

UNCLASSIFIED

AD NUMBER
AD433244
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; Mar 1964. Other requests shall be referred to U.S. Army Biological Laboratories, Fort Detrick, Frederick, MD.
AUTHORITY
BDRL D/A ltr, 27 Sep 1971

THIS PAGE IS UNCLASSIFIED

UNCLASSIFIED

AD 433244

DEFENSE DOCUMENTATION CENTER

FOR

SCIENTIFIC AND TECHNICAL INFORMATION

CAMERON STATION, ALEXANDRIA, VIRGINIA



UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

433244

FILE COPY

20

TECHNICAL MANUSCRIPT 102

DETERMINATION OF THICKNESS AND
REFRACTIVE INDEX OF THIN FILMS
AS AN APPROACH TO THE STUDY
OF BIOLOGICAL MACROMOLECULES

433244

MARCH 1964

DDC
MAR 3 1964

UNITED STATES ARMY
LABORATORIES
FORT DETRICK

Best Available Copy

5 78 750

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 102

② DETERMINATION OF THICKNESS AND REFRACTIVE INDEX OF THIN FILMS
AS AN APPROACH TO THE STUDY OF BIOLOGICAL MACROMOLECULES,

⑩ by J.B. Bateman,

Physical Sciences Division
DIRECTOR OF BIOLOGICAL RESEARCH

①
Project LA012501B028

March 1964

The work reported here was performed under Project 1A012501B028, "Basic Research in Life Sciences," Task -01, "Basic Research in Life Sciences, Biological." The expenditure order was 2037. This material was originally submitted as manuscript 5225.

The information in this document has not been cleared for release to the public.

DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this document from DDC.

Foreign announcement and dissemination of this document by DDC is limited.

ACKNOWLEDGMENTS

In preparing this paper, the author has made use of the published work and the notebooks of his former colleagues R.E. Hartman and R.D. Mattuck. He also acknowledges with thanks the cooperation of E.D. Adams, T.T. Bannister, A. Calio, E.J. Covington, Roberta S. Hartman, K. Larson, R.L. Parker, R.D. Petti, and A. Rosen, who in varying degrees have been responsible for much of the experimental work, and that of H.R. Peplow in some of the calculations. The standard tables for the stepped reflector were calculated by the Biomathematics Division, Fort Detrick; the friendly help of Mr. V.S. Palmer and Mr. G.I. Eccles is acknowledged.

ABSTRACT

Some years ago a stepped interference reflector, consisting of barium stearate multilayers on a chromium base, was devised to determine the thickness and refractive index of thin films deposited upon it. The various published descriptions of the method, and of results obtained with it, are brought together here and amplified by more detailed presentation of the theory, by additional considerations relating to selection of metal substrate, and by discussion of errors and limitations. It is pointed out that various modifications should be possible for special purposes: metals other than chromium may be found suitable; the dielectric need not be laminated. The possibility of making measurements directly at the solid-liquid interface is examined briefly.

CONTENTS

Acknowledgments	3
Abstract	3
I. INTRODUCTION	7
II. PRINCIPLES OF DETERMINATION OF THICKNESS AND REFRACTIVE INDEX OF THIN TRANSPARENT FILMS	8
A. Outline	8
B. Qualitative Sketch of Optical Principles	8
C. The Stepped Interference Reflector Method	10
III. APPARATUS AND PROCEDURES	15
A. Fabrication of Stepped Reflectors	15
IV. METHODS OF CHECKING VALIDITY OF FILM THICKNESS AND REFRACTIVE INDEX DATA	19
A. Test Criteria	19
B. Independent Determination of Thickness and Refractive Index of Thick Multilayers	19
C. Determination of Refractive Index by the Abelès Method	21
V. SOME EXPERIMENTAL RESULTS	24
A. Measurements on Thick Fatty Acid Multilayers	24
1. Illustration of Procedure	24
2. Results	26
B. Measurements with <u>s-p</u> Stepped Reflector	28
1. Transferred Monolayers of Aliphatic Acids	28
2. Transferred Monolayers and Adsorbed Films of Protein . .	28
3. Other Measurements	34
4. Summary and Discussion	34
VI. THEORY OF STEPPED INTERFERENCE REFLECTOR	37
A. Derivation of Fringe Shift Equations	37
1. Stepped Reflector without a Test Film	37
2. Stepped Reflector with Test Film	39
B. Approximations	42
1. Simple Approximate Solution to Fringe Shift Equation . .	42
2. Calibration for Determining δ_3	44
3. A More Refined Approximation	45
C. Methods of Computation; Numerical Examples	47
VII. SOME POSSIBLE SOURCES OF ERROR	51
A. Dispersion Effects	51
B. Anisotropy of Dielectric Substrate and Test Film	51
C. Film Heterogeneity	51

VIII. CONCLUSIONS	54
Literature Cited	55
Selected Bibliography	59

FIGURES

1. Illustrating the Conditions for Formation of <u>s</u> and <u>p</u> Fringes when Different Numbers of Barium Stearate Double Layers are Deposited upon Chromium	11
2. Phase Changes at Air-Dielectric (13) and Barium Stearate - Chromium (34) Interfaces	11
3. Illustrating First Condition for Formation of <u>s</u> and <u>p</u> Extinction Fringes by Compound Reflector of Barium Stearate on Metal	12
4. Chromium-Plated Glass Slide with Stepped Multilayer of Barium Stearate - Stearic Acid	12
5. Calculated Extinction Fringes Produced when Stepped Reflector of Barium Stearate on Chromium is Examined at the Correct Angle of Incidence	14
6. Simplified Scheme, Ignoring Multiple Reflections, Illustrating Displacement of Extinction Fringe when Thin Test Film is Formed upon the Dielectric Film 3	14
7. Plexiglas Trough for Monolayer Formation and Transfer	16
8. Apparatus Used for Measurement of Film Thickness and Refractive Index by the <u>s-p</u> Stepped Reflector Method	17
9. The Two Brewster Angle Methods of Abèlès for Determining Index of Refraction of Thin Films	22
10. Experimental Data Illustrating Higher-Order <u>s</u> Fringe Method of Determining Film Thickness Per Monolayer and Refractive Index	25
11. Apparent <u>s</u> Vibration Phase Change at Surface of "Clean" Chromium Reflector, Obtained by Extrapolation of Straight Line Relating Fringe Order, m , to N/λ [Equation (5)]	25
12. Thickness (Above) and Refractive Index (Below) as a Function of Number of Double Layers of Stearate Transferred to <u>s-p</u> Stepped Reflector	29
13. Results of Measuring <u>s</u> and <u>p</u> Fringe Shifts upon Transfer of Monolayers of Bovine Plasma Albumin to Stepped Reflector from Distilled Water Surfaces	32
14. Illustrating Designation of Films and Interfaces in <u>s-p</u> Stepped Reflector	37
15. Plot of d_2 Versus n_2 for $\frac{1}{2}$ Added Double Layer of Barium Stearate	50
16. Functions Used in Calculation of Film Thickness and Refractive Index from <u>s-p</u> Stepped Reflector Data	50
17. Extinction Fringe Amplitude Profiles for <u>p</u> Vibration Incident upon Metal Slides Coated with Barium Stearate	52

18.	Reflection Coefficients as a Function of Wavelength	52
19.	Film Sections Illustrating Departures from Uniformity of Thickness and Refractive Index	53

TABLES

I.	Average Variations of $b(m)$	24
II.	Spacing and Refractive Index of Thick Fatty Acid Multilayers by Measurement at Several Orders of Interference	27
III.	Spacing and Refractive Index of Thin Aliphatic Acid Films on $g-p$ Stepped Reflector	29
IV.	Summary of Data on Validity of $g-p$ Stepped Reflector Method . . .	30
V.	Thin Protein Films on $g-p$ Stepped Reflector	33
VI.	Approximate Fringe Shifts as a Function of Film Thickness and Refractive Index	47
VII.	Film Refractive Indices Calculated from Fringe Shifts by Approximate Equations	49

I. INTRODUCTION

It is perhaps trite to say that phenomena in two dimensions represent a sort of halfway house between one dimension and three, but this is nevertheless the chief reason for the interest and fascination of thin films in biology. We now realize that information is most effectively stored in one dimension, on a thread or wire; but between the abstract instructions and their three-dimensional realization we must have boundary layers, films, membranes, and septa to implement the creation of structure. Since these boundary elements are far from being mere partitions, but possess activities, specificities, structures, information-bearing capabilities of their own, their study is of the greatest importance. They are, moreover, amenable in many of their aspects to in vitro study. Such study has both intrinsic significance and significance in the investigation of the fragile threads that store genetic information, since these can be more readily preserved and manipulated by being incorporated in films.

As an ultimate accomplishment one may imagine the message of a biological linear polymer being translated in a sort of molecular wire recorder, or a minute area of interfacial film being scanned for the distribution of specific reaction foci. In the absence of any immediate prospect of such an achievement, aside from the use of the living cell itself as translator, we are left with the necessity of drawing what inferences we can from examination of macroscopic regions of the objects of interest. The value of studies using visible light in this connection rests upon the fact that while the cross section of the optical probe is necessarily very large, viewed on an atomic scale, the short dimensions of biological polymers are of the order of one per cent of a wavelength, so that polarimetric or related methods can provide significant information concerning these dimensions. This is not all, for by the same token, these methods can detect, and measure, processes involving deposition of biological macromolecules at active interfaces, or involving their release, degradation, or change of configuration.

Such considerations as these, for which we are indebted above all to the writings of Rideal^{1,2} and Langmuir,³ have inspired our interest, over a number of years, in the optical study of thin films. The remainder of this paper will be devoted to the description of a method invented by Richard Mattuck, which permits the "optical thickness," or mass per unit area, of a film to be resolved into metrical thickness and refractive index. The existence of such a method carries us a step forward in the study of biological interaction at interfaces, for there is a very clear advantage in being able to distinguish, for example, between attachment of large compact molecules to a relatively small number of reaction sites, and the formation of a thinner, but denser, film covering the entire surface.

II. PRINCIPLES OF DETERMINATION OF THICKNESS AND REFRACTIVE INDEX OF THIN TRANSPARENT FILMS

A. OUTLINE

The description of the method and of the preliminary results obtained with it is spread through several papers published from 1956 to 1961.⁴⁻⁸ Our hope in this manuscript is to consolidate that material and to present it in easily assimilable form for the interested biological scientist. The initial description will be more or less qualitative, followed by statement, without proof, of the simple approximate equations. Experimental procedures and results will then be given; the derivation of the equations, and various other matters such as limitations on validity, sources of error, and future developments, will be dealt with later.

B. QUALITATIVE SKETCH OF OPTICAL PRINCIPLES

In attempting a simple approach, consider a semi-infinite flat sheet of some material illuminated at some specified angle of incidence by a parallel beam of plane-polarized light. In general the light suffers complicated changes of amplitude (and therefore of intensity) and of phase upon reflection, which can however easily be understood by considering two simple special cases. In the first, the plane of polarization is adjusted so that the electric vector oscillates in the plane of incidence (p, or parallel vibration); in the second, the electric vector oscillates perpendicular to the plane of incidence (s, or senkrecht vibration). The changes of amplitude and phase upon reflection are in general different in the two cases; they are described by the well-known Fresnel equations, which show a different functional dependence of the reflected amplitude and phase upon the relative refractive index of the reflector. Thus, in principle, absolute measurements of the two reflection coefficients (amplitude and phase) will describe the properties of the reflector in terms of the two components of the refractive index (refraction and absorption); if the reflector is a transparent dielectric, a single measurement will suffice.

If now the plane surface is coated with a thin film, we shall have to deal in general with reflections from the two surfaces of the film, and the amplitude and phase of the reflected ray will be the result of compounding that arising from the upper surface of the film with those emerging after successive pairs of reflections within the film. Since the appropriate Fresnel coefficients will again be involved, depending as they do upon different functions of the film refractive index, it would seem that if the substrate has been adequately characterized, further measurements of the new absolute reflection coefficients and phase changes for the s and p vibrations will serve to characterize the film in terms of thickness and refractive index — remembering that the optical thickness of the film also contributes to the phase change and to the resultant amplitude.

Unfortunately, this type of procedure, so transparent in principle, suffers from severe practical drawbacks. It is quite usual in optics to try to avoid absolute measurements, and at the same time to seek conditions that will provide either qualitative distinctions, as between light and dark, or, at least, easily recognizable extrema. In the field of thin-film measurements, a useful stratagem first exploited by Drude has had a dominating influence. In illuminating the reflector we have just been discussing, the plane of polarization of the incident light can be shifted (for example) through 45 degrees, so that instead of using the s and p vibrations singly, we are in effect applying them simultaneously with equal amplitudes and exactly in phase with each other.

After reflection they no longer agree either in phase or in amplitude, but if the reflected beam is passed through suitable optical devices suitably adjusted, the agreement can be restored, and the settings of the instruments that accomplish this are simply related to the ratio of the reflected amplitudes for the s and p rays and to the difference between the two changes of phase. When the reflector is covered with a thin film, the amplitude and phase changes are modified in a manner that is dependent upon the optical parameters of the film. The sensitivity of these polarimetric quantities to changes in the film parameters can be increased in various ways. For example, it is not necessary for the reflector to be a simple semi-infinite solid slab of the kind postulated above; more typically it is laminated, a metal base coated with a dielectric layer of optimum thickness for the purpose at hand. It is this use of a compound substrate that is partly responsible for the power and versatility of Rothen's famous "ellipsometer."

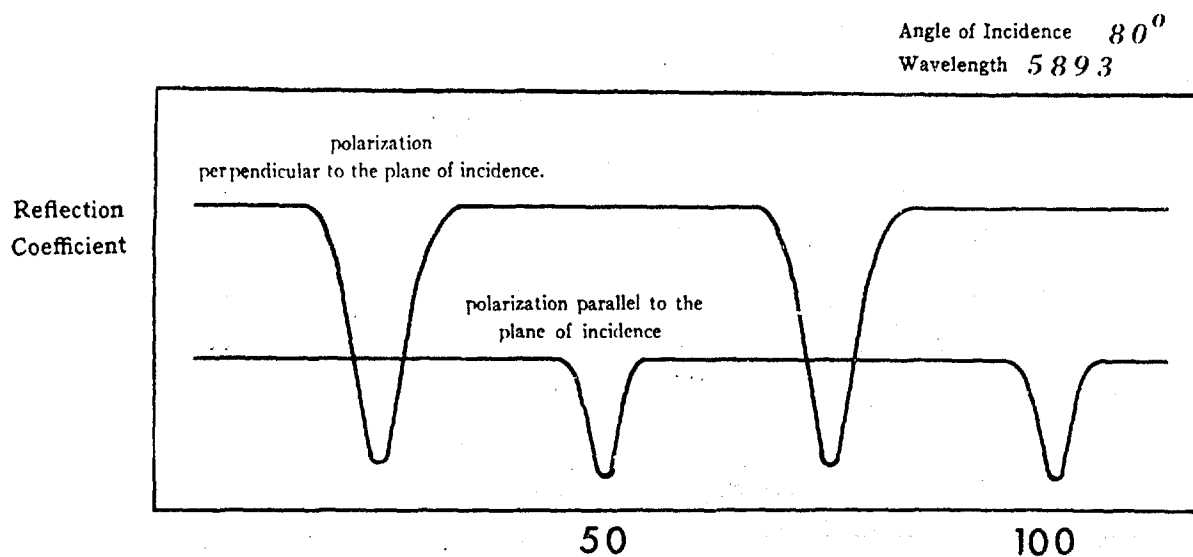
One might hope that the ellipsometric technique itself would provide a means of resolving film thickness and refractive index. Analytical approximate expressions for the effects of thin films on the reflection of polarized light, such as those of Drude⁹ and Lucy,¹⁰ provide some encouragement, but the experimentally observable quantities usually turn out to be dependent essentially upon optical thickness, with very slight second-order sensitivity to variations in refractive index per se.^{*} Attempts to get around this limitation, or to supplement the polarimetric measurement in some suitable manner,¹¹ have been discouragingly unsuccessful.¹²

* From the recent work of McCrackin et al¹³ it appears that this statement is not always correct, but our conclusion, right or wrong, provided the impetus for developing the method described here.

