It was possible at this point to isolate **2-carbomethoxy-4-cyano-4-phenylcyclohexanone**. In a separate experiment (starting with 13.7 g. of 4-cyano-4-phenylpimelonitrile) the ester was isolated to yield 13.8 g. (90%) of an oil which solidified on standing, m.p. 89–92°. Purification by evaporative distillation at 100–135° (0.4 mm.) followed by recrystallization from methanol gave a colorless crystalline product, m.p. 95–96°.

Anal. Calcd. for $C_{15}H_{15}O_2N$: C, 70.02; H, 5.88. Found: C, 70.15; H, 5.73.

Hydrolysis and decarboxylation were effected as usual with hydrochloric–acetic acids. From the ether–ethyl acetate extract there was obtained 51–66 g. (73–92%) of light-brown crystalline product, m.p. 76–91°. Recrystallization from benzene–pentane gave colorless crystalline 4-cyano-4-phenylcyclohexanone, m.p. 114.5–115°.

Anal. Calcd. for $C_{13}H_{15}ON$: C, 78.36; H, 6.57. Found: C, 78.36; H, 6.49.

The **oxime** was recrystallized from dilute methanol, m. p. 150–151°.

Anal. Calcd. for $C_{13}H_{14}ON_2$: C, 72.89; H, 6.59. Found: C, 72.83; H, 6.37.

γ-Cyano-γ-phenylpimelic acid.—A solution of 1.5 g. of dimethyl γ-cyano-γ-phenylpimelate in 20 ml. of 10% sodium hydroxide and 10 ml. of methanol was refluxed for three hours. The solution was diluted with 100 ml. of water and extracted with two 50-ml. portions of ether. Acidification of the alkaline solution gave a colorless crystalline material which was extracted with three 50-ml. portions of ether. After the ether solution had been washed with water and dried, the solvent was removed to yield 1.0 g. of colorless crystalline product, m.p. 165–167°. Recrystallization from water gave a pure sample, m.p. 171–172.5°.

Anal. Calcd. for $C_{14}H_{15}O_4N$: C, 64.35; H, 5.78; neut. equiv., 130.6. Found: C, 64.47; H, 5.62; neut. equiv., 134.

This acid was also produced in 81% yield by the alkaline hydrolysis of 2-carbomethoxy-4-cyano-4-phenylcyclohexanone.

8-Phenyl-8-cyano-1,4-dioxaspiro[4.5]decane (VIB).—From 90.0 g. (0.45 mole) of 4-cyano-4-phenylcyclohexanone there was obtained, after one recrystallization from methanol, 82.4 g. (75%) of ketal, m.p. 123–125.5°. Recrystallization was effected from ether (Dry Ice); m. p. 124–125°.

Anal. Calcd. for $C_{15}H_{17}O_2N$: C, 74.05; H, 7.04. Found: C, 73.94; H, 6.91.

8-Phenyl-1,4-dioxaspiro[4.5]decane (VIIB).—Removal of the cyano group was carried out on a 0.35-mole scale to yield 90–95% of product, m.p. 49–50°. Recrystallization from aqueous ethanol gave an analytical sample, m.p. 54–56°.

Anal. Calcd. for $C_{14}H_{18}O_2$: C, 77.03; H, 8.31. Found: C, 77.10; H, 8.68.

4-Phenylcyclohexanone (VIIIB).—Hydrolysis of the ketal gave 4-phenylcyclohexanone, m. p. 74–77°, in 69–77% yield. A recrystallized sample melted at 77–79° (reported[8] m.p. 76–77°).

(8) Fieser, Leffler, *et al.*, THIS JOURNAL, **70**, 3186 (1948).

PHILADELPHIA, PENNA. RECEIVED JUNE 29, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY]

Size and Shape of Bovine Fibrinogen[1]

BY COLIN S. HOCKING,[2] MICHAEL LASKOWSKI, JR., AND HAROLD A. SCHERAGA

Flow birefringence and viscosity measurements indicate that the bovine fibrinogen molecule may be approximated by a rigid prolate ellipsoidal model of length 670 Å. and axial ratio 18 to 1. From light scattering a molecular weight of 407,000 is obtained. A comparison of the molecular parameters of bovine and human fibrinogen indicates little difference in the size and shape of these two proteins. The dissymmetry method for determining lengths from light scattering data is not easily applicable to the present system because it is very sensitive to traces of large particle impurities.

