Rheology of Surface Films. I. Rheological Characteristics of Monomolecular Films of Ovalbumin and Serum Albumin*

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Introduction

Recent interest in the study of surface films has been focused upon the problems of high molecular substances, such as proteins or synthetic high polymers. Since the most prominent characteristics of high polymers is their forming of viscoelastic films, excellent means for measuring the mechanical properties of surface films are now greatly needed. Although classical measurements of surface viscosity, based on the canal method[1−3] or damped oscillation method,[4−8] have shown considerable merits in the film studies of simple molecules, they were not so informative when applied to the viscoelastic films of high polymers. In view of the recent development of modern rheology with three-dimensional materials, the application of the method and concept of modern rheology to the two-dimensional systems would shed new light on the field of physical chemistry of surfaces. The aim of this series of works is to provide a set of standards of surface rheology and to achieve the analyses of mechanical properties of protein or synthetic polymer films from the modern rheological standpoint.

At the beginning of this work, the first attempt was started with protein monolayers at the air-water interface. At the present stage, it appeared necessary to make a careful preliminary study with some well-defined proteins. For this purpose, crystalline ovalbumin and serum albumin were mainly used. The present paper describes the mechanical characteristics of these protein films obtained by the method based on the analyses of deformation-time relation of the film subjected to shearing stress.

Experimental

Procedures—A static mothod was employed in this preliminary study. The apparatus is principally the same as used by Schwedoff and Hatschek[9] with three-dimensional gel. The illustration is given in Fig. 1. (A) is a platinum ring of 3.7 cm. in diameter. The suspending wire is enclosed by a protector (D) which is coaxial with the torsion head (C). The rotational angle of the torsion head and the deflection of the ring are measured by means of the mirrors (E) and (F) respectively, with lamp and scale (G). Care was taken that the experimental condition might not destroy the fine structure of films. For this purpose, both the moment of intertia of the ring

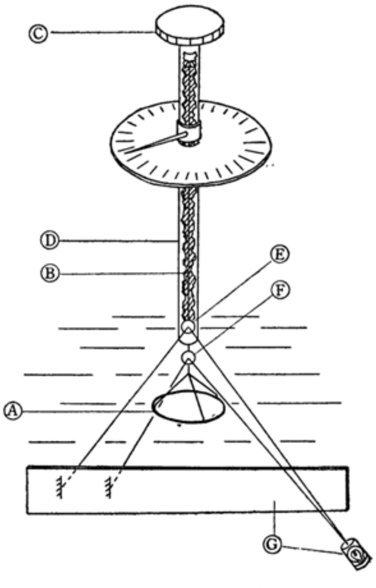

Fig. 1.—Diagrammatic illustration of the apparatus for measuring visco-elasticity of films

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* Partly presented at the Annual Meeting of the Chemical Society of Japan, April, 1952.

1) M. Joly, *J. de phys.* **11**, 471 (1937), *Kolloid-Z*, **89**, 26 (1939).

2) E. R. Washburn and H.R.R. Wakeham, *J. Am. Chem. Soc.*, **60**, 1294 (1938).

3) R. J. Meyers and W. D. Harkins, *J. chem. phys.*, **5**, 601 (1937).

4) L. Fourt and W. D. Harkins, *J. Phys. Chem.*, **42**, 897 (1938).

5) I. Langmuir, and V. J. Schaefer, *J. Am. Chem. Soc.*, **52**, 2400 (1937).

6) L. Fourt, *J. Phys. Chem.*, **43**, 887 (1939).

7) M. Joly, "Surface Chemistry" (Supplement of Research) 157 (1949).

8) C. W. N. Gumper and A. E. Alexander, *Trans. Faraday Soc.*, **46**, 235 (1950).

