{\varepsilon_1\varepsilon_2} - p + l\varepsilon_2\sin^2\chi)}{\cos\theta\sqrt{\varepsilon_1} + \cos\chi\sqrt{\varepsilon_2} + j\dfrac{2\pi}{\lambda}(l\cos\theta\cos\chi\sqrt{\varepsilon_1\varepsilon_2} + p - l\varepsilon_2\sin^2\chi)}$$

$$\frac{\vec{D}_p}{E_p} = \frac{2\sqrt{\varepsilon_1}\cos\theta}{\cos\theta\sqrt{\varepsilon_2} + \cos\chi\sqrt{\varepsilon_1} + j\dfrac{2\pi}{\lambda}[(l - q\varepsilon_2\sin^2\chi)\sqrt{\varepsilon_1\varepsilon_2} + p\cos\theta\cos\chi]} \tag{3}$$

$$\frac{\vec{D}_s}{E_s} = \frac{2\sqrt{\varepsilon_1}\cos\theta}{\cos\theta\sqrt{\varepsilon_1} + \cos\chi\sqrt{\varepsilon_2} + j\dfrac{2\pi}{\lambda}(l\cos\theta\cos\chi\sqrt{\varepsilon_1\varepsilon_2} - l\varepsilon_2\sin^2\chi + p)}$$

For sufficiently small angles of incidence $\cos\theta$ and $\cos\chi$ may be put equal to unity and $\sin^2\chi$ to zero. Also, since $l << \lambda$ there is not much error involved in ignoring terms which involve orders higher than the first in l/λ (*e.g.* see refs. 5, 6). The complex amplitude expressions derivable using Eqn. 3 then become

$$\vec{R}_p = E_p \frac{\sqrt{\varepsilon_2} - \sqrt{\varepsilon_1}}{\sqrt{\varepsilon_2} + \sqrt{\varepsilon_1}} e^{j\delta_p} \tag{4}$$

where

$$\tan\delta_p = \frac{4\pi}{\lambda}\left[\frac{\sqrt{\varepsilon_1}(p - l\varepsilon_2)}{\varepsilon_2 - \varepsilon_1}\right] \tag{5}$$

$$\vec{R}_s = E_s \frac{\sqrt{\varepsilon_1} - \sqrt{\varepsilon_2}}{\sqrt{\varepsilon_1} + \sqrt{\varepsilon_2}} e^{j\delta_s} \tag{6}$$

where

$$\tan\delta_s = \frac{4\pi}{\lambda}\left[\frac{\sqrt{\varepsilon_1}(l\varepsilon_2 - p)}{\varepsilon_1 - \varepsilon_2}\right] \tag{7}$$

$$\vec{D}_p = E_p \frac{2\sqrt{\varepsilon_1}}{\sqrt{\varepsilon_1} + \sqrt{\varepsilon_2}} e^{j\delta_p'} \tag{8}$$

where

$$\tan\delta_p' = -\frac{2\pi}{\lambda}\left[\frac{l\sqrt{\varepsilon_1\varepsilon_2} + p}{\sqrt{\varepsilon_1} + \sqrt{\varepsilon_2}}\right] \tag{9}$$

and

$$\vec{D}_s = E_s \frac{2\sqrt{\varepsilon_1}}{\sqrt{\varepsilon_1} + \sqrt{\varepsilon_2}} e^{j\delta_s'} \tag{10}$$

where

$$\tan \delta_s' = -\frac{2\pi}{\lambda}\left[\frac{l\sqrt{\varepsilon_1\varepsilon_2} + p}{\sqrt{\varepsilon_1} + \sqrt{\varepsilon_2}}\right] \tag{11}$$