C. THE STEPPED INTERFERENCE REFLECTOR METHOD

In the method now to be described, the traditional polarimetric measurements have been abandoned and replaced by observations of reflected intensity minima that occur when the s and p rays are incident upon suitable compound reflectors and then upon the same reflectors coated with the "unknown" thin film. In this respect the technique has more in common with a simple method of measuring small changes in film thickness devised years ago by Blodgett^{14,15} than with that of Drude. The basic observation is illustrated in Figure 1, adapted from Blodgett's paper. If a plate of polished metal is coated with a layer of a dielectric such as barium stearate, which can be deposited quite conveniently in successive bimolecular sheets, each adding a constant increment of thickness, the reflection coefficient for the s ray of monochromatic light at a definite angle of incidence will pass through a minimum when the thickness of dielectric is such that the optical path difference between the rays reflected at the two interfaces of the reflector is approximately 180 degrees. The word "approximately" is used because the condition for minimum reflectance is determined not only by the thickness of the dielectric but by any contribution that may result from differences between the phase changes at the two interfaces. These differences are rather small with the s ray. In the case of the p ray, on the other hand, at large angles of incidence, they approach 180 degrees, so that in order to produce minimum reflectance it is necessary for the optical path difference within the dielectric layer to approach 360 degrees: thus with increasing dielectric thickness the reflection minima will alternate in the manner shown in Figure 1. The underlying phase relationships that produce this state of affairs are illustrated in Figure 2.

The existence of a minimum in the reflected intensity depends not only upon the establishment of suitable phase relationships. It is also necessary that there should not be too great a disparity between the reflected amplitudes from the two interfaces. Obviously, if one amplitude were to be very much greater than the other, the minimum would be reduced to a mere ripple very hard to locate and to measure. Conversely, the simple equations for reflection from a dielectric-coated metal reflector show that, in principle, the minimum can be converted to an extinction if, together with the correct phase relationships, the reflected amplitudes are exactly equal. Calculations using these equations and the known optical constants of metals and barium stearate show that the necessary conditions should be readily attainable in practice. There is always an angle of incidence at which the two reflection coefficient curves intersect (see examples in Figure 3). Here, then, we see the origin of the stepped interference reflector. We have, for example, a chromium-plated glass slide, one half of which has about twenty, the other half about forty, double layers of barium stearate (Figure 4). At the correct wavelength and angle of incidence, the thinner step will give the s ray extinction; at a slightly different angle of incidence the thicker step will give the p ray extinction. Alternatively, thicker dielectric films can be used to



Double Layers of Barium Stearate

Figure 1. Illustrating the Conditions for Formation of s and p Fringes when Different Numbers of Barium Stearate Double Layers are Deposited upon Chromium. Modified from Blodgett¹⁴ by kind permission of The Williams and Wilkins Company, Baltimore, Md.

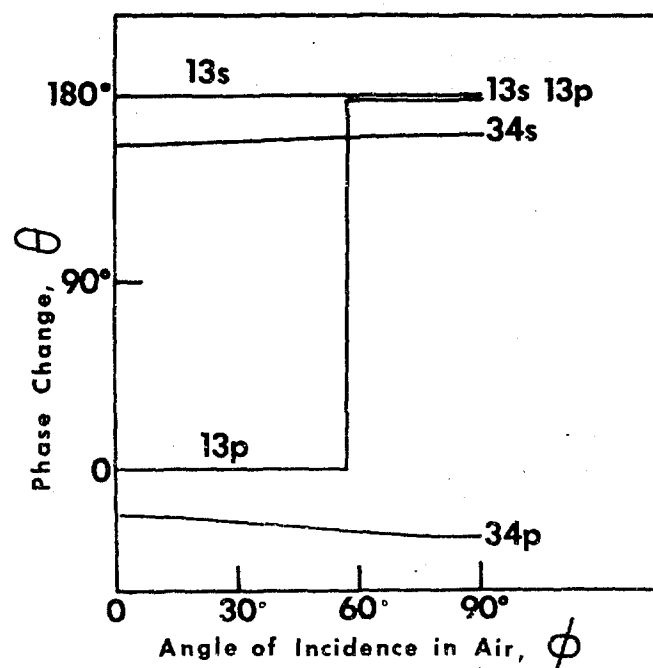


Figure 2. Phase Changes at Air-Dielectric (13) and Barium Stearate - Chromium (34) Interfaces. Letters s and p refer to plane of polarization of incident light, parallel (p) or perpendicular (s) to the plane of incidence.

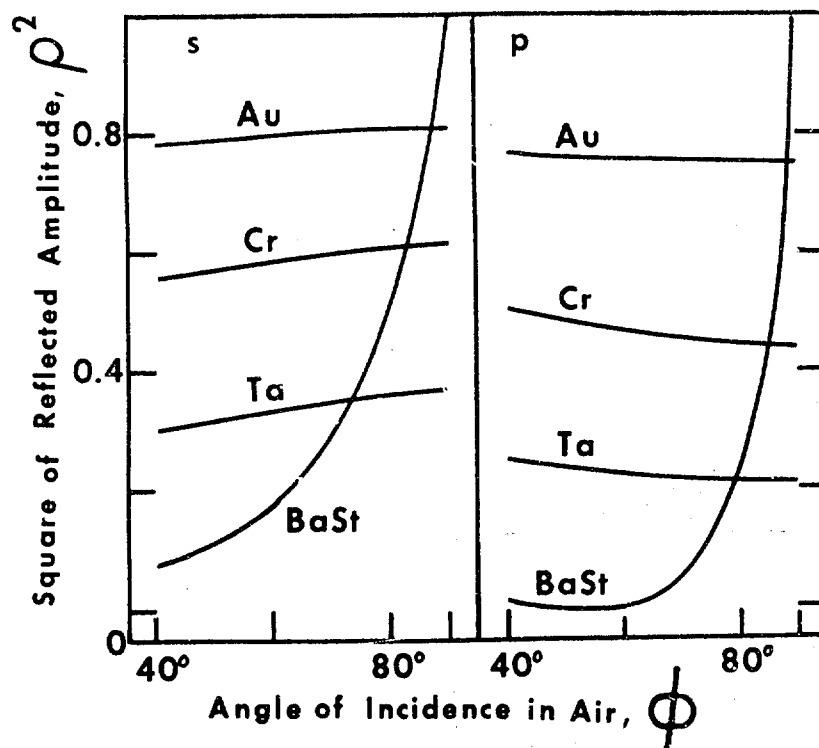


Figure 3. Illustrating First Condition for Formation of s and p Extinction Fringes by Compound Reflector of Barium Stearate on Metal. Curves labeled "BaSt" give reflected intensity at interface between air and a semi-infinite layer of barium stearate. Curves labeled Au, Cr, Ta give the corresponding reflected intensities at a barium stearate - metal interface. Wavelength about 5890 Å throughout.

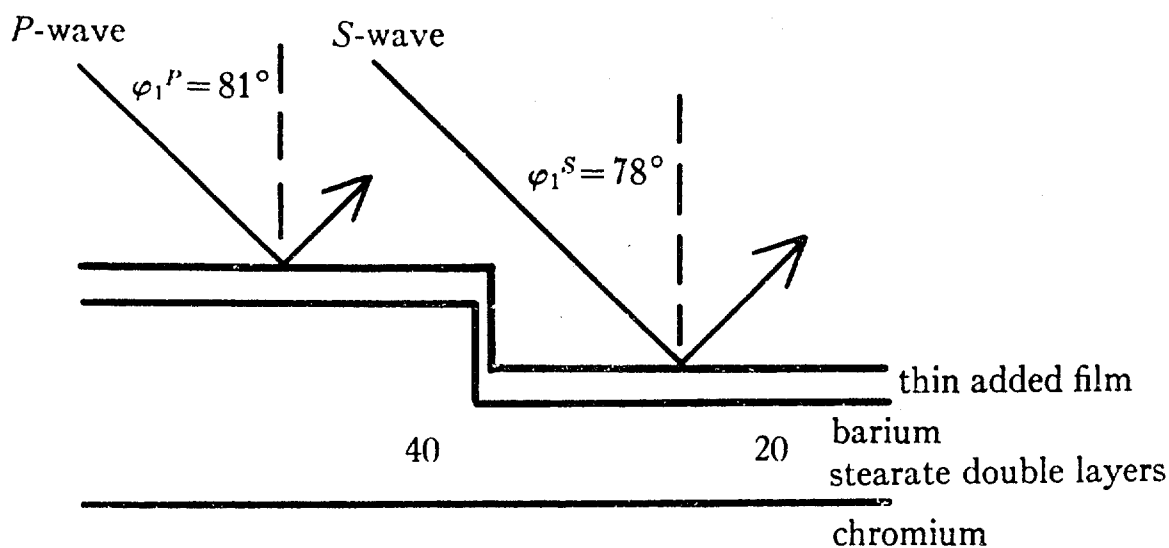


Figure 4. Chromium-Plated Glass Slide with Stepped Multilayer of Barium Stearate - Stearic Acid. (Reproduced from Journal of Physical Chemistry⁶ by kind permission of the American Chemical Society.)

give the corresponding first-order extinctions. If instead of using monochromatic light the slide is illuminated with white light and the reflected light dispersed in a spectrometer, a black band will be seen at the extinction wavelength. Figure 5 shows curves calculated for zero- and first-order s and p extinction fringes produced by barium stearate on a metal plate having optical constants resembling those of chromium, but assumed to be independent of wavelength.

What happens if a thin transparent film of arbitrary refractive index and thickness is now deposited upon both steps of the interference reflector? Clearly the reflection coefficient at the original upper surface of the dielectric substrate will be decreased; the rays reflected from this interface, now representing the boundary between the "unknown" film and the substrate (Figure 6), will continue to interfere with those reflected from the metal, with their optical path difference unchanged, and will thus produce a minimum reflected intensity at the wavelength of the original extinction. However, upon this will be imposed a second minimum produced by combining the ray reflected from the upper surface of the added film with that coming from the metal; this second minimum will occur at a new, longer, wavelength corresponding to the total optical thickness of dielectric substrate and test film (regions 2 and 3 of Figure 6). These two minima will not actually be resolved; they are introduced for purposes of exposition only. The result of combining the two hypothetical fringes will be a single rather broad minimum at an intermediate wavelength. The same process of combination will occur on both the s and p steps, but since the profiles of the two minima in each case will depend upon the values of the respective Fresnel reflection coefficients, and since these in turn will involve different functions of the refractive index of the unknown film, the wavelengths of the s and p minima will depend in distinctive ways upon film refractive index. The theory of the fringe shifts justifies this prediction and shows, furthermore, that the difference referable to film refractive index is a readily measurable quantity. After drastic approximation, the following simple equations are obtained:

$$n_2 \sim 1.5 (\delta_{3s}'\Delta_s / \delta_{3p}'\Delta_p)^{1/2} = 1.5 (\epsilon_s / \epsilon_p)^{1/2} \quad (1)$$

$$d_2 \sim 0.088 \delta_{3s}'\Delta_s / (n_2^2 - 1) \quad (2)$$

$$\epsilon_s = \delta_{3s}'\Delta_s, \quad \epsilon_p = \delta_{3p}'\Delta_p \quad (3)$$

These equations refer to the case of a film (Figure 6) deposited upon barium stearate of refractive index 1.5 and observed at an angle of incidence 81 degrees. Film refractive index and thickness are denoted by n_2 , d_2 , respectively; fringe shifts are $\Delta\lambda$; the δ_3' values are effective optical path differences for the barium stearate substrate, and are calibration constants measured as described later.

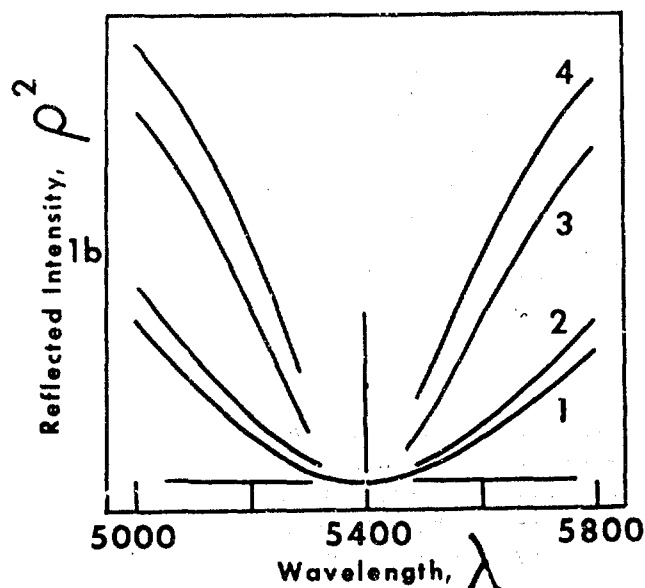


Figure 5. Calculated Extinction Fringes Produced when Stepped Reflector of Barium Stearate on Chromium is Examined at the Correct Angle of Incidence. Fringes are calculated to coincide at 5400 Å. Dispersion of metal is ignored. Curve 1: zero-order s fringe. 2: zero-order p fringe. Curves 4 and 3 are the corresponding first-order fringes respectively.

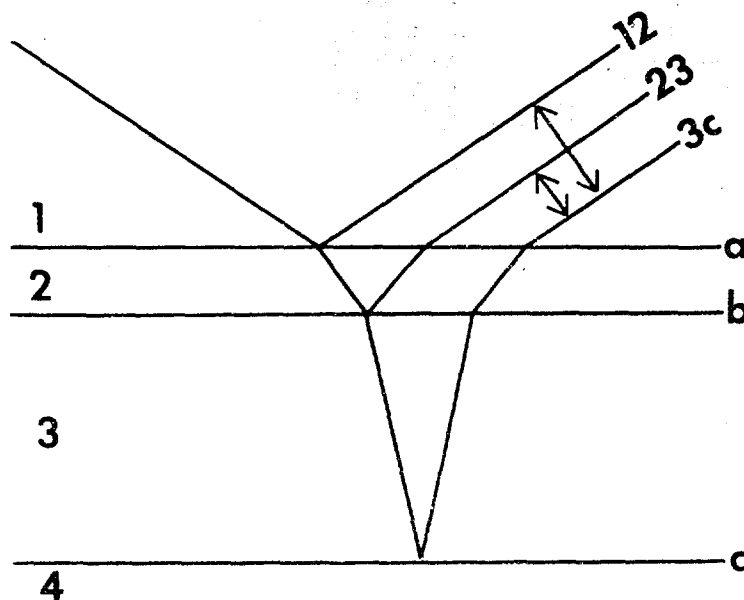


Figure 6. Simplified Scheme, Ignoring Multiple Reflections, Illustrating Displacement of Extinction Fringe when Thin Test Film is Formed upon the Dielectric Film 3. Observed fringe is compounded from fringe formed (a) by addition of rays 23 and 3c, at the wavelength λ_b of the original extinction fringe, and (b) by addition of 12 and 3c, at some longer wavelength. Resultant minimum is at an intermediate wavelength λ_a .

III. APPARATUS AND PROCEDURES

A. FABRICATION OF STEPPED REFLECTORS

Glass microscope slides coated with a thick film of chromium by vapor condensation in vacuo can be prepared in the laboratory as needed, or can be obtained from commercial sources.* Sample slides from each batch should be examined for suitability, since the optical properties of the chromium films are quite variable and occasionally will fail to give extinction fringes when coated with the usual thicknesses of stearate and examined at the usual angle of incidence. A check by this means is more satisfactory than a measurement of the optical constants of the metal film because the tolerable range of variation has not been established.

Procedures for transfer to stearate multilayers vary considerably from laboratory to laboratory and have undergone many modifications in our own laboratory. Although studied by many workers, some of the factors involved are so subtle that all-inclusive specifications cannot be given. The composition of the water upon which the stearic acid is spread, for example, is certainly of major importance, but the usual electrical criterion of purity is irrelevant, and more importance must be attached to such factors as presence of impurities picked up from ion exchange materials during the course of preparation.

Our most recent procedures have been described in some detail.⁸ The aqueous subphase is prepared from laboratory-distilled water of specific resistance around 80000 ohm-cm., and containing spectrographically detectable traces of B, Si, Al, Fe, Mg, Ca, and Cu; the salts added to this are barium chloride, 10^{-4} M; potassium bicarbonate, 2×10^{-4} M; cupric chloride, 10^{-7} M. The final pH is 6.8 and the temperature must not exceed 20°C. This solution is placed in a waxed Plexiglas film tray (Figure 7). The surface is repeatedly "swept" clean with a Plexiglas barrier, and on the clean surface is placed a polyethylene float in position 2 (Figure 7). A solution of stearic acid (Eastman, M. p. 69.5° to 70°C), 3×10^{-2} M in reagent-grade benzene, is then dropped from a micropipette upon the surface at the center of the ring 3; the ring retains the solution while permitting outward spreading of the monolayer formed as the benzene evaporates. Spreading is continued, as rapidly as possible, until the float has been propelled to position 2. A residual lens of solution is aspirated off the surface, and after a few minutes the film is compressed to 29 dynes per centimeter by applying a drop of purified oleic acid to the surface on the left (in Figure 7) of the floating barrier. Several clean chromium slides, made hydrophobic by rubbing with cotton wool, are then mounted on an automatic dipping device above the trough (position 4 in Figure 7) and dipped vertically at about five centimeters per minute. The result of repeated dipping is a very stable, uniform multilayer of barium-copper-hydrogen stearate.