9) V. E. Hatschek and R. S. Jane, *Kolloid-Z.*, **39**, 300 (1926).

and the torsion constant of a suspending wire which were chosen were sufficiently small. That is, a series of phosphor bronze wire of 25μ to 60μ in diameter and 30 cm. in length and the rotating system of moment of inertia of 0.8 g. cm^2 were used. Order of shearing stress ranged from 10^{-3} to 10^{-1} dyne per cm. The trough was 14.7 cm. wide and 60 cm. long. Wilhelmy dipping slide was applied to the one side of the trough to furnish the surface pressure-area curve (designated as F-A curve hereafter). The sensitivity of the balance was about 0.01 dyne per cm. To obtain maximum symmetry, the ring was suspended 7.3 cm. in front of the dipping slide. After the quick turn of the torsion head by a definite angle φ (less than 10 degrees), the deflection of the hanging ring ω during relaxation was measured with time t. As the surface film was distorted with the ring, ω was considered to be a strain of the film materials. When the ring was applied to a clean surface of water, the contribution of moment of intertia of the rotating system was found to be not small enough to be ignored. However, once the surface was covered by a certain amount of monolayer substance, the contribution of moment of inertia became negligible due to the increased film resistance against the motion of the ring. Under such conditions, if the film exhibits Newtonian flow, the rate of strain is given by the equation,

$$\dot{\omega} = \frac{K}{R}(\varphi - \omega) \tag{1}$$

In case of ideal elastic films, where only the instantaneous elastic deformation occurs, the following equation holds:

$$\omega = \frac{K}{E}\ (\varphi - \omega) \tag{2}$$

In above equations, K is the torsion constant of the suspending wire, R and E, the constants related to the surface viscosity coefficient η and surface shear modulus G expressed by the following formulas, (3) and (4), respectively

$$\eta = \frac{k}{4\pi}\left(\frac{1}{r_1^2} - \frac{1}{r_2^2}\right)\left(\frac{\varphi - \omega}{\dot{\omega}}\right) \tag{3}$$

$$G = \frac{k}{4\pi}\left(\frac{1}{r_1^2} - \frac{1}{r_2^2}\right)\left(\frac{\varphi - \omega}{\omega}\right) \tag{4}$$

where r_1 and r_2 are the radii of the ring, and of the circular films coaxial with the ring, respectively. In the present apparatus, r_2 may be assumed to be 7.3 cm., effective radius of the trough, which is sufficiently large compared with r_1. If the film under test is perfectly elastic, the film inside the ring would not affect the motion of the ring. Therefore, so far the instaneous elasticity is concerned, the shear modulus was calculated ignoring the film formed inside the ring. This assumption was checked to be valid within the experimental error*.

As will be described later, the mechanical properties of real films were generally neither a purely viscous, nor a purely elastic, but a more complicated one, superposed by both viscositly and elasticity. The deformation-time curve of such films were found to be quite similar to that of three-dimensional materials stressed, so that the mechanical properties of films would be analyzed by the usual method in rheology[10].

Materials—Ovalbumin was prepared from a fresh hen eggs by the sodium sulfate method and successive crystalization according to Kekwick[11]. Horse serum albumin was a crystalline product prepared by the sodium sulfate method[12] and horse hemoglobin by the alcohol method.

Results

***ω-t* Curve of Ovalbumin Film**—When the ovalbumin aqueous solution was spread on 0.1 N hydrochloric acid solution, or on 5% ammonium sulfate solution, the following ω-t curve was observed (Fig 2). When the shearing stress was imposed, an instantaneous deformation (a-b) occurred, which follows time-dependent deformation (creep, b-c-d). At a later time, if the stress was removed, the instantaneous recovery of the first deformation occurred (d-e), preceding the time-dependent recovery of the creep (e-f). Complete recovery of the overall deformation, on the removal of the stress, was not generally observed, except the case under sufficiently

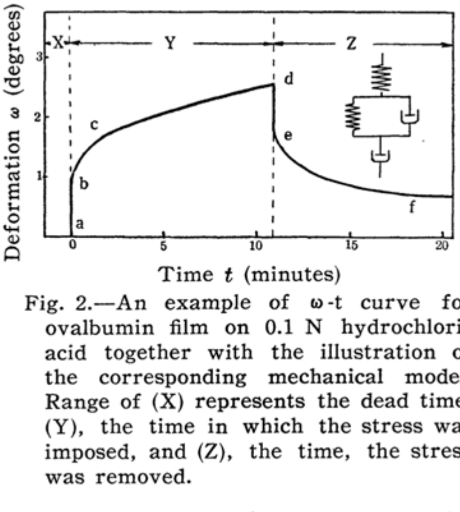

Fig. 2.—An example of ω-t curve for ovalbumin film on 0.1 N hydrochloric acid together with the illustration of the corresponding mechanical model. Range of (X) represents the dead time, (Y), the time in which the stress was imposed, and (Z), the time, the stress was removed.

