Dynamic ellipsometry, biochemical and biomedical applications

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DYNAMIC ELLIPSOMETRY
BIOCHEMICAL AND BIOMEDICAL APPLICATIONS

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Aan mijn ouders

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CHAPTER I

Introduction

The need for a better understanding of enzymatic reactions at interphases has been raised by recent developments in cell biochemistry and cell biology. However, a number of difficulties is inherent to the study of biological surfaces. Perhaps the greatest difficulty is the complex organization of biological systems and the functional continuity between various components. The living cell represents a complex of membranes and organelles, whose structural and functional properties are maintained by reactions occurring at multiple inter-related solid-gel, solid-liquid, and liquid-gel interphases. The whole cell in turn interacts with the environment by means of a highly complex outer surface. Consequently, there are both conceptual and practical difficulties in defining the precise nature and spatial limits of many interphases. If we add to this that biological systems are governed by dynamic, rather than static conditions, one can admit that identifying the properties of even the simplest biological interphase poses considerable problems.

Almost all reactions of biochemical interest at interphases studied thus far, took place at liquid-air interphases (1-23). The results have not been too encouraging, altogether, partly due to the physico-chemical behaviour of proteins. When spread at a water-air interphase, they loose part or all their biological activities (15,17,20,21). If instead a (lipid) substrate is spread at the surface and the protein (enzyme) is in the bulkphase, film penetration and unequal distribution of enzyme between the liquid phase and the film can interfere. In addition a liquid-air interphase cannot be a proper model for the natural conditions inside or on the surface of a living cell. A liquid-solid interphase would be a much better model. For instance, if the solid is a lipid or a protein, acting as a substrate, and if the enzyme is dissolved in the adjacent liquid phase, conditions might be very similar to those found in the intestinal tract. When the enzyme is on the surface of a solid phase and the substrate is dissolved in the adjacent liquid phase, the conditions resemble closely those inside and around the organelles of a living cell.

Only rather recently different physico-chemical methods have been able to study reactions at interphases which have been applied to biological systems. As we want to discuss an extension of the arsenal in this thesis, we will first briefly mention the other methods, currently available.

SPECTROSCOPIC TECHNIQUES have been applied to the study of lipid-protein interactions in membranes. One of them being NMR spectroscopy (24). The fundamentals of NMR spectroscopy are that certain nuclei (e.g. ^1H and ^{15}N) behave like minute magnetic bars and can be lined up in the presence of a magnetic field. The adsorption of radiation energy can cause the magnets to flip from one energy level to another. There exists a characteristic resonance energy for each nucleus, depending on its surroundings, e.g. whether a proton is in a methylene group, an amino group or attached to a double bond carbon, or whether the direct environment of the atom is hydrophobic or hydrophilic by nature. Interactions between lipids and proteins can be investigated by comparing spectra of mixtures, with those of lipid and protein separately. Chapman (1968) (24) and associates (25,26) have reported NMR spectra from phospholipid, bile salts, serum lipoproteins and red blood cell ghosts. Serum lipoproteins have been studied with this method by Steim et al. (27).

These studies indicate that interactions between proteins and lipids result in restriction of the mobility of the hydrocarbon chains. Interactions between lipids and proteins are believed to be of a hydrophobic character.

Other spectroscopic techniques include optical rotation dispersion (ORD) and circular dichroism (CD). The optical rotation in a protein solution is caused by the added effects of each of the optically active centers of the amino acid residues and of the three dimensional folding of the protein chain. When the rotation is measured as a function of the wavelength, one can e.g. calculate the helix content of the protein.

In the circular dichroism technique one uses two light beams, a left and a right handed circularly polarized one. These beams are unequally adsorbed by optically active materials and the difference in adsorption as a function of the wavelength gives the CD spectrum. This technique makes it also possible to evaluate conformational properties of biopolymers.

Both ORD and CD spectra of a variety of lipid-protein membranes have been reported (28-31). They suggest, on the basis of their measurements, that the membrane proteins investigated, all contain α -helical regions.

More recently, the so-called spin labelling technique has been extended to the study of biological membranes (32,33). The spin labelling

technique is based on the fact that a tag molecule (spin label), when attached to a macromolecule such as a protein, gives off a signal in the ESR spectrometer, that is dependent on its immediate surroundings and in this way the signals allow conclusions on (changes in) these surroundings. Spin labels used thus far include nitroxide type free radicals such as di-*t*-butyl nitroxide and 2.2.6.6-tetra-methyl piperidine-1-oxyl.

Except in the detecting instrumentation, the use of a spin label is very similar to that of introducing a dye into a biological material. The advantages of spin labels are their sensitivity to the local environment and their ability to measure very rapid molecular motion (33). Today, the spin label technique has been used on rabbit nerve - excitable membranes, bovine red blood cell ghosts, and artificial bilayer membrane models.

Gelfiltration has been used by Scanu et al.(34) and by Fleischer et al.(35,36) to investigate interactions of the lipid and protein components of high density lipoproteins and the interactions of γ -lipoproteins with phospholipids. This method is of value in determining binding stoichiometry between lipids and proteins.

Dialysis against a non-aqueous medium will provide information about the nature of lipid-protein interaction. This technique involves the partition of a lipid in a non-aqueous solvent and an aqueous protein solution separated by a dialyzing membrane. Materials isolated from chloroplast lamellae were studied in this way (37). Interactions between fatty acids and serum albumin were thus investigated by Spector (38) and Arvidson and Belfrage (39).

Differential scanning calorimetry (DSC) is a classical method, in contrast with the application to biological systems, which is a recent development (25,27).

The principle of DSC is that the temperature of the sample and of a thermally inert reference material in the same thermal environment are compared while the temperature of the environment is programmed. The adsorption or generation of thermal energy by the sample causes its temperature to lag momentarily or to lead the reference temperature. This difference is recorded as a function of the program or of the sample temperature, and produces a curve consisting of negative or positive peaks. These peaks indicate the existence of endothermic or exothermic thermal changes at the corresponding temperature. Phase transitions like this observed can be correlated with changes in biological properties.

For instance, Stein et al.(27) observed that the transition temperature of intact membranes of *M.Loidlowaii* was equal to that of the extracted lipids in aqueous dispersions. Using the same technique, Ladbroke et al.(25) studied the effect of cholesterol on phase transitions in phospholipids.

Heat burst calorimetry has been used for the first time in biochemistry by Kitzinger and Benzinger (40). The technique was applied by Lovrien and Anderson (41) to study the interaction between sodium dodecyl sulfate and γ -lactoglobulin and by Klopfenstein (42) to determine the enthalpy change on binding of lysolecithin to bovine serum albumin. This technique is of potential value in providing quantitative thermodynamic data, useful in the understanding of the nature of binding forces between the lipid and protein.

Another approach of the problem of lipid-protein interaction is the use of MODEL SYSTEMS. Today, only a few studies have been examining the effect of proteins on the intrinsic properties of bilayer lipid membranes (BLM). The first BLM protein interaction experiments were reported by Mueller et al.(43-44). Protein material was found to lower the resistance of a brain lipid BLM from 10^7 - 10^8 to 10^3 - 10^4 ohms/cm². Albumin modified BLMs have been studied by two different groups of investigators, yielding conflicting results. Hanai et al. (45) have investigated lecithin BLM in the presence of egg-albumin, insulin, or bovine serum albumin and did not find any effect on the BLM capacitance, unlike Tsofini et al.(46) who have found that the electrical properties of BLM are significantly altered in the presence of albumin.

Del Castillo et al.(47) developed an interesting technique to detect antigen-antibody reactions on BLMs. The interaction of BLM with bovine erythrocyte proteins was investigated by Maddy et al.(48) and by Van den Bergh (49). By adding bovine protein extract to the bathing solution, the latter reported a sudden reduction in BLM resistance.

Smekal and Tien (51) studied the interaction of a variety of proteins with oxidized cholesterol BLM using the reflectance technique of Tien and Howard. In the case of alcohol-dehydrogenase, the thickness of the membrane increased from 50 to approximately 100 Å.

The results obtained recently with fluorescent probes, in studying BLM-protein interaction should also be mentioned (51). Recently, Azzi et al.(52) and Rubalcana et al.(53) used 8-anilino-1-naphthalene sulfonate (ANS) in studies of biological membranes.

It is known that the fluorescent properties of ANS and its derivatives are dependent on the polarity and threedimensional structure of the environment (55). In aqueous solutions ANS is practically non-fluorescent, but it fluoresces with an appreciable quantumyield when dissolved in organic solvents and when associated with proteins (55). These properties make ANS an ideal probe for investigating BLM protein interactions.

These techniques all allow a considerable insight into lipid-protein interactions to be gained, and have substantially contributed to our knowledge of the biochemical reactions, occurring at lipid-water interphases. Yet they are largely indirect. This, together with the fact that many of the main unsolved problems in biochemistry, involve processes occurring at interphases, like oxydative phosphorylation, cell recognition, antibody synthesis, membrane transport, blood coagulation, enzymatic breakdown of insoluble substrates (collagen etc.) caused us to try a more direct approach.

In the study of liquid solid interphases described in this thesis, we used the ELLIPSOMETER TECHNIQUE. This technique was already known for the first time in 1895 by Paul Drude and has been reported ever since under several names (56,57) among which "ellipsometry" and "polarization spectrometry".

The theoretical basis of the method as formulated by Drude amounts to the idea that the optical properties of a bare surface can be calculated from a change in the state of polarization of a reflecting light-beam. Once these optical properties are known, it is possible to apply a similar procedure to a dielectric film on top of the surface.

Using the Maxwell theory for electromagnetic waves, Drude obtained exact equations for the interrelationship between the wavelength used, the optical constants of the system, the thicknesses of the layers and the angle of incidence. However, because these equations are hardly applicable in experimental practice, they cannot be solved in closed form for the desired parameters (refractive index and thickness of the film) in terms of the measured changes in the state of polarization. Thus trial and error and iteration methods had to be used, and routine application of the exact equations was almost impossible before the computer became available.

This is why Drude and subsequently many other workers only used the first linear terms in the Taylor expansion of the exact Drude equation and these approximating equations are often referred to as

"the Drude equations". This approximation limited application of the Drude theory to films which are very thin with respect to the wavelength of the used light beam. This is anyhow one order of magnitude smaller than can be studied by other means as interferometry or electron microscopy. Since the introduction of modern computers, the use of the exact Drude equations is a matter of routine, and the technique can be applied to films of any thickness.

Normally, each measurement, by hand, on an ellipsometer, takes 5 minutes. This is too long to follow adequately most biological adsorption processes. Only in 1952, when Trurnit (58,59) introduced an automatized so-called recording ellipsometer it became possible to follow changes in polarization relatively rapidly. As this instrument recorded the amount of light transmitted by the apparatus, the gain had to be paid for by the exchange of two interpretable parameters (An and Pol) for one, interpretable only under restrictive assumptions.

Some important contributions in the field of ellipsometry in biological systems have been made by Trurnit and Vroman. In 1952, Trurnit (58,59) studied the proteolytic activity of chymotrypsin at a liquid-solid interphase, the substrate bovine serum albumin constituting the solid phase. Depending upon buffer concentration and pH of the enzyme both adsorbed to the substrate and/or removed substrate from the solid phase. Subsequently, he studied the adsorption kinetics of chymotrypsin on solid surfaces from a theoretical point of view. On this basis he was able to describe the initial phase of the reaction qualitatively as well as quantitatively. Trurnits experiments also showed unequivocally that the recording ellipsometer could be used to measure diffusion coefficients rapidly in extremely diluted solutions. Moreover, interphasial enzyme reaction studies of this type permit one to investigate the initial reaction phase more easily and in a finer detail than by studying bulk reactions.

The work of Vroman deals with the field of blood coagulation. He and his colleagues have been using a recording ellipsometer to monitor the amount and rate of protein adsorption from citrated plasma at a solid-liquid interphase, using optically sensitive hydrophilic and hydrophobic surfaces of anodized tantalum sputtered glass slides and polished slices of silicon crystals (60,61). Vromans work has shown that exposure, of the slides to normal intact and activated plasma and various factor-deficient plasmas for only a few seconds, resulted in the adsorption of detectable material.

Ellipsometric studies have also shown that fibrinogen is an important major component of the plasma films adsorbed onto surfaces. This was demonstrated by the ability of plasma films to bind antiserum against fibrinogen (62).

The scarce literature available shows that ellipsometry is a very useful technique to study biochemical reactions at interphases (63) but that it suffers from one major drawback. Either one obtains correct values for analyzer and polarizer positions from which thickness (d) and refractive index (n) can be computed, but then the data acquisition is too slow for biochemical experiments, or one gains speed by measuring light transmission at the expense of the interpretability of the results. This brought us to develop a dynamic ellipsometer, suitable for the exact evaluation of biochemical experiments.

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CHAPTER II

The automatized ellipsometer

1. Principles of the method

An ellipsometer is a device that measures the change in the polarization state of a light beam upon reflection. It consists of an array of a light source, a polarizer, a quarter wave plate, the reflecting surface (sample), an analyzer and a light detecting device. The place of the quarter wave plate is arbitrarily between the polarizer and the surface or between the analyzer and the surface. The light has to be monochromatic. Collimating lenses, diaphragms and filters are added where necessary to modify the light and guide the light beam (fig. II.1).

The plane of polarization of the incident light beam can be varied by changing the polarizer position. In general the light will have its ellipticity and azimuth changed by reflection from the sample (fig. II.1, for precise definition see Chapter III). The analyzer will not completely extinguish elliptically polarized light but will do so with plane polarized light. By varying the plane of polarization with the polarizer and subsequently passing the beam through the fixed quarter wave plate, the ellipticity of the light incident to the reflecting surface can be adjusted in such a way that after reflection it will be plane polarized.

Whether the light is extinguished by the analyzer it is consequently a function of the position of both the analyzer and the polarizer. These two positions together with the known fixed position of the quarter wave plate will completely define the change of ellipticity on the reflecting surface. On a bare surface, this change is a function of the complex refraction index of the reflecting surface, the refraction index of the surrounding medium and the (fixed) angle of incidence. When a dielectric (i.e. a translucent) layer is deposited on the surface the thickness and refractive index of the layer will contribute to the change in ellipticity. With wavelength, angle of incidence and refractive index of the reflecting surface given, the change in ellipticity will be a function of thickness and refractive index of the dielectric layer only.

Fig. 11.1. Principle of the ellipsometer technique.

Reactions of biochemical interest at an interphase like adsorption of proteins or lipids, protein-protein-, and protein-lipid interactions, will cause deposition of and changes in the dielectric layers which can be monitored by ellipsometry. The fact that ellipsometry is only dependent on the polarization changes of light and not on adsorption causes the method to be sensitive to changes in thickness which are several orders of magnitude below the wavelength used. It also causes the method not to be restricted by constraints upon the wavelength used (Chapter III). To follow the changes in the properties of the dielectric layer with a time resolution acceptable for biochemical purposes we had to automatize the adjustment to a minimum light transmission (fig. II.2). The rest of this chapter contains a detailed description of the instrument we used.

2. Description of the instrument

2.1 Optical bank

The basic setup, consisting of a light source, polarizer, analyzer, quarter wave plate, sample mount and light detecting device mounted on optical banks is commercially available (thin film ellipsometer, type 43303-200E Ser.No. 3801, O.C.Rudolph and Sons, Fairfield, N.J. U.S.A.). It can also be realized with standard optical components. Our automated ellipsometer was basically an adaptation of the Rudolph instrument. The light source and quarter wave plate were replaced as well as the light detecting device and the sample mount (see below). The instrument was placed at a 100x80x4 cm marble slab on an ordinary laboratory table. For high precision calibration experiments a set of optical banks * were mounted on a stone slab which had a deviation in flatness of less than 5 micron per meter (150x100x20 cm) and was provided with Polaroid high precision optical components. **

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** Polaroid Corporation Cambridge, Mass. U.S.A.

Fig. II.2. Schematic representation of the automated ellipsometer.

Fig. II.3. The automated ellipsometer.

Fig. II.4. A circular polarizer is a "sandwich" consisting of a piece of linear polarizer bonded to a quarter-wave retardation sheet oriented at an angle of 45° to the transmission direction of the polarizer. This schematic diagram shows what happens as light passes through.

2.2 Light source

The use of a laser as a light source improved the signal to noise ratio by a factor 20 as compared to the conventional Hg lamps with filter setup. A helium neon laser (Spectra Physics) was used in our experiments. This source produced plane polarized monochromatic light of 632.8 nm. The laser was fixed to the support in a 6 point suspension system that allowed perfect alignment with the optical axis (fig. II.3).

2.3 Circular polarizer

As it is necessary to bring about controlled variations of the plane of polarization, the laser beam is first circularly polarized. This is done by inserting a sheet of material into the polarized beam, that selectively retards light, polarized along the slow axis of the material whereas light polarized along the fast axis at 90° to the slow axis is less retarded (circular polarizer HR CP 7 Red of the Polaroid Corporation). The thickness of this retardation

sheet is such that the retarded beam is $1/4$ of a cycle out of phase with the non retarded sheet. The sheet is called $1/4$ wave retardation plate and is bonded to a polarizing sheet (fig. II.4). The slow axis of the retarding sheet is placed at 45° to the transmission axis of the polarizer sheet. If the transmission axis of the polarizer is in the plane of the laser light, maximum intensity of circularly polarized light is obtained. If the polarizing sandwich is rotated by 90° , zero intensity is obtained. So by the rotatable circularly polarizing sandwich one can obtain circularly polarized light of any desired intensity.

2.4 The polarizer

The polarizer, a Nicol prism mounted in a graduated circle serves to polarize the light, passing the circular polarizer. By this rotatable polarizer, plane polarized light of any desired plane can be obtained. The rotation is done by hand in the conventional setup and by stepping motors (see page 27) in the automated version.

2.5 The compensator

The compensator also in a graduated circle consists of a biaxial crystal which divides the incident beam into two perpendicular components orientated in the principal directions of the device, and "retards" one component with respect to the other. The introduced phase difference is also called the compensation. In our case the compensator retards 90° , so we have a quarter wave compensator. If the compensator is not quarter wave, the calculation of thickness and refractive index is still possible but becomes more complicated (Chapter III).

In our ellipsometer the compensator is used to convert the linearly polarized light into elliptically polarized light. The light incident upon the sample has an azimuthal angle and an ellipticity predictable from the settings of the polarizer and compensator (see Chapter III). The compensator going with the Rudolph instrument is quarter wave for the $5461 \text{ \AA}$ Hg line. A compensator that was quarter wave for the He-Ne laser was purchased from Lasermetrics, U.S.A.

2.6 The apertures

The aperture is an adjustable opening allowing a variation in the area of surface examined, and the amount of light reaching the photodiode.

2.7 Sample holder

The sample holder is shown in detail in fig. II.5.

Fig. II.5 The sample holder

If the optical rails are situated in the XY plane, the role of the different micrometers can be described as follows:

- number 1,2 allow movement along the Z axis (height adjustment)
- 3 is necessary to adjust the sample parallel to the Z axis
- 4,5 allow movement of the sample in the XY plane
- 6 allows movement of the cuvet around the Z axis
- 7 revolving optical rail
- 8 allows rotation of the complete sample holder around the Z axis
- 9 allows movement of the cuvet in the XY plane
- 10 fixed optical rail.

2.8 Thermoregulation of the cuvet

For biochemical experiments it is necessary to be able to place the sample in a fluid medium of controlled temperature. The medium must be continuously stirred. Because in the study of lipids it is important to convey the range of possible transition temperatures which extends to below room temperature, cooling as well as heating is necessary. So for thermostation we chose a Peltier element. With this device it is possible to cool and heat with the same element if one side of the element is connected to a mass of high heat capacity.

A Peltier element creates a heat current which is directly proportional to the electric current passing through the element. To make efficient use of the Peltier element it is necessary to steer it by a "constant current" circuit. When the temperature of the cuvet was more than 5°C from the desired temperature, it is corrected by a switching regulation i.e. maximal heating or cooling capacity is used. Within the range of approximately 5°C from the desired temperature proportional regulation was applied in order to prevent oscillations i.e. the cooling or heating current is in proportion to the temperature gap to be bridged. With our circuit the temperature covered ranges from 0 - 50°C . The capacity is sufficient to heat the cuvet from 0 - 50°C within 2 minutes. The stability of the temperature in the cuvet is very high. The temperature varies less than 0.05°C . Small variations of temperature brought about by additions etc. are corrected in less than 20 seconds. By means of LEDs on the instrument panel it is indicated whether the right temperature is reached or not. The desired temperature is chosen by the setting of a reference voltage. The reference voltage can be given via the DU01 unit of the computer or via a potentiometer on the thermoregulation itself. The block scheme (fig. II.6) shows the elements of the thermoregulation.

2.8.1 Difference amplifier (fig. II.6)

This part detects the difference between the reference voltage and the temperature information from the thermotransducer. Via an absolute value circuit and a switching circuit which detects whether the difference is positive or negative, and a multiplier, a voltage is obtained which in sign and magnitude is proportional to the temperature gap to be bridged.

Fig. 11.6 Block scheme of the temperature regulation

2.8.2 Controlled current stabilizer (Fig. II.6)

This circuit consists of a power amplifier with symmetric Darlington output transistors. Via a resistor of 0.5 ohm a current is obtained which is in proportion to the input voltage. This circuit can give a current of approximately 5 ampères.

2.8.3 Thermometer circuit (Fig. II.6)

A bridge circuit with a negative temperature coefficient resistor.

2.8.4 Indication circuit (Fig. II.6)

Indicates whether the temperature differs more than 0.25°C with the selected one.

2.9 Apertures necessary for the alignment of the sample and cuvet.

2.10 The analyzer, the second prism in a rotative graduated circle, like the polarizer (2.4) also steered by a stepping motor.

2.11 Photodiode will be described in details together with the electronic circuits of the photodiode channel.

2.12 Alignment of the ellipsometer

The optical axis of the instrument shows an angle at point I where the reflecting surface is met (fig. II.1). The plane through the broken axis, the point I and the incident light beam define a rectangular coordinative system. The position of the polarizer, analyzer and compensator relative to this coordinative system must be exactly known. This is obtained by alignment of the ellipsometer. To obtain accurate values of the optical constants of surfaces, the alignment of the ellipsometer is very critical. The procedure for aligning the ellipsometer is typical of that generally used in optical instruments except for the adjustment of the analyzer (A) and polarizer (P), the scales of which when well aligned must read zero when the planes of transmission of the prisms are parallel to the plane of incidence. In principle, the adjustment is relatively simple. With the compensator removed and the polarizer and analyzer arms in the straight-through position, the polarizer and analyzer prisms are adjusted in their respective scales until extinction is achieved when the scale readings differ by 90° . The arms are then set for reflection from a metal surface. A minimum in the photometer reading is sought through which the scale readings differ by 90° . The planes of transmission of the two prisms should then be in the plane of incidence and normal to it.