* Evaporated Metal Films Corp., 436 W. State St., Ithaca, New York.

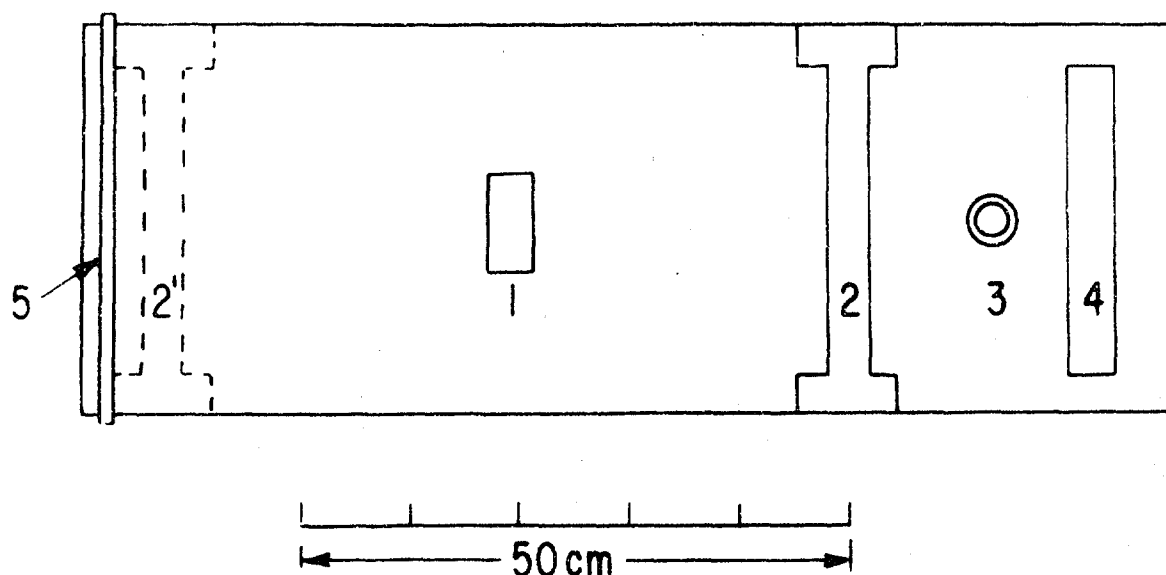


Figure 7. Plexiglas Trough for Monolayer Formation and Transfer. See text and Bateman *et al.*⁶ for Further Details. (Reproduced from the Journal of Colloid Science by kind permission of Academic Press, Inc.)

For many purposes zero-order reflectors are satisfactory, with about 20 stearate double layers on one portion and about 40 on the other. Sharper extinction fringes are obtained with first-order reflectors (61 and 80 double layers) (cf. Figure 5), with reduction of fringe shift for a given unknown film, but with an over-all gain in precision and convenience.

B. OPTICAL APPARATUS AND PROCEDURE

The various optical elements are shown in Figure 8 and identified in the accompanying caption. The arrangement is such that at an angle of incidence of about 80 degrees the area of the stepped reflector viewed by the photomultiplier is 2 mm high by 0.6 mm. The spectral band width received by the photomultiplier is estimated to be about 10 Å, representing a compromise between desirable fringe resolution and available intensity.

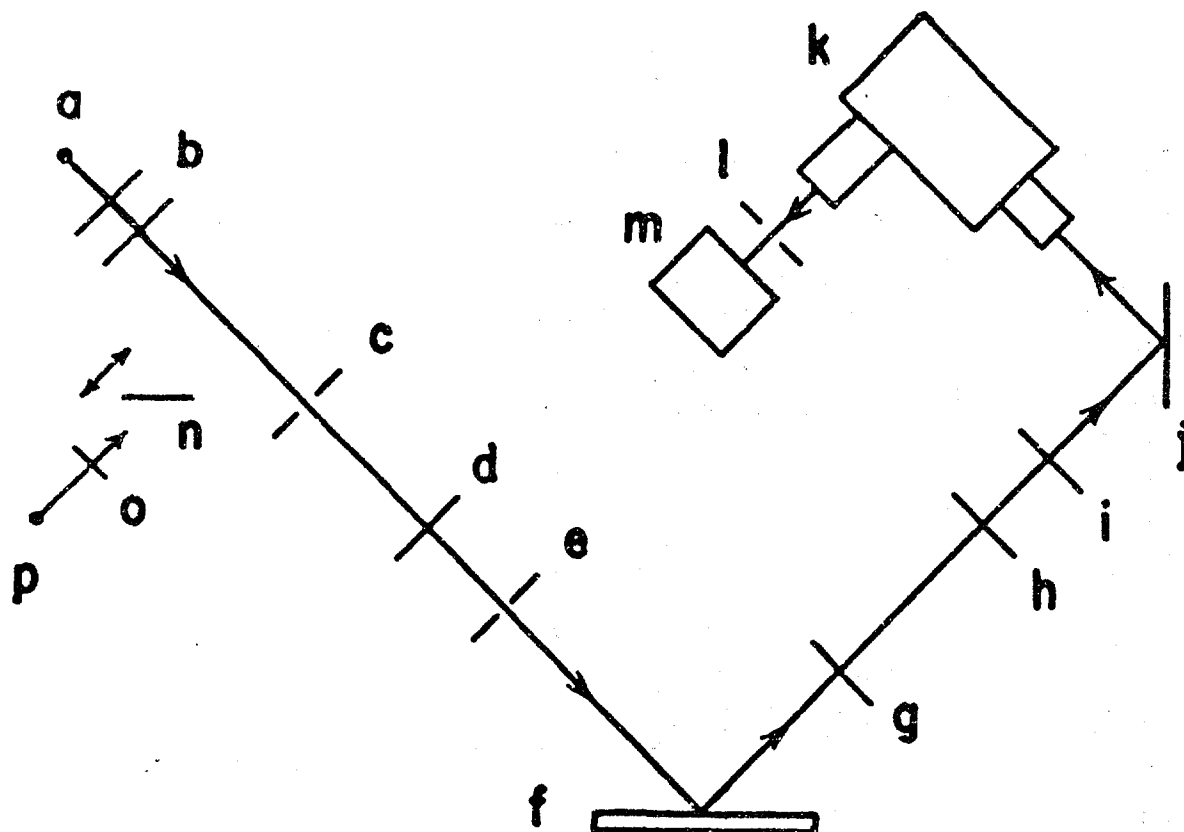


Figure 8. Apparatus Used for Measurement of Film Thickness and Refractive Index by the g-p Stepped Reflector Method. a is zirconium arc, b collimating lens, c first collimating slit, d polarizer, e second collimating slit, f stepped reflector with test film, g analyzer, h and i are focussing lenses, j is mirror, k constant deviation spectrometer mounted on turntable, l Hilger slit, m photomultiplier, n movable mirror, o collimating lens for mercury vapor lamp used for calibration. (Reproduced from Journal of the Optical Society of America by kind permission of the editor.)

Assuming the necessary alignment and other adjustments to have been checked, the first step is to locate the g and p fringe minima on the reflector. Formerly this was done at the angles of incidence for true extinction fringes, $79^{\circ}10'$ and $82^{\circ}30'$ respectively for g and p rays. A convenient practical compromise is to work at a constant angle of incidence of 81 degrees, with negligible loss of accuracy. A manual null-searching method has proved adequate for locating the fringe minima, although scale readings taken on either side of the minimum may also be useful and are convenient indicators of any unusual change in symmetry. In general, observations are made at ten places on each step of the reflector, placed in two vertical rows on either side of the axis. The uniformity of the multilayer plates is such that discrepancies between readings exceeding 2 Å are quite unusual, and within a series of established uniformity the number of observation areas may be reduced. The test films added subsequently are generally less uniform.

After locating the minima at λ_{os} and λ_{op} , calibration is done by adding a known small number of double layers of BaCuH stearate to the entire reflector and repeating the fringe measurements, which now yield the wavelength values λ_{ps} and λ_{bp} . From the four wavelength values the calibration constants δ_3 of Equations (1) through (3) can be calculated by Equation (69).

The reflector is now ready for the test film, deposition of which produces a further displacement of the fringe minima to λ_{as} and λ_{ap} . These displacements ($\lambda_{as} - \lambda_{bs}$) and ($\lambda_{ap} - \lambda_{bp}$) are the values Δ used in Equations (1) through (3). If more than a rough estimate of the test film parameters is required, more involved calculations can be carried out as indicated later.

IV. METHODS OF CHECKING VALIDITY OF FILM THICKNESS AND REFRACTIVE INDEX DATA

A. TEST CRITERIA

Independent methods are not available for determining the optical constants, or alternatively the mass per unit area and density, of the test films deposited upon the reflector. Indirect tests must be applied, recognizing that each is open to some objection. The following criteria have been used, with results that will be presented briefly in the next section:

(a) Successive transfers of monolayers of test substance: constant apparent refractive index combined with constant apparent thickness increment.

(b) Agreement of film refractive index and thickness per layer with values found using multilayers built up to several higher orders (see Section IV, B).

(c) Variation in apparent thickness of monolayers prepared from members of homologous series correlated with molecular dimensions; apparent refractive indices compared with values for organic crystals.

(d) Apparent refractive index of thin films formed by condensation of vapor compared with values for thicker films, similarly prepared, measured by the Abbe's method (see Section IV, C).

(e) Apparent refractive index of test film formed either by vapor condensation or by monolayer transfer shown to be independent of film thickness, and correlated with refractive index of substance in bulk.

(f) Demonstration that the apparent refractive index of certain porous films of very low apparent index can be increased by "filling in."

B. INDEPENDENT DETERMINATION OF THICKNESS AND REFRACTIVE INDEX OF THICK MULTILAYERS

When successive monolayers can be transferred to a chromium slide in sufficient numbers to give s ray extinction fringes of several orders, the average refractive index and thickness per monolayer can be determined by measuring the fringe wavelengths λ for various angles of incidence above and below the extinction angle.¹⁶ The fringe condition is

$$\frac{4 \pi \bar{d} N}{\lambda} (n^2 - \sin^2 \phi)^{\frac{1}{2}} + \theta = (1 + 2m)\pi \quad (4)$$

where the first term corresponds to the optical path difference in the added film. The additional phase term θ incorporates (a) phase changes on reflection at the two interfaces and (b) the retardation within any "rubbed-down" monolayer present on the metal surface. The fringe order, $\underline{m}$, is 0,1,2,..... If θ is not wavelength-dependent, the plot of N/λ against $\underline{m}$ at any angle of incidence will be a straight line:

$$\begin{aligned} \underline{m} &= 2\bar{d} (n^2 - \sin^2 \phi)^{\frac{1}{2}} (N/\lambda) - (1 - \theta/\pi)2 \\ &= b(N/\lambda) + a \end{aligned} \quad (5)$$

Equation (5) can then be solved for $\bar{d}$ and n by plotting b^2 against $\sin^2 \phi$:

$$b^2 = -4 \bar{d}^2 \sin^2 \phi + 4 n^2 \bar{d}^2 \quad (6)$$

Some dependence of θ upon wavelength would be expected. A basal layer, d_o , n_o , would contribute a term θ_f in λ^{-1} , and ϕ , while there would also be a dispersion term θ_r at the metal-dielectric interface dependent mainly on λ :

$$\theta = \theta_f + \theta_r = 4 \pi d_o (n_o^2 - \sin^2 \phi)^{\frac{1}{2}} / \lambda + \theta_r \quad (7)$$

In practice, measurements cannot be made at exactly constant wavelength; there is some variation of fringe wavelength within each set of orders at any angle of incidence, and a much larger systematic variation with changing angle of incidence, resulting in apparent dependence of the intercept $\underline{a}$ upon angle of incidence. If this is attributed to θ_f , varying according to Equation (7), it is found experimentally that d_o has a large negative value, while the observed small over-all values of θ (around 20 degrees) have to be maintained by an unreasonably large compensating value of θ_r . Thus no reliable estimate of $\theta(\phi)$ can be obtained, and it is preferable to consider it as exclusively wavelength-dependent. With this assumption, the values of $\underline{a}$ obtained at each angle of incidence can be plotted against average fringe wavelength and revised values of $\underline{a}$ can be obtained by interpolation for the fringe wavelength measured at each value of $\underline{N}$. Each measurement then yields a new value of the slope $\underline{b}$ by the equation

$$\underline{b} = (\underline{m} - \underline{a}) / (N/\lambda) \quad (8)$$

and the averaged values of $\underline{b}$ plotted against $\sin^2 \phi$ lead to the corrected estimates of $\underline{n}$ and $\bar{d}$.

If consideration is being given to the non-isotropic character of the multilayers, measurements can be made at each angle of incidence with the s ray at right angles to the direction of film deposition (the usual position of the slide) or parallel to it. In this case it is assumed that the metrical thickness of the films, $N\bar{d}$, is independent of the direction from which they are viewed.⁸ Using one prime and two primes, respectively, to denote values referable to the two mutually perpendicular directions of incidence, one obtains

$$(b')^2 + (b'')^2 = 4 \bar{d}^2 [(n')^2] - 8 \bar{d}^2 \sin^2 \phi \quad (9)$$

$$(b')^2 - (b'')^2 = 4 \bar{d}^2 [(n')^2 - (n'')^2] \quad (10)$$

C. DETERMINATION OF REFRACTIVE INDEX BY THE ABELÈS METHOD

In the Abelès method^{17,18} a portion of the surface of a plane reflector of any type is coated with the test film. The boundary between the covered and uncovered portions is illuminated with collimated monochromatic light plane polarized in the plane of incidence (p vibration) and the angle of incidence is varied until the images of the areas on either side of the boundary appear equally bright.

The reflection coefficient of the film-covered system is obtained simply by adding the multiple reflections;^{19,20**} that of the clean reflector can be expressed in terms of the film parameters by bringing the film thickness to zero:

$$r_{1b} = \frac{r_{13} + r_{3c} \exp(-i\delta_3)}{1 + r_{13} r_{3c} \exp(-i\delta_3)} \xrightarrow{\delta_3=0} \frac{r_{13} + r_{3c}}{1 + r_{13} r_{3c}} = r_{1c} \quad (11)$$

where the various reflection coefficients, which may be complex, are denoted by r . Matched fields can be obtained either by eliminating reflection at the upper film surface ($r_{13} = 0$) or at the lower surface ($r_{3c} = 0$), as shown in Figure 9. The second condition cannot readily be satisfied on metallic substrates. The first condition is generally applicable; it shows that if, by eliminating r_{13} , the multiple reflections are reduced to a single reflection r_{3c} plus complete transmission at 13 and 31, the reflected amplitude r_{3c} at an internal angle of incidence of $\sin^{-1}(\sin \phi_1/n_3)$ will be equal to that at the clean substrate surface at

* Pages 61 and 323.

** Page 55.

an angle of incidence ϕ . This is not generally the case; in a system governed by Fresnel reflection coefficients it is satisfied only when $\tan \phi_1 = n_3$. Thus the matching angle of incidence, the Brewster angle, at once gives the refractive index of the film, no matter what the optical properties of the substrate.