The complex amplitude and phase relations (Eqns. 4–11) are presented as being reasonably accurate expressions for light at near-normal incidence on an inhomogeneous region whose thickness l is much smaller than λ. If $l = 0$ they reduce to the Fresnel relations as required. Thus, since

$$\begin{aligned} \delta_p &= \delta_s = \delta_{1,2} \text{ say} \\ \delta_p' &= \delta_s' = \delta_{1,2}' \text{ say} \end{aligned} \tag{12}$$

the resultant complex amplitudes $\vec{R}$ and $\vec{D}$ may be written as

$$\vec{R} = \rho_{1,2}E \tag{13}$$

$$\vec{D} = \tau_{1,2}E \tag{14}$$

where the complex reflectance $\rho_{1,2}$ and transmittance $\tau_{1,2}$ are given by

$$\rho_{1,2} = \frac{\sqrt{\varepsilon_2} - \sqrt{\varepsilon_1}}{\sqrt{\varepsilon_2} + \sqrt{\varepsilon_1}} e^{j\delta_{1,2}} \tag{15}$$

$$\tau_{1,2} = \frac{2\sqrt{\varepsilon_1}}{\sqrt{\varepsilon_2} + \sqrt{\varepsilon_1}} e^{j\delta_{1,2}'} \tag{16}$$

where from Eqns. 5, 9 and 12

$$\tan \delta_{1,2} = \frac{4\pi}{\lambda} \frac{\sqrt{\varepsilon_1}(p - l\varepsilon_2)}{\varepsilon_2 - \varepsilon_1} = \frac{4\pi}{\lambda} \frac{\sqrt{\varepsilon_1}l(\bar{\varepsilon} - \varepsilon_2)}{\varepsilon_2 - \varepsilon_1} \tag{17}$$

and

$$\tan \delta_{1,2}' = -\frac{2\pi}{\lambda} \frac{(l\sqrt{\varepsilon_1\varepsilon_2} + p)}{\sqrt{\varepsilon_1} + \sqrt{\varepsilon_2}} = -\frac{2\pi}{\lambda} \frac{l(\sqrt{\varepsilon_1\varepsilon_2} + \bar{\varepsilon})}{\sqrt{\varepsilon_1} + \sqrt{\varepsilon_2}} \tag{18}$$

where ε is the mean value of the dielectric constant in the inhomogeneous region and is defined through the relation

$$\bar{\varepsilon} = \frac{p}{l} = \frac{1}{l}\int_1^2 \varepsilon \mathrm{d}z \tag{19}$$

When the incident ray is in the medium ε_2 the complex reflectance $\rho_{2,1}$ and transmittance $\tau_{2,1}$ are obtained by the interchanging of ε_1 and ε_2 in Eqns. 15–18. Thus

$$\rho_{2,1} = -\frac{\sqrt{\varepsilon_2} - \sqrt{\varepsilon_1}}{\sqrt{\varepsilon_2} + \sqrt{\varepsilon_1}} e^{j\delta_{2,1}} \tag{20}$$

and

$$\tau_{2,1} = \frac{2\sqrt{\varepsilon_2}}{\sqrt{\varepsilon_2} + \sqrt{\varepsilon_1}} e^{j\delta_{2,1}'} \tag{21}$$

where

$$\tan \delta_{2,1} = \frac{4\pi}{\lambda} \frac{\sqrt{\varepsilon_2}(p - l\varepsilon_1)}{\varepsilon_1 - \varepsilon_2} = \frac{4\pi}{\lambda} \frac{\sqrt{\varepsilon_2}\, l(\bar{\varepsilon} - \varepsilon_1)}{\varepsilon_1 - \varepsilon_2} \tag{22}$$

and

$$\tan \delta_{2,1}' = -\frac{2\pi}{\lambda} \frac{(l\sqrt{\varepsilon_1\varepsilon_3} + p)}{\sqrt{\varepsilon_1} + \sqrt{\varepsilon_2}} = \frac{2\pi}{\lambda} \frac{l(\sqrt{\varepsilon_1\varepsilon_2} - \bar{\varepsilon})}{\sqrt{\varepsilon_1} + \sqrt{\varepsilon_2}} \tag{23}$$

Eqns. 17, 18, 22 and 23 show that on the approximate theory of DRUDE the shifts in phase are uniquely determined by the thickness of the inhomogeneous region and by the mean value of the dielectric constant therein.