In practice, small imperfections cause a non-ideal behaviour. After having first crossed the prisms when reflecting from the surface, a minimum may be achieved either by fixing P and adjusting A or vice versa. When the light issuing from the polarizer is accurately plane polarized then the minimum achieved, by either of these two means and with the P and A scales crossed, occurs at a single value of P and A independent of the procedure followed. However, if the light issuing from the polarizer has some slight ellipticity due to imperfections in the polarizer, there are then two positions at which the prisms are crossed and minimum photometer readings are obtained depending upon whether the minimum is achieved by fixing P and adjusting A or vice versa. Moreover, the deepest minimum or most complete extinction of the reflected light now occurs at a third point where line scales are not crossed.

With certain assumptions even in this case alignment may be achieved quite accurately (McCrackin (8)).

A step by step procedure for alignment of the ellipsometer is as follows: First remove the quarterwave plate. Set the arms to the "straight through" position. Set the P scale to read zero or any convenient value. Adjust A until minimum transmission is achieved. The A scale will now in general read $(P \pm 90^\circ) + \epsilon$, ϵ being the error in the setting. Rotate the A prism in its holder until ϵ is zero. In this way we eliminate a difference in setting between P and A. The P and A scales are now crossed when the prisms are crossed. The scales should track within approximately 0.02 degrees throughout one revolution. The remaining error in the P and A settings is eliminated in the following way. With a metal surface set for reflection, place the arms to an angle ensuring high sensitivity (about 70°). Set A in such a way that the plane of transmission of the analyzer is approximately in the plane of the surface. The minimum of extinction is sought. In the ideal case $P = A \pm 90^\circ$. In this case the plane of transmission of the P prism is in the plane of incidence and the plane of transmission of the A prism is in the plane of the surface. However, the scale readings will in general not differ 90° . Let the values of P and A at minimum position, be for example $0^\circ + \epsilon'$ and $90^\circ + \epsilon'$, respectively. The quantity ϵ' is now the amount by which both scales are displaced from the true zero azimuth. In this way the absolute errors of P and A are determined. Adjust the P prism in its

holder until ϵ' is zero by turning the prism a small amount in its holder and again adjusting the P scale for minimum transmission. This will require a series of adjustments. It is imperative that the A scale and prism are not moved at this stage, and that the adjustment is done in this order. If the P scale was fixed and A adjusted, the ellipsometer would be aligned to the value of $P = A \pm 90^\circ$. Having adjusted ϵ' to zero, remove the reflecting surface and place the arms to the "straight through" position. Set the P scale at zero and adjust the A scale for minimum. The A scale will now read $90^\circ + \epsilon'$. Turn the A prism in its holder until ϵ' is zero. The ellipsometer P and A scales are now aligned.

Alignment of the compensator: Next we place the quarter wave plate between the crossed prisms. When the quarter wave plate is placed with its fast or slow axis in the plane of the polarizer, the extinction has to remain the same as without the quarter wave plate. In these positions the values on the graduated scales have to read 0° and 90° , respectively. If this is achieved, the ellipsometer is aligned. For most of the experiments the $1/4 \lambda$ plate is placed at $\pm 45^\circ$. If the $1/4 \lambda$ plate is not placed in a graduated circle, the exact $\pm 45^\circ$ positions can be obtained by placing the polarizer at $+ 45^\circ$ and the analyzer at 135° . The position of the compensator giving minimal extinction is the exact $\pm 45^\circ$ position.

3. The electronic automatization

3.1 Introduction

As explained above, the polarizer and the analyzer positions have to be changed at short intervals in order to monitor biochemical reactions in the sample. In principle this can be done by various methods (1-5).

a. Electrically; b) By an analogue circuit and servomotors; and c) by a digital circuit and stepping motors.

The principle of the system is always the same, the signal from the light detection device is coupled back to devices changing the state of polarization of the incident beam c.q. extinguishing the reflected beam to obtain a minimal output. Together with the Department of Electrotechnics of the Technological University, Delft, Holland, we did elaborate experiments in analogue and electrical automatization (9).

a. Electrically the plane of polarization can be changed by Faraday coils, or Pockel cells. In both devices the optical changes are directly dependent upon the electrical energy applied to them. This

in principle opens the possibility to construct an automated ellipsometer without moving parts (10). Also in favour of this approach is the extreme velocity of the measuring cycle (up to milliseconds). The drawback of this method lies in the fact that it remains difficult to assess precisely the angles of polarization and compensation achieved. Further work along this line is in progress.

b. An analogue circuit and servomotor system have the advantage of being relatively quick when using a custom built electronic optimization device instead of a digital computer. We experimented with a setup in which polarization and compensation were phase modulated by a Faraday coil and a Pockel cell. With us the system had a tendency to be unstable and very sensitive to optical noise. Again it was difficult to obtain reliable figures for polarizer and analyzer position with the desired degree of accuracy.

c. A digital circuit and stepping motors have the disadvantage of being relatively slow (a minimal cycle time of approximately 2 seconds can be reached) and therefore requiring a digital computer. In our hands the system worked reliably and in the very act of setting polarizer and analyzer measured the positions accurately within 0.01 of a degree. Moreover, the computer when present can be used to great advantage for other components of the system: gain controlled amplification of the light detector signal, calculations based on the exact Drude equations, time measurement etc.

3.2 The electronic steering system

Block schemes of the electronic circuits for coupling the ellipsometer and the PDP-12 computer are given in Figs. II.7 and II.8.

The electronic circuits can be separated into two independent parts:

1. the photodiode channel (Fig. II.7)
2. the stepping motor steering circuits (Fig. II.8).

3.2.1 Photodiode channel

This circuit is designed to obtain an electric signal proportional to the light intensity. The electric signal is filtered and intensified until the right level is reached to steer the analog/digital converters of the computer. The photodiode channel consists of the following parts (Fig. II.7):

1. light detector; 2. photometer amplifier; 3. amplifier correction unit; 4. filter unit, and 5. meter amplifier.

3.2.1.1 Light detector

As a light detector we used an integrated photodiode operational amplifier combination (United Detector UDT 500). We did not use a highly

Fig. II.7. Blockscheme of the photodiode channel.

Fig. II.8. Blockscheme of the hardware motordriving and timing logic.

sensitive photomultiplier tube because of the considerable noise in this combination and of the high light intensity available. Especially the very low frequent secondary emission noise of the photomultiplier tube was very inconvenient during the measuring process (Fig. 11.7).

3.2.1.2 The photometeramplifier

This amplifier consists of two parts: first a differential input to suppress the "pick up signals". Because of the low signal level a high common mode rejection (CMR) (~ 80 dB) is necessary. The input cables have to be a twisted pair of coaxial cables. The total amplification is about 4 to 5 fold. Second, an amplifier with the possibility to change the dark current. The dark current can be changed about 1 Volt.

3.2.1.3 The amplifier correction unit

In general a change in the analyzer position will not cause the same variation in the light intensity as the same change in the polarizer. The amplifier correction unit is designed to eliminate the difference in sensitivity between analyzer and polarizer angle change. In this way an optimal sample speed is achieved. The changes in sensitivity because of changes in adsorption during the measuring time are also eliminated in this way. The signal for the desired level of amplification is generated in the computer as will be explained later on. It comes out of the computer in the form of an eight bits word which is transformed by a digital/analog converter into a direct current. This direct current is connected to the Y input of a multiplexer. The photometer signal is connected via an amplifier with the X input of the multiplexer. The result is a signal, on the Z output which is of the same form as the photometer signal and which is proportional to the voltage from the A/D converter and thus to the computer signal.

Filter unit: the bandpass filter serves to limit the bandwidth of the measuring system. The bandwidth is chosen twice the bandwidth which caused an extension of the sample time by ten percents. The filter is a third order Butterworth filter with $f_0 = 100$ Hz, flank steepness 18 dB/octave.

3.2.1.4 Meteramplifier

During the initiation of the ellipsometer by hand it is necessary to have direct indication of the light intensity. This is obtained from a voltmeter served by a separate amplifier. The amplification of this unit is 15 fold. The maximum current through the meter is limited to 1.2 mA.

3.2.2 Hardware motordriving and timing logic (Fig. 11.8)

The stepping motors (PD 12) are steered via Philips steering prints (type number 9904-131-03004). To steer, one motor needs a steppulse and a direction bit. There is also the possibility of neglecting a step order by means of an "inhibit bit". This inhibit bit is transmitted to the steering print via a decoder. The pulses and bits are generated by the computer as will be described later on. The computer can give pulses much faster than the motors are able to execute their steps. Therefore, a timing circuit is included between the stepping motor equipment and the computer. After receiving a signal from the computer this circuit will generate a "non-done" signal that causes the computer not to give further pulses during a period adequately for executing the step. In order to prevent noise from pick up pulses it was necessary to disconnect the computer and the motor system electrically by means of optocouplers. The stepping motor system consequently consists of (Fig. 11.8):

3.2.2.1 Optocouplers

As opto isolators we used the Hewlett Packard high speed optically coupled isolators type 5082-4350.

3.2.2.2 Timing circuit

This circuit consists of a monostable multivibrator (SH 74121 N) which is started by the steppulse. The length of the steppulse is adjustable between 4 and 20 μ sec. A potentiometer adjusts the length of the "non done" block on about 3 msec. In this way the stepping rate is 320 steps/second.

3.2.2.3 Decoder

This circuit consists of a gate circuit which has the following properties:

1. the inhibit pulse = 0 , the steppulse does not reach the stepping motor;
 2. the inhibit pulse = 1 , the steppulse reaches to the motor.
- The switching of the inhibit cannot cause a steppuls.

3.2.2.4 Stepping motor print

This print is manufactured by Philips (type number 9904-131-03304). It is designed for steering the eight phase stepping motors. This print only receives the step order and a direction signal. To give an uncoupling of the power supply and because of the pull-in frequency, it is necessary to enlarge the motor steer voltage to 20 V and to introduce RC networks.

3.2.2.5 The stepping motor

The stepping motor is a Philips PD 12, the transmission of the motor-steps to the prism holder is constructed in such a way that one motor-step gives an angle change of about 0.01° of the prism. It is very important that the transmission is of a very high quality and that the backlash has to be reduced as much as possible. The remaining backlash is corrected by the computer program.

4 Ellipsometer steering computer program

4.1 Introduction

The ellipsometer steering computer program is designed to control the measuring process of the ellipsometer, it is carried out by a digital PDP 12 computer, which drives the polarizer and the analyzer of the ellipsometer by means of stepping motors. Both the polarizer and the analyzer have to be steered to the position where a minimum light intensity is achieved on the photodiode behind the analyzer. At the same time the position of the prisms is recorded.

4.2 Principle of the steering procedure

The light intensity at the photodiode is a parabolic function of both the polarizer and analyzer position (Fig.II.9) (Chapter III). We want to find the coordinates of the point in the analyzer-polarizer plane of minimal light intensity and to follow its changes in time. Seeking the minimum light intensity is done by moving the analyzer at a fixed polarizer value, next by fixing the analyzer at the value found and changing the polarizer again to find a minimum then again moving the analyzer etc. When one starts at a position not too far from the minimum, the real minimum is usually found after a few iterations. When the position of the minimum changes, the procedure will follow the changing minimum (6,7).

So first two polarizer positions of equal intensity are sought, and then this prism is steered to the middle in between these positions being the minimum of the parabolic function. Next the program repeats the same procedure with the motor which drives the analyzer. When the analyzer is steered to the minimum position of the function one whole measuring cycle is completed. This cycle is repeated continuously.

At the end of the measuring cycle one can optionally obtain a print-out of the momentary values of the polarizer position, analyzer position, time, temperature and pH in the cuvet. At the end of each motor-cycle, during the running back of each motor to the minimum, on the

Fig. II.9 Seeking procedure

Starting position P_1 . With fixed analyzer position the light intensity will follow the intersection $P_1P_2P_1P_3P_4$. From positions P_2 and P_3 the middle P_4 is calculated. Starting position P_4 : with fixed polarizer position the light intensity will follow intersection $P_4A_1P_4A_2A_3$. If necessary the procedure is repeated from starting position A_3 .

computer display two curves are visible. These curves consist of all the stored previous positions of polarizer and analyzer versus time. There is also a possibility to replace these curves with curves of the temperature and/or pH versus time. With a sense switch setting one transfers the computer display to a memory scope which is connected to a hard copy unit which can produce a photocopy of the displayed curve.

4.3 Steering method of the stepping motors

Technical details

The stepping motors which drive the polarizer and analyzer are

steered by the computer via the DU01 multiplexer. This unit gives command pulses via the "pulse 1-" and "pulse 2-" output channel of a M730 module to the hard ware motordriving and timing logic. The logic answers after about 2.5 msec. This logic also receives a 12-bits dataword via the same output module M730 in channel 0 of the multiplexer. By means of bit settings in this data word an indication is made which motor should be steered forwards or backwards. If bit 1=0 then the polarizer should be steered forwards, if bit 1=1 then this motor should be steered backwards. The same holds for bit 2 with the analyzer. The 12-bits dataword which goes via the M730 module of the DU01 unit to the ellipsometer, contains besides the motor steering bit 0, 1, and 2, also a digital steering of the amplifier which is coupled to the photodiode. With the seven least significant bits the gain of this amplifier is determined. The variable gain control is used to optimize the measuring time, in the cases where a big difference in sensitivity between polarizer and analyzer exists and when the sensitivity changes during the adsorption process. The whole program is controlled by a monitor program which is core resident together with a number of routines for general purpose.

4.4 The computer programs

PROGRAM DATA "ELLIPSOMETER PROGRAM"

THE ELLIPSOMETER-PROGRAM CONSISTS OF THE FOLLOWING PARTS

PART 1: BINARY ASSEMBLING OF:

SYSTEM V2

+ ARITHMETIC ROUTINES

+ INTERRUPT & SERVICE ROUTINES

+ MONITOR

(CORE RESIDENT TOGETHER)

THIS TO LOAD WITH "0700/0000; LMODE; I/O PRESET; DO; START20

PART 2: LOGIN-PROGRAM ON LINTAPE

PART 3: CALIBRATION PROGRAM IDEM

PART 4: PROCESSING PROGRAM IDEM

PART 4: FILE REPRODUCTION IDEM

MONITOR COMMANDS

(ALL COMMANDS TERMINATED BY "RETURN")

"LO":LOAD THE LOGIN PROGRAM

"YK":LOAD THE CALIBRATION PROGRAM

"PR":LOAD THE PROCESSING PROGRAM

"FR":LOAD THE FILE REPRODUCTION

"FN":LOAD THE FILE NAME LISTING

"CTRL/C" DURING ANY PROGRAM: GO TO MONITOR

"CO":CONTINUE THE CURRENT PROGRAM,INTERRUPTED BY "CTRL/C"

4.4.1 Core resident programs

The total of core resident programs consists of:

4.4.1.1 System V2 with routines for teletype printer, teletype keyboard, high speed puncher, scope subroutine, decimal conversion, and the master interrupt control.

4.4.1.2 Arithmetic routines with subroutines for double length division, multiplication, addition, subtraction and scaling.

4.4.1.3 Interrupt and service routines for the servicing of the KW 12 A clock, the stepping motors via the DU01 unit and keyboard input routine.

4.4.1.4 Monitor for guiding, steering and watching as described sub 11.4.3.

4.4.2. Calibration program (Fig. 11.10, 10a)

With this program the values of the starting position of both the polarizer and the analyzer are entered in the program. Second, the calculation factor is determined which transforms the number of steps of a stepping motor into the position change of a prism in degrees.

First we minimize the photodiode output, positioning the prism by hand; this gives the starting position. From this starting position of the polarizer, the polarizer motor is steered forward 4096 steps. The position which is reached thereupon is read in 0.01 degrees (see below) and this value is typed on the keyboard in five figures. Next the motor goes back to his starting position, this position is also read and typed on the keyboard. From the difference of both values the correction factor is calculated. The motor number is increased to motor no = 1 and the same routine is done for the analyzer position.

LO

FILE NAME=BEN/75/2

* YK

CALIBRATION OF POLARIZER

1ST POSITION IN 0.01 DEGREES=13912

2ND POSITION IN 0.01 DEGREES=08042

CALIBRATION OF ANALYZER

1ST POSITION IN 0.01 DEGREES=06460

2ND POSITION IN 0.01 DEGREES=02104

*

* PR

Fig. 11.10. Calibration program.

Fig. II.10a. Calibration program continued.

4.4.3 Processing program

Before zero time in each experiment, the polarizer and analyzer are placed in the minimum positions via the calibration program. These minimum positions are the starting positions in the processing program. The processing program starts steering forwards the polarizer motor. Meanwhile the output of the photodiode is sampled via an analog-digital converter input. When a sample value of 200 or more is reached the motor is stopped. After stopping for about 40 msec, 8 samples are taken, averaged and stored as the reference light intensity. The motor position is stored too. Next the motor is steered back. After a fixed number of steps, sampling of the photodiode output is started again. A buffer zone without sampling is needed because it is to be avoided that fluctuations in the photodiode output cause that the reference light level is reached again thus measuring two times the same intensity at one site of the minimum position. The samples are continuously compared to the reference light level. If this value is reached, the motor is steered further several steps. Now the motor is stopped about 40 msec, and started again in the reverse (forward) direction. The stop of 40 msec is necessary because the big angular acceleration in case of a sudden change of the motor direction would damage the mechanism. Without stopping the motor will slip and this causes a loss of motor steps. As the prism position is monitored by counting the steps, this would bring about an inaccuracy in the assessment of this position.

Running forward the samples of the photodiode are compared again with the first position. When this sample value is the same or less than the first position the motor is stopped and the second position is found. This position is added to the first position and divided by two. This value gives the minimum position of the function and is stored. Subtraction of motor position and the minimum position of the function gives the number of steps the motor still has to do to reach the minimum position. The polarizer measuring cycle is finished now with MOTOR N=Q. Next the polarizer position in steps is transformed to 0.01 degrees and stored.

During the time the motor goes to the minimum position the display routine displays the built file. When the motor is ready, motor N=1 is set and the whole process starts with the analyzer motor. In this case the minimum position is calculated and stored. After this cycle it is checked whether the light amplification ensures maximal efficiency. To do this the distance between the two points of maximal intensity is determined. If this value is bigger than a fixed number

of steps, the codeword for the gain control of the photodiode amplifier is lowered by 1 bit. In this way the amplification is increased. When the distance is too small, the codeword is enlarged by 1 and the amplification is lowered. Values of temperature and pH in the cuvet can also be measured. Both values are stored binary. The locations of the prisms, time, temperature and pH form together a data sample block of 10 words which is added to a datafile in the memory at the end of the analyzer cycle. The time is measured via the real time clock (KW 12) in the computer. During the measurement these five values can be printed out on the teletype by a sense switch option. When the file is as big as 15 memory blocks = 3840 locations, the whole memory field is stored on the tape labeled with the filename. After 10 measuring cycles of polarizer and analyzer the 611 memory scope is automatically enabled and displays the process. The 611 memory scope is coupled with a hardcopy unit which can give a photocopy of the display.

PROCESSING PROGRAM (Fig.II.11 a,b,c,d)

OPTIONS AFTER TYPING "CTRL/C":

"CL":CLEAR ALL DATA AND TIME,RESTART PROCESS

"FI":FINISH THE PROCESS AND STORE THE DATA

/

FOR CURVE SELECTION:

"C1":SELECT CURVE1

"C2":SELECT CURVE2

THAN:"PO","AN","TE",OR "PH"

FOR RESP.POLARIZER-,ANALYZER-,TEMPERATURE- OR ACIDITY CURVE

/

SENSE SWITCH OPTIONS:

S.S.0 HORIZONTAL CALIBRATION

S.S.1 VERTICAL CALIBRATIONS 1

S.S.2 VERTICAL CALIBRATIONS 2

S.S.3 PRINTER OUTPUT

S.S.4 CONTINUE DISPLAY

S.S.5 REFRESH MEMORY SCOPE (AUTO)

"5" IN RIGHT SWITCHES: 5 DEGR.VERTICAL MOVING WINDOW,

ELSE 2.5 DEGR.

KNOB 0: SHIFT OF X-AXIS

(ALWAYS FULLY CLOCKWISE ON START OF PROCESS)

KNOB 4: SCALING OF X-AXIS

KNOBS 1&5: Y-SHIFT OF CURVE 1 (MOVING WINDOW)

KNOBS 2&6: Y-SHIFT OF CURVE 2 (MOVING WINDOW)

File reproduction program

Handling of the data files is carried out by the file reproduction program described below.

FILE REPRODUCTION PROGRAM

DURING THE DISPLAY OF A FILE:

TYPING "+"

THE DISPLAY SHIFTS 5 TAPEBLOCKS FORWARD (INCREASING TIME)

TYPING "-":

THE DISPLAY SHIFTS 5 TAPEBLOCKS BACKWARD (AS FAR AS STARTINGBLOCK)

/

OPTIONS AFTER TYPING "CTRL/C":

/

"LS":

THE PART OF CURVE DISPLAYED IS PRINTED OUT BY TELETYPE

"HS":

THE PART OF CURVE DISPLAYED IS PUNCHED OUT BY H.S.PUNCHER

/

FOR CURVE SELECTION:

"C1":SELECT CURVE1

"C2":SELECT CURVE2

THAN:"PO","AN","TE",OR "PH"

FOR RESP.POLARIZER-,ANALYZER-,TEMPERATURE- OR ACIDITY-CURVE

/

SENSE SWITCH OPTIONS:

S.S.0 HORIZONTAL CALIBRATION

S.S.1 VERTICAL CALIBRATIONS 1

S.S.2 VERTICAL CALIBRATIONS 2

S.S.5 REFRESH MEMORY SCOPE (AUTO)

"5" IN RIGHT SWITCHES: 5 DEGR.VERTICAL. MOVING WINDOW,

ELSE 2.5 DEGR.