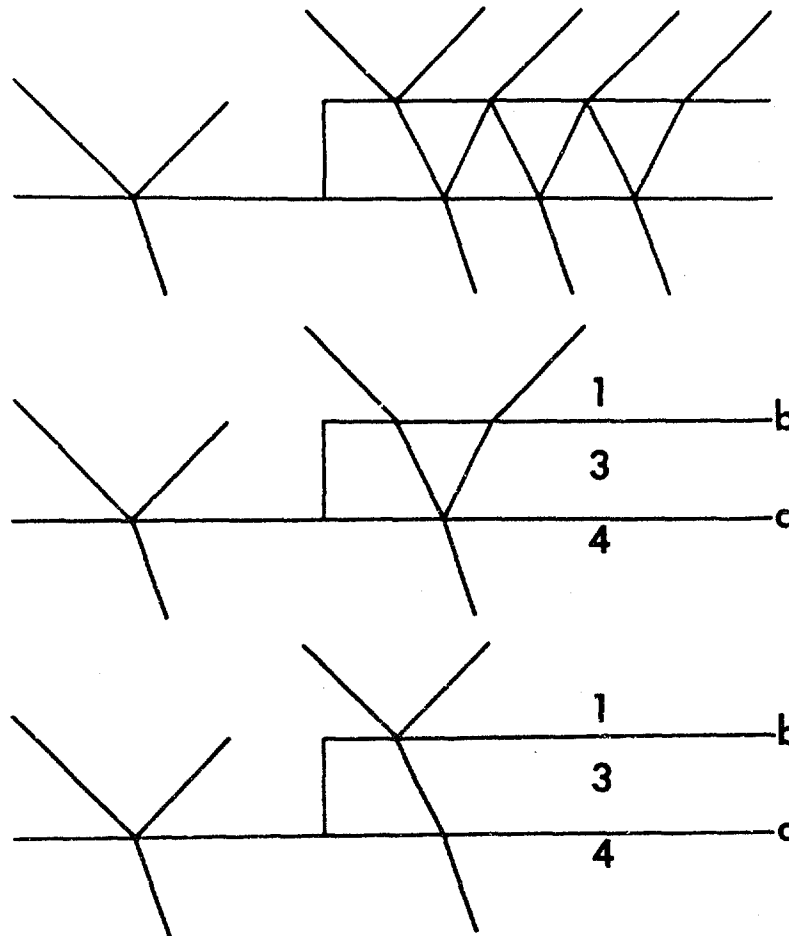


Figure 9. The Two Brewster Angle Methods of Abeles^{17,18} for Determining Index of Refraction of Thin Films. Top: Multiple reflections between surfaces b and c of a dielectric film on an arbitrary base. Middle: Multiple reflections abolished when light (p vibration) is incident at Brewster angle for interface b. Bottom: Film on dielectric substrate. Multiple reflections abolished when incidence corresponds to Brewster angle for interface c.

Abeles¹⁷ remarks that there may be an optimum film thickness for the detection of the matching angle, and it has been noted⁵ that when a metallic base is used, the condition for maximum contrast in the vicinity of the matching angle is periodic in film thickness, with δ_3 , the optical path difference in the film, equal to π , 3π , 5π ; this is, remembering the additional phase difference close to π between r_{13} and r_{34} , the condition for maximum r_{1b} . The contrast is much less in the vicinity of the p ray extinction. The upshot of this is that the minimum film thickness for satisfactory measurements is about $1000 \text{ \AA}$. The method, in this form, is not suitable for direct check of results obtained by the stepped reflector method, but furnishes an interesting comparison of thin and thick films deposited under similar conditions.*

The apparatus used consisted of a sodium vapor source, collimating lens, two collimating irises, Nicol prism, Spencer student spectrometer table with added slide holder, and a viewing telescope focused on a dust particle at the center of the slide. The matching condition was established visually by simultaneous manual rotation of the slide holder and telescope arm, 10 to 20 such readings being taken.

* More recent work on the method is referred to in the review by Abeles.²¹

V. SOME EXPERIMENTAL RESULTS

A. MEASUREMENTS ON THICK FATTY ACID MULTILAYERS

1. Illustration of Procedure

The method outlined in Section IV, B has been used for measurements of double-layer spacing and refractive index of barium stearate, barium copper stearate, and barium copper nonadecanoate mixed in unknown proportions with the free acids. An illustration of the primary fringe wavelength measurements has been given in an earlier paper;¹⁶ the data used for the initial calculations from Equations (5) and (6) are shown graphically for a typical case in Figure 10. Examples of the graphs used to obtain corrected values of $a(\lambda)$, for application of Equation (8), are given in Figure 11. The resulting individual values of $b(m, \phi)$ are then averaged for each ϕ and Equation (6) is again applied for calculation, by least squares, of final values of n and $\bar{d}$. The values of $b(m)$ prove to be compatible with a straight line passing through the origin, but they nevertheless show some non-random variation that seems to depend upon the nature of the film substance. Illustrations of this slight systematic variation, which suggests a variation of film properties with thickness, are given in Table I.

TABLE I. AVERAGE VARIATIONS OF $b(m)^a$

Film	$m = 1$	2	3 b/b_1	4	5	No. of Slides Examined
BaCuH Stearate	1	0.99583	0.99464	0.99667	0.99834	3
BaCuH Stearate, checkerboard	1	0.99915	0.99980	0.99963	-	1
BaCuH Nonadecanoate	1	0.99974	1.00061	0.99974	0.99850	2

a. Values averaged over five angles of incidence.

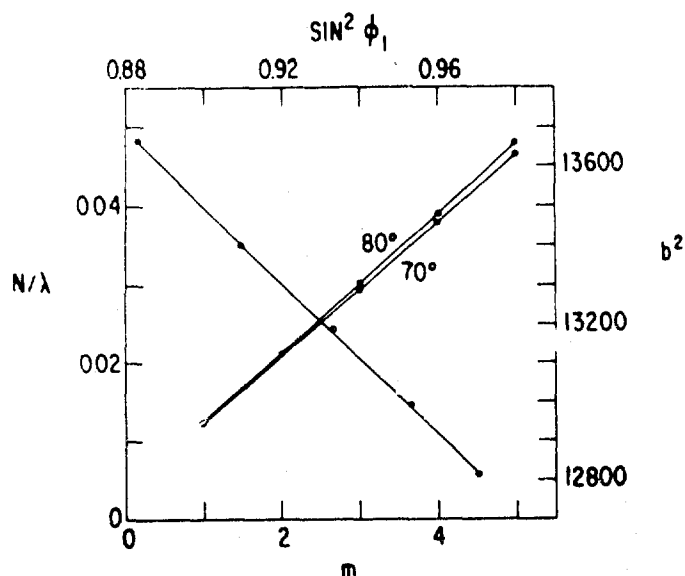


Figure 10. Experimental Data Illustrating Higher-Order $\frac{1}{2}$ Fringe Method of Determining Film Thickness Per Monolayer and Refractive Index. BaCuH multilayers on chromium plated glass. Open circles: N/λ as function of fringe order, m [text Equation (5)] for angles of incidence 70 and 80 degrees. Closed circles: $b^2(\sin^2 \phi)$ [text Equation (6)] for angles of incidence 70.0, 72.5, 75.0, 77.5, and 80.0 degrees. (Reproduced from the Journal of Colloid Science⁸ by kind permission of Academic Press, Inc.)

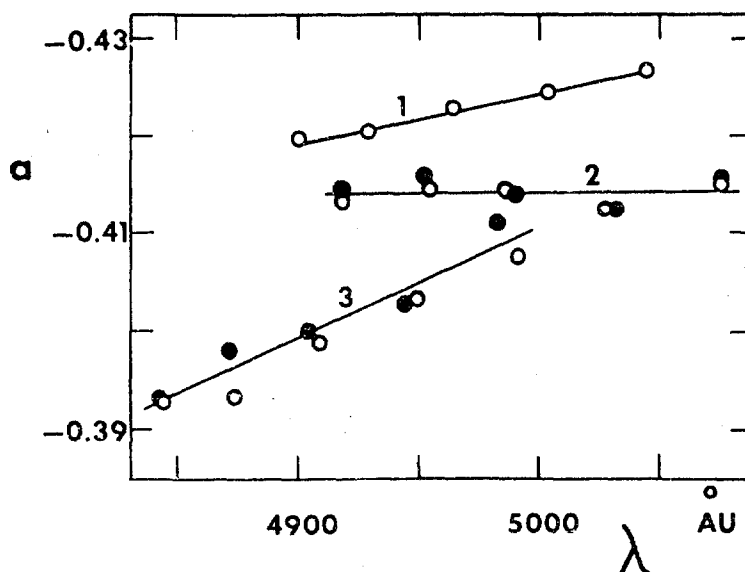


Figure 11. Apparent $\frac{1}{2}$ Vibration Phase Change at Surface of "Clean" Chromium Reflector, Obtained by Extrapolation of Straight Line Relating Fringe Order, m , to N/λ [Equation (5)]. Abscissa: Average fringe wavelength for first- to fifth-order fringes. Ordinate: a , equal to $-0.5(1 - \theta/\pi)$, where θ is phase change at "clean" surface. If $\theta = 0$, $a = -0.5$. Curve 1: For BaCuH stearate slide III; 2: BaCuH stearate checkerboard slide V; 3: BaCuH nonadecanoate slide II. See Table II.

2. Results

The measurements are summarized in Table II. The variation from slide to slide is rather greater than might be hoped, apparently because of variations in the nature of the multilayer rather than of poor reproducibility of the optical measurements themselves. The average spacings for stearate and nonadecanoate are compatible with a vertical orientation of the extended chains, although the extended chain lengths themselves are open to uncertainty as to the effect of the barium and copper atoms and the percentage of free acid in the films. The values of the refractive index of stearate are compatible with the assumption that the index that is effective for the s vibration is the ordinary index n_0 if the hydrocarbon chains are normal to the slide. In the case of the nonadecanoate films, it is apparent that the chains are either more loosely packed than is the case for stearate, or that there is a certain proportion of vacancies throughout the film. An indication of this can be obtained from the values of $(n^2 - 1)/(n^2 + 2)$, since

$$\frac{n^2 - 1}{n^2 + 2} = \frac{1}{\rho} \cdot \frac{R}{M} \quad (12)$$

where R is molecular refractivity, M is molecular weight, and ρ is density of the solid-air mixture having refractive index n .^{*} The highest value found was 3.07 for the BaCuH stearate checkerboard slide (qv); for stearate transferred in the usual manner, 2.90 to 3.02; for nonadecanoate, 2.76 to 3.00.

A test for vertical orientation was done by examining some of the film-coated slides after turning them through 90 degrees in the slide holder. In the usual measurements the vertical axis of the slide coincides with the direction of dipping during film transfer, and is perpendicular to the plane of incidence; in this case, if the molecular chains are not exactly perpendicular to the dipping axis, the refractive index for the s vibration will be a little larger than the ordinary index, n_0 . Rotating the slide through 90 degrees will then bring the refractive index back to n_0 . The expected difference, $n' - n''$, would be about 0.0010 for a tilt of five degrees, 0.0015 for 10 degrees, 0.0040 for 15 degrees. Thus the observed differences, 0.0019 for stearate and 0.0012 for nonadecanoate (Table II), suggest a tilt of about 6 to 10 degrees.

* Although R/M is not exactly constant for the different fatty acids, the difference is probably less than one per cent; e.g., 0.3054 and 0.3079 for palmitic and stearic acids, respectively, at 80°C.²⁴

TABLE II. SPACING AND REFRACTIVE INDEX OF THICK FATTY ACID MULTILAYERS BY MEASUREMENT AT SEVERAL ORDERS OF INTERFERENCE

Slide	Reference	Substance	Fringe Type	$\bar{d}$	n'	n''	$n' - n''$
-	22*	BaH stearate	s	45.7	1.508	-	-
-	23*	BaH stearate	s	47.575	1.498	-	-
I	8	BaCuH stearate	s	51.326	1.492	-	-
I	-	BaCuH stearate	p	-	1.493	-	-
II	8	BaCuH stearate	s	49.485	1.510	1.508	0.00200
III	8	BaCuH stearate	s	49.256	1.515	-	-
V	8	BaCuH stearate checkerboard	s	48.535	1.52250	1.52269	-0.00019
I'	8	BaCuH nonadecanoate	s	51.409	1.5108	1.5125	-0.00170
II'	8	BaCuH nonadecanoate	s	54.225	1.4661	1.4649	0.00120
II'	8	BaCuH nonadecanoate 2 months later	-	53.908	1.4679	1.4667	0.00120
Averages:		BaH stearate	s	46.638	1.503		
		BaCuH stearate ⁺	s	49.762	1.508		
		BaCuH stearate checkerboard	s	48.535	1.522		
		BaCuH nonadecanoate ⁵	s	52.738	1.488		

$\bar{d}$ = Thickness per double layer, ÅU.

n' = Refractive index for light incident at right angles to monolayer transfer direction.

n'' = Value for incidence parallel to transfer direction.

* = Values as given in Reference. Remaining values have been revised as described in Section IV, B. Differences from values given in Literature Cited Item 7 are small except for slide II' remeasured after two months; values given there were $\bar{d} = 52.53$, $n' = n'' = 1.491$.

+ = Weighted average, with double values assigned to II and III, since edge effects between orders were suspected in slide I.

5 = Weighted average, with double values to I'' and single values to each measurement of II'.

A further test was made by building a stepped multilayer in the usual way and then adding to this a second series of steps at right angles, producing a checkerboard effect for which the refractive indices n' and n'' should be equal and, in the presence of molecular tilting, slightly larger than n_0 . This slide proved to be superior to all others in that the intercept a was independent of wavelength, the values of b showed no dependence on fringe order (Table I), and the difference between n' and n'' was reduced by a factor of 10 (Table II). At the same time, the film density was higher than usual, suggesting that transfer in two directions may be advantageous in the production of uniformly packed multilayers.

B. MEASUREMENTS WITH s-p STEPPED REFLECTOR

1. Transferred Monolayers of Aliphatic Acids

Since stearate is used in determining the calibration constants δ' and δ'' , Equation (1), calculation of apparent refractive index and thickness $3p$ of subsequently added stearate amounts to a further calibration and should yield the same values as those initially assumed. The fairly satisfactory check obtained is shown in Figure 12. The results for stearic acid transferred from a distilled water substrate show equally satisfactory constancy of double-layer spacing and refractive index, with values that are significantly different from the calibration values for barium stearate.

Measurements with a few other long-chain saturated acids are shown in Table III; the values of $\bar{d}$ are compared in Table IV with the actual molecular dimensions obtained in X-ray diffraction studies. It is of interest that while the experimental values of $\bar{d}$ show the correct overall trend, there are considerable irregularities and even a reversal of the expected order. This reversal is avoided if the amounts of material per monolayer are estimated in terms of a "Lorentz-Lorenz" function, $(n^2 - 1) \bar{d} / (n^2 + 2)$: the values of this quantity, with increasing chain length, are 11.934, 13.705, 14.817, 16.045, 16.619. Some irregularity remains, but this may be due to differences in structure of films formed from the odd and even members of the aliphatic series. The improvement resulting from calculation of the Lorentz-Lorenz function is probably an example of the $n - d$ "coupling" discussed in the next paragraph.

2. Transferred Monolayers and Adsorbed Films of Protein

Two distinct procedures in the study of protein films, and their results,^{6,7} are relevant to the present display of the stepped reflector method.

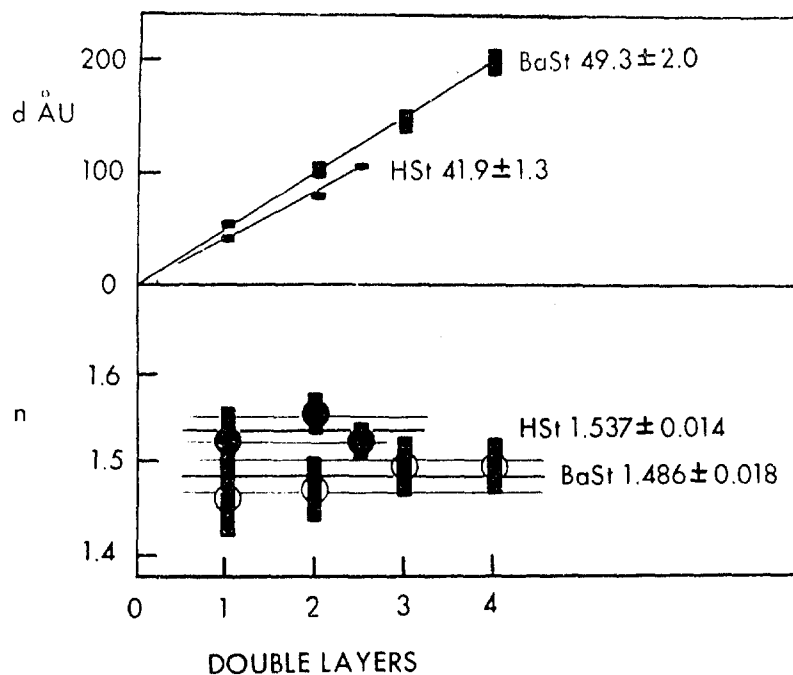


Figure 12. Thickness (Above) and Refractive Index (Below) as a Function of Number of Double Layers of Stearate Transferred to s - p Stepped Reflector. Vertical bars indicate standard deviations of each value. Over-all thicknesses per double layer, $\bar{d}$, and refractive index, $\bar{n}$, are given at right, together with standard deviation for each series. BaSt indicates barium hydrogen stearate. H St is free stearic acid.

