

Laboratory Notes on:  
Langmuir-Vroman Techniques

by

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Leo Vroman has successfully developed a number of extremely simple, "kitchen-sink" techniques that can be used to accurately study protein adsorption at an interface. Three of his main techniques, interference colors, vapor patterns and metal oxide patterns, are discussed.

A. Interference Colors

Interference is said to occur where two or more trains of waves cross one another. The brilliant colors often seen when light is reflected from a soap bubble or from a thin layer of oil floating on water are produced by interference effects between the two trains of light waves reflected at opposite surfaces of the thin film of soap solution or oil. (See Figure 1). In Figure 1, the line AB is one ray in a beam of light incident on the upper surface of a thin film. A part of the incident light is reflected at the first surface as indicated by ray BC, and a part, represented by BD, is transmitted. At the second surface the light is again reflected and emerges as ray EF. If the beam has appreciable width the wave fronts will overlap and the reflected wave trains can produce interference effects. To understand how Vroman uses this effect, a look at the work of Irving Langmuir and co-workers is necessary.

Even films whose thickness is of atomic dimensions have a marked effect on the light reflected from the surface. Obviously, if the thickness of the film is of the order of half a wavelength of the light, interference phenomena will occur between the light reflected from the air-film and from the film-substrate interfaces. The only requirement is that the refractive index of the substrate (usually glass or metal) be sufficiently different from the refractive index of the film so that light is plentifully reflected from the optical boundary between the film and the substrate. In that case, then light changes in film thickness are visible as changes in interference color. For example, Katharine B. Blodgett found that nine layers of barium stearate on glass show a faint yellow-brown color, due to the diminished intensity of blue light. As the number of layers is increased the color of the film changes, corresponding to the change of the absent color to the successive longer wave-lengths green, yellow, red. As still more layers are added the spectrum is repeated<sup>(1)</sup>.



On this basis, Blodgett and Langmuir developed two methods for estimating the thickness of monolayers, both based on the use of built-up multilayer films<sup>(2)</sup>. For substances which can themselves be deposited as multilayers, Blodgett<sup>(1)</sup> showed that the interference of monochromatic light between the top and bottom of the multilayer permits a measurement of the thickness. The refractive index of the multilayer,  $n$ , is estimated by measuring the Brewster angle,  $\theta$ ; i.e., the angle of incidence for which reflected light is completely plane-polarized, given by Brewster's law, found experimentally by Sir David Brewster in 1812,

$$\tan \theta = \frac{n'}{n}$$

where  $n'$  is the refractive index of the substrate. Under monochromatic light, the film shows alternating intensity maximums and minimums with increasing thickness, rather than showing a number of successive colors from yellow to blue (as the absent color ranges from blue to red) as in white light (Figure 2). Then the thickness can be calculated from the equation

$$t = \frac{(2n + 1) \lambda}{4n} \quad (1)$$

where  $t$  is the thickness of the layer giving maximum interference (or minimum intensity) at perpendicular incidence,  $\lambda$  is the wavelength and  $n$  is an integer with values of 0, 1, 2 . . . and is odd or even depending on the relative refractive indices of the film and substrate. Equation (1) applies only to cases where  $n$  is less than  $n'$ . In a subsequent paper<sup>(2)</sup>, it was pointed out that this simple equation requires modification, when the film is birefringent. For the measurement of the thickness of a single layer, however, such discrepancies are small.

The second method of estimating a monolayer thickness consists in depositing on a chromium-plated slide enough layers of a reference film, 35 monolayers of barium stearate, for example<sup>(3)</sup>. The added thickness produces an optical path slightly less than  $\lambda/2$  of the light used, which is reflected at an angle of incidence between  $60^\circ$  and  $81^\circ$ . Additional layers are then deposited stepwise, so that the difference in thickness between two adjacent steps is equal to one double layer of barium stearate. Measurements consist of determining the angle of incidence at which two adjacent steps appear equally dark in monochromatic light. When this occurs, it means that, exactly midway between the two equally dark steps, there is half a wavelength of phase difference between the light reflected from the slide and that from the film<sup>(4)</sup>. If the equally dark steps are those consisting of 47 and 49 monolayers of barium stearate, then the maximum interference occurs for a thickness corresponding to 48 monolayers.