Application of theory to lipid membranes

Let us suppose that in a bimolecular lipid membrane the profile for the dielectric constant is symmetrically disposed about the centre of the membrane and varies from a value of ε_1 in the aqueous electrolyte phases to a value of ε_2 in the interior of the membrane (Fig. 4). The regions lying between the loci of those points where ε first attains the values ε_1 and ε_2 constitute optically inhomogeneous regions of thickness l which is much smaller than the wavelength of visible light.

Let d_e denote the distance measured perpendicular to the surface of the membrane over which $\varepsilon = \varepsilon_2$. Writing

$$\beta = \frac{4\pi}{\lambda} \sqrt{\varepsilon_2}\, d_e \tag{24}$$

it is evident that the complex reflectance ρ of the membrane is given by

$$\rho = \rho_{1,2} + \tau_{1,2}\tau_{2,1}(\rho_{2,1}e^{j\beta} - \rho_{2,1}^{3}e^{j.2\beta} + \rho_{2,1}^{5}e^{j.3\beta} + \ldots) \tag{25}$$

Since $|\rho_{2,1}| < 1$ the series in Eqn. 25 converges, yielding

$$\rho = \rho_{1,2} + \tau_{1,2}\tau_{2,1} \frac{\rho_{2,1}e^{j\beta}}{1 - \rho_{2,1}^2 e^{j\beta}} \tag{26}$$

Eqn. 26 is presented as the most exact formulation for the complex reflectance of a lipid membrane which is derivable by using the DRUDE theory. This equation can be evaluated for any membrane model once the variation of ε in the inhomogeneous regions together with the values of ε_1, ε_2, λ and d_e are specified. The equation, however, is not in a suitable form for illuminating in a simple way the effect that the introduction of the inhomogeneous regions has on the formula for the energy reflectance.

It is proposed now to seek modified equations for the complex reflectance which, while less general than Eqn. 26 as regards application to specific membrane models, are in a form that is much easier to handle. The modified treatment is limited to membranes that satisfy two assumptions. The first of these is:

Assumption 1. There is not much error involved in ignoring the many inter-reflections which occur across the region where $\varepsilon = \varepsilon_2$. Thus, from Eqn. 25

$$\rho = \rho_{1,2} + \tau_{1,2}\tau_{2,1}\rho_{2,1}\,\mathrm{e}^{\mathrm{j}\beta} \tag{27}$$

approximately.

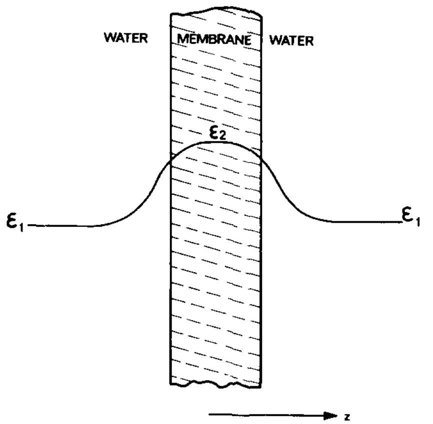


Fig. 4. Scheme showing a possible profile for the dielectric constant in a membrane.

Before the second assumption is stated it is pointed out that from Eqns. 18 and 23 $\tan \delta_{1,2}' = \tan \delta_{2,1}'$ is necessarily of order $2\pi l/\lambda$. Thus to good approximation

$$\tan \delta_{1,2}' = \tan \delta_{2,1}' = \delta_{1,2}' \tag{28}$$

For the reflected rays, however, there exists the possibility of a rather specialized variation in ε which leads to large values for $\delta_{1,2}$ and $\delta_{2,1}$, as can be seen from Eqns. 17 and 22. The second assumption excludes these cases.

