Optical determination of the thickness of single planar thin lipid films using the symmetrical three-layer model

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Abstract: The total thickness of thin lipid films can be determined by the method of absolute or relative reflectance measurements. The film is described by the symmetrical three-layer model. By assuming equal refractive indices for the hydrocarbon layer and for the organic solution, the thickness and refractive index of the head group layer can be estimated.

Key words: Membrane, thin liquid film, bilayer, optical method, three-layer model, lipid film.

1. Introduction

Since the 1960s, planar lipid bilayers have been used as model systems for biological membranes. The thickness is very important for biological membranes and thin lipid films. The thickness of single planar lipid films are obtained from optical reflectance and electrical capacitance measurements.

The widespread use of the electrical technique to determine thickness is due to the simplicity of measurement and the clear interpretation of experimental results. However, this method estimates only the thickness of the hydrophobic core of a lipid film. A further difficulty lies in the need for assuming the dielectric constant of the hydrocarbon layer, since there is no possibility of measuring it directly. The possibility of calculating dielectric constants has been considered by Ohki [1].

The total thickness of lipid bilayers, the thickness of the polar and of the hydrophobic part, can be determined by optical methods. The first optical apparatus and extensive measurements on free soap films were performed by Derjaguin and coworkers [2]. From this work, the determination of film thickness has developed in two directions. Scheludko and coworkers [3–7], Mysels and coworkers [8, 9] and Lyklema and coworkers [10] determined reflectance by comparing the intensity of the reflected light of a black film with that of a film giving the maximum reflectance before the transition to the thin state. The basic idea of the measurement has been previously described by Perrin [11]. Many scientists have employed this method of relative reflectance in investigating soap films. Huang and Thompson [12] and later Tien and Dawidowicz [13] applied the method, in different forms, to lipid bilayers.

Van den Tempel [14], Corkill and coworkers [15] and Duyvis [16] proceeded in a second direction; determined reflectance by comparing the reflected light with the incident light. The basic idea of the measurement has been previously described by Wells [17]. This method of absolute reflectance has been employed on soap films and later on lipid bilayers. The refractive index of the bilayer must be known to a quite high precision. A successful determination of the refractive index and the thickness of lipid bilayers were firstly obtained by Cherry and Chapman [18, 19]. A critical comparison of electrical and optical methods applied for the determination of lipid film thicknesses is given in Ref. [20]. The generally accepted molecular structure of a black lipid film in water consists of a symmetrical three-layer model in which the polar and hydrocarbon regions are considered as separate regions, in contrast to one-layer or isotropic models. The symmetrical three-layer model was used by Duyvis [16],
Cherry and Chapman [19], Tien [21] and Smart and Senior [22]. In Refs. [21, 22] it is pointed out that the optical measurements from the method of absolute reflectance are unable to distinguish between a one-layer model and a three-layer model. That the model is three-layer is suggested by the structure of the lipid film, however. For this reason the three-layer model was also used in Ref. [22] to determine additional optical parameters of thin foam films with the help of absolute reflectance and infra-red absorption measurements.

In Ref. [19] an equation for the determination of the reflectivity of lipid film is presented. This equation has been modified by approximations so that an equation for a quasi-one-layer model arises which is good enough for use.

This paper deals with the consistent application of the symmetrical three-layer model to improve precision of, and to extend the method of determining additional parameters of the thin lipid film. Calculation will be made for the method of absolute reflectance as well as for the method of relative reflectance.

2. The symmetrical three-layer model

The generally accepted molecular structure of a black lipid film in water [16,19,21,22] is shown schematically in Fig. 1.

At the top of the figure, the polar head groups of the lipids are in contact with the aqueous phase, while the interior of the film consists of the hydrocarbon chains oriented approximately perpendicular to the plane of the film. Below is shown the variation of the refractive index across the thickness of the film. Here \( n_h, d_h \) and \( n_p, d_p \) are the refractive indices and the thicknesses of the inner and outer layer, respectively, and \( n_o \) is the refractive index of the surrounding phase. The total thickness of the film is

\[
d = 2d_p + d_h.
\]

In Refs. [19, 21] it was assumed that the maximum mean value of \( n_p \) is equal to 1.7. In the following, \( n_h \) will be assumed to be identical to the value of an appropriate bulk phase of the organic liquid [20], which is surely a good approximation for solvent rich films.

3. Reflection of light by a symmetrical three-layer film

The optical properties of liquid films in aqueous solution are in principle the same as those of thin transparent solid or soap films. Hence, many of the theoretical considerations of thin films can be applied directly. Many problems have been solved regarding these structures [23].

As a first approximation, the incidence of light is nearly normal and the absorption and the multiple reflectance can be neglected. The amplitude of the reflected light \( A_R \) as a function of the amplitude of incident light \( A_o \) is given by

\[
A_r = r_1 A_o + r_2 \exp (-i\alpha \phi_1) A_o + r_3 \exp (-i\alpha \phi_2) A_o + r_4 \exp (-i\alpha \phi_3) A_o
\]

where

\[
a = \frac{4\pi}{\lambda}; \quad \phi_1 = n_p d_p; \quad \phi_2 = n_p d_p + n_h d_h;
\]

\[
\phi_3 = 2n_p d_p + n_h d_h
\]

and

\[
r_n = \frac{n_j - n_h}{n_j + n_h}
\]

with \( \lambda \) as the wavelength of incident light and \( r_n \) as the Fresnel coefficient (see Fig. 2).