A slide coated in such a way is called an optical gage. It shows a great difference in intensity between the individual steps in the neighborhood of total interference.

When a film of unknown thickness is transferred onto such a gage, the angle of incidence must be changed in order to re-establish equal darkening of two adjacent steps which are not necessarily those matched before the unknown film was deposited. The thickness of the unknown film deposited on top of the optical gage is calculated from this shift of angle incidence. If the index of refraction of the film is about the same as that of barium stearate (1.495) the following equation can be used for the calculation:

$$t = 24.3 \left( N_S \frac{\cos r_S}{\cos r} - N \right) \quad (2)$$

where  $t$  is the thickness in Angstroms of the unknown film,  $N_S$  is the number of barium stearate monolayers for which there is a minimum intensity in the reflected light at an angle of refraction  $r_S$  before the deposition of the unknown film (generally  $N_S = 48$ ) and  $N$  and  $r$  are the corresponding values after the deposition of the unknown film. The corresponding  $r_S$  values for sundry angles of incidence are given in Table 1. The constant 24.3 is the thickness in Angstroms of one monolayer of barium stearate. If the unknown film is 48 Å thick, the angle of incidence remains the same, but matching occurs for the pair of steps directly below the one matched before the transfer of the film (for example, 45 - 47 instead of 47 - 49).

Best results are obtained with monochromatic sodium arc or mercury arc light filtered for  $\lambda = 5461 \text{ Å}$ , linearly polarized with the electric vector vibrating perpendicular to the plane of incidence. Under these conditions, this method should permit measurements to less than 1 Å<sup>(4)</sup>. This method was used extensively by Langmuir's group, especially to examine protein films<sup>(6)</sup>. A more recent development based on a similar principle is the measurement of white-light interference fringe displacement by monolayers deposited on stepped multilayer plates<sup>(7,8)</sup>. The greatest drawback of the method is the necessity of building up some 50-odd layers of barium stearate for each determination.

A simpler variation of this technique is created by using an optical step gage such as obtained from the General Electric Company or Millis Research. The estimation of thickness of an unknown film is made by matching the color of the slide coated with the film with the color of a step of the gage, if white light is used. With monochromatic light the intensities of the reflected light are matched instead of the colors. The light does not have to be polarized<sup>(9)</sup> and no measurement of angle of incidence is necessary. Measurements are accurate to less than about 3 Å. Both methods pioneered by Langmuir have been expanded by Rothen<sup>(10,11,12)</sup>.



In adapting these methods for his studies, Leo Vroman first made use of the barium stearate step gage as reported in his extremely readable *Blood*<sup>(10)</sup>. However, he moved to tantalum-coated glass slides, covered with anodic oxide films, which exhibit beautiful interference colors<sup>(14)</sup>. The hue of the colors depends on the thickness of the oxide which is determined by the potential applied during the electrolysis<sup>(15)</sup>. Rothen<sup>(16)</sup> mentions tantalum-coated slides, but says that, on the whole, rhodium-coated glass slides are the best.

Vroman's tantalum-coated slides were prepared at Bell Telephone by a thorough cleaning of the glass to remove all intro-compounds, followed by a tantalum sputtering and a heat-oxidation by temperatures of 300 - 500°C. The oxidation with  $Ta_2O_5$  follows by suspension of the tantalum sputtered slides in 0.01% nitric acid, while connected to a DC power supply. The oxide layer forms a uniform color depending upon the voltage applied. At 21 volts, the layer appeared orange; at 23 volts, wine red; at 25 volts, purple; at 26 volts, a reddish tan; at 27 volts, bluish violet. The orange and violet surfaces appeared best for observations in air and in buffer respectively<sup>(14)</sup>. Such slides withstand water rinses and flaming from a Bunsen flame.