/

KNOB 0: SHIFT OF X-AXIS

(ALWAYS FULLY CLOCKWISE ON START OF PROGRAM)

KNOB 4: SCALING OF X-AXIS

KNOB 1&5: Y-SHIFT OF CURVE 1 (MOVING WINDOW)

KNOB 2&6: Y-SHIFT OF CURVE 2 (MOVING WINDOW)

Working up of the particular program options executed by the MONITOR after "CTRL/C" typing a command code.

Fig.II.11. Processing program

Fig.II.11a. Processing program (continued)

Fig. II.11b. Processing program (continued)

Fig. II.11c. Processing program (continued)

Page
3

Repeat for Analyzer

Fig. II.11d. Processing program (continued)

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CHAPTER III

Optical basis of the method

1.1 Definitions and basic principles of polarized light

Interpretation of ellipsometer data, trouble shooting in the instrument and recognition of possible artefacts is not well possible without understanding the working principles. We therefore give here the relevant parts of the optical theory on polarized light.

Ellipsometry is based on the analysis of plane polarized light resulting from the reflection of incident elliptically polarized light from a metal surface. The concept of elliptically polarized light is best understood when it is considered as resulting from the interaction of two beams of plane polarized light.

Consider a light wave travelling along the Z-axis of a coordinate system. The electric vector of the wave can be given by:

$$\begin{aligned} E_x &= A \cos(\tau + \delta_1) \\ E_y &= B \cos(\tau + \delta_2) \end{aligned} \quad (1)$$

where $\tau = \omega(t - \frac{Z}{v})$, ω and v are the angular frequency and linear velocity of the light, respectively, and the total amplitude is the vectorsum of A and B.

The polarization can be described by the amplitudes A and B and the phase difference $\delta = \delta_1 - \delta_2$ of the components.

Elimination of τ gives the following relation:

$$\left(\frac{E_x}{A}\right)^2 + \left(\frac{E_y}{B}\right)^2 - 2 \frac{E_x E_y}{AB} \cos \delta = \sin^2 \delta \quad (2)$$

which is the equation of a conic section, an ellipse in this special case. From the equation one can see:

of $\delta \approx 0$

$$\left(\frac{E_x}{A}\right)^2 + \left(\frac{E_y}{B}\right)^2 - \frac{2E_x E_y}{AB} = 0 \quad (3)$$

$$\frac{E_x}{A} = \frac{E_y}{B} \longrightarrow \text{plane polarized light.}$$

The same is true for $\delta = \pi, 2\pi$

If $\delta = 90^\circ, 270^\circ$

$$\left(\frac{E_x}{A}\right)^2 + \left(\frac{E_y}{B}\right)^2 = 1$$

Only if $A=B$ we get $E_x^2 + E_y^2 = A^2$ which is the general formula for a circle.
In the next picture all possible situations are given:

Fig. III.1. Cases of polarization for various phase differences Δ .

From fig. III.1 we see that the ellipticity is a function of δ . The relationship between an ellipse and its components in the reference planes is given below.

Fig. III.2 Representation of monochromatic elliptically light

The components E_x and E_y of the electric vector E have amplitudes A_x and A_y . The relevant parameters are:

1. azimuth ρ (angle formed by the positive X-axis and the long axis of the ellipse)
2. ellipticity $\chi = \pm \arctan \left(\frac{b}{a} \right)$

The + sign denotes a clockwise orbit of the electric vector

3. The ratio $\frac{A_y}{A_x}$ expressed by $\alpha = \arctan \frac{A_y}{A_x}$
4. the phase difference δ between E_x and E_y .

These quantities satisfy the equations of Partington

$$\begin{aligned} \tan 2\rho &= \tan 2\alpha \cdot \cos \delta \\ \sin 2\chi &= \sin 2\alpha \cdot \sin \delta. \end{aligned} \quad (4)$$

As seen from the last relation, plane polarized light is obtained for zero phase difference ($\delta=0$) between E_x and E_y .

1.2 Complex notation

To describe a lightwave, one can use the following notation:

$$E_x = A \sin \left[\omega \left(t - \frac{z}{v} \right) + \delta_1 \right], \quad E_y = B \sin \left[\omega \left(t - \frac{z}{v} \right) + \delta_2 \right] \quad (5)$$

However, calculation with these quantities becomes clearer and simpler if a symbolic expression by means of complex numbers is used. If we denote:

$$\omega(t - \frac{z}{v}) = \tau,$$

then according to Euler's relation

$$e^{i\tau} = \cos\tau + i\sin\tau$$

it follows that $\cos\tau$ is the real part and $\sin\tau$ the imaginary part of the complex expression $e^{i\tau}$. The real part of the final expression is understood to represent the physical quantity in question (1).

The equations $E_x = A \sin \omega t$, $E_y = B \sin \omega t$ can then be written in the form

$$E_x = \bar{A} e^{i\tau} \quad E_y = \bar{B} e^{i\tau} \quad (6)$$

where the amplitudes $\bar{A}$ and $\bar{B}$ can be given generally by complex numbers, for instance the complex amplitude $\bar{A}$ can be written in the form:

$$\bar{A} = A' + iA'' = \sqrt{A'^2 + A''^2} e^{i\delta_1} = A e^{i\delta_1}$$

where

$$\tan \delta_1 = \frac{A''}{A'}$$

Equations (eq. 5) can then be written in the form

$$E_x = \bar{A} e^{i\tau} = A e^{i(\tau + \delta_1)} \quad E_y = \bar{B} e^{i\tau} = B e^{i(\tau + \delta_2)} \quad (7)$$

The method of writing

$$E_x = A \sin[\omega(t - \frac{z}{v}) + \delta_1] = \bar{A} e^{i\tau} \quad \text{and}$$

$E_y = B \sin[\omega(t - \frac{z}{v}) + \delta_2] = \bar{B} e^{i\tau}$ shows that E_x and E_y are components of the vector harmonic wave which are generally written in the form (1).

$$\vec{E} = \vec{E}_0 e^{i\tau} \quad (8)$$

where $\vec{E}_0$ is the complex vector of amplitude which has the following components

$$E_{0,x} = A e^{i\delta_1}, \quad E_{0,y} = B e^{i\delta_2}, \quad E_{0,z} = C e^{i\delta_3}$$

The change from one form of polarized light to another is brought about by reflection and refraction of the light by particular materials and the type of change is determined by the optical properties of these materials. In order to understand the measurements of thin films by ellipsometry it is necessary to consider the changes in polarized light which occur on reflection and refraction.

1.3 The reflection and refraction of light on a boundary between two isotropic dielectrics

When studying the reflection and refraction of light on a boundary between two isotropic homogeneous dielectrics we orientate the coordinate system so that the XY plane lies in the plane boundary of the two media. The X-axis is the projection of the plane boundary of the two dielectrics, while the Y-axis is normal to the plane of drawing. The plane of incidence in which the incident reflected and refracted rays ly is in the plane of drawing, i.e. in the XZ plane. The medium from which a plane light wave is incident on the refracting boundary has refractive index n_0 ; similarly the other medium into which the light wave is refracted, has refractive index n_1 . The vector plane wave of the incident light is written in the complex form

$$\vec{E}_0^d = \vec{A}_0 e^{i\tau_0^d} \quad (9)$$

similarly the vector reflected wave is given by

$$\vec{E}_0^r = \vec{R}_0 e^{i\tau_0^r} \quad (10)$$

and the vector refracted wave by

$$\vec{E}_1^d = \vec{A}_1 e^{i\tau_1^d} \quad (11)$$

Fig. III.3 Reflection and refraction of light at plane boundary between two dielectrics with orientation of the vectors $\vec{E}_0^d$, $\vec{E}_0^r$, and $\vec{E}_1^d$ and its component $A_{0,p}$, $A_{0,s}$, $R_{0,p}$, $R_{0,s}$, $A_{1,p}$, $A_{1,s}$.

In the above equations the exponent of the function e in the incident wave is:

$$\tau_0^d = \omega \left(t - \frac{x \sin \rho_0 + z \cos \rho_0}{v_0} \right) \quad (12)$$

in the reflected wave:

$$\tau_0^r = \omega \left(t - \frac{x \sin \rho_0' + z \cos \rho_0'}{v_0} \right) \quad (13)$$

in the refracted wave:

$$\tau_1^d = \omega \left(t - \frac{x \sin \rho_1 + z \cos \rho_1}{v_1} \right) \quad (14)$$

where t is the time and ω the circular frequency of the light wave, ρ_0 the angle of incidence, ρ_0' the angle of reflection and ρ_1 the angle of refraction.

The vector of the amplitude of the incident light wave $\bar{A}_0$ is divided into two components - the component $A_{0,p}$ lying in the plane of incidence, and the component $A_{0,s}$ normal to the plane of incidence. Similarly in a reflected light wave the vector of the amplitude $\bar{R}_0$ is divided into the components $R_{0,p}$ and $R_{0,s}$ and in a refracted light wave the amplitude $\bar{A}_1$ is divided into $A_{1,p}$ and $A_{1,s}$.

If we write out the above vector light waves into components along the coordinate axes, the components of the amplitude s are normal to the plane of incidence and thus lie in the direction of the Y -axis, while the components of p lie in the plane of incidence, and have projections into the X and Z axis. We thus obtain for the components of the light vectors:

for incident light:

$$E_{0,x}^d = A_{0,p} \cos \rho_0 e^{i\tau_0^d}$$

$$E_{0,y}^d = A_{0,s} e^{i\tau_0^d}$$

$$E_{0,z}^d = -A_{0,p} \sin \rho_0 e^{i\tau_0^d}$$

for reflected light:

$$E_{0,x}^r = R_{0,p} \cos \rho_0' e^{i\tau_0^r}$$

$$E_{0,y}^r = R_{0,s} e^{i\tau_0^r}$$

$$E_{0,z}^r = -R_{0,p} \sin \rho_0' e^{i\tau_0^r}$$

for refracted light:

$$E_{1,x}^d = A_{1,p} \cos \rho_1 e^{i\tau_1^d}$$

$$E_{1,y}^d = A_{1,s} e^{i\tau_1^d}$$

$$E_{1,z}^d = -A_{1,p} \sin \rho_1 e^{i\tau_1^d}$$

(15)

On the boundary between the two dielectrics the tangential components of the electric vector vary continuously. We thus have (2):

$$\begin{aligned} E_{0,x}^d + E_{0,x}^r &= E_{1,x}^d \\ E_{0,y}^d + E_{0,y}^r &= E_{1,y}^d \\ E_{0,z}^d + E_{0,z}^r &= E_{1,z}^d \end{aligned} \quad (16)$$

These conditions must be satisfied for the boundary $z=0$ in every time t . These boundary conditions are satisfied if two basic conditions are satisfied for the boundary $z=0$.

1.4 Phase conditions

The phase condition which leads to relations between the exponents

$$\tau_0^d = \tau_0^r = \tau_1^d \quad (17)$$

From the phase condition for $z=0$ we obtain the first relation by comparing the coefficients of x

$$\frac{\sin \rho_0}{v_0} = \frac{\sin \rho_0'}{v_0} \quad \text{i.e. } \sin \rho_0 = \sin \rho_0' \quad (18)$$

since the reflected and incident rays have different directions $(\rho_0' \neq \rho_0), \rho_0' = \pi - \rho_0$ i.e. $\cos \rho_0' = -\cos \rho_0$.

This is the law of reflection.

By further comparison of the coefficient of x we obtain a second relation

$$\frac{\sin \rho_0}{v_0} = \frac{\sin \rho_1}{v_1} \quad \text{or} \quad \frac{\sin \rho_0}{\sin \rho_1} = \frac{v_0}{v_1} = \frac{n_1}{n_0}$$

This is Snell's law of refraction, normally written in the form

$$n_0 \sin \rho_0 = n_1 \sin \rho_1 \quad (20)$$

The amplitudes $\bar{A}_0, \bar{R}_0, \bar{A}_1$ are generally complex numbers

$$\bar{A}_0 = A_0 e^{i\delta_0^d} \quad \bar{R}_0 = R_0 e^{i\delta_0^r} \quad \bar{A}_1 = A_1 e^{i\delta_1^d}$$

The phase condition then gives the following relations:

$$\delta_0^d = \delta_0^r = \delta_1^d$$

So the light reflected or refracted at the boundary between two dielectrics has no change of phase or changes by $\pm \pi$. When the light incident on the boundary between two dielectrics is plane polarized then the reflected or refracted light is also plane polarized.

If we write the equations for the exponent more general: ω_0^d for the incident wave, ω_0^r for the reflected wave and ω_1^d for the refracted wave instead of ω , then we have from the phase condition:

$$\omega_0^d = \omega_0^r = \omega_1^d = \omega \quad (22)$$

So on a static boundary $z=0$ the frequency of the light at reflection and refraction does not vary.

1.5 Amplitude conditions (Fresnel's formulae)

The amplitude conditions which lead to relations between the components of the amplitudes in incident, reflected and refracted light ($\cos \rho_0' = -\cos \rho_0$) have the following form:

$$\begin{aligned} (A_{0,p} - R_{0,p}) \cos \rho_0 &= A_{1,p} \cos \rho_1 \\ (A_{0,p} + R_{0,p}) n_0 &= A_{1,p} n_1 \\ A_{0,s} + R_{0,s} &= A_{1,s} \\ (A_{0,s} - R_{0,s}) n_0 \cos \rho_0 &= A_{1,s} n_1 \cos \rho_1 \end{aligned} \quad (23)$$

From the last four equations we can calculate Fresnel's formulae which give the relations between the amplitudes of reflected and incident light and the relations between the amplitudes of refracted and incident light, both for the p and the s component.

By calculation we obtain for the amplitude of reflected light:

$$\begin{aligned}
 R_{o,p} &= \frac{n_1 \cos \rho_0 - n_0 \cos \rho_1}{n_1 \cos \rho_0 + n_0 \cos \rho_1} & A_{o,p} &= \frac{\tan(\rho_0 - \rho_1)}{\tan(\rho_0 + \rho_1)} A_{o,p} \\
 R_{o,s} &= \frac{n_0 \cos \rho_0 - n_1 \cos \rho_1}{n_0 \cos \rho_0 + n_1 \cos \rho_1} & A_{o,s} &= -\frac{\sin(\rho_0 - \rho_1)}{\sin(\rho_0 + \rho_1)} A_{o,s}
 \end{aligned} \quad (24)$$

and for the ratio of the reflected and incident amplitudes:

$$\begin{aligned}
 r_p &= \frac{R_{o,p}}{A_{o,p}} = \frac{n_1 \cos \rho_0 - n_0 \cos \rho_1}{n_1 \cos \rho_0 + n_0 \cos \rho_1} = \frac{\tan(\rho_0 - \rho_1)}{\tan(\rho_0 + \rho_1)} \\
 r_s &= \frac{R_{o,s}}{A_{o,s}} = \frac{n_0 \cos \rho_0 - n_1 \cos \rho_1}{n_0 \cos \rho_0 + n_1 \cos \rho_1} = -\frac{\sin(\rho_0 - \rho_1)}{\sin(\rho_0 + \rho_1)}
 \end{aligned} \quad (25)$$

Similarly one can calculate the amplitude of light transmitted through a boundary of dielectrics.

2.1 Total phase difference by reflection of a monochromatic lightwave on an isotropic adsorbing substrate covered by a thin absorbing layer

Fig. III.4. Phase difference introduced by reflection on a substrate covered by a thin layer.

Medium 0 is the surrounding medium $n_0=1$ (air)

Medium 1 : thin absorbing layer, thickness d_1 , optical constant $\bar{n}_1$

Medium 2 : substrate, optical constant $\bar{n}_2$.

The optical constant $\bar{n}_j$ is defined in the following way:

$$\bar{n}_j = n_j - ik_j \quad (26)$$

$\bar{n}_j$ = optical constant medium j

n_j = refractive index medium j

k_j = absorbing coefficient medium j.

In the figure you can see that the difference in optical path between two successive reflected beams equals:

$$|O_1C| + |O_2C| - |O_1A|$$

The resulting phase difference introduced by this path is calculated below.

$$|O_1A| = a \sin \rho_0 \text{ (in cm)} = a / \lambda_0 \sin \rho_0 \text{ (in wave length)}$$

λ_0 = wavelength in medium 0

$$|O_2B| = a \sin \rho_1 \text{ (in cm)} = a / \lambda_1 \sin \rho_1 \text{ (in wave length)}$$

λ_1 = wavelength in medium 1

Upon reflection and refraction the frequency does not change, so

$$1) \quad v_0 = f \lambda_0$$

$$v_1 = f \lambda_1$$

$$v_2 = f \lambda_2$$

$$\frac{v_0}{\lambda_0} = \frac{v_1}{\lambda_1} = \frac{v_2}{\lambda_2}$$

$$2) \quad n_0 \sin \rho_0 = \bar{n}_1 \sin \rho_1 \text{ (Snell's law)}$$

$$3) \quad n_0 v_0 = \bar{n}_1 v_1 = \bar{n}_2 v_2 \text{ (definition of } n)$$

}

$$n_0 \lambda_0 = \bar{n}_1 \lambda_1 = \bar{n}_2 \lambda_2$$

$$\frac{a}{\lambda_1} \sin \rho_1 = a \frac{n_0}{\bar{n}_1 \lambda_1} \sin \rho_0 = \frac{a}{\lambda_0} \sin \rho_0$$

$$\text{so the optical path } |O_1A| = |O_2B|$$

$$|O_1C| + |O_2C| - |O_1A| = \frac{1}{2} |O_1A| + |DF| + |FE| + \frac{1}{2} |O_1A| - |O_1A| =$$

$$2d \cos \rho_1 \text{ (in cm)} = \frac{2d_1 n_1 \cos \rho_1}{\lambda_0} \text{ (in wavelength)} = \frac{4\pi d_1 n_1 \cos \rho_1}{\lambda_0} \text{ (in degrees)}$$

So the total phase difference is $\frac{4\pi d_1 n_1}{\lambda_0} \cos \rho_1 = X$ (27)

X is real if the thin layer d_1 does not absorb for only then the optical constant is real.

2.2 Multiple reflection (Drude equation)

Thus far we calculated the effect of passing one boundary of two media. In reality the lightwave has multiple reflections and refractions in the thin layer. For this situation we sum up all effects and calculate the total reflection coefficient.

Fig. III.5 Multiple reflection and refraction on an absorbing substrate covered by a thin absorbing layer. Light waves that enter the substrate are supposed not to come out of the substrate. The p component is parallel the plane of incidence, the s component is perpendicular on the plane of incidence.

From the figure we see:

$$\left. \begin{aligned} r_{1p} + \tau_{1p} &= 1 \\ -r_{1p} + \tau_{1p}' &= 1 \end{aligned} \right\} \rightarrow \tau_{1p}\tau_{1p}' = 1 - r_{1p}^2 \quad (28)$$

$$\left. \begin{aligned} r_{1p} &= \frac{\bar{E}_{1p}(r)}{\bar{E}_{1p}(i)} = \frac{n_1 \cos \rho_0 - n_0 \cos \rho_1}{n_1 \cos \rho_0 + n_0 \cos \rho_1} \\ r_{1s} &= \frac{\bar{E}_{1s}(r)}{\bar{E}_{1s}(i)} = \frac{n_0 \cos \rho_0 - n_1 \cos \rho_1}{n_0 \cos \rho_0 + n_1 \cos \rho_1} \end{aligned} \right\} \text{ Fresnel's reflection coefficients}$$

Now we define the total amplitude reflection coefficient where the behaviour of the light waves inside the thin layer is also included:

$$R_p = \frac{\bar{E}_p(r)}{\bar{E}_p(i)} = r_{1p} + (r_{1p}^2) r_{2p} e^{-ix} - (1 - r_{1p}^2) r_{1p} r_{2p}^2 e^{-i2x} + \dots \quad (29)$$

$$R_s = \frac{\bar{E}_s(r)}{\bar{E}_s(i)} = r_{1s} + (1 - r_{1s}^2) r_{2s} e^{-ix} - (1 - r_{1s}^2) r_{1s} r_{2s}^2 e^{-i2x} + \dots$$

Except for the first term this is a converging geometric progression with a common ratio: $-r_{1p} r_{2p} e^{-ix}$, so

$$R_p = r_{1p} + \frac{(1 - r_{1p}^2) r_{2p} e^{-ix}}{1 + r_{1p} r_{2p} e^{-ix}} = \frac{r_{1p} + r_{2p} e^{-ix}}{1 + r_{1p} r_{2p} e^{-ix}} \quad (30)$$

similarly

$$R_s = \frac{r_{1s} + r_{2s} e^{-ix}}{1 + r_{1s} r_{2s} e^{-ix}} \quad (31)$$

We write $\bar{E}_p = A_p e^{i\delta_p}$ and $\bar{E}_s = A_s e^{i\delta_s}$

$$\frac{\bar{R}_p}{R_s} = \frac{E_p^r \cdot E_s^i}{E_p^i \cdot E_s^r} = \left(\frac{A_p}{A_s} \right)^{(r)} / \left(\frac{A_p}{A_s} \right)^{(i)} \cdot e^{i[(\delta_p - \delta_s)^{(r)} - (\delta_p - \delta_s)^{(i)}]} \quad (32)$$

This may be expressed in terms of the relative attenuation and phase shift of the parallel component with respect to the perpendicular component that occurs, represented by the azimuthal angle ψ and relative phase shift Δ by:

$$\Delta \equiv (\delta_p - \delta_s)^{(r)} - (\delta_p - \delta_s)^{(i)} \quad \text{tg} \psi \equiv \left(\frac{A_p}{A_s} \right)^{(r)} / \left(\frac{A_p}{A_s} \right)^{(i)} \quad (32a)$$

$$\text{tg} \psi e^{i\Delta} = \frac{r_{1p} + r_{2p} e^{-ix}}{1 + r_{1p} r_{2p} e^{-ix}} \cdot \frac{1 + r_{1s} r_{1p} e^{-ix}}{r_{1s} + r_{2s} e^{-ix}} \quad (33)$$

$$\text{where } x = \frac{4\pi d_1 n_1}{\lambda_0} \cos \rho_1 \quad (34)$$

This equation was derived for the first time by Drude in 1889 (3-5).

2.3 System of several layers

For a system of several layers the Drude equation can be generalized according to several methods (6-8).

The generalizations are based on the conception that the reflection of light from one film (i.e. at two optical boundaries) can be replaced by the reflection of light at one hypothetical boundary, which gives the same amplitude and phase, i.e. the same complex amplitude. The mathematical basis for the conception was given by Murmann (9). The conception is clear from fig. III.6 which schematically shows the reflection of light both from clean glass and from glass with one film.