Recent results for the length and molecular weight,[3] and for the intrinsic viscosity and sedimentation constant[4] of bovine fibrinogen raise the question of possible differences in the size and shape of bovine and human fibrinogen. Since much attention is being directed to the study of the reactions of these proteins, it was considered desirable to determine some of the molecular parameters of bovine fibrinogen using viscosity, flow birefringence and light scattering techniques, and to compare these parameters with those for human fibrinogen.[5]

Experimental Methods and Results

Materials.—Experimental work was carried out both with fibrinogen samples prepared from fresh steer blood and also from Armour bovine Fraction I. The results reported here were obtained by refractionation of the Armour material using Laki's procedure[6] which gave preparations which were 91–94% clottable with thrombin. All measurements were carried out on freshly fractionated material without drying the protein. The purified fibrinogen solutions were dialyzed against a solution made by mixing 85% by volume of 0.45 *M* NaCl with 15% of sodium citrate buffer of ionic strength 0.45. The final *p*H of the samples was 6.3.

Clottable protein was determined by the gravimetric method of Morrison[7] using Parke, Davis thrombin and the per cent. clottability by that of Laki.[6]

Viscosity.—Ostwald type viscometers having a flow time of two to three minutes with buffer at 25.00 ± 0.02° were used. From a plot of reduced specific viscosity, η_{sp}/c, against concentration of *fibrinogen* in g./100 ml. an intrinsic viscosity of 0.25 was obtained; this value corresponds to an axial ratio of 18 for an unhydrated prolate ellipsoid of revolution[8] if a partial specific volume of 0.725 is assumed.[9]

This value for the intrinsic viscosity confirms that obtained by Nanninga[10] (0.27 at *p*H 7) but is lower than the value 0.34 obtained by Shulman and Ferry[4] who have pointed out that their value may be too high because of a small contribution from non-clottable proteins.

Flow Birefringence.—The apparatus used for the flow birefringence studies has already been described.[11,12]

The orientation theory of Peterlin and Stuart[13] gives the extinction angle, χ, as a function of the parameter α =

(1) This work was supported by contract N6-onr 26414 between Cornell University and the Office of Naval Research.

(2) Rotary Foundation Fellow, 1950–1951.

(3) R. F. Steiner and K. Laki, THIS JOURNAL, **73**, 882 (1951).

(4) S. Shulman and J. D. Ferry, *J. Phys. Colloid Chem.*, **55**, 135 (1951).

(5) J. T. Edsall, J. F. Foster and H. Scheinberg, THIS JOURNAL, **69**, 2731 (1947).

(6) We wish to thank Dr. Laki for sending us the details of his purification and assay procedures.

(7) P. R. Morrison, THIS JOURNAL, **69**, 2723 (1947).

(8) R. Simha, *J. Phys. Chem.*, **44**, 25 (1940).

(9) S. H. Armstrong, Jr., M. J. E. Budka, K. C. Morrison and M. Hasson, THIS JOURNAL, **69**, 1747 (1947).

(10) L. Nanninga, *Arch. Neerland. Physiol.*, **28**, 241 (1946).

(11) J. T. Edsall, C. G. Gordon, J. W. Mehl, H. Scheinberg and D. W. Mann, *Rev. Sci. Instruments*, **15**, 243 (1944).

(12) H. A. Scheraga and J. K. Backus, THIS JOURNAL, **73**, 5108 (1951).

(13) A. Peterlin and H. A. Stuart, *Hand. u. Jahrb. d. Chem. Physik, Bd. 8, Abt. IB* (1943).

G/Θ, where G is the velocity gradient across the gap and Θ is the rotary diffusion constant. Simultaneous measurements of χ and G enable one to evaluate Θ. Tables for use in this connection have recently been compiled.[14]

On the assumption that the protein is representable as an elongated, rigid prolate ellipsoid of revolution the molecular length is obtainable from the equation of Perrin,[15] using a value of 18 for the axial ratio.

Measurements were made at 25.0° on clarified fibrinogen solutions in glycerol–water–salt mixtures at various protein and glycerol concentrations. The range of glycerol concentrations was 45 to 60 weight per cent. and that of protein concentrations was 0.09 to 0.30 g./100 ml. The extinction angle χ as a function of $G\eta$ is shown in Fig. 1. The curves are theoretical ones for lengths of 600, 650 and 700 Å. There is a concentration dependence which becomes negligible below 0.16% protein. It may also be seen that the best value for the molecular length is 670 Å.