Vroman uses these slides for ellipsometry and does not usually measure thickness with the interference color technique. However, such a method is very useful to check for uniformity of a film and as a simple way to follow thickness changes. For example, he reports<sup>(17)</sup> that albumin adsorbed out of water onto tantalum-sputtered slides (oxidized at 26 volts) showed a deep purple color against the reddish-tan slide. Then upon exposure to plasma, this adsorbed layer decreased in thickness as shown by a color shift to the red. Upon subsequent exposure to rabbit anti-human albumin serum caused a thickness increase, demonstrated by a light blue color. Thus the interference color technique is a very useful method that can obtain results as good as some sophisticated instruments can.

The second main technique of Vroman is the water vapor pattern method. Once again, at least some of the original inspiration for this technique must go to Irving Langmuir. In 1937, Langmuir with Schaefer<sup>(9)</sup> reported that "a sensitive indication of the uniformity of an initial layer (of barium stearate) may be obtained by cooling the back of a plate with running water and breathing on the front side so as to form a fog-like deposit of minute water drops. Lack of uniformity is made apparent by this 'breath figure'." Rothen used this technique in studying proteolytic action of enzymes on adsorbed protein layers<sup>(18)</sup>.

Vroman adapted this two-dimensional Wilson cloud chamber-idea for his work in 1962<sup>(19)</sup>. He was able to see changes in water wettability since the rounder droplets forming on less water wettable areas (greater contact angle areas) will scatter light more than will droplets with a lower contact angle and will thus appear lighter under



proper illumination. He was able to assess the relative hydrophobicity of thrombin and fibrinogen adsorbed onto glass and Lucite by water vapor patterns. Each test plate was refrigerated and then placed face-down over a 50°C water bath. The pattern was recorded by holding the plate a short distance in front of a black velvet cloth and photographing with an oblique flash. The technique has been only slightly changed up to the present<sup>(14)</sup>.

One interesting use of the vapor pattern method is in connection with the antisera test<sup>(20)</sup>. Solutions of proteins adsorbed on tantalum-sputtered slides were treated with drops of several antisera; upon testing with the vapor test, the protein films appeared darkest (hence presumably most wetted) where they had been exposed to their matching antiserum. This then allows Vroman to identify changes at interfaces, for example, at a plasma-solid interface<sup>(21)</sup>.

The delicacy of such a simple technique is demonstrable in that concentrations of thrombin as low as 0.2 mg % could change the wettability of a glass slide<sup>(14)</sup>.

However, recently Vroman writes that initially water vapor condensation does not reflect wettability. The reaction site of a protein and its antiserum, although quite wettable, may appear white on initial exposure to vapor, but will turn black in a few minutes, after the contact angle of the water droplets becomes the determining factor in the scattering of light<sup>(22)</sup>. He is also studying the complex factors involved in the evaporation rate of the droplets by phase microscopy, causing condensation under the objective from a wash bottle filled with warm water through which air is passed with an aquarium pump. On glass slides first rubbed with ferric stearate and then step-coated with barium stearate, water will condense rather evenly (with high contact angles on all areas) but will evaporate very rapidly from ferric stearate and very slowly from the first double layer step of barium stearate.

Therefore, another clever Vroman trick produces interesting information which can be used to identify short-term changes on a molecular level, such as those found with albumin exposed to glass for less than 5 seconds<sup>(17)</sup>.

The third Vroman technique is the metal oxide pattern method. Adsorbed films demonstrate their presence by simple exposure to a metal oxide. Vroman<sup>(14)</sup> gives the directions to obtain such a reaction: the treated surfaces are covered with a suspension of about 1.2 gram metal oxide in 10 ml of water, gently rocked for ten seconds and rinsed. After drying, the patterns of adsorbed powder could be lifted off the surface with "Scotch" tape and stored.

He also found that the glass from which a powder pattern had been removed by tape retained a residue to which fresh powder suspensions adhered even more rigidly. When tape was stuck over part of an area on which protein was adsorbed but which was only treated with powder after the tape was removed, the part of the pattern previously exposed to tape showed a markedly greater contrast.