Fig. III.6 Schematic representation of reflection of light a) from clean glass, b) from glass with one thin film and the equivalent reflection from a hypothetical medium.

In the theory of reflection of light from two films, the resultant amplitude $r_2 e^{i\delta_2}$ can be calculated in two ways:

1. we first consider reflection from the upper film (i.e. at the first two boundaries with basic amplitudes r' and $r'' e^{-ix_1}$) and obtain the equivalent amplitude $r_1 e^{i\delta_1}$ we then deal with the third (lower) boundary having basic amplitude $r''' e^{-i(x_1 + ix_2)}$.

This procedure can be schematically represented as follows:

It is shown graphically in fig. III.7a.

Fig.III.7 Schematic representation of reflection of light from two thin films on glass, the equivalent reflection from one thin film and the equivalent reflection from a hypothetical medium.

2. The second possible way of calculating the reflection of light from two thin films is by first calculating for the lower film (i.e. for the second and third boundaries with the basic amplitudes $r''e^{-ix_1}$ and $r'''e^{-(x_1+x_2)}$) and getting the equivalent amplitude $\bar{r}_2e^{i\delta_2}$ to which we add the upper boundary with basic amplitude r' . This procedure can be schematically written as follows

$$\begin{array}{c} r''' \\ r'' \\ r' \end{array} \begin{array}{l} \nearrow \\ \nearrow \\ \nearrow \end{array} \begin{array}{l} \bar{r}_2e^{i\delta_2} \\ \bar{r}_2e^{i\delta_2} \\ r_2e^{i\delta_2} \end{array}$$

and is graphically represented in fig. 7b.

For a system with an arbitrary number of parallel isotropic films the total reflection coefficients R_p and R_s are found by an iterative summation process (8). For the case of a surface covered with two layers the result is for both p and s components:

$$R = \frac{r_1 + R' \exp(-ix_1)}{1 + r_1 R' \exp(-ix_1)}$$

$$R' = \frac{r_{11} + r_{111} \exp(-ix_2)}{1 + r_{11} r_{111} \exp(-ix_2)} \quad (34)$$

2.4 Influence of very thin transitional films (approximated Drude theory)

For very thin films ($d_1 \ll \lambda$) Drude derived equations which numerically represent the influence of thin transitional films which are spontaneously formed on the surface of metal for example by oxydation, on the measured optical quantities in reflected light, i.e. the azimuth ψ and the phase difference Δ . If the quantities measured on the clean surface of metal (without a superficial film) are denoted ψ' and Δ' for an angle of incidence ϕ_0 and the same quantities measured on the same metal having a thin superficial film ψ and Δ and the layer thickness is L , then Drude's formulae have the form:

$$2\psi = 2\psi' + \sin 2\psi' \frac{4\pi \cos \phi_0 \sin^2 \phi_0 a'}{\lambda (\cos^2 \phi_0 - a')^2 + a'^2} \int_0^L (1 - n_1^2 \cos^2 \phi_0) \left(1 - \frac{1}{n_1^2}\right) dL \quad (35)$$

$$\Delta = \Delta' - \frac{4\pi \cos \phi_0 \sin^2 \phi_0 (\cos^2 \phi_0 - a')}{\lambda (\cos^2 \phi_0 - a')^2 + a'^2} \int_0^L \left(1 - \frac{1}{n_1^2}\right) dL \quad (36)$$

$$\text{where } a' = \frac{1 - k^2/n^2}{n^2(1 + k^2/n^2)^2} \quad a' = \frac{2(k/n)}{n^2(1 + k^2/n^2)^2} \quad (37)$$

If a transitional film of thickness d_1 and refractive index n_1 is regarded as homogeneous, then the above equations can be written in the form

$$\frac{2\psi - 2\psi'}{\sin 2\psi'} = \frac{4\pi d_1 \cos \phi_0 \sin^2 \phi_0 a'}{\lambda (\cos^2 \phi_0 - a')^2 + a'^2} (1 - n_1^2 \cos^2 \phi_0) \left(1 - \frac{1}{n_1^2}\right) \quad (38)$$

$$\Delta - \Delta' = - \frac{4\pi d_1 \cos \phi_0 \sin^2 \phi_0 (\cos^2 \phi_0 - a')}{\lambda (\cos^2 \phi_0 - a')^2 + a'^2} \left(1 - \frac{1}{n_1^2}\right) \quad (39)$$

Tronstad generalized Drudes formulae for the case when the first medium is not air with refractive index 1, but has generally the refractive index n_0 . The Tronstad-Drude formulae have the form (10,10a)

$$\Delta - \Delta' = - \frac{4\pi d_1 \cos \phi_0 \sin^2 \phi_0 (n_1^2 - n_0^2)}{\lambda (\cos^2 \phi_0 - n_0^2 a)^2 + n_0^4 a'^2} \times$$

$$\times [(\cos^2 \phi_0 - n_0^2 a)(1/n_1^2 - a) + n_0^2 a'^2] , \quad (40)$$

$$2\psi - 2\psi' = \sin 2\psi' \frac{4\pi d_1 \cos \phi_0 \sin^2 \phi_0 (n_1^2 - n_0^2)}{\lambda (\cos^2 \phi_0 - n_0^2 a)^2 + n_0^4 a'^2} \times$$

$$\times [n_1^2 a' (1/n_1^2 - a) - (\cos^2 \phi_0 - n_0^2 a)a] \quad (41)$$

The Tronstad-Drude formulae are only valid for very thin films ($d_1 \ll \lambda$). These formulae can also be derived from the exact Drude equations by expanding the formulae according to the Taylor series.

3 Determinations of ψ and Δ from ellipsometer scale readings

3.1 Introduction

By equation 33) the reflection of light from a surface is characterized by the complex reflection coefficient $\tan \psi e^{i\Delta}$, and hence by the two quantities ψ and Δ . However, the determination of ψ and Δ from polarizer and analyzer readings merits some discussion, for this is not always a perfectly direct matter.

For any given surface there is a multiplicity of polarizer, analyzer and compensator scale settings that produce extinction by the analyzer of the reflected light from the surface, and the problem rises of how to exactly determine the values of ψ and Δ from these various readings. In order to explain how these numerous readings arise and how ψ and Δ may be computed from them it is well to keep two facts in mind:

- all azimuthal angles are measured positive counter-clockwise from the plane of incidence when looking into the light beam, and
- the compensator which may be set at any azimuth is generally set so that its fast axis is in an azimuth of $\pm \pi/4$.

The present discussion is for an instrument with the compensator before reflection. If the compensator is placed after reflection, the reasoning has to be changed accordingly. The various readings fall into four sets called zones, two with the fast axis of the compensator set at $\pi/4$, numbered 2 and 4 and two with it set at $-\pi/4$, numbered 1 and 3. In each zone there is one independent set of polarizer and analyzer readings, making four independent sets of P and A readings in all. However, since both analyzer and polarizer may be rotated by π without affecting the result, there are 16 polarizer and analyzer settings falling into four independent zones. Since the compensator may also be rotated by π without affecting the results, there are 32 possible sets of readings on the ellipsometer. Rather than calculating Δ and ψ directly from the P and A values, it is useful to calculate three other quantities, p , a_p , and a_s from the P and A values, p being related to the P readings, a_p related to the A readings in zones 1 and 4, and a_s related to the A readings in zones 2 and 3.

3.2 The relationship of p , a_p , and a_s to the P and A readings observed on the ellipsometer

The relationship of p , a_p , and a_s to the P and A readings observed on the ellipsometer is derived below.

Fig. III.8 Schematic representation of the light beam, when standing between the sample and the compensator.

In fig. III.8 we are standing between the compensator and the reflecting surface and we are looking into the beam. The slow axis of the compensator is fixed at $+45^0$. The components of the unit light vector $\vec{p}$ along the axis of the compensator are

F component: $-\sin(45^0-P)$

L component: $\cos(45^0-P)$.

After passing the compensator, the phase of the L component is δ degrees behind the phase of the F component.

$$e^{-i\delta} = \cos\delta - i\sin\delta$$

The components along the p and s axis are now (fig. III.8):

$$\bar{E}_s^{(i)} = \underbrace{-\sin(45^0-P)\cos 45^0}_{\text{(F component)}} + \underbrace{\cos(45^0-P)\sin 45^0}_{\text{(L component)}}(\cos\delta - i\sin\delta)$$

$$\bar{E}_p^{(i)} = \sin(45^0-P)\sin 45^0 + \cos(45^0-P)\cos 45^0(\cos\delta - i\sin\delta)$$

$$\begin{aligned} \left(\frac{E_p}{E_s}\right)^{(i)} &= \frac{1 + \cos(45^0-P)\cos\delta - i\cot(45^0-P)\sin\delta}{-1 + \cot(45^0-P)\cos\delta - i\cot(45^0-P)\sin\delta} = \\ &= \frac{\cot^2(45^0-P) - 1 + 2i\cot(45^0-P)\sin\delta}{\cot^2(45^0-P) + 1 - 2\cot(45^0-P)\cot\delta} \end{aligned} \quad (42)$$

We already defined eq. 32a)

$$\Delta = (\delta_p - \delta_s)^{(r)} - (\delta_p - \delta_s)^{(i)}$$

To have complete extinction, the light reaching the analyzer has to be planely polarized, so

$$(\delta_p - \delta_s)^{(r)} = 0 \quad \rightarrow \quad \Delta = -(\delta_p - \delta_s)^{(i)}$$

$$\text{We can say } \frac{\bar{E}_p}{\bar{E}_s} = \tan L e^{-i\Delta}$$

$\tan L$ equals the ratio of the amplitudes of the p and s components of the incident beam.

So with eq. 42)

$$\frac{\cot^2(45^0-P) - 1 + 2i\cot(45^0-P)\sin\delta}{\cot^2(45^0-P) + 1 - 2\cot(45^0-P)\cos\delta} = \tan L e^{-i\Delta} \quad (43)$$

Separation of the real and imaginary parts give

$$\frac{\cot^2(45^\circ - P) - 1}{\cot^2(45^\circ - P) + 1 - 2\cot(45^\circ - P)\cos\delta} = \tan L \cos \Delta = \frac{\cos(90^\circ - 2P)}{1 - \sin(90^\circ - 2P)\cos\delta} \quad (44)$$

$$\frac{2\cot(45^\circ - P)\sin\delta}{\cot^2(45^\circ - P) + 1 - 2\cot(45^\circ - P)\cos\delta} = -\tan L \sin \Delta = \frac{\sin(90^\circ - 2P)\sin\delta}{1 - \sin(90^\circ - 2P)\cos\delta} \quad (45)$$

From eq. 44) en eq. 45) we get by division

$$-\tan \Delta = \sin \delta \tan(90^\circ - 2P)$$

$$\text{so } \tan \Delta = \sin \delta \tan(90^\circ + 2P) \quad \text{first zone} \quad (46)$$

Squaring and adding eq. 44) and eq. 45) gives

$$\tan^2 L = \frac{\cos^2(90^\circ - 2P) + \sin^2(90^\circ - 2P)\sin^2\delta}{1 + \sin^2(90^\circ - 2P)\cos^2\delta - 2\sin(90^\circ - 2P)\cos\delta} =$$

$$= \frac{1 + \sin(90^\circ - 2P)\cos\delta}{1 - \sin(90^\circ - 2P)\cos\delta}$$

$$+ \sin(90^\circ - 2P)\cos\delta = \frac{\tan^2 L - 1}{\tan^2 L + 1} = -\cos 2L$$

$$\text{so } \cos 2L = -\cos \delta \cos 2P \quad \text{first zone} \quad (47)$$

In the third zone the formulas are

$$\tan \Delta = \sin \delta \tan(90^\circ + 2(90^\circ + P)) = \sin \delta \tan(90^\circ + 2P) \quad \text{third zone} \quad (48)$$

$$\cos 2L' = -\cos \delta \cos 2(90^\circ + P) = \cos \delta \cos 2P \quad \text{third zone} \quad (49)$$

From formulas eq. 47) and eq. 49) it follows

$$\cos 2L' = -\cos 2L = \cos(180^\circ + 2L) \rightarrow L' = 90^\circ + L$$

$$\text{so } \tan L' = -\cot L. \quad (50)$$

$$\text{Since } \tan \psi = \left| \frac{r_p}{r_s} \right| = \left| \frac{E_p^{(r)}/E_p^{(i)}}{E_s^{(r)}/E_s^{(i)}} \right| = \frac{A_p^{(r)}}{A_s^{(r)}} \bigg/ \left| \frac{E_p^{(i)}}{E_s^{(i)}} \right| = \frac{A_p^{(r)}/A_s^{(r)}}{\tan L}$$

where A_p and A_s are the amplitudes of the reflected components (here we use the amplitudes because after reflection the light has to be plane polarized to have complete extinction.)

To obtain complete extinction, the analyzer setting has to be perpendicular to the plane of polarization. It is easy to see that

$$\frac{A_p}{A_s} = \cot(90^\circ + A)$$

that is why

$$\tan \psi = \frac{\cot(90^\circ + A)}{\tan L}$$

$$\left. \begin{array}{ll} \text{first zone} & \tan \psi = \frac{\cot(90^\circ + A_1)}{\tan L} \\ \text{third zone} & \tan \psi = \frac{\cot(90^\circ + A_3)}{\tan L'} \end{array} \right\} \tan^2 \psi = -\tan A_1 \tan A_3$$

$$\text{set } A_3 = 180^\circ - a_s$$

$$A_1 = a_p$$

$$\text{this gives } \tan^2 \psi = \tan a_p \tan a_s \quad (51)$$

Second and fourth zones are obtained by changing the places of the fast and slow axis

$$\begin{aligned} \left(\frac{E_p}{E_s} \right)^{(i)} &= \frac{1 + \cot(45^\circ - P) \cos \delta + i \cot(45^\circ - P) \sin \delta}{-1 + \cot(45^\circ - P) \cos \delta + i \cot(45^\circ - P) \sin \delta} \\ &= \frac{\cos(90^\circ - 2P) - i \sin(90^\circ - 2P) \sin \delta}{1 - \sin(90^\circ - 2P) \cos \delta} = \tan L e^{-i\Delta} \end{aligned}$$

$$\tan \Delta = \sin \delta \tan(90^\circ - 2P)$$

second zone

$$= \sin \delta \tan(90^\circ - 2(90^\circ + P))$$

fourth zone

$$\tan^2 L = \frac{\cos^2(90^\circ - 2P) + \sin^2(90^\circ - 2P) \sin^2 \delta}{(1 - \sin(90^\circ - 2P) \cos \delta)^2}$$

$$\cos 2L = \cos \delta \cos 2P$$

second zone

$$\cos 2L = \cos \delta \cos 2P$$

fourth zone

These calculations can also be done for the second, third, and fourth quadrant. In this way we get 16 possible combinations for extinction.

The relations for the different quadrants are summarized in the next table; with this table we determine p , a_s , and a_p from the polarizer and analyzer settings P and A . The introduction of the small "p" in the table is for the simplification of the relation. The formulas eq. 52) and eq. 53) give the relations between ψ , Δ , and p , a_s , and a_p .

If the compensator is exactly quarterwavelength then we have $\delta = 90^\circ$, this gives:

$$\Delta = \frac{1}{2}\pi + 2p \quad (52)$$

$$\psi = a_p = a_s \quad (53)$$

Relation of P and A readings to p , a_p , and a_s

zone	compensator	P	A
1	$-\pi/4$	p	a_p
		$p + \pi$	a_p
		p	$a_p + \pi$
		$p + \pi$	$a_p + \pi$
3	$-\pi/4$	$p + \pi/2$	$\pi - a_s$
		$p + 3\pi/2$	$\pi - a_s$
		$p + \pi/2$	$2\pi - a_s$
		$p + 3\pi/2$	$\pi - a_s$
2	$+\pi/4$	$\pi/2 - p$	a_s
		$3\pi/2 - p$	a_s
		$\pi/2 - p$	$a_s + \pi$
		$\pi/2 - p$	$a_s + \pi$
4	$+\pi/4$	$\pi - p$	$\pi - a_p$
		$2\pi - p$	$\pi - a_p$
		$\pi - p$	$2\pi - a_p$
		$2\pi - p$	$2\pi - a_p$

(54)

Exact compensator	1) $\Delta = \frac{1}{2}\pi + 2p$
	2) $\psi = a_p = a_s$
Non-exact compensator	1) $\tan\Delta = \sin\delta \tan(90^\circ + 2P)$
	2) $\cos 2L = -\cos\delta \cos 2P$
	3) $\tan^2\psi = +\tan a_p \tan a_s$
	4) $\tan^2 L = \tan a_p \cot a_s$

The meanings of p , a_p , and a_s in the four zones are as follows:

Zone 1: The fast axis of the compensator is set at $-\pi/4$. The polarizer plane of transmission makes an angle of $+p$ with the plane of incidence. The analyzer plane of transmission makes an angle of $+a_p$ with the plane of incidence.

Zone 2: The fast axis of the compensator is set at $+\pi/4$. The polarizer plane of transmission makes an angle of $-p$ with the surface (an angle of $\pi/2 - p$ with the plane of incidence). The analyzer plane of transmission makes an angle of $+a_s$ with the plane of incidence.

Zone 3: The fast axis of the compensator is set at $-\pi/4$. The polarizer plane of transmission makes an angle of $+p$ with the surface (an angle of $p + \pi/2$ with the plane of incidence). The analyzer plane of transmission makes an angle of $-a_s$ with the plane of incidence.

Zone 4: The fast axis of the compensator is set at $+\pi/4$. The polarizer plane of transmission makes an angle of $-p$ with the plane of incidence. The analyzer plane of transmission makes an angle of $-a_p$ with the plane of incidence.

The meanings of p , a_p and a_s are now clear. The first is the angle between the polarizer plane of transmission and either the plane of incidence or the plane of the surface, while a_p and a_s represent the angle between the analyzer plane of transmission and the plane of incidence, being labeled either a_p or a_s according to whether p is the angle between the polarizer plane of transmission and the plane of incidence or the plane of the surface, respectively.

In practice, the values of p , a_p , and a_s found from the P and A readings in the various zones are not identical. Mc.Crackin et al.(11) pointed out the discrepancy and suggested a simple averaging technique to adjust experimental data. They show that the average of the observed value of p in zones 1 and 3 is the same as the average of the value obtained in zones 2 and 4. The source of this discrepancy is explained by Archer et al.(12) who show that the deviation is due to a difference of trans-

mittance for components of the polarized light with electric vectors coincident with the fast and slow axes of the quarter wave plates.

Influences of non-ideal polarizer, compensator and analyzer on the measurements are treated thoroughly by W.R. Hunter et al. (13) and many others (14-21).

4 Determination of n and k on a clean surface

If the surface is not covered with a thin film we can simplify the Drude equations. In this case $d_1=0$, n_2 and k_2 are unknown.

$$\operatorname{tg} \psi e^{\Delta i} = \frac{\bar{R}_p}{\bar{R}_s} \rightarrow \frac{1 - \operatorname{tg} \psi e^{i\Delta}}{1 + \operatorname{tg} \psi e^{i\Delta}} = \frac{R_s - R_p}{R_s + R_p} =$$

according to eq. 24) and eq. 25)

$$\frac{(n_0 \cos \phi_0 - \bar{n}_1 \cos \phi_1)(\bar{n}_1 \cos \phi_0 - n_0 \cos \phi_1) - (n_0 \cos \phi_0 + \bar{n}_1 \cos \phi_1)(n_1 \cos \phi_1 - n_0 \cos \phi_1)}{(n_0 \cos \phi_0 - \bar{n}_1 \cos \phi_1)(\bar{n}_1 \cos \phi_0 + n_0 \cos \phi_1) + (n_0 \cos \phi_0 + \bar{n}_1 \cos \phi_1)(\bar{n}_1 \cos \phi_0 - n_0 \cos \phi_1)} =$$

$$= \frac{n_0^2 \cos \phi_0 \cos \phi_1 - \bar{n}_1^2 \cos \phi_0 \cos \phi_1}{n_0 \bar{n}_1 \cos^2 \phi_0 - n_0 \bar{n}_1 \cos^2 \phi_1} \left. \vphantom{\frac{(n_0 \cos \phi_0 - \bar{n}_1 \cos \phi_1)(\bar{n}_1 \cos \phi_0 - n_0 \cos \phi_1) - (n_0 \cos \phi_0 + \bar{n}_1 \cos \phi_1)(n_1 \cos \phi_1 - n_0 \cos \phi_1)}{(n_0 \cos \phi_0 - \bar{n}_1 \cos \phi_1)(\bar{n}_1 \cos \phi_0 + n_0 \cos \phi_1) + (n_0 \cos \phi_0 + \bar{n}_1 \cos \phi_1)(\bar{n}_1 \cos \phi_0 - n_0 \cos \phi_1)}} \right\} \rightarrow$$

$$\text{Snellius} \rightarrow \cos \phi_1 = \frac{1}{\bar{n}_1} \sqrt{\bar{n}_1^2 - n_0^2 \sin^2 \phi_0}$$

$$= \frac{\cos \phi_0 \sqrt{\bar{n}_1^2 - n_0^2 \sin^2 \phi_0}}{n_0 \sin^2 \phi_0} \left. \vphantom{\frac{\cos \phi_0 \sqrt{\bar{n}_1^2 - n_0^2 \sin^2 \phi_0}}{n_0 \sin^2 \phi_0}} \right\} \rightarrow \frac{\sqrt{n_1^2 - k_1^2 - n_0^2 \sin^2 \phi_0 - 2i n_1 k_1}}{n_0^2 \operatorname{tg}^2 \phi_0 \sin^2 \phi_0}$$

$$\bar{n}_1 = n_1 - i k_1$$

$$= \frac{\sqrt{n_1^2 - k_1^2 - n_0^2 \sin^2 \phi_0 - \sin^2 k_1}}{n_0^2 \operatorname{tg}^2 \phi_0 \sin^2 \phi_0} = \frac{1 - \operatorname{tg} \psi \cos \Delta - i \operatorname{tg} \psi \sin \Delta}{1 + \operatorname{tg} \psi \cos \Delta + i \operatorname{tg} \psi \sin \Delta} =$$

$$\frac{1 - \operatorname{tg}^2 \psi - 2i \operatorname{tg} \psi \sin \Delta}{1 + \operatorname{tg}^2 \psi + 2i \operatorname{tg} \psi \cos \Delta} = \frac{\cos 2\psi - i \sin^2 \psi \sin \Delta}{1 + \sin^2 \psi \cos \Delta}$$

Separation of the real and imaginary part gives

$$n_1^2 - k_1^2 - n_0^2 \sin^2 \psi_0 = n_0^2 \tan^2 \phi_0 \sin^2 \phi_0 \frac{\cos^2 2\psi - \sin^2 2\psi \sin^2 \Delta}{(1 + \sin 2\psi \cos \Delta)^2}$$

$$2n_1 k_1 = n_0^2 \tan^2 \phi_0 \sin^2 \phi_0 \frac{2 \cos 2\psi \sin 2\psi \sin \Delta}{(1 + \sin 2\psi \cos \Delta)^2}$$

If we measure in air $n_0 = 1$

we get

$$n_1^2 - k_1^2 = \tan^2 \phi_0 \sin^2 \phi_0 \frac{\cos^2 2\psi - \sin^2 2\psi \sin^2 \Delta}{(1 + \sin 2\psi \cos \Delta)^2} + \sin^2 \phi_0 \quad (56)$$

$$2n_1 k_1 = \tan^2 \phi_0 \sin^2 \phi_0 \frac{2 \cos 2\psi \sin 2\psi \sin \Delta}{(1 + \sin 2\psi \cos \Delta)^2} \quad (57)$$

For the calculation of the numerical values see the computer Fortran program Virgin (Chapter IV).