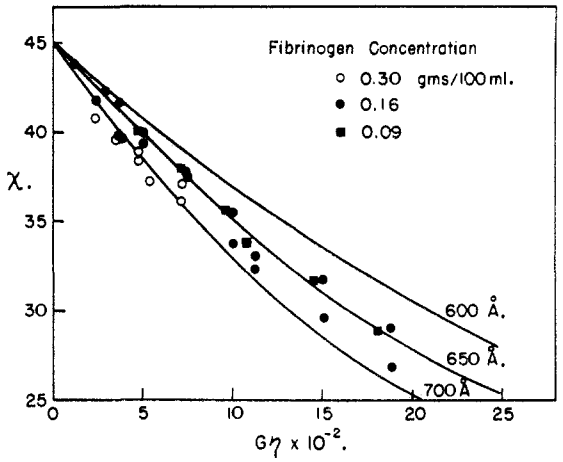

Fig. 1.—Extinction angle, χ, as a function of $G\eta$ for several concentrations of bovine fibrinogen. The curves are theoretical ones for the lengths indicated.

The solutions are relatively monodispersed as can be seen in Fig. 2 where the length L is plotted as a function of $G\eta$. If the solutions were polydispersed then L would vary as the gradient changes.

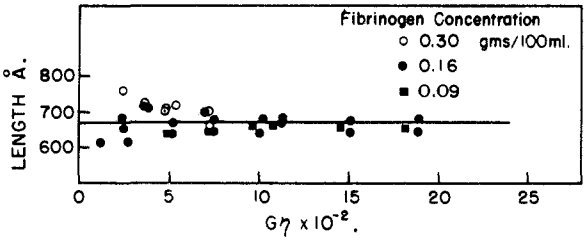

Fig. 2.—Dependence of length on gradient. The horizontal line, corresponding to a length of 670 Å., indicates that the system is relatively monodispersed.

The magnitude of the birefringence was also determined; a knowledge of this quantity, together with the rotary diffusion constant and refractive increment reported below, enables one to calculate the principal indices of refraction of the fibrinogen molecule.[16] The resulting values are $n_1 = 1.64$ and $n_2 = n_3 = 1.69$. Since n_1 is less than n_2, as found for human fibrinogen and other asymmetric proteins of plasma,[17] bovine fibrinogen possesses a negative intrinsic birefringence, indicating that the polarizability of the protein is greatest in a direction perpendicular to its long axis. The implications of this result with respect to the arrangement of the chains in the molecule is being explored at present in this Laboratory. The calculation of the depolarization factor from n_1 and n_2 is indicated below.

Light Scattering.—The instrument used was designed by A. M. Bueche and described by Debye and Anacker.[18] In order to remove dust and other large particles the solutions were centrifuged for five hours in a Sorvall angle centrifuge at 20,000 g.

Measurements were made with mercury blue light, 4358 Å.; the appropriate correction factors for scattering volume and for unpolarized light were applied. The angular dependence of the scattering from carbon tetrachloride agreed with the theoretical values within 1.3% which was used as a cell correction.

From the values of n_1, n_2, n_3 reported above, it is possible to calculate the mean polarizability of the particle and also its optical anisotropy.[19] These quantities are related to the depolarization factor[20,21] which, in this case, is 4×10^{-4}. The measured value for the depolarization, extrapolated to zero concentration, was essentially zero within the experimental error, in agreement with this calculation. Therefore, no depolarization correction was required.

The angular dependence of scattering was determined on solutions containing from 0.1 to 5 mg./ml. of protein and found to be independent of concentration in this range. Figure 3 represents a typical case of the relative intensity of scattering from a solution containing 2 mg./ml. The angular distribution of the solvent scattering was approximately 1.5–2.0% of the total observed intensity of scattered light from a 0.1% solution indicating that the solvent was essentially free of scattering impurities.

It is apparent that the scattering curve represents a polydispersed system and one is led to suspect the presence of a few large aggregates which scatter much light at the lower angles. This means that calculations made by using the relations for a monodispersed system are incorrect and, in this instance, give values which are definitely too high. For rod shaped particles of negligible diameter the angular dependence of intensity of the scattered light is given by an equation developed by Debye.[18,20,22,23] In particular, the ratio of the intensity of light scattered at 45° to that at 135° is a convenient manner in which to characterize the major dimension. Tables relating this ratio, known as the dissymmetry, with the length, L, have been presented by Cashin[24] and by Doty and Steiner.[25] Using these relations the apparent length of the fibrinogen molecule has been calculated from the observed dissymmetry. Values in the range 800–840 Å. were obtained, in agreement with those reported by Steiner and Laki[3]; however, since the curve of Fig. 3 is not representative of a monodispersed system, the lengths calcuated in this manner are incorrect but are reported here to emphasize the necessity for examining the complete angular scattering curve.