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* The ring, coated with paraffin, was applied to the elastic films of ovalbumin, and the shear modulus of instantaneous elasticity was obtained by equation (4). Next, after the film inside the ring was removed by being absorbed with filter paper, the same measurement was made again. Both results obtained were found to coincide each other within the experimental error.

[10] T. Alfrey, "Mechanical Behaviors of High Polymers," Interscience Publishers (1948).

[11] R. A. Kekwick and R. K. Cannon, *Biochem. J.*, **30**, 227 (1936).

[12] R. A. Kekwick, *Biochem. J.*, **32**, 552 (1938).

small stress. Such mechanical behaviors may be approximately represented by the mechanical model of four elements with spring (elasticity) and dash pot (viscosity) as illustrated in Fig. 2. Such behavior was in common covering nearly the whole range of film areas. The instantaneous elasticity is the most definable in mechanical terms, since it mainly involves biased strain, and not any flow. So, with ovalbumin films, the main attention was directed toward the instantaneous elasticity of the film.

Relation between Stress and Instantaneous Elasticity—At a definite area, the stress-strain relation was obtained. As shown in Fig. 3, the strain was found to be proportional to the stress imposed. Such linearity holds covering almost the whole range of film areas. This means that the instantaneous elasticity obeys Hooke's law. When the shear stress was over a certain limit, however, the stress-strain curve became bent with the convex curvature against the stress axis. Further increase in the stress rendered the instantaneous elastic deformation obscure, causing the destruction of film structure (inset of Fig. 3). When the structure was once destroyed, reformation of the film took an appreciable time. In other words, ovalbumin film shows two-dimensional "thixotropy".

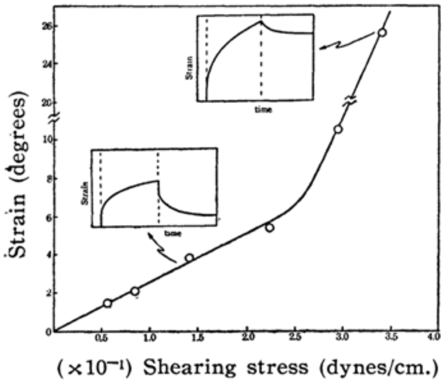

Fig. 3.—Stress-strain curve for ovalbumin films on 5% ammonium sulfate solution at 9.5 sq. m. per mg. Temperature, 10°C. Insets give the ω-t curves under the stress indicated by arrows.

Relation between Instantaneous Elasticity and Film Area—In a sufficiently large area, ovalbumin film did not show any perceptible resistance against the motion of the ring. As the area was reduced, at a definite area, the ring suddenly received an appreciable resistance. In the case, the ω-t curve was not typical as shown in Fig. 2 and instantaneous elastic deformation was obscure. If the film was compressed a little more, however, the ω-t curve converted to give a typical instantaneous elastic response as shown in Fig. 2.

Shear modulus of instantaneous elasticity is obtained from the equation (4) at various areas, and the modulus vs. area curve (designated as G-A curve hereafter) was obtained. A typical example of the G-A curve is given in Fig. 4. The aqueous solution of 0.08 mg. per cc. was spread on 0.1 N hydrochloric acid at the initial spreading concentration of 0.2 mg. per sq. m. Twenty minutes were allowed to insure complete spreading. The stepwise compression was extended to about 2 hours. Compressibility*-area curve, obtained from the F-A curve, was depicted in the same figure. It was found that the G-A curve starts at about 5 sq. m. per mg. (point A) and surface modulus increased