Assumption 2. There is not much error involved in putting

$$\tan \delta_{1,2} = \delta_{1,2} \qquad \tan \delta_{2,1} = \delta_{2,1} \tag{29}$$

It then follows that for membranes satisfying these two criteria, using Eqns. 15, 16, 20 and 21

$$\rho = R_\mathrm{f}\,\mathrm{e}^{\mathrm{j}\delta_{1,2}}(1 - B\,\mathrm{e}^{\mathrm{j}(4\pi\Delta/\lambda)}) \tag{30a}$$

where

$$R_\mathrm{f} = \frac{\sqrt{\varepsilon_2} - \sqrt{\varepsilon_1}}{\sqrt{\varepsilon_2} + \sqrt{\varepsilon_1}} \tag{30b}$$

$$B = \frac{2\sqrt{\varepsilon_1}}{\sqrt{\varepsilon_1} + \sqrt{\varepsilon_2}}\,\frac{2\sqrt{\varepsilon_2}}{\sqrt{\varepsilon_1} + \sqrt{\varepsilon_2}} \tag{30c}$$

and

$$\frac{4\pi\Delta}{\lambda} = 2\delta_{1,2}' + \delta_{2,1} - \delta_{1,2} + \beta \tag{30d}$$

The phase angle $4\pi\Delta/\lambda$ may be evaluated by substituting in Eqn. 30d for the component phase angles $\delta_{2,1}$ *etc.* from Eqns. 17, 18, 22 and 23. This procedure gives

$$\frac{4\pi\Delta}{\lambda} = \frac{4\pi}{\lambda}(\Delta_1 + \Delta_2) \tag{31a}$$

where

$$\Delta_1 = \frac{2\sqrt{\varepsilon_2}(p - l\varepsilon_1)}{\varepsilon_2 - \varepsilon_1} = \frac{2\sqrt{\varepsilon_2}\,l(\bar{\varepsilon} - \varepsilon_1)}{\varepsilon_2 - \varepsilon_1} \tag{31b}$$

and

$$\Delta_2 = \sqrt{\varepsilon_2}\,d_c \tag{31c}$$

Multiplication of Eqn. 30a by its complex conjugate then yields for the energy reflectance

$$\begin{aligned} |\rho|^2 &= R_f^2\left[1 + B^2 - 2B\cos\frac{4\pi}{\lambda}(\Delta_1 + \Delta_2)\right] \\ &= R_f^2\left[(1 - B)^2 + 4B\sin^2\frac{2\pi}{\lambda}(\Delta_1 + \Delta_2)\right] \end{aligned} \tag{32}$$

Many systems satisfying Assumption 1 above will also satisfy an additional assumption, namely

Assumption 3. There is not much error involved in assuming that $B = 1$

For these systems Eqn. 32 yields

$$|\rho|^2 = 4R_f^2\sin^2\frac{2\pi}{\lambda}(\Delta_1 + \Delta_2) \tag{33}$$

Eqns. 32 and 33 are presented as approximate relations for the energy reflectance which apply for a restricted range of membrane models.

Comparison with an exact treatment

A partial test for the validity of the present treatment is to compare the value calculated for the energy reflectance of the symmetrical trilayer shown in Fig. 5 using Eqns. 26, 32 and 33 with values predicted by a multilayer theory for homogeneous dielectrics. This has been done for cases where $d_c + 2l$ equals 50, 140 and 200 Å for a range of values of $\sqrt{\varepsilon_1}$, $\sqrt{\varepsilon_2}$ and $\sqrt{\bar{\varepsilon}}$.

TABLE I

CALCULATIONS OF ENERGY REFLECTANCE FOR THE SYMMETRICAL TRILAYER DEPICTED IN Fig. 5

Calculations have been performed for $\sqrt{\varepsilon_1} = 1.33$, $\sqrt{\bar{\varepsilon}} = 1.51$, $l = 20$ Å, $d_c = 100$ Å and $\lambda = 6328$ Å. R_1, R_2, R_3 are the reflectivities calculated by using Eqns. 33, 32 and 26, respectively. R_{ex} is the exact value for the reflectivity as calculated using multilayer theory.