5. Ellipsometric theories in the submonolayer range

5.1 Introduction

Apart from considering the thin layer as homogeneous the optical behaviour of thin films may be considered from another viewpoint. From studies on the structure of the films formed by most available methods it is found that films generally consist of aggregates of crystallites of variable size and orientation and with different extent of accupation. Description of the optical behaviour of such a system is essentially a scattering problem. One can ask the question of how much of optical theory is applicable to this situation.

A number of measurements in the monolayer range has been reported. The adsorption of relatively long polar organic molecules, tagged with ^{14}C (22,23) or tritium (24) on metal substrates was studied simultaneously using ellipsometry and radiotracer methods. The measurements showed at least qualitatively a linear relationship between optical and radiochemical readings, suggesting that the ellipsometric signal is proportional to the amount adsorbed even for films with an incomplete cover. Hayfield and White (25) used controlled vacuum evaporations of calcium fluoride on aluminum and metal oxide surfaces to obtain non-uniform (partly) covered surfaces. They found that the

change in ellipticity followed the average thickness of the film. Clausen and Archer determined ellipsometric adsorption isotherms (ellipsometric effect versus pressure) at room temperature for water and a number of small organic molecules on variously treated silicon surfaces (26,27).

In their interpretation of the data both authors assume that the values of the "index of refraction" of the adsorbed layers are constant and equal to those of the corresponding bulk liquid phases. They found that in most cases the ellipsometric thickness of the monolayer was smaller than the molecular diameter of the adsorbed molecules. Archer ascribed this partly to differences in reactivity of the surfaces and partly to surface irregularities on an atomic scale whereas Clausen preferred the first explanation, implying a non-complete cover of the surface and thus a lower value of the "index of refraction".

The interpretation of the ellipsometric measurements in the (sub) monolayer range is not yet clear. Within the scope of a three-dimensional model in which the layer is characterized by the macroscopic parameters of an index of refraction (n) and a thickness (d) one usually assumes that an incomplete monolayer in which only the fraction θ of the possible sites is occupied behaves like a continuous film (n constant) of effective thickness d_m (d_m : diameter of the molecules). The proportionality between ellipsometric effect and θ then follows immediately from the approximate Drude equations. Another possibility is that the thickness (d_m) is constant and that the index of refraction varies with θ . In general, this no longer implies a linear relationship between ellipsometric effect and θ .

According to Hall (28) one would expect the ellipticity to vary as the square root of the fractional cover.

5.2 Short review of the ellipsometer theories (29, 30, 35)

In this section I shall give a short review of the ellipsometer theories, especially with regard to the submonolayer range. The following subjects will be treated:

1. General equations (macroscopic theory).
2. Approximation used in the macroscopic theory.
3. Some theories in the submonolayer range.
4. Some theories of inhomogeneous films.

5.2.1 General equations

We already found that upon reflection there is generally a change both in the amplitudes A and the phases δ of the two components

$$E_p = A_p \exp(i\delta_p) \text{ and } E_s = A_s \exp(i\delta_s).$$

This change was described by the two angles ψ and Δ defined as eq. 32a).

$$\Delta = (\delta_p^{(s)} - \delta_s^{(s)}) - (\delta_p^{(i)} - \delta_s^{(i)})$$

$$\tan \psi = \frac{A_p^{(r)}/A_s^{(r)}}{A_p^{(i)}/A_s^{(i)}}$$

The total effect upon reflection summarized in equation 32):

$$\frac{\bar{R}_p}{\bar{R}_s} = \frac{\bar{E}_p^{(r)}/\bar{E}_p^{(i)}}{\bar{E}_s^{(r)}/\bar{E}_s^{(i)}} = \tan \psi e^{i\Delta}$$

Here R_p and R_s are the amplitude reflection coefficients which we will use for a reflecting system in general and r_p and r_s for one interphase. Three special cases of the exact Drude theory will be given now.

5.2.1a Clean surface (Fig. III.9a)

For the clean surface the amplitude reflection coefficients take the form of the Fresnel coefficients:

$$r_p = \frac{\bar{n}_1 \cos \phi_0 - n_0 \cos \bar{\phi}_1}{\bar{n}_1 \cos \phi_0 + n_0 \cos \bar{\phi}_1} \quad \text{and} \quad r_s = \frac{n_0 \cos \phi_0 - \bar{n}_1 \cos \bar{\phi}_1}{n_0 \cos \phi_0 + \bar{n}_1 \cos \bar{\phi}_1}$$

$$\text{with } n_0 \sin \phi_0 = \bar{n}_1 \sin \bar{\phi}_1$$

$$\text{and } \bar{n}_1 = n_1 - ik_1$$

In this case the measurement of ψ and Δ yields the real and imaginary parts, n_1 and k_1 of the index of refraction of the substrate (eq.56 and 57)).

5.2.1b Surface covered with one layer (fig. III.9b)

If a film is present on the surface, multiple reflections will occur within the film which for both components lead to extra phase differences. For one passage these are equal to

$$x_1 = \frac{4\pi}{\lambda} n_1 d_1 \cos \phi_1 \quad (\text{eq.27})$$

The total reflection coefficients are derived directly and they are (eq. 30),31))

$$R_p = \frac{r_{1p} + r_{2p} \exp(-ix_1)}{1 + r_{1p} r_{2p} \exp(-ix_1)} \quad \text{and} \quad R_s = \frac{r_{1s} + r_{2s} \exp(-ix_1)}{1 + r_{1s} r_{2s} \exp(-ix_1)}$$

where r_{1p} denotes the Fresnel coefficient for boundary 1 etc.

Fig. III.9 Schematic representation of the reflection of polarized light

- a: clean surface
- b: surface covered with one layer
- c: surface covered with two layers.

5.2.1c Surface covered with several layers (fig.III.9c)

For a system with an arbitrary number of parallel isotropic films the total reflection coefficients R_p and R_s are found by an iterative summation process as described by Crook (8). For the case of fig. III.9c the result is, for both p and s components:

$$R = \frac{r_1 + R' \exp(-ix_1)}{1 + r_1 R' \exp(-ix_1)}$$

$$R' = \frac{r_2 + r_3 \exp(-ix_2)}{1 + r_2 r_3 \exp(-ix_2)}$$

For the computation we make use of a Fortran program called Elli 2, KC, CA, written for a PDP-8 computer. With this program we can calculate values of Δ and ψ for every desired combination of λ, ϕ_0 , and the optical parameters of the reflecting system.

It is also possible to give an explicit expression of the Drude equation in d . The procedure to invert the Drude equation is given in 5.2.1d.

5.2.1d Inversion of Drude's exact ellipsometric equation (43)

The formula of 5.2.1c can be written as follows:

$$\rho \equiv \tan \psi e^{i\delta} = \frac{\left[\frac{r_{1p} + r_{2p} e^{-2i\delta}}{1 + r_{1p} r_{2p} e^{-2i\delta}} \right]}{\left[\frac{1 + r_{1s} r_{2s} e^{-2i\delta}}{r_{1s} + r_{2s} e^{-2i\delta}} \right]},$$

where the Fresnel reflection coefficients are given by:

$$r_{jp} = \frac{n_{j+1} \cos \phi_j - n_j \cos \phi_{j+1}}{n_{j+1} \cos \phi_j + n_j \cos \phi_{j+1}}$$

$$r_{js} = \frac{n_j \cos \phi_j - n_{j+1} \cos \phi_{j+1}}{n_j \cos \phi_j + n_{j+1} \cos \phi_{j+1}}$$

where j denotes interphases 1 or 2 between media n_0 and n_1 or media n_1 and n_2 , respectively.

Let us rewrite the fundamental equation of ellipsometry into a form suitable for the present numerical method. Let $\eta = e^{-2i\delta}$

then the fundamental equation can be expressed as:

$$\rho[r_{1s} + (r_{1p}r_{1s}r_{2p} + r_{2s})\eta + r_{1p}r_{2p}r_{2s}\eta^2] = r_{1p} + (r_{1p}r_{1s}r_{2s} + r_{2p})\eta + r_{1s}r_{2p}r_{2s}\eta^2$$

or more simply as

$$A\eta^2 + B\eta + C = 0$$

where:

$$A = (\rho r_{1p} - r_{1s})r_{2p}r_{2s}$$

$$B = r_{1p}r_{1s}(\rho r_{2p} - r_{2s}) + (\rho r_{2s} - r_{2p})$$

$$C = \rho r_{1s} + r_{1p}$$

The solutions of the quadratic equation are simply:

$$\eta = [-B \pm (B^2 - 4AC)^{\frac{1}{2}}] / 2A$$

Substitution of the Fresnel coefficients give

$$d = (\lambda/4\pi) [i \ln \eta / (n_1^2 - n_0^2 \sin^2 \phi_0)^{\frac{1}{2}}]$$

Because the left-hand side of the above equation is real and that of the right hand side is complex, the above complex equation can be written as

$$d = [\lambda/4\pi (n_1^2 - n_0^2 \sin^2 \phi_0)^{\frac{1}{2}}] \operatorname{Re}(i \ln \eta)$$

$$\operatorname{Im}(i \ln \eta) = 0$$

where Re and Im denote real and imaginary parts respectively of the quantity in the {} brackets. η is a function of ϕ , Δ , ψ , n_0 , n_1 , n_2 . Because λ , ϕ , n_0 , Δ , and ψ are known from the experimental measurements the film thickness is a function of three unknown optical parameters n_1 , n_2 , and k_2 only. This function can numerically be calculated by standard procedures.

5.2.2 Approximations (macroscopic theory)

Although the computer permits the use of exact equations, approximations are sometimes preferred for reasons of clarity and comparison.

5.2.2a Approximated Drude equations for thin films (50-100 Å) (Fig. III.9b) (4,5,10,31,33)

When a very thin film $d_1 \ll \lambda$ is present on a clean substrate (characterized by $\bar{\Delta}$ and $\bar{\Psi}$) one gets from equation 33) using the approximations $e^{ix} = 1 - ix$ $1/(1 - ix) = 1 + ix$ for $n_0 = 1$ and $k_1 = 0$

$$\tan \psi e^{i\Delta} = \tan \bar{\psi} e^{i\bar{\Delta}} \left\{ 1 - \frac{iF4\pi\bar{n}_2^2 \cos \phi_0 \sin^2 \phi_0}{\lambda(\bar{n}_2^2 - 1)(\bar{n}_2^2 \cos^2 \phi_0 - \sin^2 \phi_0)} \right\} \quad (58)$$

The parameter F contains the information about the layer (d_1, n_1) and is given in table 1 (first column).

	Drude	Strachan	Sivukhin
F	$d_1(n_1^2 - 1) \left(\frac{1}{n_1^2} - \frac{1}{\bar{n}_2^2} \right)$	$4\pi(\tau_z - \frac{\tau_x}{\bar{n}_2^2})$	$(\gamma_2 - \gamma_x) \left(1 - \frac{1}{\bar{n}_2^2} \right)$
L	$d_1(n_1^2 - 1) \left(\frac{1}{n_1^2} - a_2 \right)$	$4\pi(\tau_z - \tau_x a_2)$	$(\gamma_2 - \gamma_x)(1 - a_2)$
M	$d_1(n_1^2 - 1)$	$4\pi\tau_z$	$(\gamma_2 - \gamma_x)$

Table III.1 Expressions for the parameters F, L, and M in equations 58), 59), and 60) for the Drude, Strachan and Sivukhin models.

Separating the real and imaginary parts at both sides of equation (58) gives with introduction of:

$$\delta\Delta = \bar{\Delta} - \Delta$$

$$\delta\psi = \bar{\psi} - \psi$$

The expressions for $\delta\Delta$ and $\delta\psi$ read:

$$\delta\Delta = 4\pi\cos\phi_0\sin^2\phi_0 \times$$

$$\times \frac{L\{\cos^2\phi_0 - a_2 + \sin^2\phi_0(a_2^2 - a_2'^2)\} + Ma_2'^2(1 - 2a_2\sin^2\phi_0)}{\lambda[(\cos^2\phi_0 - a_2 + \sin^2\phi_0(a_2^2 - a_2'^2))^2 + a_2'^2(1 - 2a_2\sin^2\phi_0)^2]} \quad (59)$$

and

$$\delta\psi = -2\pi\cos\phi_0\sin^2\phi_0\sin 2\bar{\psi}a_2' \times$$

$$\times \frac{L(1 - 2a_2\sin^2\phi_0) - iH(\cos^2\phi_0 - a_2 + \sin^2\phi_0(a_2^2 - a_2'^2))}{\lambda[(\cos^2\phi_0 - a_2 + \sin^2\phi_0(a_2^2 - a_2'^2))^2 + a_2'^2(1 - 2a_2\sin^2\phi_0)^2]} \quad (60)$$

$$\text{where } a_2 = \frac{1 - k_2^2/n_2^2}{n_2^2(1 + k_2^2/n_2^2)^2}$$

$$\text{and } a_2' = \frac{2k_2/n_2}{n_2^2(1 + k_2^2/n_2^2)^2}$$

The expressions for the parameters L and M are given in the first column of table III.1. It should be noted that values of $\delta\psi$ calculated with (59) and (60) can deviate already considerably from the exact values for thicknesses of less than $10 \text{ \AA}$ (32,33).

5.2.2b Multiple film systems (Fig.III.9c)

For very thin films (total thickness $\ll 50\text{--}100 \text{ \AA}$) the change due to any film is the same as that which would occur if the film were present as a single layer (27).

Mertens et al.(34) proposed a method of accounting for the presence of the first layer on the substrate by calculating the apparent optical

constants (N_2 and K_2) of substrate and layer from the measured Δ and ψ values (at $d_1=0$). The effect of the following layer is then calculated with these optical parameters.

This method was checked by C.A. Bootsma and F. Meyer (35) who used the method of Crook (8). The differences between their values and those obtained with the Mertens method were never more than 3%.

5.2.3 Theories for the submonolayer range

5.2.3a Extrapolated macroscopic theory (Drude model)

The optical properties of a system consisting of a large number of spherical metal particles of a diameter that is small compared to the wavelength of the light used and embedded in a dielectric medium were investigated in 1904-1906 by J.C. Maxwell Garnet (36). In the light of the results of structure examinations of thin films it is clear that the thin film approximates better the system considered by Maxwell Garnet than to a parallel-sided homogeneous slab of bulk metal. The proposed model envisaging spherical particles possessing properties identical to those of the bulk metal is a considerable simplification of the real thing; the agreement between the predictions of the simple theory and the experimental results, although in some instances not quantitatively impressive, is sufficient to demonstrate the essential correctness of this approach to the problem (37).

When an electromagnetic wave traverses such a system, the spherical crystallites will be polarized by the electric field E of the wave and will thus produce an additional field E_s . This field may be calculated in precisely the way in which the polarization field in a dielectric is calculated. In this model we can use the Lorentz relation:

$$E^e = E + \frac{4\pi P}{3} \quad (61)$$

for the effective local field E^e at an atom. In this case one can obtain the wellknown Clausius Mosotti relation (Born) (2)

$$\frac{\epsilon - 1}{\epsilon + 2} = \frac{4\pi\alpha}{3a^3}$$

where ϵ is the dielectric constant, α is the atomic polarizability and a^3 is the atomic volume. If the Maxwell relation $n = \sqrt{\epsilon\mu}$ is used,

and we assume $\mu=1$, we get $\epsilon=n^2$, so

$$\frac{n^2-1}{n^2+2} = \frac{4\pi\alpha}{3a^3} \quad (62)$$

known as the Lorenz-Lorentz equation (Born). This relation is seen to be a bridge which connects Maxwell's phenomenological theory with the atomic theory of matter. Extrapolation of this equation to a monolayer with formal index of refraction n_m occupied by molecules with diameter d_m and cross-section c_m gives

$$\frac{n_m^2-1}{n_m^2+2} = \frac{4\pi}{3} \frac{\alpha}{d_m c_m} \quad (63)$$

The subscript m is used for parameters characteristic for the complete monolayer. For a further extrapolation into the submonolayer range, in which only the fraction $\theta < 1$ of the possible sites is occupied, we consider the following possibilities:

1. constant index of refraction (n_m) varying average thickness ($d_m \theta$). Here eq. 62) is no longer valid for $\theta < 1$.
2. Varying index of refraction (n_θ), constant thickness (d_m).

In both cases the dependence of $\delta\Delta$ and $\delta\psi$ on θ is again described by the equations eq. 59) and eq. 60). In case 1. one has to replace d_1 by $d_m \theta$ and n_1 by n_m in L and M of Table III.1. In case 2. on the assumption of the validity of eq. 61) for the sub-monolayer range too, one gets upon replacing c_m by c_m/θ in eq. 63).

$$\frac{n_\theta^2-1}{n_\theta^2+2} = \theta \frac{n_m^2-1}{n_m^2+2} \quad (64)$$

In L and M thickness d_1 has to be replaced by d_m and n_1 by n_θ . The relationship between $\delta\Delta$ and θ can be derived from equations 59) and 64).

Schopper (37) modified this Maxwell Garnet theory by the assumption of ellipsoidal particles instead of spherical particles. This theory gives in the case of goldfilms even better agreement with the experimental results.

Another suggestion for the dependence of $\delta\Delta$ on θ in the submonolayer

range was given by Hall (28) by assuming that an incomplete monolayer scatters incoherently, he proposes that the ellipsometric signals vary as the square root of coverage.

5.2.3b Quasi microscopic theory (Strachan's model) (38)

In treating the optical effect of submonolayers a physically more appropriate model is that of a two-dimensional distribution of Hertzian oscillators. In Strachan's model the film is characterized by the scattering indices τ_x , τ_y and τ_z in the three directions x , y (both parallel to the surface) and z (normal to the surface). These indices are defined as proportionality factors between the x , y , and z components of the oscillator strength per unit surface area and the components of the total electric field. According to Strachan an element dS of the surface becomes a Hertzian oscillator of strengths $dS(\tau_x A_x, \tau_y A_y, \tau_z A_z)$ (65).

By correcting for the field produced by the oscillators on both sides of the surface, new boundary conditions are found, from which by keeping only first order terms Strachan derives the Fresnel coefficients R_p and R_s . From these expressions we get for $R_p/R_s, \delta\Delta$ and $\delta\psi$ equations 32), 59), and 60) with F , L , and M as given in the second column of table III.1 for $\tau_x = \tau_y$. Strachan's theory is of a quasi-microscopic nature. Strachan does not give a microscopic interpretation of the τ 's in terms of structure and molecular polarizabilities. In cases of low oscillator strengths per unit area, where the influence of the dipole fields of the other adsorbed molecules can be neglected, one can identify the τ with polarizability per surface area

$$\tau_i = \alpha_i / c \quad i=x,y,z \quad (66)$$

This is equivalent to a linear dependence of τ on coverage θ , and therefore implies also a linear relationship between $\delta\Delta$ and θ . For higher oscillator strengths relation eq. 66) may no longer be used.

5.2.3c Microscopic theory (Sivukhin's model) (39,40)

Using the results of earlier calculations by Ewald, Sivukhin also derived expressions for R_p and R_s . In his theory the transition layer at the surface is characterized by the parameters γ_x, γ_y (parallel to the surface) and γ_z (normal to the surface). These parameters are defined as

$$\gamma_i = \frac{\tau_i}{P_i} \quad i=x,y,z$$

Here τ is the dipole moment induced in the transition layer per unit surface area and P the polarization vector of the homogeneous substrate. The resulting expressions for R_p/R_s and also for $\delta\Delta$ and $\delta\psi$ are again given by eq. 32), eq. 59) and eq. 60) with F , L , and M as given in the third column of table I (for $\gamma_x = \gamma_y$).