TABLE III. SPACING AND REFRACTIVE INDEX OF THIN ALIPHATIC ACID FILMS ON s - p STEPPED REFLECTOR

Ref.	Substance	C atoms	Fa / dynes/cm	N	$\bar{d}$	$\bar{n}$	$\bar{d}-\bar{d}(\text{cal})$	$\bar{n}-\bar{n}(\text{cal})$
5	BaH stearate	18	29	1-5	-	-	1.73	-0.012
8	BaCuH stearate		29	2	-	-	-0.099	0.002
				6	-	-	-0.319	0.001
8	BaCuH stearate		16	4	47.89	1.518	-	-
	BaCuH stearate		9	4	45.16	1.528	-	-
5	Stearic acid	18	29	1-2.5	41.9	1.537	-	-
			29	2	41.0	1.535	-	-
-	BaCuH pentadecanoate	15	9	4	39.7	1.513	-	-
-	BaCuH palmitate	16	29	6	46.6	1.500	-	-
8	BaCuH nonadecanoate	19	29	4	56.1	1.487	-	-
-	BaCuH arachidate	20	29	2	53.9	1.529	-	-
				4	53.7	1.531	-	-

a. F = film surface pressure at time of transfer to stepped reflector.

N = number of double molecular layers used in measurement.

$\bar{d}(\text{cal})$, $\bar{n}(\text{cal})$ refer to values for stearic acid used in stepped reflector calibration.

$\bar{d}(\text{cal}) = 47.575 \text{ Å}$, $\bar{n}(\text{cal}) = 1.498$.⁵

$\bar{d}(\text{cal}) = 49.499 \text{ Å}$, $\bar{n}(\text{cal}) = 1.510$.⁸

TABLE IV. SUMMARY OF DATA ON VALIDITY OF s-p STEPPED REFLECTOR METHOD

Substance	Stepped s-p Reflector		Higher Order s - Fringes		Abelès Method		Other Values	
	n_{sp}	d, δ	n_s	δ	n_p	δ	n bulk	n film
Stearic acid	1.537 ± 0.014 1.535 ± 0.023	41.9 ± 1.3 41.0 ± 1.4			1.549 ± 0.006^a 1.508 ± 0.014	48.84^b (~ 41 ?)		1.51^c 1.4917^d 1.551
BaH stearate			1.503	46.63 ₈				
BaCuH soaps						(41.22) ^e		
pentadecanoate	1.513	39.7				(43.76)		
palmitate	1.500	46.6				(48.84)		(1.559) ^g
stearate	1.487	56.1	1.5225^f 1.489	48.535^f 52.74		(51.38) (53.92)		
nonadecanoate								
arachidate	1.530	53.8						
Bovine plasma albumin								
Adsorbed ^h	1.589	29.0				$>22^i$	1.6^j	
Monolayers ^k	1.592	9.6 ₃				10.0^m		
Botulinum toxins								
Adsorbed, type A	1.588	24.9						
Adsorbed, type B	1.526	30.1				25.5^n		
Fused ring compound ^o								
Monolayers	1.676 ± 0.028				1.728 ± 0.014		1.7^p	
CaF ₂	1.362 ± 0.015	258(± 10)			1.250 ± 0.022		1.434^q	$1.23-1.28^r$
Cryolite, Na ₃ AlF ₆	1.393 ± 0.009	106-197			1.405 ± 0.006		1.364^s	$1.30-1.31^t$
MgF ₂	1.411 ± 0.014	63,246			1.412 ± 0.006		1.384^u	1.400 ± 0.002
CaCO ₃	1.563 ± 0.020	150			1.596 ± 0.006		1.572^v	
ZnS	1.51 ± 0.06 $1.50 \pm (0.04)$	93 ~ 100			1.98 ± 0.02		2.37^w	

- n_{sp} = refractive index determined by stepped reflector method. Calibration with BaCuH stearate for all BaCuH compounds using $n_3 = 1.510$, $d_3 = 49.499$ AU. For remaining experiments, calibration with BaH stearate using $n_3 = 1.498$, $d_3 = 47.575$ AU.
- n_s = refractive index by s-fringe method.
- n_H = refractive index by Abelès method with directions of incidence and reflection parallel to long axis of film slide.
- d_p = film thickness (evaporated films).
- d = thickness per double layer (fatty acids) or monolayer (proteins and ring compounds).

Footnotes

- a Cf Literature Cited Item 8.
- b Stearic acid unit cell: $c = 48.84$ Å, $c \sin \beta = 43.75$ or 39.75 .²⁵
- c From Blodgett et al.¹⁵
- d n_o and n_e respectively.¹⁵
- e Values in this column obtained by adding 2.54 Å per carbon atom to extended chain length of stearic acid. No allowance made for metal atoms.
- f Values obtained with checkerboard reflector, with equal numbers of double layers deposited at right angles.
- g n_e for BaPbH stearate.
- h Adsorbed from HCl solution at pH 5.
- i Molecule $22 \times 49 \times 106$ Å²³ if deposited in orientation similar to that in unit cell would form film about 22 Å thick.²⁸
- j Calculated for solid protein from refractive index increment of solutions, using Lorentz-Lorenz equation.
- k Protein spread on dilute $ZnCl_2$.²⁸
- m Side chain spacing in extended β -form.
- n From sedimentation and diffusion measurements, axial ratio ~ 18 , molecular weight $\sim 10^6$, consistent with ellipsoid of revolution $460 \times 25-5$ Å.²⁹
- o 8, 9, 10, 11 - tetrahydro-benzanthracene 8 - malonic acid obtained from the laboratory of Dr. W.E. Bachmann through the Chemical Biological Coordination Center of the National Research Council.³⁰
- p Assumed from structural resemblance to other fused-ring compounds of refractive index around 1.7.
- q From Literature Cited Item 22.
- r Range of values given for very thin films.²⁰
- s Value for β index.²²
- t n_p determined by Abelès.¹⁸
- u Average of n_o , 1.378 and n_e , 1.390 .²²
- v Average of values given for calcite: 1.6583 , β , 1.4864 . Value for CaO = 1.838 .²²
- w Average of 2.36 and 2.38 .

a. Direct Adsorption

The stepped reflector is immersed in a solution of protein, the free remaining protein is washed away in a stream of solvent, the solvent in turn is displaced by water, and the reflector with adsorbed protein is then dried. The entire process may be repeated.

b. Monolayer Transfer

A monolayer of protein is formed upon an aqueous surface and the monolayer is transferred by dipping, in the manner used in preparation of stearate films. According to the conditions of transfer the films produced may be "AB," double layers produced by attachment of the monolayer during both downward and upward passage of the slide through the interfaces, or "B," with attachment only during emergence.

A complete protocol of an experiment on direct adsorption of protein has been published.⁶ Figure 13 shows that, with successive transfers of bovine plasma albumin monolayers, the relative s and p fringe displacements remain constant, as does the corresponding increment in thickness for each added monolayer. Other data on the thickness and refractive index of adsorbed and transferred films are given in Table V. In instances where several successive monolayer or double-layer transfers were made to the same reflector or pair of reflectors, a single value of refractive index was obtained by calculating the linear regression lines of ϵ_s on ϵ_p . Individual values of d were then calculated for each experimental point.

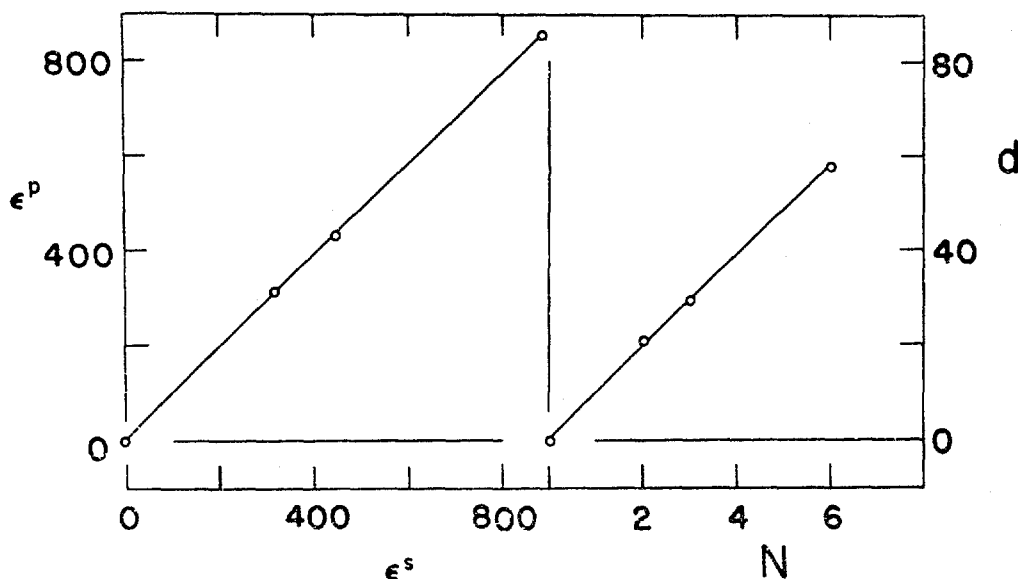


Figure 13. Results of Measuring s and p Fringe Shifts upon Transfer of Monolayers of Bovine Plasma Albumin to Stepped Reflector from Distilled Water Surfaces. Film pressure, 9.5 dynes/cm. Left side: Abscissa, ϵ_s ; ordinate, ϵ_p . Straight line is regression line that gives rough estimate of film refractive index by text Equations (89) or (90). Right side: Apparent film thickness d and number of transferred monolayers N . Slope of regression line gives average thickness per monolayer, $\bar{d}$. (Diagram reproduced from Proceedings of the First National Biophysics Conference⁷ by kind permission of Yale University Press.)

TABLE V. THIN PROTEIN FILMS ON S-P STEPPED REFLECTOR

	X	n	d, Å	$\bar{d}$, Å
<u>Adsorbed Films of Bovine Plasma Albumin</u>				
Solvent	pH	Conc., per cent		
Water	-	0.67	29.2 ± 1.6	-
Water	-	0.67	27.8 ± 2.0	-
Acetate buffer, protein removed with water	4.7	0.0067 - 10.00	20.2 ± 10.6	-
Water	5.5 - 6.0	0.05 - 5.00	30.5 ± 5.8	-
HCl	5.0	0.1	28.4	-
(a) HCl	3.0	0.1	14.5	-
(b) Water, 2nd exposure		0.1	26.6	-
Averages for water and HCl pH 5		1.589		29.0
<u>Adsorbed Films of Botulinum Toxins</u>				
Type A in 0.05 M acetate	3.38	0.357	24.5, 26.1, 24.2	
		avg. 1.588	avg. 24.9	
Type B in 0.2 M succinate	5.5	0.06	31.1, 29.3, 29.9	
		avg. 1.526	avg. 30.1	
<u>Transferred Monolayers of Bovine Plasma Albumin</u>				
Liquid Substrate	F	Type		
Water	9	AB	20.9	10.45
		AB ₂	29.2	9.73
		AB ₅	57.6	9.60
Dilute zinc chloride	9	AB	19.97, 19.07	9.99, 9.54
		(AB) ₂	36.10, 35.93	9.03, 8.98
Dilute zinc chloride	16	AB	17.67, 24.25	8.84, 12.13
		(AB) ₂	36.22, 36.76	9.06, 9.19
		(AB) ₃	54.35,	9.06
Averages for ZnCl ₂		1.592		9.63

n = film refractive index.

d = total thickness.

d = average thickness per monolayer.

X = number of slides.

F = surface pressure at time of transfer, dynes/cm.

A indicates film transferred during downward motion of slide.

B indicates transfer during emergence of slide from film tray.

A notable feature of the measurements on protein films is a marked coupling between film thickness and refractive index, of such a nature that, under similar conditions of deposition, rather wide variations of $\bar{n}$ and $\bar{d}$ are associated with highly reproducible values of the surface density, γ mg/m². For a protein film regarded as a mixture of solid protein and air, γ is given by the Lorentz-Lorenz equation in the form

$$\frac{n^2 - 1}{n^2 + 2} = \frac{\gamma}{d} \cdot \frac{1}{\rho} \cdot \frac{n_o^2 - 1}{n_o^2 + 2} \quad (13)$$

where $\rho \sim 1.30^{28}$ and $n_o \sim 1.60^{26,27}$ for solid protein, giving

$$\frac{\gamma}{d} = 0.38 \frac{n^2 - 1}{n^2 + 2} \quad (14)$$

The $n - d$ coupling has been confirmed by using 25 sets of data in which duplicate slides were coated with protein under various conditions. For each pair of slides, with protein films n_1, d_1, γ_1 and n_2, d_2, γ_2 , the angle θ given by

$$\tan \theta = \delta \left(0.38 \frac{n^2 - 1}{n^2 + 2} \right) / \delta (\gamma_1/d) \quad (15)$$

is a useful measure of the degree of coupling; in absence of coupling the average value will be zero, and with perfect coupling, 45 degrees. The value obtained for the 25 pairs of slides was 38.3 degrees.

3. Other Measurements

Several substances were deposited upon stepped reflectors by condensation in vacuo, and monolayers of one organic substance of high bulk index of refraction were successfully transferred. The results of these miscellaneous measurements are included in Table IV.

4. Summary and Discussion

The evidence for the validity of the stepped reflector method rests upon the accumulation of data that can be considered in connection with auxiliary information that is not strictly comparable. For example (Table IV):

(a) Using calibration constants that are fairly close to values obtained by the s fringe method, measurements of the thickness per monolayer of several other aliphatic soap films are in fair agreement with values anticipated on stereochemical grounds.

(b) The corresponding indices of refraction are reasonable since (i) low values for the odd carbon acid, nonadecanoic, are found by both stepped reflector and s fringe methods; (ii) some uncertainty as to the actual values arises from the presence of Ba and Cu, and from the fact that the ordinary and extraordinary indices are appreciably different.

(c) Protein adsorbed films and monolayers prepared under optimum conditions appear to have refractive indices close to 1.60, the value for dry protein in bulk.

(d) The thicknesses of adsorbed films of two different proteins are consistent with known molecular dimensions, while transferred monolayers have the thickness of extended polypeptide chains.

(e) The refractive indices of several substances deposited in vacuo, and of a fused-ring organic compound transferred in monolayer form, were in satisfactory agreement with values obtained on much thicker films prepared by similar methods and examined by the Abelès method. Agreement to be expected with bulk values is limited by the fact that the substances are not always isotropic, and by a possible decrease of refractive index in very thin layers.

Two discrepancies exist:

(a) Zinc sulfide, of bulk index ~ 2 , appeared to be much less dense ($n \sim 1.5$) in very thin layers, although the Abelès method gave an intermediate value. Zinc sulfide is used routinely as a highly refractive material in preparing interference filters³¹ and we know of no precedent for these low values.

(b) The apparent refractive index of stearic acid is unexpectedly high (1.536) by the stepped reflector method, and still higher (1.549) by the Abelès method. The first result has been explained⁸ by calculating the s and p indices as a function of angle of molecular tilt and showing that the value 1.537 is compatible with the values of n_p and n_s expected when the molecules are tilted at an angle 30.3 degrees to the slide normal. The still higher value obtained by the Abelès method becomes reasonable when one remembers that the slide was viewed parallel to the monolayer dipping direction at an angle of incidence $\tan^{-1} 1.549$ or 56.45 degrees. The incident beam is nearly parallel to the molecular chains and a value approaching the extraordinary index, 1.55, is to be expected.

A final point of relevance to these tests is the coupling between n and d , which suggests, under carefully duplicated conditions, the formation of films having the same mass but different thicknesses. A result of this tendency, which has been noted with the aliphatic acids as well as the proteins, is that the reproducibility is less than that calculated for the known accuracy with which the fringe wavelengths can be measured. Mattuck⁴ gives the following standard deviations upon the latter basis: for films 12 Å thick, $\sigma_n = \pm 0.054$, $\sigma_d = \pm 1.4$ Å; for films 142.5 Å thick, $\sigma_n = \pm 0.010$, $\sigma_d = \pm 3.2$ Å. Larger standard deviations in n and d in replicate experiments, with small deviations in γ , apparently show that with extremely thin films the orientation is far from perfect, although on the average the same number of molecules is always needed to "saturate" a receptor surface. This question needs further study, but for the moment we shall assume that it does not point to any shortcomings of the optical observations.

VI. THEORY OF STEPPED INTERFERENCE REFLECTOR

A. DERIVATION OF FRINGE SHIFT EQUATIONS

Having presented evidence of the usefulness of the s-p stepped reflector method, we now sketch the derivation of the fringe shift equations for those who are interested; the original derivations are spread over three papers^{4,11,12} and a brief self-contained statement may be of value.

1. Stepped Reflector Without a Test Film

We refer to the diagram of the stepped reflector given in Figure 14, with the interfaces represented by lower case letters and the substances forming them by numbers. The letters s and p indicate the portion of the reflector under consideration, and the plane of polarization of the light impinging upon it.