$\sqrt{\varepsilon_2}$	R_1	R_2	R_3	R_{ex}
1.0	$0.425 \cdot 10^{-3}$	$0.425 \cdot 10^{-3}$	$0.444 \cdot 10^{-3}$	$0.445 \cdot 10^{-3}$
1.1	$0.172 \cdot 10^{-3}$	$0.171 \cdot 10^{-3}$	$0.175 \cdot 10^{-3}$	$0.176 \cdot 10^{-3}$
1.2	$0.214 \cdot 10^{-4}$	$0.214 \cdot 10^{-4}$	$0.220 \cdot 10^{-4}$	$0.221 \cdot 10^{-4}$
1.3	$0.217 \cdot 10^{-4}$	$0.217 \cdot 10^{-4}$	$0.186 \cdot 10^{-4}$	$0.211 \cdot 10^{-4}$
1.4	$0.212 \cdot 10^{-3}$	$0.212 \cdot 10^{-3}$	$0.211 \cdot 10^{-3}$	$0.214 \cdot 10^{-3}$
1.5	$0.640 \cdot 10^{-3}$	$0.638 \cdot 10^{-3}$	$0.644 \cdot 10^{-3}$	$0.645 \cdot 10^{-3}$
1.6	$0.134 \cdot 10^{-2}$	$0.133 \cdot 10^{-2}$	$0.136 \cdot 10^{-2}$	$0.136 \cdot 10^{-2}$
1.7	$0.234 \cdot 10^{-2}$	$0.231 \cdot 10^{-2}$	$0.241 \cdot 10^{-2}$	$0.241 \cdot 10^{-2}$
1.8	$0.369 \cdot 10^{-2}$	$0.361 \cdot 10^{-2}$	$0.384 \cdot 10^{-2}$	$0.384 \cdot 10^{-2}$
1.9	$0.539 \cdot 10^{-2}$	$0.526 \cdot 10^{-2}$	$0.571 \cdot 10^{-2}$	$0.570 \cdot 10^{-2}$
2.0	$0.749 \cdot 10^{-2}$	$0.725 \cdot 10^{-2}$	$0.807 \cdot 10^{-2}$	$0.806 \cdot 10^{-2}$

TABLE II

CALCULATIONS OF ENERGY REFLECTANCE FOR THE SYMMETRICAL TRILAYER DEPICTED IN Fig. 5

Calculations are performed for $\sqrt{\varepsilon_1} = 1.33$, $\sqrt{\bar{\varepsilon}} = 1.51$, $l = 10$ Å, $d_c = 30$ Å and $\lambda = 6328$ Å. The symbols R_1, R_2, R_3 and R_{ex} are defined in Table I.

$\sqrt{\varepsilon_2}$	R_1	R_2	R_3	R_{ex}
1.0	$0.221 \cdot 10^{-4}$	$0.297 \cdot 10^{-4}$	$0.230 \cdot 10^{-4}$	$0.230 \cdot 10^{-4}$
1.2	$0.177 \cdot 10^{-7}$	$0.360 \cdot 10^{-7}$	$0.163 \cdot 10^{-7}$	$0.166 \cdot 10^{-7}$
1.4	$0.353 \cdot 10^{-4}$	$0.353 \cdot 10^{-4}$	$0.353 \cdot 10^{-4}$	$0.354 \cdot 10^{-4}$
1.6	$0.158 \cdot 10^{-3}$	$0.157 \cdot 10^{-3}$	$0.160 \cdot 10^{-3}$	$0.160 \cdot 10^{-3}$
1.8	$0.393 \cdot 10^{-3}$	$0.395 \cdot 10^{-3}$	$0.411 \cdot 10^{-3}$	$0.411 \cdot 10^{-3}$
2.0	$0.762 \cdot 10^{-3}$	$0.798 \cdot 10^{-3}$	$0.827 \cdot 10^{-3}$	$0.827 \cdot 10^{-3}$

TABLE III

CALCULATIONS OF ENERGY REFLECTANCE FOR THE SYMMETRICAL TRILAYER DEPICTED IN Fig. 5

Calculations are performed for $\sqrt{\varepsilon_1} = 1.00$, $\sqrt{\bar{\varepsilon}} = 1.51$, $l = 50$ Å. $d_c = 100$ Å and $\lambda = 6328$ Å. For the meaning of the symbols R_1, R_2, R_3, R_{ex}, see Table I.