By electrostatic calculations Sivukhin related the γ 's to the structure and molecular polarizabilities of substrate and layer. His considerations for the case of a monolayer on a homogeneous isotropic substrate (a simple cubic lattice) will be summarized here. The layer consists of atoms with polarizability α_0 and mutual distance a_0 for the substrate these parameters are denoted by α and a . The effective field E_0^e working at an atom in the origin of the layer is written as the sum of three terms

$$E_0^e = E^{\text{hom}} + E^s + E^{\text{inh}} \quad (67)$$

The first term E^{hom} is the homogeneous field produced by the incident wave and the dipoles of the substrate lattice. This field is equal to the average field in the macroscopic Maxwell theory

$$E_{x,y}^{\text{hom}} = E_{x,y} \quad E_z^{\text{hom}} = E_z + 4\pi P_z \quad (68)$$

The second term in eq. 67) E^s represents the field of the dipoles in the adsorbed layer and is calculated by a two-dimensional summation, resulting in

$$E_{x,y}^s = \frac{A_0}{2a_0^3} P_{0,x,y} \quad E_z^s = -\frac{A_0}{a_0^3} P_{0,z} \quad (69)$$

Here A_0 is the lattice sum depending only on the symmetry of the layer. $A_0 = 9.035$ for a simple cubic lattice and 11.034 for a hexagonal lattice (41) and P_0 is the atomic dipole moment ($P_{0i} = \tau_{ic}$). The third term in eq. 67) E^{inh} the inhomogeneous field of incident wave and substrate lattice is zero at a great distance from the surface Sivukhin derives for E^{inh} the following expression

$$E_{x,y}^{\text{inh}} = -\frac{1}{2} B P_{x,y} \quad E_z^{\text{inh}} = B P_z \quad (70)$$

For a simple cubic lattice where eq. 61) is valid

$$B = \left(\frac{A}{Z} - \frac{4\pi}{3} \right) \exp\{2\pi(a - a_1)/a\} \quad (71)$$

Here A is the lattice sum for a plane in the substrate lattice parallel to the surface and a_1 is the distance between the plane of the layer and the first plane in the substrate lattice. Insertion of eq. 68), eq. 69), and eq. 70) in eq. 67) yields with

$$P_i = \alpha_i E_i^e \quad P_{0i} = \alpha_{0i} E_{0i}^e \quad (72)$$

The following expressions for $\gamma_{x,y}$ and γ_z :

$$\gamma_{x,y} = \frac{\alpha_{ox,y}}{c} \left(\frac{a^3}{\alpha} - \frac{4\pi}{3} - \frac{B}{2} \right) / \left(1 - \frac{A_0}{2a_0^3} \alpha_{ox,y} \right)$$

$$\gamma_z = \frac{\alpha_{0,z}}{c} \left(\frac{a^3}{\alpha} + \frac{8\pi}{3} + B \right) / \left(1 + \frac{A_0}{a_0^3} \alpha_{0,z} \right) \quad (73)$$

The dependence of $\gamma_z - \gamma_x$ and therefore of $\delta\Delta$ on the degree of coverage θ is rather complicated as both the cross-section c and the distance between the atoms in the layer a_0 change with θ .

6 Computation methods for some inhomogeneous films (42)

Only non-adsorbing films are considered which are such that their refractive index is a function of the coordinate z only, the direction O_z being the normal to the planes which limit the film.

Two different methods of computation are discussed below:

- the use of the Maxwell differential equation;
- the replacement of the inhomogeneous layer by a number of homogeneous layers.

These two methods are general which means that they are not restricted to special experimental circumstances but can be employed in any circumstance.

6.1 Maxwell differential equation

The thin film is bound by the planes $z=0$ and $z=d$. It is comprised between two semi-infinite media, the refractive indices of which are n_0 (for $z<0$) and n_s (for $z>d$). Light is incident on the plane $z=0$ and we shall generally have $n_0=1$. The refractive index under study $\tilde{n}(z)$ is a complex function of z which means that it can be absorbing. It is convenient to use the dielectric constant of the film $\epsilon(z)=\tilde{n}^2(z)$. The refractive index of the substrate, n_s , may be complex which occurs when the medium is adsorbing.

The law of refraction will be written:

$$n_0 \sin \phi_0 = n_s \sin \phi_s = S \quad (74)$$

In the layer the fields verify a wave equation which is a direct consequence of Maxwell's equations. First the s vibration is discussed. We shall choose the xoy coordinates in such a way as to have $E = (E_x, 0, 0)$ which means that the plane yoz is the plane of incidence. We are looking for solutions of Maxwell's equations which are:

$$E_x = U(z) \exp(i(\omega t - kSy))$$

$$H_y = V(z) \exp(i(\omega t - kSy))$$

Here the notation $k = 2\pi/\lambda$ is used, λ being the wavelength of the incident light in vacuo.

It can be shown that

$$V(z) = \frac{i}{k\mu} \frac{dU}{dz} \quad (75)$$

μ being the magnetic permittivity.

U verifies the second order differential equation (wave equation).

$$\frac{d^2 U}{dz^2} - \frac{d \log \mu}{dz} \frac{dU}{dz} + k^2 (\epsilon \mu - S^2) U = 0 \quad (76)$$

for the p vibration

$$\frac{d^2 U}{dz^2} - \frac{d \log \epsilon}{dz} \frac{dU}{dz} + k^2 (\epsilon \mu - S^2) U = 0 \quad (77)$$

let $U_1(z)$ and $U_2(z)$ be two arbitrary solutions of the wave equation. The general integrals of eq. 75) and eq. 76) are

$$U = aU_1(z) + bU_2(z) \quad (78)$$

$$V = \frac{i}{k\mu(z)} (aU'_1(z) + U'_2(z)) \quad (79)$$

a and b being the arbitrary constants which must be chosen in order to satisfy the boundary conditions. On the plane $z=0$ we must have

$$E_0 = aU_1(0) + bU_2(0) \quad (80)$$

$$H_0 = \frac{i}{k\mu(0)} (aU'_1(0) + bU'_2(0)) \quad (81)$$

E_0 and H_0 being the tangential components of the fields in the first medium on the plane $z=0$. The equations 80) and 81) may be written:

$$\begin{bmatrix} E_o \\ H_o \end{bmatrix} = \begin{bmatrix} U_1(0) & U_2(0) \\ iU'_1(0) & iU'_2(0) \\ k\mu(0) & k\mu(0) \end{bmatrix} \begin{bmatrix} a \\ b \end{bmatrix} = [k(0)] \begin{bmatrix} a \\ b \end{bmatrix} \quad (82)$$

In the same way the boundary conditions on the plane $z=d$ are

$$\begin{bmatrix} E_t \\ H_t \end{bmatrix} = [k(d)] \begin{bmatrix} a \\ b \end{bmatrix} \quad (83)$$

E_t and H_t being the tangential components of the fields in the substrate on the plane $z=d$. Combining we may write:

$$\begin{bmatrix} E_o \\ H_o \end{bmatrix} = [k(0)] [k(d)]^{-1} \begin{bmatrix} E_t \\ H_t \end{bmatrix} = [M] \begin{bmatrix} E_t \\ H_t \end{bmatrix} \quad (84)$$

This shows that an inhomogeneous film may be characterized by a two-by-two matrix like a homogeneous one. The advantage of this representation is that the complex reflected and transmitted (if it exists) amplitudes will have the same expression as for homogeneous films, namely:

$$r_s = \rho_s \exp(i\delta_{rs}) \frac{y_o(m_{11} + y_s m_{12}) - (m_{21} + y_s m_{22})}{y_o(m_{11} + y_s m_{12}) + (m_{21} + y_s m_{22})} \quad (85)$$

where $[M] [m_{ij}]$ ($i, j=1, 2$) $y_o = n_o \cos \phi_o$ $y_s = n_s \cos \phi_s$

In the same way the expression for r_p is found.

6.2 An inhomogeneous film replaced by a pile of homogeneous ones

It is possible to replace an inhomogeneous film by a superposition of homogeneous films of different refractive indices. In that case the problem pertaining to the solution of the differential field equations is eliminated. The only difficulty is the estimation of the number of homogeneous layers which is necessary for the replacement of the inhomogeneous one. In practice this is done by successive

trials. The inhomogeneous film is replaced first by a unique layer having an average refractive index

$$\bar{n} = \frac{1}{d} \int_0^d n(z) dz \quad (86)$$

and the computation is done.

Then the film is replaced by two, three etc. homogeneous films and the values of ψ and Δ are computed. The refractive index of each layer is an average of the refractive indices of the inhomogeneous layer it replaces. The satisfactory result will be obtained when ψ_j, Δ_j , and ψ_{j+1}, Δ_{j+1} do not differ from each other to the desired accuracy (the index indicates the number of layers which replace the inhomogeneous film). There are no practical difficulties when using a large and rapid computer. The computation may take some time when the variation of the refractive index is rapid.

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CHAPTER IV

Calculations of thickness and refractive index from analyzer and polarizer data

1 Introduction

The use of the exact Drude equations involves calculations which can not be solved without the aid of the computer. We, therefore, made computer programs which could convert analyzer- and polarizer data into thickness and refractive index. The following programs were developed:

1. Virgin: this program calculates the effective refractive index of the reflecting surface on which the adsorption experiments are to be carried out.
2. Ellis: the program Ellis simulates the theoretical behaviour of any set of layers on any surface. Because the Drude equations are difficult to solve in a closed form we calculated analyzer and polarizer settings to a large series of thicknesses and refractive indices. In this way a map was created from which the thickness and refractive index could be read from any analyzer and polarizer setting to be expected.
3. Ellip: the program Ellip gives the graphical representation of values calculated by Ellis.

2 Computer programs

These three computer programs will now be discussed in detail.

2.1 Virgin

The computer program Virgin was developed to calculate the optical constants n and k of the reflecting surface, on which the processes we wanted to study, took place. These values of n and k are essential for the input of the computer program Ellis. The calculations of the Virgin program are based on the formulae for a clean surface derived in Chapter III. As the reflecting surface is immersed in a dielectric medium a correction has to be applied to formulas (56,57) as they are derived from a reflecting surface in air.

Input: Read δ , ψ , angle of incidence, refractive index of the medium.

DELTA,PSI,FIO(3F7.2): 108.20 23.02 68.00
 REFRACTIVE INDEX OF THE MEDIUM(F6.4):1.3335

Program

```

5  READ(1,10)DELTA,PSI,FIO
10 FORMAT('DELTA,PSI,FIO(3F7.2):'3F7.2)
   READ(1,15)RNO
15  FORMAT('INDEX OF REFRACTION OF THE MEDIUM(F6.4):'F6.4)
   PI=3.14159
   RAD=2.*PI/360
   DELTA=DELTA*RAD
   PSI=PSI*RAD
   FIO=FIO*RAD
   U=(TAN(FIO)*SIN(FIO))*2
   V=(1.+SIN(2.*PSI)*COS(DELTA))*2
   W=COS(2.*PSI)*2-(SIN(2.*PSI)*SIN(DELTA))*2
   X=SIN(4.*PSI)*SIN(DELTA)
   Y=U/V
   Z=W*Y+SIN(FIO)*2
   HI=0.5*SQRT(Z*2+(X*Y)*2)
   REN=SQRT(0.5*Z+HI)
   CON=X*Y/2./REN
   REN=RNO*REN
   CON=RNO*CON
   WRITE(1,20)REN,CON
20  FORMAT('N='F7.4,' K='F7.4)
   GOTO 5
   END

```

Output: n=2,8852 k=-2,5639

2.2 Ellis

The ellipticity of light reflected on a surface can be changed by a number of parameters. Thickness and refractive index of a dielectric layer are of interest as they are the variables we want to follow in a given experiment. Others as n and k of the bare surfaces (see above), angle of incidence, wavelength of the light and refractive index of the medium can be kept constant in one experiment. Ellis calculates the dependency of azimuth and ellipticity on thickness and refractive index in a given experimental setup: over a range in which the experimental changes are to be expected. It thus maps out the (n,d) pairs on a (ψ,Δ) plane.

These maps are used to convert experimental data (ψ, Δ) into the variables of biochemical interest (n, d). These maps will be called Conversion Maps.

The use of Ellis is not restricted to one variable dielectric layer on a bare surface. Any number of dielectric layers can be taken into account, and anyone of these layers can be treated as the variable one, once the optical constants of the others are given.

Description of the program.

As an example we give the input of a system, consisting of three layers: air, barium stearate and chromium. On this chromium layer we simulate the growth of a layer of barium stearate in steps of $25 \text{ \AA}$ till a thickness of $500 \text{ \AA}$ is reached. Angle of incidence $\phi = 68^\circ$ degrees. Wavelength of the laser light is $632,8 \text{ nm}$.

The input is given in the following form

N=3

V(1,1)=1.0000 V(2,1)=0.0000 D(1)=0.0

V(1,2)=1.4800 V(2,2)=0.0000 D(2)=0.0

V(1,3)=2.1092 V(2,3)=-2.3685

DR=0.0000 DC=0.0000 DD=25.0

F=68.0000 DF=0.0000 W=6328.0

LR=2, LC=1, LD=2, NR=1, NC=1, ND=20, NF=1

Explanation is given in table IV.1.

READ DATA

N=TOTAL NUMBER OF LAYERS

V(1,J)=REAL PART REFRACTION INDEX LAYER J

V(2,J)=COMPLEX PART REFRACTION INDEX LAYER J

D(J)=THICKNESS LAYER J IN ANGSTROM

LR,LC,LD=NUMBER OF LAYER TO BE INCREMENTED

DR,DC,DD=INCREMENT OF V(1,LR),V(2,LC),D(LD)

NR,NC,ND=NUMBER OF INCREMENTS DR,DC,DD

CI=COS INCIDENT ANGLE LAYER I

RP,RN=FRESNEL COEFF.

TRP,TRN=TOTAL REFLECTION COEFF.(MC.CRACKIN)

PSI(J)=CALCULATED VALUE OF PSI

DEL(J)=CALCULATED VALUE OF DELTA

T1,T2=STORAGE VARIABLES

W=WAVELENGTH IN ANGSTROM

F=ANGLE OF INCIDENCE

DF=INCREMENT OF F

NF=NUMBER OF INCREMENT DF

Table IV.1. Explanation of the input variables of Ellis.

Because of the complexity of this program which has several subroutines like "Complex Arithmetic" (CA) and "Reflection Coefficient" (RC) we only give here the flowchart of the program in fig. IV.2.

Fig. IV.2. Flowchart of Ellis.

Output

The output is given in the following form: the first five rows are the ψ values, the last five ones the Δ values. With the given input the following output was obtained:

26.56	27.03	27.51	27.99	28.47	113.48	110.80	108.25	105.81	103.48
28.95	29.44	29.92	30.41	30.89	101.26	99.15	97.15	95.25	93.46
31.38	31.87	32.36	32.86	33.36	91.77	90.18	88.69	87.30	86.01
33.87	34.39	34.92	35.46	36.02	84.82	83.74	82.76	81.89	81.13

This output is also given in the form of a paper tape. This paper tape gives the values of ψ and Δ with a higher accuracy and can be used as input for the program Ellip.

2.3 Ellip

The data obtained by the program Ellis can be plotted by the program Ellip. This program requires as accessory input a specification of the graphical output format of the conversion map.

3. Applications

We will now illustrate the type of variation of ψ and Δ with n and d under various simulations of experimental circumstances.

3.1 The growth of layers of different real parts of refractive index onto a silicon surface

In fig. IV.3. a system consisting of a silicon reflecting surface with layers of varying refractive index and various thicknesses is represented. The refractive indices vary between 1.2 and 3.5 as is indicated in the graphs. The lines show the effect of changing thickness at constant refractive index. The marks on each line represent a change in thickness of 25 Å. This figure shows the differences in sensitivity of both analyzer and polarizer in different regions of the plane.

In region A e.g. Δ varies with d and ψ with n ; in region B the opposite is true. In region C ψ varies with d and Δ is hardly sensitive to n . In region B a change in d of 25 Å causes a change in Δ of 70°, whereas in region B the sensitivity is more than ten times less.

From this type of graph it is clear that

- a simulation of the situation to be expected experimentally should be carried out in order to see if indeed the experimental setting is adequate c.q. how it can be changed to become adequate;
- any method, using a fixed polarizer setting and estimating analyzer changes by measuring the intensity of the transmitted light is applicable only under very restricted circumstances.

Fig. IV.3. Map of Δ, ψ on n, d for a silicon surface ($n=4,05$ $k=0,028$). Angle of incidence: 68° ; refractive index of the medium $n=1$ (air); wavelength $6328 \text{ \AA}$ (see text).

With the different refractive indexes the periodicity of the function is reached at a thickness which is shown in the next table.

$n=1,1$	$d_p = \pm 5500$	$\text{\AA}$
1,2	4400	$\text{\AA}$
1,3	3750	$\text{\AA}$
1,4	3250	$\text{\AA}$
1,5	2750	$\text{\AA}$
1,6	2500	$\text{\AA}$
1,8	2250	$\text{\AA}$
2,0	2000	$\text{\AA}$
2,5	1500	$\text{\AA}$
3,0	1250	$\text{\AA}$
3,5	1000	$\text{\AA}$

These periodicities set a practical limit to the thickness of the layers that can be measured.

3.2 The influence of the optical properties of the reflecting surface

In fig. IV.4. the variation of ψ and Δ with n and d of a Ba-stearate layer ($n=1,47$) is shown with silicon or with chromium, as the underlying reflective surface.

These graphs show clearly the difference in behaviour of both systems. The change in Δ caused by a step of $0.50 \text{ \AA}$ in the silicon system is 10.28 degrees, the change in ψ in this case is only 0.15 degrees. In the chromium system these changes are respectively 6.06 degrees and 0.89 degrees.

Consequently in the silicon system we have a higher sensitivity to changes in thickness but in the chromium system we can differentiate better between several refractive indices. The curves obtained on chromium at different refractive indices diverge at a smaller thickness than in the case of silicon.

The influence of varying the complex part of the refractive index of the reflecting surface on the conversion map of barium stearate (fig. IV.5.) shows that there is hardly any difference in sensitivity unless the complex value is very small. A difference is observed between $n=2,8-0,05 i$ and $n=2,8-0,55 i$ only.

Fig. IV.4. Conversion map for Ba-stearate ($n=1,47$) onto silicon ($n=4,05$ $k=0,028$), and on chromium ($n=2,8$ $k=2,5$) $\phi=68^\circ$ $\lambda=6328 \text{ \AA}$. The figure indicates d in Ångström.

Fig. IV.5. The influence of varying the complex part of the refractive index of the reflecting surface on the conversion map of barium stearate. Optical constants: chromium $n=2,8$ $k=0,05$ - $5,05$ in 10 steps of $0,5$; barium stearate $n=1,47$, thickness $0-750 \text{ \AA}$ in 15 steps of $50 \text{ \AA}$. Angle of incidence $\phi=68^\circ$.

3.3 The influence of the angle of incidence (fig. IV.6.)

The graph shows a growth of a barium stearate layer ($n=1,47$) in steps of $125 \text{ \AA}$ until a thickness of $600 \text{ \AA}$ is reached on a chromium surface ($n=2,8 - 2,3 i$) at 10 different angles of incidence ($55-82^\circ$).

In the graph the difference is indicated in Δ value caused by one step of $125 \text{ \AA}$. An optimal sensitivity is found between $73-67$ degrees.

3.4 The influence of wavelength on the sensitivity of $n=1,47$ layers on chromium

In fig. IV.7. five graphs of d versus ψ , Δ at $n=1,47$ ($d=10 \times 50 \text{ \AA}$) are plotted for wavelengths of 2328 , 4328 , 6328 , 8328 , and $10,000 \text{ \AA}$. The reflecting surface is chromium ($n=2,8-2,3 i$). In this figure one notices that the five curves completely overlap, the only difference lies in the sensitivity of the thickness. $500 \text{ \AA}$ at $2328 \text{ \AA}$ wavelength gives about the same effect at $1200 \text{ \AA}$ at $6328 \text{ \AA}$. The differences caused by a change of $50 \text{ \AA}$ of $n=1,47$ are plotted in fig. IV.8.

3.5 The influence of varying real parts of refractive index of the immersion medium on the chromium barium stearate system (fig. IV.9.)

The reflecting surface is chromium ($n=2,8-2,3 i$); $n=1,50$; $d=10 \times 40 \text{ \AA}$. The refractive index of the immersion medium varies between 1.0 and 1.9 . Obviously the sensitivity is nil for $n=1,5$, i.e. when no difference between the refractive indexes of the medium and the substrate exist. The sensitivity increases with the difference between the refractive indices.

Fig. IV.6. Influence of angle of incidence on the sensitivity at a change in thickness of $125 \text{ \AA}$ at $n=1.47$ on chromium ($n=2.8-2.3 i$). The different angles ($55^\circ-82^\circ$) are indicated in the graph. Each mark indicates a $125 \text{ \AA}$ step in thickness.

Fig. IV.7. Influence of wavelength on the sensitivity of the growth of barium stearate layers upon chromium (see text). The abscissa gives the correct value for $\lambda=2328 \text{ \AA}$ only. The other graphs are shifted to the right 10 mm per graph. Plotted normally, they fall within less than 0.5 mm of the $\lambda=2328 \text{ \AA}$ graph.

Fig. IV.8. The changes caused by $50 \text{ \AA}$ barium stearate on chromium measured at different wavelengths.

Fig. IV.9. The influence of varying the real parts of the refractive index of the immersion medium on the chromium barium stearate system (see text).

CHAPTER V

Experimental calibrations

1 Introduction

In order to be able to check the functioning of the instrument and the precise characteristics of the optical components it is necessary to calibrate these components. If necessary corrections can be introduced in the equations, from which n and d are calculated to accommodate the non ideal behaviour of the optical components. Consequently they have to be checked against predictable observations (1-6), i.e. the instrument has to be calibrated using known standards.

Three different sets of experiments were carried out:

1. Exact determination of the angle of incidence from the reflection on a dielectric of a known refractive index.
2. Variation of the angle of incidence to obtain an optimal response from the reflexion of five barium stearate monolayers on a chromium surface.
3. Calibration of the instrument with 1-101 layers of barium stearate transferred to a chromium surface.

In the last two systems the calibration was done by means of multilayers of barium stearate on a chromium covered glass slide. For this reason we will first describe this technique of preparation of multilayers on solid surfaces.

1.1 Preparation of monolayers

A monomolecular barium stearate film is formed on water by putting a small amount of stearic acid, dissolved in benzene, on a clean surface of buffer containing bariumsalts (3×10^{-5} M BaCl_2 , 4×10^{-4} M KHCO_3 , pH 7.0 - 7.2, temperature 22°C). The stearic acid spreads rapidly in a monomolecular film. In this pH range the Ba^{2+} ions in the water combine with the carboxylgroups of the spread molecules, converting the film to barium stearate.

Langmuir, Blodgett and many other investigators have demonstrated (7-15, 20) that the fatty acids tend to orient themselves on a water surface with the $-\text{COOH}$ "head" of each molecule in contact with the water and the chain tilted upwards in a more or less steep angle to the plane of the water surface.

1.2 Transfer of the monolayers to solid surfaces

In our experiments we used thoroughly cleaned chromium coated glass slides as the solid support. The cleaning was done with Rogipal * and twice distilled water. A good quality of distilled water is compulsory. The clean chromium surface is hydrophobic. Chromium surfaces treated with chromic acid - H_2SO_4 give hydrophilic chromium oxide films.

The film is transferred from water to chromium by lowering a clean slide slowly into the water on which the film is spread by means of a motor driven device. By lowering the hydrophobic surface into the water, the fatty acid chain bound to the surface and the layer is transferred to chromium with the hydrocarbon tails attached to the surface. The exposed upper surface of the film is composed of closely packed $-(\text{COO})_2 \text{Ba}$ groups. By raising this slide slowly out of the water, a second layer attaches with the polar head groups bound to the polar head groups of the already adsorbed fatty acid molecules. The slide emerges from the water completely dry. Now the exposed upper surface of the film is composed of closely packed hydrocarbon groups. So, when starting with a hydrophobic surface one gets even numbers of layers. While starting with a hydrophilic surface one gets odd numbers of layers.