In addition to the above results calculations were also made of the molecular weight. From measurements of turbidity and refractive increment the molecular weight is obtainable from the equation, $\frac{1}{M} = H\frac{c}{\tau}$, where $\frac{c}{\tau}$ is the value of the *total* protein concentration in g./ml. divided by the excess turbidity extrapolated to zero concentration; H is a refractive constant given by $\frac{32\pi^3}{3}\frac{n_0^2}{N\lambda^4}\left(\frac{n - n_0^2}{c}\right)$ where n_0 is the refractive index of the solvent, 1.35, λ is the wave length *in vacuo*, 4358×10^{-8} cm., N is the Avogadro number, 6.024×10^{23}, and $(n - n_0)/c$ is the refractive increment, 0.199, as determined in a differential refractometer which was a modified version of that described by P. P. Debye.[26] Substitution gives a value for H of 10.99×10^{-6}.

A sample of polystyrene supplied by Debye and Bueche and having an excess absolute turbidity over that of toluene of 3.51×10^{-3} cm.$^{-1}$ for a 0.5% solution has been used as a

(14) H. A. Scheraga, J. T. Edsall and J. O. Gadd, *J. Chem. Phys.*, **19**, 1101 (1951).
(15) F. Perrin, *J. Phys. Radium*, [7] **5**, 497 (1934).
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(18) P. Debye and E. W. Anacker, *J. Phys. Colloid Chem.*, **55**, 644 (1951).
(19) H. A. Stuart, *Hand. u. Jahrb. d. Chem. Physik*, **10**, 27 (1939).
(20) G. Oster, *Chem. Revs.*, **43**, 319 (1948).
(21) P. Debye and E. S. Elyash, private communication.
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(23) P. Debye, *J. Phys. Colloid Chem.*, **51**, 18 (1947).
(24) W. M. Cashin, private communication.
(25) P. Doty and R. F. Steiner, *J. Chem. Phys.*, **18**, 1211 (1950).
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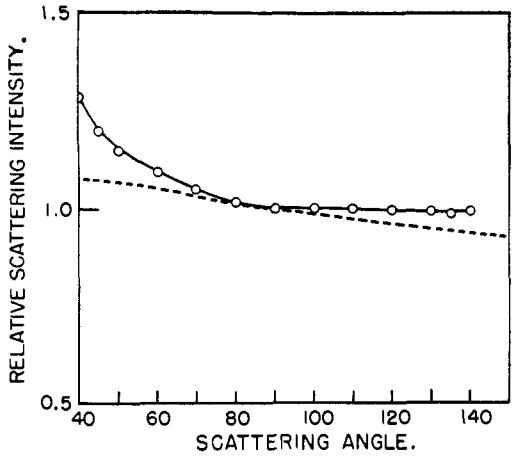

Fig. 3.—Angular scattering curve for a 0.2% solution. The dashed curve is a theoretical one for a rod of 670 Å. length. From a comparison of the experimental and theoretical curves it is apparent that the system is polydispersed.

standard for our turbidity measurements. Carr and Zimm[27] have published data on the absolute intensity of scattering of materials suitable for standards in this work. These latter workers report a value of 3.48×10^{-3} for this standard while Doty and Steiner[25] report a value of 3.50×10^{-3}.

The values of $H\frac{c}{\tau_{90}}$ at various concentrations are plotted in Fig. 4, the value extrapolated to zero concentration being 2.68×10^{-6}. The dissymmetry correction is discussed below.

Discussion

Several experimental problems are involved in the application of the methods used here. In flow birefringence studies it is necessary to add glycerol in order to increase the viscosity of the solvent. Also high rates of shear are required to make measurements on relatively small macromolecules. However, the errors resulting from the presence of dust and denatured molecules are of lesser significance than in the case of light scattering. It was originally hoped that a comparison could be made of the dissymmetry and flow birefringence methods for determining particle lengths. However, as will be pointed out, the dissymmetry method does not appear to be easily applicable to this protein and the validity of the flow birefringence method will have to be based upon its success in previously studied systems.