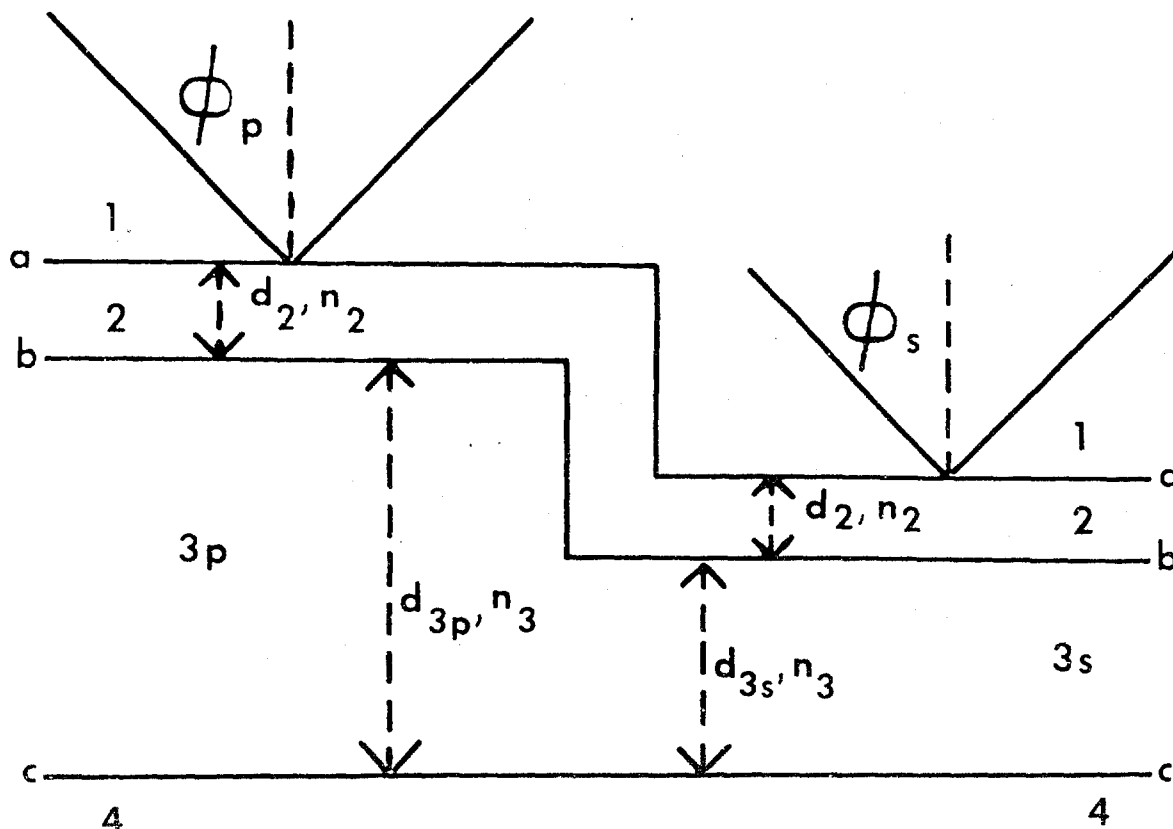


Figure 14. Illustrating Designation of Films and Interfaces in s-p Stepped Reflector. Medium 1 is air; 2, the test film; 3s, barium copper stearate of thickness suitable for production of s extinction fringe in absence of film 2; 3p, barium stearate of thickness suitable for production of p extinction fringe in absence of film 2; 4 is chromium or chromium-plated glass. ϕ_s and ϕ_p are angles of incidence appropriate to the extinction conditions; in the theoretical development given here, $\phi_s = \phi_p = \phi$; in Mattuck⁴ the distinction is maintained. Reflection at a single interface separating two semi-infinite media is designated with a numerical suffix. Reflection at an interface within the actual system shown in the diagram is designated by the number of the medium containing the incident beam, followed by the letter designating the interface - e.g. r_{1a} , the reflection coefficient for the whole reflector; r_{1c} , the reflection coefficient for a clean metal-coated glass slide in air.

Considering first the case of a dielectric film, 3, between a metal plate, 4, and air, 1, we repeat Equation (11):

$$r_{1b} = \frac{r_{13} + r_{3c} \exp(-i\delta_3)}{1 + r_{13} r_{3c} \exp(-i\delta_3)} \quad (16)$$

where

$$r_{13s} = \frac{\cos \phi - (n_3^2 - \sin^2 \phi)^{\frac{1}{2}}}{\cos \phi + (n_3^2 - \sin^2 \phi)^{\frac{1}{2}}} \quad (17)$$

$$r_{13p} = \frac{n_3^2 \cos \phi - (n_3^2 - \sin^2 \phi)^{\frac{1}{2}}}{n_3^2 \cos \phi + (n_3^2 - \sin^2 \phi)^{\frac{1}{2}}} \quad (18)$$

$$\delta_3 = (4\pi/\lambda) d_3 (n_3^2 - \sin^2 \phi)^{\frac{1}{2}} \quad (19)$$

while r_{3c} is given by equations similar to (17) and (18) using

$$\frac{\Lambda}{n_4} = n_4 - ik_4 \quad (20)$$

For present purposes it is sufficient to write

$$r_{3c} = \rho_{3c} \exp(i\theta_{3c}) \quad (21)$$

giving

$$r_{1b} = \frac{r_{13} + \rho_{3c} \exp i(\theta_{3c} - \delta_3)}{1 + r_{13} \rho_{3c} \exp i(\theta_{3c} - \delta_3)} = \rho_{1b} \exp i\theta_{1b} \quad (22)$$

At a wavelength λ_b and a film thickness d_3' giving a phase change δ_3' at a suitable angle of incidence, the reflected amplitude will be zero (extinction fringe) if

$$r_{13} = \rho_{13} \exp i180^\circ = \rho_{3c} \quad (23)$$

and

$$\theta_{3c} - \delta_3' = 2\pi m \quad (24)$$

$$(m = 0, 1, 2 \dots)$$

The condition for Equation (23) has already been illustrated in Figure 3; it is found empirically to be satisfied when ϕ is about 78 degrees for the s and 81 degrees for the p vibration in the case of barium stearate films on chromium-plated glass. The second condition is satisfied when the s layer has about 20 or 60 barium stearate double layers; about 40 or 80 are required for the p layer, because of the different value of θ_{3c} illustrated in Figure 2.

2. Stepped Reflector with Test Film

If a film of substance 2, thickness d_2 , is placed on the step reflector, the reflected amplitudes will now be given by

$$r_{1a} = \frac{r_{12} + r_{2b} \exp(-i\delta_2)}{1 + r_{12} r_{2b} \exp(-i\delta_2)} \quad (25)$$

where

$$r_{2b} = \frac{r_{23} + r_{3c} \exp(-i\delta_3)}{1 + r_{23} r_{3c} \exp(-i\delta_3)} \quad (26)$$

and δ_2, δ_3 are values appropriate to the wavelength of observation, λ , by equations similar to (19). By suitable manipulation the new fringe condition can now be calculated by differentiating Equation (25) with respect to wavelength.

The original fringe condition can be used to define r_{3c} by Equations (16), (23), and (24) and applying this to any wavelength λ by assuming no dispersion of the reflection coefficients:

$$r_{3c} = -r_{13} \exp i\delta_3' \quad (27)$$

$$r_{2b} = \frac{r_{23} - r_{13} \exp i(\delta_3' - \delta_3)}{1 - r_{23} r_{13} \exp i(\delta_3' - \delta_3)} \quad (28)$$

Substitution in Equation (25) and introducing some abbreviations then leads to

$$r_{1a} = \frac{a - b \exp(-i\epsilon) + c [\exp(-i\xi) - ab \exp(-i\eta)]}{1 - ab \exp(-i\epsilon) + c [a \exp(-i\xi) - b \exp(-i\eta)]} \quad (29)$$

where

$$a = r_{12}$$

$$b = r_{13}$$

$$c = r_{23}$$

$$\epsilon = \delta_2 + \delta_3 - \delta_3' = \xi + \eta$$

$$\xi = \delta_2$$

$$\eta = \delta_3 - \delta_3'$$

Multiplying r_{1a} by its complex conjugate we then obtain

$$r_{1a} \cdot r_{1a}^* = \frac{(a - b \cos \epsilon + c \cos \xi - abc \cos \eta)^2 + (b \sin \epsilon - c \sin \xi + abc \sin \eta)^2}{(1 - ab \cos \epsilon + ac \cos \xi - bc \cos \eta)^2 + (ab \sin \epsilon - ac \sin \xi + bc \sin \eta)^2} \quad (30)$$

$$= \frac{A + f(\epsilon)}{B + f(\epsilon)} = \frac{A + f}{B + f} \quad (31)$$

where

$$A = a^2 + b^2 + c^2 + a^2 b^2 c^2 \quad (32)$$

$$B = 1 + a^2 b^2 + a^2 c^2 + b^2 c^2 \quad (33)$$

$$f = f(\epsilon) = -2ab [\cos \epsilon + c^2 \cos (\xi - \eta)] + 2c [a(1 + b^2) \cos \xi - b(1 + a^2) \cos \eta] \quad (34)$$

The variables ξ and ϵ are readily expressed in terms of ϵ by introducing the phase shift δ_2' due to the new film at the original fringe wavelength λ_b and noting that

$$\delta_3/\delta_3' = \delta_2/\delta_2' \quad (35)$$

One finds

$$\xi = \frac{\delta_2'(\epsilon + \delta_3')}{\delta_2' + \delta_3'} \quad (36)$$

$$\eta = \frac{\delta_3'(\epsilon - \delta_2')}{\delta_2' + \delta_3'} \quad (37)$$

The fringe condition is now given by

$$\frac{d(r_{1a} \cdot r_{1a}^*)}{d\epsilon} = \frac{B - A}{(B + f)^2} \cdot \frac{df}{d\epsilon} = 0 \quad (38)$$

remembering that

$$d\epsilon/d\lambda \neq 0.$$

Hence

$$df/d\epsilon = 0. \quad (39)$$

Differentiation of Equation (34), using (36) and (37), and applying the argument used in deriving Equation (11) by placing

$$c = (b - a)/(1 - ab) \quad (40)$$

then gives

$$\sin \epsilon - \frac{b - a}{1 - ab} \left[\frac{1 + b^2}{b} \cdot \frac{\delta_2'}{\delta_3' + \delta_2'} \sin \xi + \frac{1 + a^2}{a} \cdot \frac{\delta_3'}{\delta_3' + \delta_2'} \sin \eta + \frac{b - a}{1 - ab} \cdot \frac{\delta_3' - \delta_2'}{\delta_2' + \delta_2'} \sin (\eta - \xi) \right] = 0 \quad (41)$$

It is now convenient to eliminate ϵ , ξ , and η , using the definitions, Equation (29), together with

$$\lambda_b/\lambda_a = \delta_3/\delta_3' = \delta_2/\delta_2' \quad (42)$$

where λ_a is the new fringe wavelength. It is also convenient to designate the two unknowns x and y :

$$x(n_2, d_2) \equiv \delta_2' \quad (43)$$

$$y(n_2) \equiv r_{12} \equiv a \quad (44)$$

and to define the fringe shift Δ :

$$\Delta = \lambda_a - \lambda_b \quad (45)$$

Equation (41) then becomes

$$(\delta_3' + x) \sin(\omega x - \theta) + f_1 (\delta_3' - x) \sin(-\omega x - \theta) + f_2 x \sin \omega x + f_3 \delta_3' \sin(-\theta) = 0 \quad (46)$$

where

$$\omega = \lambda_b / \lambda_a = 1 - \Delta / \lambda_a \quad (47)$$

$$\theta = \delta_3' \Delta / \lambda_a \quad (48)$$

$$f_1 = [(b - y)/(1 - by)]^2 \quad (49)$$

$$f_2 = - (b - y) (1 + b^2) / b(1 - by) \quad (50)$$

$$f_3 = (b - y) (1 + y^2) / y(1 - by) \quad (51)$$

B. APPROXIMATIONS

1. Simple Approximate Solution to Fringe Shift Equation

An exact analytical solution to Equation (46) has not been obtained, but expansion of the trigonometric functions and neglect of square and higher powers of ωx and θ and their products leads to a quadratic in x that can be solved. The result is

$$x \approx \delta_3' \frac{1 - \omega}{\omega} \cdot \frac{b}{1 - b^2} \cdot \frac{1 - y^2}{y} \quad (52)$$

and the approximation is equivalent to specifying that x must be less than six degrees and the film thickness less than 150 Å. Also, by an equation similar to (14),

$$x = 4\pi d_2 \beta_2 / \lambda_b \quad (53)$$

where

$$\beta_2^2 = n_2^2 - \sin^2 \phi \quad (54)$$

Combining Equations (52) and (53) one finds

$$d_2 = \frac{\Delta}{4\pi\beta_2} \delta_3' \frac{b}{1 - b^2} \cdot \frac{1 - y^2}{y} \quad (55)$$

where, it will be recalled, β_2 and y are both functions of the refractive index of the test substance, n_2 ; Δ is measured experimentally; δ_3' is determined by calibration; and b can be calculated from the refractive index of substance 3 and the angle of incidence.

Equation (55) applies to both s and p vibrations, and it must now be shown that, after inserting the appropriate reflection coefficients, d_2 can be eliminated from the two resulting equations and an explicit solution for n_2 obtained.

Substituting [Equations (17) and (18)]

$$y_s = (\cos \phi - \beta_2)/(\cos \phi + \beta_2) \quad (56)$$

$$y_p = (n_2^2 \cos \phi - \beta_2)/(n_2^2 \cos \phi + \beta_2) \quad (57)$$

one obtains

$$d_2 \sim \frac{1}{\pi} \frac{\cos \phi}{1 - n_2^2} \cdot \frac{b_s}{1 - b_s^2} \delta_{3s}' \Delta_s \quad (58)$$

$$d_2 \sim \frac{1}{\pi} \cdot \frac{n_2^2 \cos \phi}{n_2^4 \cos^2 \phi - n_2^2 + \sin^2 \phi} \cdot \frac{b_p}{1 - b_p^2} \delta_{3p}' \Delta_p \quad (59)$$

Neglecting $n_2^4 \cos^2 \phi$ and placing $\sin^2 \phi \sim 1$,

$$n_2^2 = \frac{b_s}{1 - b_s^2} \cdot \frac{1 - b_p^2}{b_p} \frac{\delta_{3s}'}{\delta_{3p}'} \cdot \frac{\Delta_s}{\Delta_p} \quad (60)$$

which gives, on substitution for b_g and b_p , Equations (17) and (18),

$$n_2^2 = \frac{n_3^2}{1 + (1 + n_3^2) \cos^2 \phi} \cdot \frac{\delta'_{3s} \Delta_s}{\delta'_{3p} \Delta_p} \quad (61)$$

If n_3 is 1.5 and ϕ is 81 degrees (values used in the most recent work), we find, calculating the term in n_3 or placing it nearly equal to n_3 , respectively,

$$n_2 \sim 1.444 (\delta'_{3s} \Delta_s / \delta'_{3p} \Delta_p)^{\frac{1}{2}} \sim 1.50 (\delta'_{3s} \Delta_s / \delta'_{3p} \Delta_p)^{\frac{1}{2}} \quad (62)$$

while, from Equation (58)

$$d_2 \sim 0.088 \delta'_{3s} \Delta_s / (n_2^2 - 1) \quad (63)$$

2. Calibration for Determining δ'_3

The values of δ'_3 cannot be calculated reliably because the thickness of the dielectric film 3 is not determined solely by the number of double layers transferred; calibration, however, is a simple matter. This is done by measuring the fringe shifts produced by the last one or more substrate double layers prior to deposition of the test film. Preparation of the stepped reflector is stopped when fringes can be observed at some suitable wavelengths λ_{os} , λ_{op} . By Equation (19), and abbreviating with

$$\beta_3 = (n_3^2 - \sin^2 \phi)^{\frac{1}{2}} \quad (64)$$

we find

$$\delta'_3 = 4\pi d_{30} \beta_3 / \lambda_o \quad (65)$$

Upon transfer of N additional double layers of thickness $N\bar{d}$ to both steps of the reflector, the fringe wavelength shifts to the higher value λ_b in order to keep δ'_3 constant:

$$\delta'_3 = 4\pi(d_{30} + N\bar{d})\beta_3 / \lambda_b \quad (66)$$

$$= 4\pi d_3 \beta_3 / \lambda_b \quad (67)$$

Eliminating d_{30} and placing

$$\lambda_b - \lambda_o = \Delta_o \quad (68)$$

$$\delta'_3 = 4\pi N \bar{d} \beta_3 / \Delta_o \quad (69)$$

Typically, if n_3 is 1.5 and $\bar{d}$ 50 ÅU,

$$\delta'_3 = 709 N / \Delta_o \quad (70)$$

where the fringe shift is in ÅU and δ'_3 in radians; δ'_{3s} is somewhat less than 180 degrees and δ'_{3p} somewhat less than 360 degrees (again, compare Figure 2).