A film in which the molecules are orientated with the hydrocarbon groups outwards will be unwettable. The property of shedding water is an important factor in these experiments. As the chromium slide is raised out of the water on which the film is spread, the water recedes from the glass wherever the film attaches to the solid surface. If the slide is withdrawn from the water rather slowly (1-3 cm per minute) it will emerge completely dry. This enables us to select a lifting speed at which the film is deposited uniformly over the entire surface.

In this method it is important that the film is kept under constant pressure while the chromium is being raised. This pressure can be kept constant in two ways, described under 1.2.1 and 1.2.2.

1.2.1 Piston oil method

This method was first described by Langmuir (1, 6-19) who pointed out that pressure could be most conveniently applied by placing a tiny drop of castor oil, or oleic acid, or any kind of hydrophilic oil, at one end of the trough. A hydrophilic oil has the property that the molecules of the oil spread on water until they have covered all the available area with a monomolecular layer and crowd into this layer until they have established an equilibrium surface pressure.

*) Rogier Bosman B.V., Rotterdam, Holland.

The equilibrium pressure exerted by an oil is characteristic for each individual pure oil, e.g. oleic acid exerts approximately twice the pressure of castor oil. After the surface is covered with a monomolecular layer, the surplus oil remains gathered in many droplets. If, however, new space becomes available on the water surface, the oil spreads instantaneously from these lenses, and covers the new area with a monomolecular layer too. Thus a drop of oil placed at one end of a trough on which a barium stearate film has been spread, acts as a surface piston to hold the stearate film under constant pressure at any instant. Oil for this purpose is called piston oil. The barium stearate molecules are separated from the piston oil molecules by means of a waxed thread.

1.2.2 Automatic recording Langmuir trough (Fig. V.1.)

The Langmuir trough is a device to measure film pressure as a function of a surface area available to the film. It consists of an inert thermostated container for the subphase (water, c.q. buffer solution) shaped in such a way that a large surface is available. This surface is divided into two compartments by a floating barrier. The pressure exerted by a film present on one side of the barrier is electronically recorded. At the side of the barrier where the film is applied, a motor driven second barrier can change the surface available to the film.

The difference of the surface tension:

$$F = \tau - \tau_0$$

τ_0 = surface tension of the clean subphase

τ = surface tension of the film covered subphase

is measured with an electronic tension measuring system (C) multiplied (D) and recorded on the Y channel of an X/Y recorder (E). The spread molecules are in the area (A) between the measuring barrier (F) and the moving barrier (G). The latter is propelled by a motor and changes the area A. In a smaller area (film compression) the surface tension F increases in a characteristic way. A potentiometer connected to the moving barrier transduces the area to a proportional current that is connected to the X-channel of an X/Y recorder.

The Langmuir trough we used was a modified analytical trough. The modification consisted of the lowering of a part of the teflon coated trough in order to allow the dipping of the chromium slide. The trough was of the type: Lauda filmwaage, Messgeräte Werk Lauda. P.O. Box 350697 Lauda/Tauber. Our first experiments were carried out with the piston oil method. The remaining experiments were performed with the Langmuir trough.

We measured at 14 different angles, starting at 55° in steps of two degrees. The results are shown in fig. V.2. The curve of ΔP versus ϕ shows a maximum of about 68° which compared well to the optimum calculated theoretically.

2.3 Calibration of the ellipsometer with monolayers of barium stearate on chromium in air.

By transferring monolayers of barium stearate to a solid surface, one obtains a very useful system to compare theory and experiment in a range between zero and several thousands of angströms of a layer of known refractive index. The transfer of the monolayers was done with the Langmuir trough as described above. Up to 101 layers of barium stearate were used.

Fig. V.2. The differences of polarizer readings ΔP , between a chromium slide covered with five monolayers bariumstearate and a bare chromium slide, as a function of the angle of incidence ϕ .

2.3.1 Procedure and results

All measurements were performed in two zones. The optical constants of a bare chromium slide were determined from the ψ and Δ readings via the program Virgin (page 85). Starting from these values we calculated and plotted conversion graphs in the relevant ranges of n and d via the programs Ellis and Ellip. The chromium slide was covered then with varying layers of barium stearate and ψ and Δ were measured. The values of ψ and Δ thus found experimentally were plotted in the conversion graph. Measured in this way n and d can be compared to the n and d which a multilayer of Ba stearate should theoretically have.

The result is given in Fig. V.3. The black curves are the theoretical curves. Each curve gives the changes in Δ and ψ when a layer of given refractive index grows on the chromium surface until a thickness of 3000 Å in steps of 25 Å each is reached. The four different curves have refractive indices of $n=1,47$; $n=1,49$; $n=1,51$; and $n=1,53$. The red curve is the experimental curve. This one shows the values of 101 monolayers of barium stearate.

The experimental curve fits the theoretical ones reasonably well, however, there are still some differences.

1. The first part of the curve does not exactly fit in with the theoretical curves.
2. The refractive index is a function of the thickness. These changes are summarized in Fig. V.3.

Fig. V.3. The effect of 101 layers of barium stearate on a chromium surface. $n=2.4152 - 2.4737i$ (red curve). Black curves are the theoretical curves of layers of $n=1.47$; $n=1.49$; $n=1.51$; $n=1.53$. Angle of incidence $\phi=68^\circ$ (see text).

3 Discussion

If the non ideal behaviour of the optical components would contribute significantly to the readings obtained it could also be expected that systematic differences would be found between the angle of incidence set, and the angle of incidence calculated from the reflexion at a glass surface. The angle of optimal sensitivity would be shifted too. This appeared not to be the case. We came to the conclusion that the Δ and ψ values as read by the instrument were sufficiently close to the real values. The similarity between the experimental and the theoretical curves obtained with Ba-stearate layers of increasing thickness could not be improved either by assuming a different angle of incidence as will be shown in fig. V.6.

Yet there is a deviation of the readings obtained with multilayers of Ba stearate and the readings to be expected from the known thickness of these layers and the known refractive index. The thickness of one monolayer is cited in the literature to be $24.4 \text{ \AA}$ (7,12). A 1-fold multilayer will therefore be $1 \times 24.4 \text{ \AA}$. The refractive index of Ba-stearate is given as 1.46 (7,12).

If the differences found in Δ and ψ cannot be blamed on imperfections of the instrument, they must necessarily be explained in terms of non-ideal behaviour of the layers.

In fig. V.4. it is shown that the experimental findings can be accommodated when the first three layers have a refractive index that is much higher than that of bulk Ba-stearate. This assumption appeared to be rather fruitful, as can be seen from fig. V.5. An almost perfect similarity is found from 3 - 50 layers when it is assumed that the first three layers have a refractive index that is higher than 1.48. For the first layer e.g. we find a thickness of 8 and a refractive index of 2.3. The physical basis of this phenomenon is unclear; maybe that after all the molecular configuration of Ba-stearate layers deposited on chromium is very different from the type of monolayers at an air-water interphase. It might also be possible that the effect is caused by uniaxial anisotropy in the adsorbate (Den Engelsen, 21).

Starting with an effective refractive index of the chromium and the first three layers (Virgin) we find: (fig. V.5.)

After the first three layers we find:

0 - 200 Å	refr.ind. $n=1,47$	thickness of a monolayer	27.0
200 - 500 Å	refr.ind. $n=1,46$	thickness of a monolayer	26.6
500 - 800 Å	refr.ind. $n=1,47$	thickness of a monolayer	26.6
800 - 1400 Å	refr.ind. $n=1,48$	thickness of a monolayer	26.5.

Ba-stearate layers of more than ~ 1400 Å i.e. of more than about 50 monolayers, start to deviate from the theoretical values again.

Fig. V.4. The behaviour of the first monolayers of bariumstearate on chromium. Each mark indicates ten angström. Refractive index $n=1,5 - n=2,3$. See text.

Fig. V.5. Bariumstearate layers on the effective surface of chromium and the first three layers. Refractive index $n_{\text{eff.}} = 2.1092 - 2.3685i$ (Virgin).

To obtain a better fit the effect of small changes in the angle of incidence was simulated. Three angles: $\phi=67.5$; 68.0 ; 68.5 are simulated in this system as is shown in fig. V.6. These changes, however, did not cause a better fit.

The experimental curve is not a closed one so we tried the effect of assuming a small complex part of the refractive index of Ba-stearate as well. The result is shown in fig. V.7.

This figure shows that this assumption would not improve the similarity between the theoretical curve and the observed curve. In our experiment these thick layers, however, were several hours old on account of the long procedure necessary to deposit the monolayers. Perceptible disturbances of the layers can be observed ("blisters and patches"). We therefore think that the cause of the non-ideal behaviour is very trivial at least in our case.

4. Calibration in the cuvet

In our ellipsometer we want to observe biological processes. These processes take place in a liquid surrounding so we have to carry out the measurement in a cuvet. To minimize the influence of the cuvet on the measurement we had trapezoid cuvetts made, which allowed a perpendicular angle of incidence on the windows of the cuvet. The angles of the cuvet were 68 degrees. Special care had to be taken to prevent birefringence in the windows because of stress.

To measure possible influences of the cuvet we first compared the measurements of bare chromium in air with and without cuvet. Second, we measured the optical constants of bare chromium in the cuvet filled with media of different refractive indices. Although the refractive indices differed a little, no systematic influences of the cuvet were measured. The differences of the refractive indices in air and in the media were already described by Mc.Crackin et al. (22). We also measured the barium stearate calibration in the cuvet filled with water. In this case we only checked a limited number of experimental points in the cuvet because the monolayers of barium stearate were not completely stable when transferred through the air-water interphase of the cuvet. The experimental points are shown in fig. V.8. In this experiment too, we find a predictable behaviour of the barium stearate layers. The refractive index of the layer, however, is about $n=1.51$ and the thickness after 35 layers is about $890 \text{ \AA}$, which gives a thickness of about $25.4 \text{ \AA}$ per monolayer. The thickness in this case is lower than

the one we found in air. A possible explanation may be that some material from the monolayer is lost by going through the air-water interphase of the cuvet several times.

The higher refractive index is in accordance with the experiments of Mc.Crackin et al.(22) who found differences in the refractive index of several surfaces when measured in air or in water. A good explanation cannot be given, although as will be seen in the next chapter, we always find an increase in refractive index when we have a decrease in thickness. Consequently, it may be a matter of packing of the layers.

From these experiments, we conclude that the cuvet does not substantially influence the experimental results, at least when care is taken that the incident and reflected beams are normal to the cuvet windows.

Fig. V.6. The effect of different angles of incidence on the barium stearate calibrations (see text and V.5).

Fig. V.7. The effect of a small complex part of the refractive index of bariumstearate (see text and V.5).

Fig. V.8. Barium stearate calibration in the cuvet.
 Refractive index chromium $n=2,5680 - 2,4371i$.
 Refractive index medium $n=1,3335$.
 Each mark indicates 50 angström.

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CHAPTER VI

Applications

6.1 Introduction

As an application of dynamic ellipsometry in studying protein adsorption and protein-protein interaction, the following experiments are presented:

1. Adsorption of human fibrinogen onto different surfaces like chromium-chromium oxide and silicon-silicon oxide.
2. Adsorption of human albumin on a hydrophilic chromium surface.
3. Interaction of these proteins with their antibodies.
4. Replacement of proteins by fibrinogen.
5. The relation between refractive index and thickness of a monolayer of protein on a chromium-chromium oxide surface with varying hydrophobicity.

These experiments will be interpreted according to the methods given in chapters IV and V.

6.2 Materials

As a reflecting surface we used: chromium sputtered glass slides manufactured by Stabilix, The Hague, Holland. Silicon slides were a gift of Philips Natuurkundig Laboratorium, Eindhoven, Holland.

Human fibrinogen (lyophilized, grade L) was obtained from Kabi A.B., Stockholm, Sweden. Human serum albumin was obtained from Calbiochem. Anti-albumin (agglutinating antiserum Batch no. KHO₂-15-A01) and anti-fibrinogen (precipitating antiserum Batch KH18-05-P0₂) were obtained from Centraal Laboratorium Bloedtransfusiedienst van het Nederlandse Rode Kruis, Amsterdam, Holland. Serum was obtained from normal human blood by clotting during 2 hours, and centrifugation during 30 min 12,000 g.

6.3 Adsorption of fibrinogen

6.3.1 Hydrophilic chromium surface

The chromium sputtered glass slides which are hydrophobic can be made hydrophilic by treating them with hot chromic acid. In this way a polar chromic oxide layer is formed which binds water. The cuvet was filled with buffer (Tris-HCl, 0.01 M pH 7.4) and the slides were measured in two different zones to determine their initial optical properties. After about 800 seconds, 0.1 mg of fibrinogen was added (100 μ l of a 1 mg/ml solution). After this addition

the polarizer (Δ) and analyzer (ψ) changes are recorded in time (fig. VI.1). After the moment of addition, the polarizer and analyzer change rather fast and an end level is reached after about 600 seconds for both analyzer and polarizer. Further addition of fibrinogen does not cause any further changes. Interpretation of the curves in terms of thickness (d) and refractive index (n) is done via the conversion graphs of the Δ and ψ values corresponding to the n and d values (fig.VI.2). The black lines give the theoretical curves of refractive indices 1.4 - 1.8 in steps of 0.1. The marks on each graph represent a change in thickness of $10 \text{ \AA}$. The calculated behaviour of n and d as a function of time is given in fig. VI.3. This figure shows that the refractive index does not change much during the adsorption. The thickness follows the saturation behaviour of a monolayer adsorption (type I of Winterbottom (1)). After about 4000 seconds the thickness stabilizes around $130 \text{ \AA}$. The refractive index is then 1.38.

Fig. VI.1. Fibrinogen adsorption.

Hard copy of the memory scope recording of the change in analyzer and polarizer after two additions (1,2) of $100 \mu\text{l}$ of fibrinogen 1 mg/ml .

Surface: chromium oxide: $n = 2,7525 - 2,6544$ i.

Buffer: Tris-HCl 0.01 M pH 7.0.

Fig. VI.2. Conversion graph of fibrinogen adsorption.
 Red line gives the experimental points.
 Black lines are the theoretical points.
 Further details: see text.

Adsorption of Fibrinogen on a chromiumoxide (hydrophilic) surface.

conc. Fibrinogen $10 \mu\text{g./ml.}$
buffer $0.01\text{M. Tris-HCl pH. 7.0}$

Fig. VI.3. Behaviour of thickness and refractive index as a function of time during the adsorption of fibrinogen ($10 \mu\text{g/ml}$) in 0.01 M Tris-HCl buffer pH 7.0 onto a chromium oxide (hydrophilic) surface (see text).

6.3.2 Hydrophobic chromium surface

The adsorption of fibrinogen on chromium which is hydrophobic is done under the same experimental conditions as in the foregoing paragraph. The adsorption behaviour of analyzer and polarizer are shown in fig. VI.4. In this graph one observes immediately that at 1,600 seconds the analyzer does not change anymore, whereas the polarizer is not yet stable. This indicates that other processes than adsorption of a layer of constant refractive index must be going on. Fig. VI.5 shows the relevant $\psi\Delta \rightarrow n.d$ map with the experimental findings.

The black curves represent the theoretical curves of the refractive indices $n=1.4 - 1.8$ in steps of 0.1 . The marks indicate changes of thickness of $10 \text{ \AA}$. The refractive index of the chromium was $n=2.6645$ $k=2.4782$.

Fig. VI.4. Fibrinogen adsorption on hydrophobic chromium.
(see text).

The experimental curve apparently does not follow one of the theoretical black curves of constant refractive index. In fig. VI.6 n and d are plotted as a function of time. During the first 150 seconds the thickness grows at constant refractive index $n=1,8$. Then the refractive index lowers and the thickness still increases. The thickness grows till about $68 \text{ \AA}$ while the refractive index lowers till about 1.5. From this point on the layer gets thinner and optically denser. The layer stabilizes at $d=35 \text{ \AA}$ and $n=1,8$.

Fig. VI.5. Conversion graph of fibrinogen adsorption onto hydrophobic chromic surfaces (see text).

Adsorption of Fibrinogen on a chromium (hydrophobic) surface.

conc. Fibrinogen $10 \mu\text{g./ml.}$

buffer 0.01 M. Tris-HCl pH 7.0

Fig. VI.6. Behaviour of thickness and refractive index as a function of time during adsorption of fibrinogen ($10 \mu\text{g/ml}$) in 0.01 M Tris-HCl buffer pH 7.0 onto chromium (hydrophobic) surfaces.

6.3.3 Adsorption on silicon-silicon oxide surfaces

Silicon slides had to be treated during one hour with chromic acid to become hydrophilic, for hydrophobic surfaces it took several hours to stabilize in buffer. By treating the silicon with chromic acid the silanon structures of the surface are hydrolized to silanol structures which are hydrophilic. The same process takes place in buffer but in this case the process takes much more time. Stable hydrophobic surfaces cannot be obtained with silicon.

In fig. VI.7 the experimental conditions were the same as in fig. VI.4 but for the surface which was silicon-silicon oxide. In this figure one sees that the changes in analyzer and polarizer markedly differ from those obtained with the chromium surfaces because of the different refractive index of the reflecting surface (see chapter IV). In this experiment adsorption of fibrinogen causes the polarizer to change about 6 degrees whereas the analyzer changes only about 0.4 degrees. This illustrates the sensitivity of the technique. About 120 Å thickness causes a change in polarizer of about 6 degrees while the accuracy of the instrument is about 0.01 of a degree, so changes in thickness of about 0.2 of an Angström can be seen. Interpretation of the analyzer and polarizer curves by the method used before shows identical adsorption behaviour of the protein as on hydrophilic chromium.

FIBRINOGEN adsorption on silicon.

Fig. VI.7 Adsorption of fibrinogen onto silicon.

6.3.4 Conclusions from the fibrinogen adsorption experiments

The difference in character of adsorption on hydrophobic and hydrophilic surfaces depends upon the interaction of the protein and the surface. The interaction with hydrophobic surfaces is much stronger than with hydrophilic ones. This is to be expected as a hydrophilic surface to a certain extent resembles the natural surroundings of a protein in solution. Our explanation of the adsorption behaviour is as follows:

The first fibrinogen molecules adsorb onto the surface with the whole length of the molecules fixed to the surface. This gives a thin layer which is optically very thick so we have a high refractive index. After some time the surface is partly covered and it is not possible anymore for the molecule to attach to the surface directly over its whole length. So we get a layer of random orientated fibrinogen molecules partly attached to the surface. This results in a thick layer which is loosely packed i.e. a layer with a high water content and a low refractive index. These random orientated molecules reorientate giving a layer which is closely packed with a high refractive index and a thickness of about $40 \text{ \AA}$. This thickness indicates a layer of one molecule thick if we accept the dimension of the molecule being length 375-450 $\text{\AA}$; mean transverse diameter 60-90 (2,3,12,13,14).

6.4 Adsorption of human albumin

The adsorption of human albumin on hydrophilic chromium surfaces was studied. The experimental conditions were the same as described in 6.3.1. The result is shown in fig. VI.8. The figure shows that in the beginning of the adsorption there is a very thick layer of very loosely packed albumin molecules. The "thickness" is about $400 \text{ \AA}$ with a refractive index which is only slightly higher than the refractive index of water.

Then rearrangement takes place and a layer which has an average thickness of about $15 \text{ \AA}$ is formed. The refractive index is about $n=1,55$. This thickness correlates well with the reported dimensions of albumin $21,5 \times 50 \times 106 \text{ \AA}$ (4,5). As will be shown in 6.7 it is very difficult, however, to compare our results with those in literature.

6.5 Protein antibody interactions

6.5.1 Fibrinogen-antifibrinogen interaction

In the study of these interactions we used the same experimental conditions and surfaces as described in 6.3.1. We first adsorbed the fibrinogen as sub 6.3.1. When the analyzer and polarizer did

not change anymore, the content of the cuvet was washed twice with new buffer. During this washing the polarizer and analyzer did not change so the fibrinogen layer remained unchanged on the chromium. After this washing the antifibrinogen was added and the polarizer and analyzer started to change (fig.VI.9). The results calculated as shown before are given in fig. VI.10. In this figure we see a thickness of the fibrinogen of $100 \text{ \AA}$ at a refractive index of 1.40. Addition of antifibrinogen gives a change in thickness of about $400 \text{ \AA}$ with refractive index of 1.366.

Fig. VI.8. Adsorption of albumin into a chromium (hydrophilic) surface. Concentration of albumin: $10 \text{ \mu g/ml}$. Buffer $0.01 \text{ M Tris-HCl pH } 7.0$.

Fig. VI.9. Fibrinogen-antifibrinogen interaction (see text).

Fig. VI.10. Fibrinogen-antifibrinogen interaction (see text).

Fig. VI.11. Albumin-antialbumin interaction (see text).

6.5.2 Albumin-antialbumin interaction

This experiment is exactly comparable to the fibrinogen-antifibrinogen experiment in 6.5.1. Final albumin concentration was $10 \mu\text{g/ml}$. The result is shown in fig. VI.11. In this figure we see a thickness of the albumin layer of about $22 \text{\AA}$ with a refractive index of $n=1,867$. After washing, addition of anti-albumin gives a Langmuir like adsorption with constant refractive index. The thickness in the end is $300 \text{\AA}$

6.6 Displacement of several proteins by fibrinogen

6.6.1 Displacement of albumin by fibrinogen on a chromium surface

These experiments were carried out under the same experimental conditions as in 6.3.1. Final albumin concentration $10 \mu\text{g/ml}$, final

fibrinogen concentration 10 $\mu\text{g/ml}$. After the first addition of albumin, a monolayer is formed. Further addition of albumin causes no further deposits. Addition of fibrinogen after washing gives a big change in polarizer and analyzer. After calculation we find a thickness of 80 Å with a refractive index of $\pm 1,515$. These data are compatible with a fibrinogen monolayer. Addition of antifibrinogen caused a big change in thickness and refractive index as shown in fig. VI.12. The layer thus presumably is fibrinogen that replaced the albumin. This is further supported by

1. addition of anti-albumin after the fibrinogen adsorption, which caused only a very small change, and
2. adsorption of albumin after a fibrinogen adsorption which gave no reaction at all.