Metal oxides most suitable for pattern formation on glass and plastic are (in order of increasing sensitivity):  $\text{WO}_3$ ,  $\text{Mn}_3\text{O}_4$ ,  $\text{Ta}_2\text{O}_5$ , Co oxides,  $\text{Fe}_3\text{O}_4$  and  $\text{Fe}_2\text{O}_3$ ,  $\text{Cr}_2\text{O}_3$ . Later<sup>(17)</sup>, it was found that sequential exposure to Mn and Cr oxide suspensions stained the most avidly attracting surfaces brown, while less stainable films colored green.

None of this should be surprising in view of the fact that organic films can be stained with heavy atoms by deposition into chemically preferred locations in the film<sup>(23)</sup>, and that adsorbed films can specifically bind ions such as those present in ferric oxide<sup>(24)</sup>. Some films can also be made water-wettable by treatment with solutions of certain polyvalent cations<sup>(9)</sup>.

The intensity of the staining of adsorbed films generally corresponds with the intensity of wettability change caused by the film being studied. Properly cleaned substrate surfaces did not adsorb the powder. The lower limit of this test is about at 0.05 % dilutions of normal plasma in contact with the surface for about one second. Lower concentrations, however, leave a track which can be shown on a "second printing". The possible uses of this method are well demonstrated<sup>(14)</sup>.

MORAL: EXPENSIVE EQUIPMENT DOES NOT A GENIUS MAKE.

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FIGURE 1

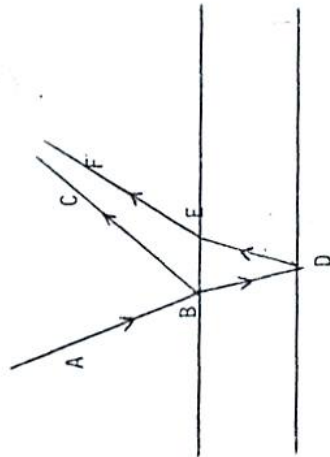


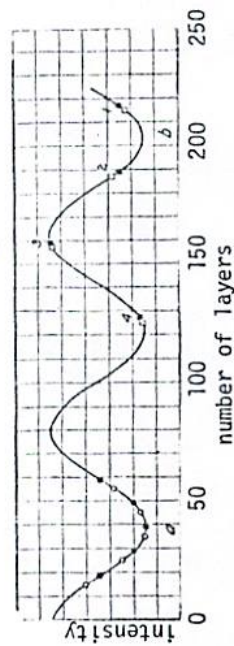
TABLE 1

—COSINES OF ANGLE OF REFRACTION  $r$  FOR  
ANGLE OF INCIDENCE  $i$  ON AIR-BARIUM SILICATE  
BOUNDARY

ANGLE OF INCIDENCE, $i$	ANGLE OF REFRACTION, $r$	Cos $r$
60°	35° 21'	0.8156
61°	35° 45'	0.8116
62°	36° 09'	0.8075
63°	36° 32'	0.8035
64°	36° 54'	0.7997
64° 30'	37° 03'	0.7978
65°	37° 16'	0.7959
65° 30'	37° 26'	0.7941
66°	37° 36'	0.7922
66° 30'	37° 47'	0.7903
67°	37° 57'	0.7885
67° 30'	38° 07'	0.7868
68°	38° 16'	0.7851
68° 30'	38° 26'	0.7834
69°	38° 35'	0.7817
69° 30'	38° 44'	0.7801
70°	38° 53'	0.7784
70° 30'	39° 02'	0.7768
71°	39° 10'	0.7752
71° 30'	39° 19'	0.7736
72°	39° 27'	0.7722
72° 30'	39° 34'	0.7708
73°	39° 42'	0.7694
73° 30'	39° 50'	0.7680
74°	39° 57'	0.7667
74° 30'	40° 04'	0.7653
75°	40° 11'	0.7640
76°	40° 24'	0.7615
77°	40° 26'	0.7592
78°	40° 48'	0.7570
79°	40° 59'	0.7550
80°	41° 08'	0.7531
81°	41° 17'	0.7514

taken from (5), p. 768.

FIGURE 2



taken from (1), p. 1013.