Since the light scattering data indicate the presence of traces of large particles which scatter considerably at low angles, it is not valid to calculate the length from the dissymmetry, I_{45}/I_{135} on the basis of a monodispersed system of rigid rods. The lengths determined in this manner are, therefore, too high and are thus only an upper limit. Presumably, if these traces could be removed, it is conceivable that the angular scattering curve would have the appearance calculated from a rigid rod model. The flow birefringence data are not significantly affected, Fig. 2 indicating a relatively monodispersed system. One must, therefore, attach greater significance to the length obtained from the latter method, *viz.*, 670 Å.

It should be pointed out that it is possible to account for the observed angular dependence data

(27) C. I. Carr and B. H. Zimm, *J. Chem. Phys.*, **18**, 1616 (1950).

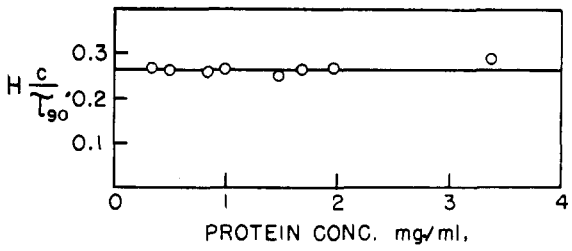

Fig. 4.—Concentration dependence of $H\frac{c}{\tau_{90}}$.

and, therefore, for this discrepancy between the two methods, by assuming the presence of a small number of spherical particles of a radius of 2,000 Å. among the fibrinogen rods. One can postulate that such particles might be gel-like or denatured agglomerates in which the protein material occupies about 1/100 of the total volume of the particle. By virtue of their low density, they would not be removed by the centrifugal clarification procedure. Such particles need be present only to the extent of 0.01% to account for the observed departure of the angular scattering curve from that for a rigid rod. Such a small concentration of big particles would escape detection in the ultracentrifuge and, if spherical as assumed, would not be oriented in a flow birefringence experiment.

The postulated size of the extraneous agglomerates of protein, which affect the scattering at low angles, is such that a minimum occurs in their scattering curve at 90°. Thus, the molecular weight as determined from the 90° scattering is not appreciably affected by their presence. It is also, therefore, valid to correct the 90° scattering by a dissymmetry factor corresponding to rods of 670 Å. length. From Cashin's tables[24] the factor for correcting the turbidity for rods of such length is 1.09 giving a value of $H\frac{c}{\tau_0}$ of 2.46×10^{-6}. A molecular weight of 407,000 may, therefore, be assigned to the bovine fibrinogen. Nanninga's value[10] of 441,000 from osmotic pressure may be cited here for comparison.

In Table I are listed some molecular parameters of bovine fibrinogen on the basis of this discussion together with some of the published data on human fibrinogen. The data on the human material have been extensively discussed by Edsall, Foster and Scheinberg[5] who concluded that human fibrinogen molecules in solution can be approximated by rigid, hydrated ellipsoids of revolution. This is also true for the bovine material and is in agreement with the electron microscope studies of Hall[28]

TABLE I

COMPARISON OF MOLECULAR PARAMETERS OF BOVINE AND HUMAN FIBRINOGEN

	Bovine	Human[a]
$\Theta_{20,w}$, sec.$^{-1}$	39,400	35,000
Molecular weight	407,000	580,000
Length, Å.	670	700
H_0	0.25	0.25
$S_{20,w} \times 10^{13}$	8.4[b]	8.5–9

[a] Edsall, Foster and Scheinberg.[5] [b] Shulman and Ferry.[4]

(28) C. E. Hall, *J. Biol. Chem.*, **179**, 857 (1949).

who found that both human and bovine fibrinogen molecules are rod-like, filamentous structures.

On the basis of the data listed in Table I, there does not appear to be too great a difference in the size and shape of bovine and human fibrinogen. The discussion of other similarities or differences for these two proteins is beyond the scope of this paper.

The authors wish to thank Dr. F. Bueche for helpful discussions in connection with the light scattering results.

ITHACA, NEW YORK RECEIVED JUNE 7, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MICHIGAN]

Wetting Characteristics of Cellulose Derivatives. I. Contact Angles Formed by Water and by Organic Liquids[1]

BY F. E. BARTELL AND B. ROGER RAY[2]

The wetting characteristics of each of a fairly extensive series of cellulose derivatives were determined by measuring the angle of contact of water and of various organic liquids against these derivatives. Fibers, rods, foils, films and coatings of the derivatives were used. A regular and systematic increase in the hydrophobic nature of the fatty acid esters was found to occur with increase in length of hydrocarbon chain; also, with the acetate derivatives, increase occurred with increase in acetyl content. Qualitatively, the increase in hydrophobicity with substituent groups was in the order: hydroxyl, formyl, acetyl, hydrogen phthalate, ethoxy, nitro, propionyl and benzoyl. The degree of polymerization appeared to have little influence on the wetting characteristics of the two sides. Organic liquids of high surface tensions tended to give finite angles of contact on the derivatives. Hysteresis of contact angle ranged from approximately 10° to over 40°. When cosines of the contact angles of water on a series of the fatty acid triesters were plotted against either the tensile strengths or the densities of the respective esters, a linear relationship was observed.