6.6.2 Displacement of serum proteins by fibrinogen on a chromium surface

The serum protein was added to a final concentration in the cuvet of 10 $\mu\text{g/ml}$. After the adsorption of serum, the fibrinogen was added. In fig. VI.13 we see the changes in polarizer and analyzer. Fig. VI.14 shows the thickness and refractive index as a function of time. Addition of serum gives an adsorption of about $d=180$ Å and $n=1,36$. After refreshing the contents of the cuvet the optical constants remain the same. Addition of fibrinogen causes a change in polarizer and analyzer (fig. VI.13) that must be interpreted (fig. VI.14) as a change of the refractive index only. After washing, addition of antifibrinogen causes a large change in thickness. This indicates that fibrinogen replaced the adsorbed serum proteins. This is further supported by the observation that antifibrinogen does not interact with the adsorbed serum proteins (first layer) and anti-whole serum does not interact with the layer generated after addition of fibrinogen (second layer).

6.7 Relation between thickness and refractive index in a monolayer of protein

During the adsorption of albumin or fibrinogen onto different chromium-chromium oxide slides, monolayers of different thickness and refractive index are obtained.

we have already seen in 6.3.1 and 6.3.2 that under experimental conditions differing only in the hydrophobicity of the chromium surface, layers of $d=130$ Å, $n=1,38$ till $d=35$ Å $n=1,8$ are formed.

Fig. VI.12. Displacement of albumin by fibrinogen on a chromium surface. Fibrinogen-antifibrinogen interaction. Concentration albumin 10 $\mu\text{g/ml}$. Concentration fibrinogen 10 $\mu\text{g/ml}$. Buffer 0.01 M Tris-HCl, pH 7.0.

In Fig. VI.15 and VI.16 the relation between the values of the thickness and refractive index of monolayers of albumin and fibrinogen are plotted.

The curves resemble an orthogonal hyperbola with the refractive index of the medium as one asymptote. This is suggestive of a relation of the type $d \cdot \Delta n = \text{constant}$ (87)

where $\Delta n = n_{La} - n_m$

n_{La} = refractive index adsorbed layers

n_m = refractive index medium.

Fig.VI.13. Displacement of serum proteins by fibrinogen

1. Addition of serum proteins. Final conc. 10 $\mu\text{g/ml}$.
2. Addition of fibrinogen. Final conc. 10 $\mu\text{g/ml}$.
3. Addition of antifibrinogen.

Fig.VI.14. Displacement of serum proteins by fibrinogen .

Fig. VI.15. Relation between n and d in a monolayer of albumin on chromium surfaces, differing in hydrophobicity.

Fig. VI.16. Relation between n and d in a monolayer of fibrinogen on chromium surfaces differing in hydrophobicity.

To test the validity of this formula we plotted d against $1/\Delta n$ (Fig. VI.16; fig.VI.17).

The figures show that formula (87) indeed can be taken as a first approximation of the relation between d and Δn . Further experiments are necessary.

6.8 Discussion

This last chapter shows a number of possible applications of the technique. Some of these experiments have been done before by using the recording ellipsometer (6-9), however, in those cases it was not possible to measure the effects in a quantitative way. The supposition in those experiments that the refractive index of the layer is a constant during the adsorption (10,11) is valid only under very limited experimental conditions as is shown in this thesis. In our experiments we were able to quantitate protein adsorption. In this way we found a series of interesting phenomena like rearrangement of layers as discussed in 6.3.4, quantitation of protein-antibody interaction, and the replacement of proteins by fibrinogen. Because of our possibilities to quantitate both d and n we found a possible relation between these parameters in adsorbed monolayers. A possible physical background may be the fact that in proteins the refractive index of the protein solution changes about 0.2 per gram protein per millilitre. So Δn is something like concentration, d is a thickness, consequently the product will be an amount of protein per surface unit. In all experiments done we presumably obtained a monolayer adsorption so in all those cases the amount of protein per surface unit has to be the same. To interpret these data in terms of the Lorenz-Lorentz equation (chapter III) or the other theories mentioned for the submonolayer systems, we have to wait until we have enough experimental data on different proteins.

Fig. VI.17. Relation between $1/\Delta n$ and d of a monolayer of albumin (see text).

Fig. VI.18. Relation between $1/\Delta n$ and d of a monolayer of fibrinogen (see text).

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SUMMARY

This thesis describes the development of an ellipsometer useful for biochemical experiments.

The ellipsometer is an optical instrument that can be used to measure the thickness and the refractive index of thin transparent layers deposited on a reflecting surface. A big number of unsolved medical, biochemical and biophysical problems concern processes occurring at interphases. Some examples are membrane transport, nerve conduction, oxydative phosphorylation, thrombogenesis etc.

Consequently it is very important to have an apparatus that can measure thickness and refractive index of layers of biological molecules at an interphase. The ellipsometric technique in principle allows such measurements, however, the major part of the apparatuses were until now not able to do so within 5 minutes, which is too slow for biochemical experiments. The developed recording ellipsometer which could follow the change in light intensity reaching the photodiode continuously, could measure biochemical experiments fast enough but in this case it was not possible to discriminate between polarizer and analyzer changes, consequently these experiments can in general not be interpreted in terms of thickness or refractive index.

We developed a dynamic ellipsometer which follows both analyzer and polarizer automatically in time.

Chapter I summarizes the techniques available in the study of protein-protein- and protein-lipid-interactions and reviews some of the obtained results.

Chapter II describes the measuring principle, electronic circuits and the computer steered stepping motors.

In Chapter III a general survey of the optical theory, necessary to understand the apparatus and the interpretation of the results is given.

Subjects of Chapter IV are the theoretical analysis of the influence of the different parameters on the sensitivity of the measurements and the computer program for the interpretation of these experiments.

Chapter V gives the development of some standard experiments that can be used to calibrate the instrument and to calculate the corrections necessary to compensate the non-ideal behaviour of the optical components.

Chapter VI gives a number of illustrations of the technique applied for the unraveling of the following medical-biological problems:

1. Adsorption of a.o. fibrinogen and albumin onto different surfaces.

All proteins tested adsorb onto chromium and silicon until a monolayer is obtained. The thickness of the layer varies with the water content.

The water content of the layer is influenced by the hydrophobicity of the surface. There is a relation between the thickness of the layer and the found refractive index. This relation is hyperbolic in a good approximation.

2. Interaction of proteins with their antibodies. By means of ellipsometry it is possible to observe a reaction of a monolayer of protein ($< 10^{-11}$ grammol) with a monolayer of antibody ($< 10^{-11}$ grammol).

3. Fibrinogen displaces proteins adsorbed before, like albumin and serum proteins from chromium surfaces.

ZUSAMMENFASSUNG

Diese Dissertation beschreibt die Entwicklung eines Ellipsometers für biochemische Zwecke. Das Ellipsometer ist ein optisches Instrument, das verwendet wird um die Dicke und den Brechungsindex dünner durchsichtiger Schichten auf einer reflektierenden Oberfläche zu messen. Viele ungelöste medizinische, biochemische und biophysikalische Probleme betreffen Prozesse, die sich an Grenzflächenabspielen. Beispiele dafür sind Membrantransport, Reizleitung, oxydative Phosphorylierung, Thrombogenese usw. Deshalb ist es sehr wichtig, ein Instrument zu haben, das Dicke und Brechungsindex biologischer Molekülschichten auf einer Grenzfläche messen kann. Die ellipsometrische Technik ermöglicht dies. Die vorhandene Apparatur war aber nicht geeignet für diese Zwecke. Man hatte nur ein statisches Ellipsometer zur Verfügung, das genau die Änderungen des Lichtpolarisationszustandes messen kann und in dieser Weise genaue Bestimmungen der Dicke und des Brechungsindex ermöglicht, aber nur eine Messung pro 5 bis 10 Minuten durchführt, was für dynamische biochemische Prozesse nicht genügt. Daneben gab es ein Ellipsometer, das kontinuierlich nur die Lichtintensität des von dem Instrument durchgelassenen Lichtes mass, so dass nur ungefähre Schätzungen der Dicke oder des Brechungsindex möglich waren. Wir entwickelten ein Instrument, das beide Parameter automatisch misst mit Unterbrechungen von $2\frac{1}{2}$ bis 5 Sekunden.

Kapitel I beschreibt im kurzen die bis heute verwendeten Techniken zur Untersuchung von Protein-Protein- und Protein-Lipid Interaktionen und gibt eine Übersicht der damit erreichten Ergebnisse.

Wirkungsprinzip, elektronische Eigenschaften, Lenkung der Stufenmotoren und das vom Computer gelenkte Messverfahren werden im Kapitel II beschrieben.

Kapitel III gibt eine kurze Übersicht der Theorie, die man braucht zur Deutung der Wirkung des Apparats, und der Ergebnisse. Die theoretische Analyse des Einflusses der verschiedenen Variablen auf die Empfindlichkeit der Messungen und der Computerprogramme für die Beobachtungsdeutung findet man im Kapitel IV. Kapitel V beschreibt die Entwicklung von Normalversuchen zur Eichung des Instruments und zur Berechnung von Korrekturen für nicht-ideale Wirkungen der optischen Komponenten.

Kapitel VI beschreibt eine Anzahl von mit dieser Technik bearbeiteten biomedizinischen Problemen z.B.

1. Adsorption von Fibrinogen und Albumin an verschiedenen Oberflächen. Es stellt sich heraus dass alle geprüften Proteine an Chrom oder Silicon adsorbiert werden, bis sich eine monomolekulären Schicht hergestellt hat. Die Schichtdicke variiert mit dem Wassergehalt. Der Wassergehalt der Schichten wird beeinflusst von der Hydrophilität der Oberfläche. Zwischen der Schichtdicke und dem Brechungsindex besteht ein Zusammenhang der fast hyperbolisch ist.
2. Interaktionen von Proteinen mit ihren Antikörpern. Es ist möglich, mit Hilfe der Ellipsometertechnik, unmittelbar die Reaktion einer Proteinmonoschicht ($< 10^{-11}$ Grammmol) mit einer Antikörpermonoschicht ($< 10^{-11}$ Grammmol) zu beobachten.
3. Fibrinogen verdrängt adsorbierte Proteine wie Albumin und Serumproteine von Chromoberflächen.

RÉSUMÉ

L'objet de cette thèse est la description des modifications d'un ellipsomètre. Ces modifications ont été rendues nécessaires pour permettre des études biochimiques.

L'ellipsomètre est un instrument d'optique qui permet de mesurer l'épaisseur et l'indice de réfraction de couches minces et transparentes se trouvant sur des surfaces réfléchissantes. De nombreux problèmes non résolus qui se posent dans les domaines médicaux, biochimiques, biophysiques et biomédicaux, concernent des processus qui se passent au niveau des interfaces liquide - solide, par exemple: les transports transmembranaires, la conduction nerveuse, la phosphorylation oxydative, la thrombogénèse. Il est donc important de posséder un instrument qui soit capable de mesurer l'épaisseur et l'indice de réfraction de couche de molécules de substances biologiques se trouvant à ces interfaces.

La technique ellipsométrique permet de telles mesures. Cependant les appareils existants n'étaient pas aptes à le faire. En effet, on ne disposait que d'ellipsomètres statiques qui pouvaient mesurer exactement des modifications de lumière polarisée et permettaient ainsi de déterminer d'une manière précise l'épaisseur et l'indice de réfraction. Avec ces instruments le temps nécessaire pour effectuer une mesure était compris entre 5 et 10 minutes ce qui est beaucoup trop long pour suivre un processus biochimique. Il était également possible d'effectuer des mesures d'une manière continue mais seulement de l'intensité lumineuse transmise par l'instrument de telle sorte que soit l'épaisseur soit l'indice de réfraction pouvait être estimé d'une manière grossière.

Nous avons développé un instrument capable de suivre automatiquement ces 2 paramètres à des intervalles variant entre 2½ et 5 secondes.

Le chapitre I résume les techniques utilisées jusqu'à présent pour l'étude des interactions protéines-protéines et protéines-lipides aux interfaces et il donne également une vue générale des résultats obtenus.

La description de l'appareil en ce qui concerne son principe de fonctionnement, son schéma électronique, son mode d'emploi et le procédé de mesure dirigé par computer est traité dans le chapitre II.

Le chapitre III donne une vue globale de la théorie nécessaire à la compréhension du fonctionnement de l'appareil et à l'interprétation des résultats. L'analyse théorique de l'influence des différentes variables sur la sensibilité des mesures et celles des programmes du computer pour l'interprétation des résultats est traitée dans le chapitre IV.

Dans le chapitre V on développe des expériences standards qui permettent d'étalonner l'instrument et de tenir compte de corrections nécessaires lors du comportement non idéal des composants optiques.

Le chapitre VI donne une série d'illustrations de l'application de cette technique aux problèmes biologiques et médicaux suivants:

1. L'adsorption du fibrinogène et de l'albumine sur différentes surfaces.

Il semble que toutes les protéines testées s'adsorbent sur le chrome ou le silicium sous la forme d'une couche mononucléaire. L'épaisseur de la couche varie avec la teneur en eau.

La teneur en eau de la couche est influencée par la nature hydrophyle de la surface. La relation entre l'épaisseur de la couche et l'indice de refraction est de nature hyperbolique.

2. L'interaction entre les protéines et leurs anticorps.

Il semble possible à l'aide de l'ellipsomètre d'observer directement la réaction entre une couche monomoléculaire de protéine ($< 10^{-11}$ molécule gramme) et une couche monomoléculaire ($< 10^{-11}$ molécule gramme).

3. Le fibrinogène décolle d'une surface en chrome des protéines adsorbées auparavant sur celle-ci telle l'albumine des protéines du sérum.

KOMPENDIUM

Dees dissertasie besjreif wie mèt vaöl belump 'nen ellipsometer woord ineingefisternöld dee ziech lient veur biochemisch gebruik. U'n ellipsometer is 'n optisch instrumint wat in staot is de dikte en brekingsindex te mete vaan dun, doorziechtige laögskes die op e reflekterend oppervlak zitte.

'n Groete struip oonopgelosde medische, biochemische, biofysische en biomedische probleme regardere processe die ziech aon grensvlakke aofspeule, zoe wie beveurbeeld membraantransport, de geleijing vaan steekskes door de zenuwbaone, oxydatieve fosforylering, 't oontstoon vaan thromboos en zoe mie. 't Is daorum vaan importantie 'n instrumint te höbbe, wat dikte en brekingsindex (bezettingsgraad) vaan laoge vaan biologische molekule in e grensvlak kin mete. De ellipsometrische techniek maak dit in prinsiep meugelek. De bestaonde appretuur dougde dao evels neet veur. Me had naomelek wel de statische ellipsometer, dee puntelik de veranderinge in de polarisatietowstand vaan 't leech kin mete en zoe de eksakte bepaoling vaan dikte en brekingsindex meugelek maak, meh boe mer ein meting um de 5 à 10 minute mèt kôs weure gedoon, wat veur dynamische biochemische processe neet genög is.

Ouch had me ellipsometers die kontinu allein mër de intensiteit mete vaan 't leech wat door 't instrumint weurd doorgelaote, boedoor óf vaan dikte óf vaan brekingsindex allein mer groof taxaties meugelek zien.

Veer höbbe 'n instrumint oetgeprakkezeerd wat allebey de parameters otomatisch vervolg mèt intervallen vaan 2½ tot 5 sekonde.

Hoofstök I gief 'n korte inventarisatie vaan de technieke, die tot noe tow veurnaomelek zien gebruik veur 't bestudere vaan eiwit-eiwit en eiwit-lipide interaksies aon interfases en gief 'n euverziech vaan de rizzeltaote die daomèt zien behaold. De besjreiving vaan 't apperaat wat betröf wërrekingssprinsiep, elektronische regeling, 't sture vaan de stepke-veur-stepke drejjende motors en de door de kompjoeter bestuurde meetproceduur weure in hoofstök II besjreve.

In hoofstök III weurd e kort globaal euverziech gegeve vaan de theorie die nudig is um 'n inziech te kinne krieve in de wërreking vaan 't apperaat en um de rizzeltaote te kinne interpreteere.

De theoretische besjowwing euver de inwërreking vaan de versjeije variabele op de geveuligheid vaan de metinge en daobij ouch de kompjoeter-programma's veur de interpretasie vaan de observasies weure in hoofstök IV behandeld.

In hoofstök V weure standaardeksperimente oet de deuk gedoon die gooien deens kinne doen bij 't ijke vaan de klømmel en bij de besiefering vaan de korrekties veur 't geval 't gedrag vaan de optische komponente neet ideaal is.

Hoofstök VI gief 'n hamfel illustrasies vaan d'n techniek, towgepas op de volgende medisch-biologische probleme:

1. Adsorptie vaan o.a. fibrinogeen en albumine aon versjeije oppervlakke. Daobij bliek tot alle beproefde eiwitte op chroom- en siliciumoppervlakke weure vasgehawwe totdat 'n laag is oontstande die monomolekulaair is. De dikte vaan de laag varieert mèt 't watergehalte. 't Watergehalte vaan de laag weurd o.a. mètbepaold door de wateraontrèkkingskrach vaan 't oppervlak. Dao is e verband tösse laagdikte en brekingsindex, wat - es alles good zit - hyperbolisch is.
2. Interaksies vaan eiwitte mèt hun anti-liechame. 't Bliek meugelek um m.b.v. ellipsometrie direk de reaksie vaan 'n monolaog eiwit ($< 10^{-11}$ grammol) mèt 'n monolaog antiliechaam ($< 10^{-11}$ grammol) te konstatere.
3. Fibrinogeen verdringk al ieder geadsorbeerde eiwitte, zoe wie albumine en serumeiwitte, vaan chroomoppervlakke.

SAMENVATTING

Dit proefschrift beschrijft de ontwikkeling van een ellipsometer geschikt voor biochemisch gebruik. De ellipsometer is een optisch instrument dat in staat is de dikte en brekingsindex te meten van dunne doorzichtige laagjes die zich op een reflektierend oppervlak bevinden. Een groot aantal onopgeloste medische, biochemische, biofysische en biomedische problemen betreffen processen die zich aan grensvlakken afspelen. Men denke b.v. aan membraantransport, prikkelgeleiding, oxydatieve fosforylering, thrombogenese etc. Het is daarom van groot belang om te beschikken over een instrument dat dikte en brekingsindex (bezettingsgraad) van lagen van biologische molekulen in een grensvlak kan meten. De ellipsometrische techniek maakt dit in principe mogelijk. De bestaande apparatuur was echter niet geschikt. Men beschikte nl. over de statische ellipsometer, die nauwkeurig de veranderingen in de polarisatietoestand van het licht kan meten en zodoende exakte bepaling van dikte en brekingsindex mogelijk maakt, maar slechts één meting per 5 à 10 minuten toestaat, hetgeen voor dynamische biochemische processen onvoldoende is of over ellipsometers die kontinu slechts de lichtintensiteit van het door het instrument doorgelaten licht meten waardoor of van dikte of van brekingsindex slechts ruwe schattingen mogelijk zijn. Wij ontwikkelden een instrument dat beide parameters automatisch vervolgt met intervallen van $2\frac{1}{2}$ à 5 seconden.

Hoofdstuk I somt in het kort de technieken voor het bestuderen op, die tot nu toe voornamelijk gebruikt zijn voor het bestuderen van eiwit-eiwit- en eiwit-lipide interacties aan interfases en geeft een overzicht van de daarmee bereikte resultaten.

De beschrijving van het apparaat wat betreft werkingsprincipe, elektronische regelingen, sturing van de stappenmotoren en de computer gestuurde meetprocedure worden in hoofdstuk II beschreven.

In hoofdstuk III wordt een kort globaal overzicht gegeven van de theorie die nodig is om een inzicht te kunnen krijgen in de werking van het apparaat en om de resultaten te kunnen interpreteren. De theoretische analyse van de invloed van de verschillende variabelen op de gevoeligheid van de metingen en de computerprogramma's voor de interpretatie van de waarnemingen worden in hoofdstuk IV behandeld. In hoofdstuk V worden standaardexperimenten ontwikkeld die kunnen dienen om het instrument te ijken en om correcties voor niet-ideaal gedrag van de optische componenten te berekenen.

Hoofdstuk VI geeft een aantal illustraties van de techniek toegepast op de volgende medisch-biologische problemen:

1. Adsorptie van o.a. fibrinogeen en albumine aan verschillende oppervlakken. Het blijkt dat alle geteste eiwitten op chroom en silicium adsorberen tot een monomoleculaire laag verkregen is. De dikte van de laag varieert met het watergehalte. Het watergehalte van de laag wordt o.a. beïnvloed door de hydrofilititeit van het oppervlak. Er is een verband tussen laagdikte en brekingsindex dat in goede benadering hyperbolisch is.
2. Interakties van eiwitten met hun antilichamen. Het blijkt mogelijk om met behulp van ellipsometrie direkt de reactie van een monolaag eiwit ($< 10^{-11}$ grammol) met een monolaag antilichaam ($< 10^{-11}$ grammol) waar te nemen.
3. Fibrinogeen verdringt reeds eerder geadsorbeerde eiwitten zoals albumine en serumeiwitten van chroomoppervlakken.

CURRICULUM VITAE

Op verzoek van de Faculteit der Geneeskunde volgt hier een overzicht van mijn academische studie.

Ik 1966 liet ik mij na het behalen van het UL0-B en HBS-B diploma inschrijven aan de Gemeente Universiteit van Amsterdam. Daar behaalde ik in 1969 het kandidaatsexamen scheikunde letter S_2 . De doctoraalstudie verliep met hoofdvak biochemie onder leiding van de hoogleraar Dr.E.C.Slater. Het bijvak neurophysiologie verliep onder leiding van Prof.Dr.J.Ariens Kappers op het Nederlands Centraal Instituut voor Hersenonderzoek. Als speciale richting werd wiskunde gedaan. In februari 1971 werd het doktoraal examen afgelegd.

Van 1971 tot maart 1974 was ik als wetenschappelijk medewerker verbonden aan het Laboratorium voor Cardiobiochemie en Bloedstollingsbiochemie van het Academisch Ziekenhuis in Leiden, onder leiding van Dr.H.C.Hemker. Vanaf 1973 werden door mij de colleges Pathologische Scheikunde voor vijfde en zesde jaars studenten van de Vrije Universiteit Brussel gegeven. Sinds maart 1974 ben ik als wetenschappelijk medewerker verbonden aan de Rijksuniversiteit Limburg.

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