3. A More Refined Approximation

For a given value of the fringe shift Δ the exact Equation (46) and the simple analytical approximation, Equation (52), both represent curves $x(y)$ corresponding to pairs of values of n_2 and d_2 . For the special case when the test film has the same refractive index, 1.5, as the substrate (i.e. $y = b$), the exact slope of this curve can be written in simple form and compared with the approximate slope obtained from Equation (52). The latter is then modified to conform to the equation for the exact slope, and the modified equation (assumed also to be valid for other values of n_2) is integrated. The resulting improved approximation gives a curve $x(y)$ that (a) coincides with the exact curve when $n_2 = 1.5$, (b) has the same slope as the exact curve when $n_2 = 1.5$, and (c) has the same form as Equation (52) in the limit as x approaches zero.

Differentiating Equation (46) and setting $y = b$, one finds

$$f_1 = f_2 = f_3 = 0 \quad (71)$$

$$\sin(\omega/x - \theta) \sim 0 \quad (72)$$

$$\cos(\omega/x - \theta) \sim 1 \quad (73)$$

$$df_3/dn_2 = - df_2/dn_2 \quad (74)$$

$$df_2/dn_2 = - df_3/dn_2 = \frac{1}{b} \cdot \frac{1 + b^2}{1 - b^2} \cdot \frac{dy}{dn_2} \quad (75)$$

giving finally

$$-\left(\frac{dx}{dn_2}\right)_{1.5} = \frac{1}{b} \cdot \frac{1+b^2}{1-b^2} \cdot \frac{1}{\omega} (\sin \theta) \left(\frac{dy}{dn_2}\right)_{1.5} \quad (76)$$

The result of a similar calculation with Equation (52) is the same as Equation (76) except for the replacement of $\sin(\theta)$ by its argument. Assuming Equation (76) to be approximately correct for any n_2 and integrating, we get

$$x = \frac{b}{1-b^2} \frac{1-y^2}{y} \cdot \frac{\sin \theta}{\omega} + K \quad (77)$$

and finally, evaluating K by placing n_2 equal to 1.5,

$$x \sim \frac{1}{\omega} \frac{1-y^2}{y} \cdot \frac{b}{1-b^2} \cdot \sin \theta + \theta - \sin \theta \quad (78)$$

As before, separate expressions for d_2 are now obtained by substituting for y_s and y_p :

$$d_2 = \frac{\lambda_a}{4\pi\beta_2} (\beta_2 F \sin \theta + \theta - \sin \theta) \quad (79)$$

where for the s step we use λ_{as} , θ_s , and F_s , and for the p step the corresponding p terms;

$$F_s = \frac{b_s}{1-b_s^2} \cdot \frac{4 \cos \phi}{1-n_2^2} \quad (80)$$

$$F_p = \frac{b_p}{1-b_p^2} \cdot \frac{4 n_2^2 \cos \phi}{n_2^2 \cos^2 \phi - n^2 + \sin^2 \phi} \quad (81)$$

Eliminating d_2 and proceeding without further approximations,

$$\frac{F_s}{F_p} = \frac{D_p}{D_s} + B \cdot \frac{E_p - E_s}{D_s} \quad (82)$$

where

$$D = \lambda_a \sin \theta \quad (83)$$

$$E = \lambda_a (\theta - \sin \theta) \quad (84)$$

$$B = 1/\beta_2 F_p \quad (85)$$

Equation (82) is quite suitable for calculation of n_2 from the measured fringe shifts, since an approximate value can be obtained by equating D_p/D_s to F_s/F_p , using this to calculate B (approx.) and then applying the complete equation to obtain a second, more accurate value of n_2 . The film thickness can then be obtained by Equation (79), which may be abbreviated:

$$4\pi d_2 = DF + E/\beta_2 \quad (86)$$

C. METHODS OF COMPUTATION; NUMERICAL EXAMPLES

The fact that the s and p fringe shifts are sufficiently different to permit resolution of film thickness and refractive index is evident from the first crude approximation, Equation (62), and is illustrated graphically in Figure 15. That the magnitudes of the fringe shifts are sufficient for experimental purposes is evident from the data already presented; some typical sets of values for quick reference are collected in Table VI.

TABLE VI. APPROXIMATE FRINGE SHIFTS AS A FUNCTION OF FILM THICKNESS AND REFRACTIVE INDEX

Calculated by Equations (62) and (63) Using
 $\Delta_s = 420$ and $\Delta_p = 220$ for Calibration

$n_2 =$		1.30		1.50		2.0	
d (Å)		Δ_s	Δ_p	Δ_s	Δ_p	Δ_s	Δ_p
23.7		59	40.7	105	55	257	77
95.0		236	163	420 ^{a/}	220 ^{a/}	1026	300

a. Assumed values.

The choice of the approximation to use will depend upon the accuracy desired and the time available for computation. For convenience we restate the several approximate equations for the refractive index and present examples of results obtained by the use of each:

$$F_s/F_p = (D_p/D_s)^2 + B(E_p - E_s)/D_s \quad (87)$$

$$n_2^2 = n_2^2 (F_s/F_p) (\epsilon_s/\epsilon_p) \quad (88)$$

$$n_2^2 = b_s(1 - b_p^2) \epsilon_s/b_p(1 - b_s^2) \epsilon_p \quad (89)$$

$$n_2^2 = 2.25 \epsilon_s/\epsilon_p \quad (90)$$

In Equations (87) through (89),

$$F_s = [b_s/(1 - b_s^2)] [4 \cos \phi / (1 - n_2^2)] \quad (91)$$

$$F_p = [b_p/(1 - b_p^2)] [4 n_2^2 \cos \phi / (n_2^4 \cos^2 \phi - n_2^2 + \sin^2 \phi)] \quad (92)$$

$$D = \lambda_a \sin \theta \quad (93)$$

$$E = \lambda_a (\theta - \sin \theta) \quad (94)$$

$$B = 1/\beta_2 F_p \quad (95)$$

$$\epsilon = \delta_3' \Delta = \delta_3' (\lambda_a - \lambda_b) \quad (96)$$

$$\theta = \delta_3' \Delta / \lambda_a = \epsilon / \lambda_a \quad (97)$$

$$b \equiv r_{13} [\text{Equations (17) and (18)}]$$

The use of successive approximations with Equation (87) has been outlined already. Equations (88) and (89) can be used similarly; (88) is simply, in other notation, the result of combining Equations (58) and (59) without omitting $n_2^4 \cos^2 \phi$ and $\sin^2 \phi$, and can be used after a preliminary value of n_2 has been obtained by (89). Equation (90) is of little value because the numerical factor in Equation (89) can always be used.

Examples of calculated values of n_2 for several values of Δ_s and Δ_p are collected in Table VII. The most reliable values are those in the final column, since Mattuck¹² has shown by direct comparison with the exact Equation (46) that Equation (87) is reliable to three decimal places in n_2 . If permanent tables or graphs (Figure 16) of F_s/F_p are available, some time is saved by using Equations (89) and (88); (90) or (89) alone give only a crude estimate.

TABLE VII. FILM REFRACTIVE INDICES CALCULATED FROM FRINGE SHIFTS BY APPROXIMATE EQUATIONS

Δ_s	Δ_p	Δ_s/Δ_p	λ_{as}	λ_{ap}	Eq. (90)	Eq. (89)	n_2 Eq. (88)	Eq. (87)	
								1	2
59.0	40.7	1.4496	5059	5048	1.2770	1.3405	1.2956	1.2970	1.2967
105	55	1.0991	5105	5055	1.4655	1.5383	1.4759	1.4773	1.4772
420	220	1.9091	5420	5220	1.4655	1.5383	1.4759	1.4785	1.4768
257	77	3.3377	5257	5077	1.9378	2.0340	1.9055	1.9120	1.9159

$n_3 = 1.510$ and $\phi = 81$ degrees throughout

$$b_s/(1 - b_s^2) = -1.791102$$

$$b_p/(1 - b_p^2) = -0.722482$$

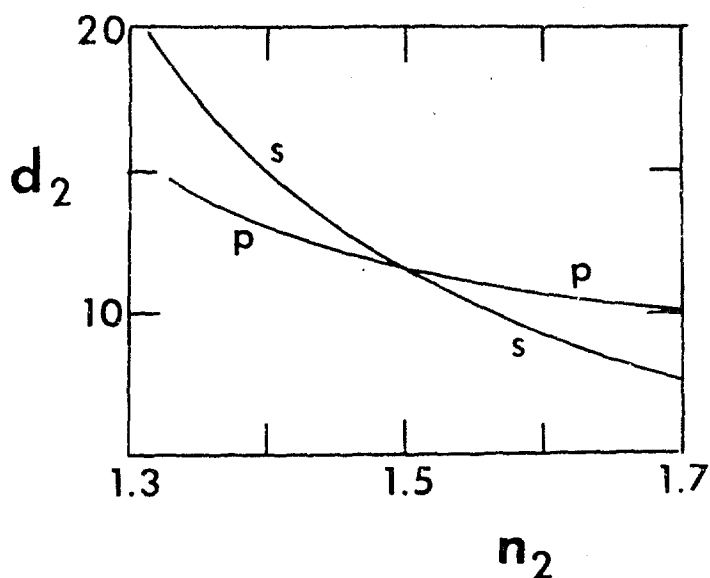


Figure 15. Plot of d_2 Versus n_2 for $\frac{1}{4}$ Added Double Layer of Barium Stearate. Letters s and p indicate curves obtained from s fringe and p fringe equations. Each curve represents the solutions $d_2(n_2)$ that are compatible with a constant fringe shift, 52.5 ÅU for the s step and 26.25 ÅU for the p step. (Adapted from Mattuck, Figure 2.⁴ Permission to reproduce the diagram was kindly granted by the Editors of the Journal of the Optical Society of America.)

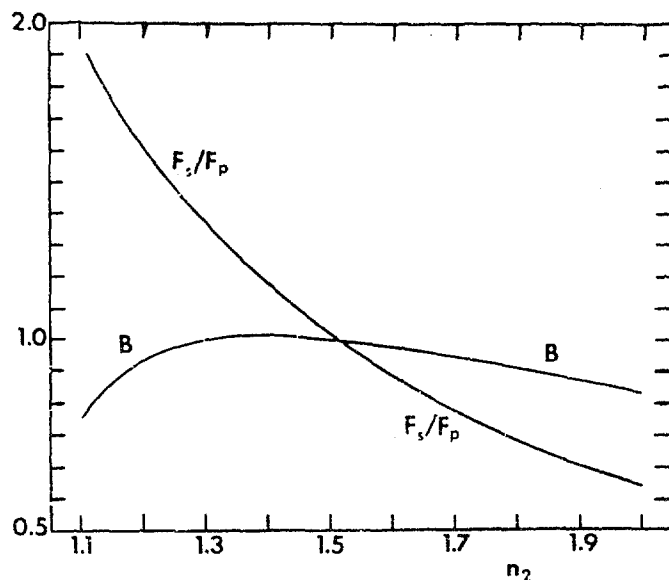


Figure 16. Functions Used in Calculation of Film Thickness and Refractive Index from s - p Reflector Data, Using Equation (87), Defined in Equations (91), (92), and (95). Calculated for films of refractive index n_2 on dielectric of refractive index n_3 equal to 1.51. Angle of incidence, 81 degrees. Ordinate represents either F_s/F_p or B .

VII. SOME POSSIBLE SOURCES OF ERROR

A. DISPERSION EFFECTS

The dispersion of the metal base has been neglected in the development of the s-p stepped reflector. In this respect the choice of chromium appears to have been fortunate; moreover, it can be assumed that if the optical constants of the metal vary in a regular manner over the range of wavelengths used, the calibration procedure will minimize dispersion errors. When less regular variations occur, the fringes may become quite unsymmetrical, as shown in the case of gold in Figure 17; the effect of this on the fringe shifts has not yet been calculated. Ideally, the angle of incidence for extinction fringes should be independent of wavelength; the extent of actual deviations from this is illustrated in Figure 18.

B. ANISOTROPY OF DIELECTRIC SUBSTRATE AND TEST FILM

The theory has been developed for isotropic films, but it is certain that the refractive index of barium stearate, at an angle of incidence 81 degrees, for the p vibration is about 0.18 higher than for the s vibration. Mattuck⁴ has given approximate fringe shift equations appropriate to this difference and has concluded that errors arising from birefringence will usually be one per cent or less. The effects are nevertheless detectable, and some observations on aliphatic acid films already discussed in Section V have been analyzed in terms of molecular orientation.⁸

C. FILM HETEROGENEITY

It has been pointed out⁶ that the theory envisages plane-parallel homogeneous films with sharp boundaries. However, the existence of (a) skeleton films of stearate,¹⁵ (b) incomplete or mosaic-like films of protein,⁶ (c) changes in optical properties with thickness,²⁰ and (d) the n-d coupling already discussed, all suggest that various types of irregularity may be encountered (Figure 19) and that it may be difficult in practice to detect these or to characterize them. Chemical nonuniformity may exist in the stepped reflector itself; Schaefer³² has observed periodic variations in the surface properties of BaH stearate multilayers, and it has been noted⁸ that, with respect to certain polycyclic organic compounds, the s and p surfaces behave quite differently, so that optical measurements on these substances were impossible.

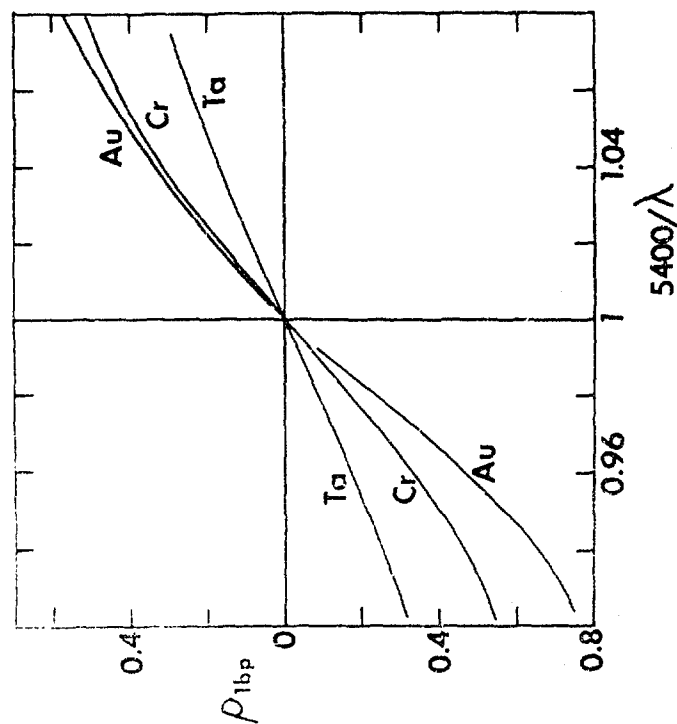


Figure 17. Extinction Fringe Amplitude Profiles for P Vibration Incident upon Metal Slides Coated with Barium Stearate. Calculated from Equation (23); values for complex Fresnel reflection coefficients r_{3c} calculated from optical constants by equations given by Born and Wolf (p. 624 ff).¹⁹ In absence of dispersion effects, fringes are nearly parabolic in vicinity of the extinction position, giving a straight line when plotted in opposite quadrants as in diagram. This is true for Cr and Ta, which give fairly symmetrical fringes, but not for Au, which gives grossly distorted fringes. All extinctions normalized to occur at 5400 Å. Abscissa, $5400/\lambda$. Ordinate, ρ_{1bp} .