Soft solids such as cellulose derivatives and other polymers and resins, characterized as they are by low free surface energies, provide especially desirable surfaces for wettability investigation. Complications due to adsorption are at a minimum, and a number of liquids can be found which give finite and measurable values for both advancing and receding angles. These measured angles appear to be stable and closely reproducible. The available data on the wettability of cellulose derivatives, however, are limited to a few water–air–solid contact angle values, most of which are unreliable or ill defined. Some investigators have chosen to ignore the phenomenon of hysteresis of contact angle when making measurements on cellulose derivatives. Others have attempted to circumvent its effects. The hysteresis effect, *i.e.*, the difference between the angle formed when a liquid has been caused to advance over the solid and the angle formed when the liquid has been caused to recede, may be absent or negligible in some systems, but in most systems it is appreciable, and in the case of the cellulose derivatives it must be taken into account.

The specific objectives of the present research were to measure both the advancing and receding contact angles of water and of several organic liquids on representative cellulose derivatives and to correlate the data with the physical and chemical structures of these solids.

Experimental Details

Methods Used.—The angles were measured by the vertical-rod, the tilting plate, or the controlled-drop-volume method (a modification of the usual drop-on-plate method) depending upon the nature of the solids to be studied. Numerous comparisons showed complete agreement among these methods.

In the vertical-rod method[3] either a fiber of the polymer, or a rod or a plate coated with a film of it, or a foil[4] was partially immersed in the liquid. A magnified silhouette of the liquid–solid interface was projected onto a screen or a photographic plate from which the angle could be measured directly. In the well-known tilting-plate method[5,6] the plate or rod was tilted until the liquid interface on one side remained perfectly horizontal up to the liquid–solid contact point. The angle of tilt gave the contact angle. In the controlled-drop-volume (or sessile drop) method[7,8] the angle was determined either by erecting a tangent upon the enlarged image of the drop or by calculations involving drop shape factors.

The vertical-rod and controlled-drop-volume methods proved to be superior because of the ease with which they enabled enclosure of the system so as to prevent vaporization, the convenience of observation of time effects, the facility with which advancing and receding conditions could be attained, and the accuracy possible over the entire range of 0 to 180°.

An air thermostat regulated to 25 ± 0.1° enclosed the contact angle device and cell and was supported independently so as to introduce no vibration. For vertical-rod (or vertical plate) and tilting-plate measurements a special supporting device and sample holder utilizing fine rack-and-pinion elements, made possible a sensitive and regular movement of the sample in the horizontal and vertical planes and also permitted a rotating movement about a fixed point which could be so adjusted that the center of rotation of the sample remained exactly at the interface of the liquid. Magnifications of from 5 to 1000× were used.

The controlled-drop-volume method was found to be the most suitable for some measurements on foils or on films. A critical requirement of this method is the sensitive and positive control of the drop volume accomplished by use of a pipet with a very fine tip (approximately 0.02 mm. o.d.) from which the liquid is either forced out by air pressure to

(1) The data in this paper were taken from a portion of a thesis of B. Roger Ray, submitted to the School of Graduate Studies of the University of Michigan in partial fulfillment of the requirements for the Ph.D. degree, December, 1945.

(2) Minnesota Mining and Manufacturing Co. Fellow, 1943–1945.

(3) F. E. Bartell, J. L. Culbertson and M. A. Miller, *J. Phys. Chem.*, **40**, 881 (1936).

(4) The term "foil" is used throughout to designate a sheet-like material of considerable thickness and rigidity and without a supporting base. The term "film" is reserved for a very thin layer of material attached to a supporting base.

(5) F. M. Fowkes and W. D. Harkins, THIS JOURNAL, **62**, 3377 (1940).

(6) N. K. Adam and H. L. Shute, *J. Soc. Dyers and Col.*, **53**, 121 (1937).

(7) F. E. Bartell and P. H. Cardwell, THIS JOURNAL, **64**, 494 (1942).

(8) F. E. Bartell and K. E. Bristol, *J. Phys. Chem.*, **44**, 86 (1940).