$\sqrt{\varepsilon_2}$	R_1	R_2	R_3	R_{ex}
1.0	$0.282 \cdot 10^{-14}$	$0.282 \cdot 10^{-14}$	$0.344 \cdot 10^{-14}$	$0.389 \cdot 10^{-2}$
1.2	$0.667 \cdot 10^{-2}$	$0.661 \cdot 10^{-2}$	$0.652 \cdot 10^{-2}$	$0.702 \cdot 10^{-2}$
1.4	$0.113 \cdot 10^{-1}$	$0.110 \cdot 10^{-1}$	$0.117 \cdot 10^{-1}$	$0.119 \cdot 10^{-1}$
1.6	$0.173 \cdot 10^{-1}$	$0.166 \cdot 10^{-1}$	$0.189 \cdot 10^{-1}$	$0.189 \cdot 10^{-1}$
1.8	$0.251 \cdot 10^{-1}$	$0.236 \cdot 10^{-1}$	$0.288 \cdot 10^{-1}$	$0.288 \cdot 10^{-1}$
2.0	$0.347 \cdot 10^{-1}$	$0.322 \cdot 10^{-1}$	$0.420 \cdot 10^{-1}$	$0.418 \cdot 10^{-1}$

TABLE IV

CALCULATIONS OF ENERGY REFLECTANCE USING THE SMART AND SENIOR TREATMENT

Columns (a), (b) and (c) relate to the trilayers specified in Tables I, II and III, respectively.

$\sqrt{\varepsilon_2}$	$R_{s,s}$ (a)	$R_{s,s}$ (b)	$R_{s,s}$ (c)
1.0	$0.568 \cdot 10^{-3}$	$0.355 \cdot 10^{-4}$	$0.325 \cdot 10^{-2}$
1.1	$0.226 \cdot 10^{-3}$		
1.2	$0.321 \cdot 10^{-4}$	$0.883 \cdot 10^{-7}$	$0.684 \cdot 10^{-2}$
1.3	$0.178 \cdot 10^{-4}$		
1.4	$0.214 \cdot 10^{-3}$	$0.348 \cdot 10^{-4}$	$0.122 \cdot 10^{-1}$
1.5	$0.654 \cdot 10^{-3}$		
1.6	$0.137 \cdot 10^{-2}$	$0.160 \cdot 10^{-3}$	$0.194 \cdot 10^{-1}$
1.7	$0.240 \cdot 10^{-2}$		
1.8	$0.379 \cdot 10^{-2}$	$0.396 \cdot 10^{-3}$	$0.290 \cdot 10^{-1}$
1.9	$0.555 \cdot 10^{-2}$		
2.0	$0.775 \cdot 10^{-2}$	$0.768 \cdot 10^{-3}$	$0.409 \cdot 10^{-1}$

Some typical results are given in Tables I–III. For the 100-Å trilayer (Table I) the more exact formula (Eqn. 26) yields energy reflectance values mostly lying within 10 % of the exact values. (The agreement declined somewhat as the ratio $l:d_c$ was increased.) The most significant discrepancies occur when the value for $\sqrt{\varepsilon_2}$ lies within about 10 % of that for $\sqrt{\varepsilon_1}$ and when the ratio $\sqrt{\varepsilon_2}:\sqrt{\varepsilon_1}$ is less than unity. Similar remarks apply to the more approximate formulae (Eqns. 32 and 33). However, in these cases there are also significant discrepancies when the ratio $\sqrt{\varepsilon_2}:\sqrt{\varepsilon_1}$ is large. These discrepancies appear to be due to the usage of Assumptions 1 and 3 (see above).

The results for the 50-Å and 200-Å trilayers showed the same trends as those for the 100-Å case, and some typical results have been included in Tables II and III. These and other results have shown that if a 10 % fit is an acceptable criterion then it seems that, except in the extreme cases referred to above, Eqns. 26, 32 and 33 provide reasonable predictors for the energy reflectance.

Comparison with the treatment of SMART AND SENIOR

SMART AND SENIOR[12] have shown that the reflectivity of a thin symmetrical trilayer in air is almost equal to that of a homogeneous film of the same thickness and of a refractive index equal to the mean refractive index of the trilayer. It is fairly easy to show that if their treatment is generalized to the case where the film may be immersed in any medium the reflectivity is given, in the present notation (*cf.* ref. 12, Eqn. 11), by

$$R_{s,s} = \frac{(2l + d_c)^2 \dfrac{\pi^2}{\lambda^2}\left[\left(\dfrac{\overline{N}}{\sqrt{\varepsilon_1}}\right)^2 - 1\right]^2 \varepsilon_1}{1 + (2l + d_c)^2 \dfrac{\pi^2}{\lambda^2}\left[\left(\dfrac{\overline{N}}{\sqrt{\varepsilon_1}}\right)^2 - 1\right]^2 \varepsilon_1}$$

when $\overline{N}$ is the mean refractive index in the film.