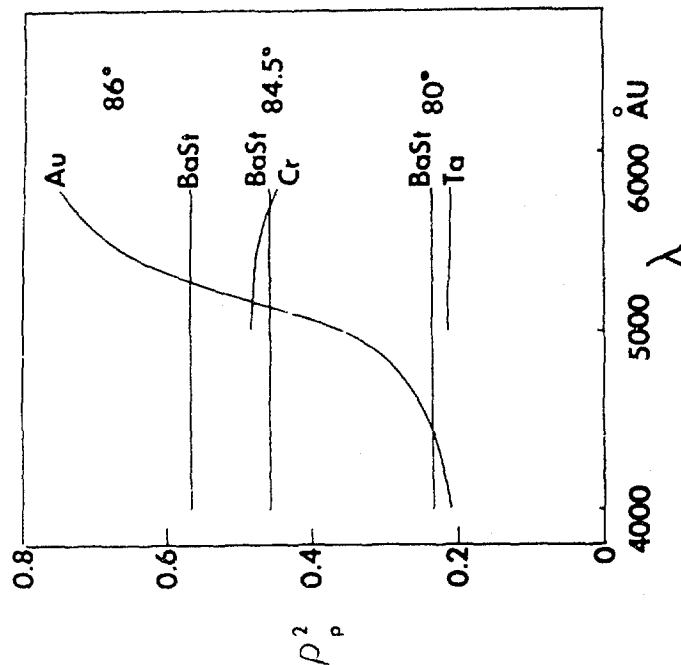


Figure 18. Reflection Coefficients as a Function of Wavelength. The diagrams show that as a result of dispersion the first extinction fringe condition [$r_{13} = \rho_{3c}$, Equation (23)] may be dependent upon wavelength. The assumption that this is not so, used in the theory of the stepped reflector, is very nearly true for tantalum over the range 5000 to 6000 Å, and for chromium at any wavelength between 4000 and 5800 Å. Horizontal lines give $r_{13}^2 (= \rho_{13}^2)$ for the air - barium stearate interface; closed circles give ρ_{3c}^2 , assumed equal to ρ_{34}^2 , for the stearate-metal interface. Optical constants of the metals from International Critical Tables and Landolt-Boernstein "Tabellen."

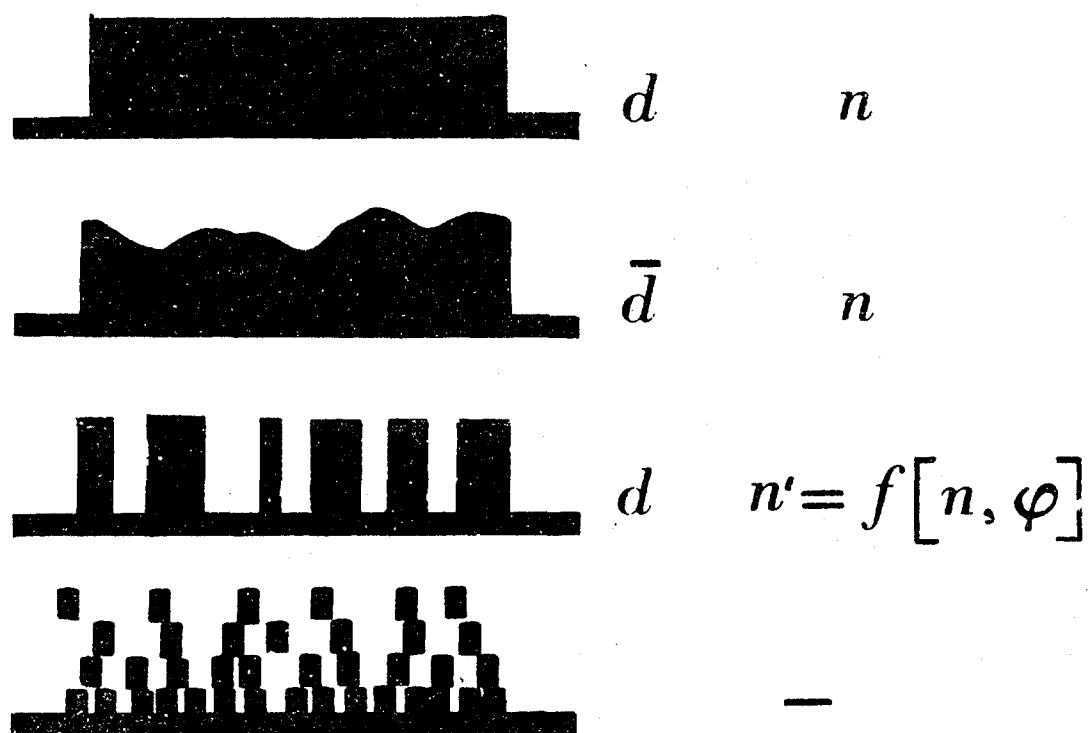


Figure 19. Film Sections Illustrating Departures from Uniformity of Thickness and Refractive Index. Second from top, homogeneous film with irregular surface. Third, film with random pores. Fourth, film density decreasing with distance from substrate. (Reproduced from the Journal of Physical Chemistry⁶ by kind permission of the American Chemical Society.)

VIII. CONCLUSIONS

The outcome of these investigations has been a method that, within certain clearly defined limits, should be very serviceable for the study of dielectric films of thickness greater than a few Angstroms, and of index of refraction between 1.1 and 2.0. Certain technical improvements and certain other developments are of course to be expected. The former should include development of stepped reflectors with isotropic dielectric layers, preferably with zero- and first-order steps on the same slide as an aid to the detection of differences of surface reactivity with changing thickness.

For the biologist two matters will be of special interest. The first is the need, in many types of investigation, to characterize thin test films placed upon the stepped reflector and then to observe their ability to take up additional material by adsorption or chemical attachment. The determination of the optical constants of a second or a third test film requires a modification of the fringe equations that has not yet been attempted, although the basic theory of multiple layers is available.²⁰

The second matter of biological interest is the possibility of characterizing films at the liquid-solid interface. This presents special difficulties because the extinction fringe condition ($r_{13} = r_{3c}$) occurs at rather higher angles of incidence than in air: $\phi_s \sim 88$ degrees, $\phi_p \sim 83$ degrees for the water, barium stearate, chromium system. A solution to the problem exists in principle; if the reflector is composed of a dielectric material (medium 4 in Figure 14) of refractive index equal to that of water, r_{13} is equal to r_{34} at all angles of incidence, so that there should be no difficulty in obtaining extinction fringes with suitable thicknesses of barium stearate. In practice, the only solid substance said to have a sufficiently low refractive index, polytetrafluoroethylene, also possesses undesirable optical and chemical properties.

LITERATURE CITED

1. Rideal, E.K. "Discussion on surface phenomena," Proc. Roy. Soc. (London) A155:684-690, 1936.
2. Rideal, E.K. "Surface action in biology," Nature 140:671-672, 1937.
3. Langmuir, I. "The collected works of Irving Langmuir," Vol. 9, "Surface phenomena," New York, Pergamon Press, 1961.
4. Mattuck, R.D. "A stepped interference reflector for determining the optical constants of non-absorbing unimolecular films. I. Theory," J. Opt. Soc. Amer. 46:621-628, 1956.
5. Mattuck, R.D.; Petti, R.D.; and Bateman, J.B. "A stepped interference reflector for determining the optical constants of non-absorbing unimolecular films. II. Experimental," J. Opt. Soc. Amer. 46:782-789, 1956.
6. Bateman, J.B., and Adams, E.D. "The thickness and refractive index of plasma albumin films on stearate," J. Phys. Chem. 61:1039-1049, 1957.
7. Bateman, J.B. "Continuous and disperse films of bovine plasma albumin on stearate," In: Proc. First Natl. Biophysics Conf. New Haven, Conn., Yale University Press, 1959. pp. 298-313.
8. Bateman, J.B., and Covington, E.J. "Molecular tilt in fatty acid multilayers," J. Colloid Sci. 16:531-548, 1961.
9. Drude, P. "Ueber Oberflaechenschichten. II Theil," Ann. Physik u. Chem., Neue Folge 36:865-897, 1889.
10. Lucy, F.A. "Studies of surface film by reflection of polarized light. I. A more rigorous algebraic method for computing results," J. Chem. Phys. 16:167-174, 1948.
11. Hartman, R.E. "The fringes of equal reflection coefficient ratio and their application to the determination of the thickness and refractive index of monomolecular films. I. Theory," J. Opt. Soc. Amer. 44: 192-196, 1954.
12. Mattuck, R.D. "Recent attempts to determine the thickness and refractive index of unimolecular films," J. Opt. Soc. Amer. 46: 615-620, 1956.

13. McCrackin, F.L.; Passaglia, E.; Stromberg, R.R.; and Steinberg, H.L. "Measurement of the thickness and refractive index of very thin films and the optical properties of surfaces by ellipsometry," J. Res. Natl. Bur. Stds. 67A:363-377, 1963.
14. Blodgett, K.B. "Properties of built-up films of barium stearate," J. Phys. Chem. 41:975-984, 1937.
15. Blodgett, K.B., and Langmuir, I. "Built-up films of barium stearate and their optical properties," Phys. Rev. 51:964-982, 1937.
16. Hartman, R.E.; Hartman, R.S.; Larson, K.; and Bateman, J.B. "The fringes of equal reflection coefficient ratio and their application to the determination of the thickness and refractive index of monomolecular films. II. The determination of the thickness and refractive index of barium stearate double layers," J. Opt. Soc. Amer. 44:197-198, 1954.
17. Abelès, F. "Sur la détermination des indices et des épaisseurs des couches minces," Compt. Rend. Acad. Sci., Paris 228:553-555, 1949.
18. Abelès, F. "La détermination de l'indice et de l'épaisseur des couches minces transparentes," J. Phys. Radium 11:310-314, 1950.
19. Born, M., and Wolf, E. "Principles of optics," New York, Pergamon Press, 1959.
20. Heavens, O.S. "Optical properties of thin solid films," New York, Academic Press, 1955.
21. Abelès, F. "Methods for determining optical parameters of thin films," Progress in Optics, 2:249-288, 1963.
22. "Handbook of chemistry and physics," 43rd ed., Cleveland, Ohio, Chemical Rubber Publishing Co., 1961-62.
23. Anderegg, J.W.; Beeman, W.W.; Shulman, S.; and Kaesberg, P. "An investigation of the size, shape, and hydration of serum albumin by small-angle X-ray scattering," J. Amer. Chem. Soc. 77:2927-2937, 1955.
24. Deuel, H.J. "The lipids," Vol. I, "Chemistry," New York, Interscience Publishers, Inc., 1951. p. 103 ff.
25. Schöön, T. "Polymorphe Formen kristalliner Kohlenstoffverbindungen mit langen gestreckten Ketten (Nach Strukturuntersuchungen durch Elektronenbeugung)," Z. Physiol. Chem. 39B:385-410, 1938. Also: Strukturber. 6:238-240, 1938.

26. Putzeys, P., and Brosteaux, J. "L'indice de réfraction des protéides," Bull. Soc. Chim. Biol. 18:1681-1703, 1936.
27. Doty, P., and Geiduschek, E.P. "Optical properties of proteins," In: Neurath, H., and Bailey, K., ed. "The proteins," Vol. I, Part A. New York, Academic Press, 1953.
28. Low, B.W. "The structure and configuration of amino acids, peptides and proteins," In: Neurath, H., and Bailey, K., ed. "The proteins," Vol. I, Part A., New York, Academic Press, 1953.
29. Wagman, J., and Bateman, J.B. "The behavior of the botulinus toxins in the ultracentrifuge," Arch. Biochem. Biophys. 31:424-430, 1951.
30. Bachmann, W.E. "The synthesis of compounds related to 1,2-benzanthracene and cholanthrene," J. Org. Chem. 3:434-447, 1938.
31. Turner, A.F. "Some current developments in multilayer optical films," J. Phys. Radium 11:444-460, 1950.
32. Schaefer, V.J. "Studies of surface properties by the light scattering of deposited liquid films," J. Phys. Chem. 45:681-701, 1941.

SELECTED BIBLIOGRAPHY

- Abelès, F. "Sur la détermination des indices et des épaisseurs des couches minces," Compt. Rend. Acad. Sci., Paris 228:553-555, 1949.
- Abelès, F. "La détermination de l'indice et de l'épaisseur des couches minces transparentes," J. Phys. Radium 11:310-314, 1950.
- Abelès, F. "Methods for determining optical parameters of thin films," Progress in Optics, 2:249-288, 1963.
- Anderegg, J.W.; Beeman, W.W.; Shulman, S.; and Kaesberg, P. "An investigation of the size, shape, and hydration of serum albumin by small-angle X-ray scattering," J. Amer. Chem. Soc. 77:2927-2937, 1955.
- Bachmann, W.E. "The synthesis of compounds related to 1,2-benzanthracene and cholanthrene," J. Org. Chem. 3:434-447, 1938.
- Bateman, J.B., and Adams, E.D. "The thickness and refractive index of plasma albumin films on stearate," J. Phys. Chem. 61:1039-1049, 1957.
- Bateman, J.B. "Continuous and disperse films of bovine plasma albumin on stearate," In: Proc. First Natl. Biophysics Conf. New Haven, Conn., Yale University Press, 1959. pp. 298-313.
- Bateman, J.B., and Covington, E.J. "Molecular tilt in fatty acid multilayers," J. Colloid Sci. 16:531-548, 1961.
- Blodgett, K.B. "Properties of built-up films of barium stearate," J. Phys. Chem. 41:975-984, 1937.
- Blodgett, K.B., and Langmuir, I. "Built-up films of barium stearate and their optical properties," Phys. Rev. 51:964-982, 1937.
- Born, M., and Wolf, E. "Principles of optics," New York, Pergamon Press, 1959.
- Deuel, H.J. "The lipids," Vol. I, "Chemistry," New York, Interscience Publishers, Inc., 1951. p. 103 ff.
- Doty, P., and Geiduschek, E.P. "Optical properties of proteins," In: Neurath, H., and Bailey, K., ed. "The proteins," Vol. I, Part A. New York, Academic Press, 1953.
- Drude, P. "Ueber Oberflaechenschichten. II Theil," Ann. Physik u. Chem., Neue Folge 36:865-897, 1889.

"Handbook of chemistry and physics," 43rd ed., Cleveland, Ohio, Chemical Rubber Publishing Co., 1961-62.

Hartman, R.E. "The fringes of equal reflection coefficient ratio and their application to the determination of the thickness and refractive index of monomolecular films. I. Theory," J. Opt. Soc. Amer. 44: 192-196, 1954.

Hartman, R.E.; Hartman, R.S.; Larson, K.; and Bateman, J.B. "The fringes of equal reflection coefficient ratio and their application to the determination of the thickness and refractive index of monomolecular films. II. The determination of the thickness and refractive index of barium stearate double layers," J. Opt. Soc. Amer. 44:197-198, 1954.

Heavens, O.S. "Optical properties of thin solid films," New York, Academic Press, 1955.

Langmuir, I. "The collected works of Irving Langmuir," Vol. 9, "Surface phenomena," New York, Pergamon Press, 1961.

Low, B.W. "The structure and configuration of amino acids, peptides and proteins," In: Neurath, H., and Bailey, K., ed. "The proteins," Vol. I, Part A., New York, Academic Press, 1953.

Lucy, F.A. "Studies of surface film by reflection of polarized light. I. A more rigorous algebraic method for computing results," J. Chem. Phys. 16:167-174, 1948.

Mattuck, R.D. "Recent attempts to determine the thickness and refractive index of unimolecular films," J. Opt. Soc. Amer. 46:615-620, 1956.

Mattuck, R.D. "A stepped interference reflector for determining the optical constants of non-absorbing unimolecular films. I. Theory," J. Opt. Soc. Amer. 46:621-628, 1956.

Mattuck, R.D.; Petti, R.D.; and Bateman, J.B. "A stepped interference reflector for determining the optical constants of non-absorbing unimolecular films. II. Experimental," J. Opt. Soc. Amer. 46:782-789, 1956.

McCrackin, F.L.; Passaglia, E.; Stromberg, R.R.; and Steinberg, H.L. "Measurement of the thickness and refractive index of very thin films and the optical properties of surfaces by ellipsometry," J. Res. Natl. Bur. Stds. 67A:363-377, 1963.

Putzeys, P., and Brosteaux, J. "L'indice de réfraction des protéides," Bull. Soc. Chim. Biol. 18:1681-1703, 1936.

- Rideal, E.K. "Discussion on surface phenomena," Proc. Roy. Soc. (London) A155:684-690, 1936.
- Rideal, E.K. "Surface action in biology," Nature 140:671-672, 1937.
- Schaefer, V.J. "Studies of surface properties by the light scattering of deposited liquid films," J. Phys. Chem. 45:681-701, 1941.
- Schoon, T. "Polymorphe Formen kristalliner Kohlenstoffverbindungen mit langen gestreckten Ketten (Nach Strukturuntersuchungen durch Elektronenbeugung)," Z. Physiol. Chem. 39B:385-410, 1938. Also: Strukturber. 6:238-240, 1938.
- Turner, A.F. "Some current developments in multilayer optical films," J. Phys. Radium 11:444-460, 1950.
- Wagman, J., and Bateman, J.B. "The behavior of the botulinus toxins in the ultracentrifuge," Arch. Biochem. Biophys. 31:424-430, 1951.