Calculations of $R_{s,s}$ for the symmetrical trilayers treated in Tables I–III are given in Table IV. For these systems $R_{s,s}$ is superior to R_1 and R_2 when the external medium is air (Table III) and here it is comparable to R_3. It may however be considered as being roughly comparable in reliability to the more approximate formulae R_1 and R_2 when the external medium is water (Tables I and II) and in these cases is inferior to R_3.

Generally the reliability of the $R_{s,s}$ formula improves as the difference $\sqrt{\varepsilon_2}-\sqrt{\bar{\varepsilon}} \to 0$. It also improves for fixed values of $\sqrt{\bar{\varepsilon}}$ and $\sqrt{\varepsilon_2}$ as the value of $\sqrt{\varepsilon_1} \to 1$. These trends are as expected on the assumed single layer approximation.

DISCUSSION

In the theoretical section an attempt has been made to apply the DRUDE theory for the transition layer to a thin membraneous system, in which the dielectric constant varies in an unknown way, in order to obtain useful expressions for the reflectivity of the system. The formalism employed is sufficiently general to ensure freedom from specific membrane models. It has been shown that the treatment has a fairly wide range of validity when it is applied to symmetrical trilayers.

Suppose now that the dielectric constant in a bilayer membrane varies in the way shown in Fig. 5. For discussion purposes the otherwise unwarranted supposition

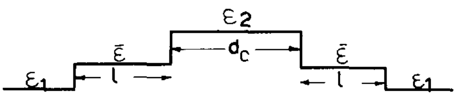


Fig. 5. Scheme of symmetrical trilayer. Light is incident from the medium ε_1.

will be adopted that the refractive index value derived from the angle of incidence for which the energy reflectance is a minimum, characterizes the central portion of the membrane, *i.e.* where $\varepsilon = \varepsilon_2$ in Fig. 5. Experimental data may now be analysed using any of the reflectance formulae, Eqns. 26, 32 or 33. For convenience the data of HUANG AND THOMPSON[1] will be analysed using the approximate reflectance formula (Eqn. 33). These workers obtained an equivalent single layer thickness of 72 Å for $\sqrt{\varepsilon_1} = 1.33$ and $\sqrt{\varepsilon_2} = 1.66$.

Substituting into Eqn. 33 gives

$$3.32l(\bar{\varepsilon} - 1.73) + 1.66d_c = 120 \tag{34}$$

where $\bar{\varepsilon}$ is the mean value of the dielectric constant in the transition regions of length l, and d_c is the distance over which $\varepsilon = \varepsilon_2$. If it is supposed that $\bar{\varepsilon} = 2.3$ say (*i.e.* the arithmetical mean of ε_1 and ε_2), then Eqn. 34 yields

$$1.69l + 1.66d_c = 120 \tag{35}$$

It is evident that if $l = 0$, the single layer model results, and the membrane thickness d becomes equal to d_c and has a value of 72 Å.

If we denote by α that fraction of the width (l) of the transition layers which appears within the membrane it is evident that when $l >> d_c$ as $\alpha \to 1$, $d_c \to 0$ and $d \to 2l$ which from Eqn. 35 then has a value of around 140 Å. On the other hand if $\alpha \to 0$, $d \to d_c$ and the thickness might then be less than 72 Å if a significant part of the phase shift occurs in the transition layers.

It would seem, therefore, that in the present state of knowledge, optical measurements by themselves do not permit a conclusive determination of membrane thickness. However, it seems possible that optical data used in conjunction with information derived from other techniques such as low frequency capacitance, electron microscopy and X-ray diffraction might provide evidence favourable or otherwise to a particular membrane model.

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