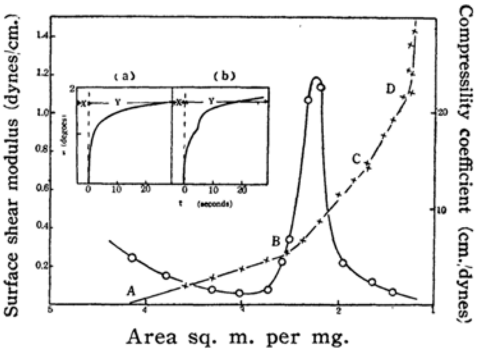

Fig. 4.—Surface shear modulus-area curve (crosses) and compressibility-area curve (circles) of ovalbumin film on 0.1 N hydrochloric acid, at 21°C. Insets give the ω-t curves. The region of (X) represents the dead time, and (Y), the time the stress is imposed.

TABLE I

FLUCTUATION OF BOTH VALUES OF CRITICAL AREA (A) AND CRITICAL SHEAR MODULUS (G) AT BREAKS (A, B, C, D) OF SHEAR MODULUS-AREA CURVE OF OVALBUMIN FILMS ON 0.1 N HYDROCHLORIC ACID, AT 14–15°C.

	A	B	C	D
a (m^2/mg)	5.3	2.25	1.65	1.30
g (*dynes/cm*)		0.20	0.35	0.81
a (m^2/mg)	4.8	2.15	1.65	1.35
g (*dynes/cm*)		0.35	0.57	0.86
a (m^2/mg)	5.3	2.10	1.55	1.35
g (*dynes/cm*)		0.45	0.75	1.15
a (m^2/mg)	5.3	2.55		
g (*dynes/cm*)		0.37		

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* Surface compressibility is defined as $-1/A(dA/dF)$, where F is the surface pressure in dynes per cm. and A, the film area in sq. m. per mg.

linearly with the decrease of the surface area, exhibiting several breaks at about 2.3 sq. m. per mg. (point B), 1.7 sq. m. per mg. (point C) and 1.3 sq. m. per mg. (point D). The extent of fluctuation of values of these breaks, obtained under the same condition, was shown in Table I. Although absolute values of shear modulus corresponding to the breaks were more or less scattered, the areas of breaking points were considerably reproducible.

It is to be noted that the minimum point of the compressibility curve appeared around the area corresponding to the breaks (point B) of the G-A curve. In addition, the particular state of the film phase in B-C range was also found from the corresponding ω-t curve. In A-B range, the ω-t curve was of such a type as in the inset (a) of Fig. 4, whereas it was converted into the type as (b) when the film was compressed to B-C range. Further compression down to C-D range made the ω-t curve into the same type as that in A-B range. From these facts it is strongly suggested that compression of the film exercises several steps of transformation of film phase which involves different modes of packing of film molecules.

In higher pressure region, the G-A curve was also obtained together with the compressibility-area curve (Fig, 5). Here, the shear modulus was found to increase steeply around the area of minimum compressibility. The type of the ω-t curve was almost similar to that of the low pressure region.

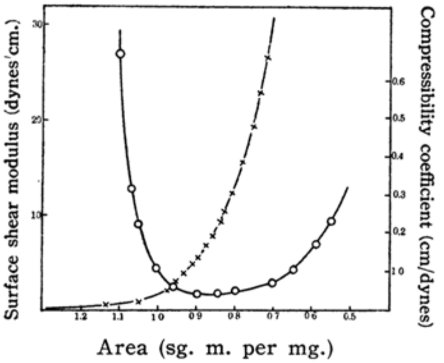


Fig. 5.—Surface shear modulus-area curve (crosses) and compressibility-area curve (circles) of ovalbumin film in high pressure region. Substrate solution, 0.1 N hydrochloric acid, and temperature, 13–14°C.

Effect of Various Experimental Conditions.—It was found that the ovalbumin film on 0.1 N hydrochloric acid, at elevated room temperature, did not exhibit an instantaneous elasticity until it was compressed down to the area of 1.7 sq.m. per mg. That is, elastic ovalbumin films at large areas underwent "melting" above certain temperature around 20°C. The film, once it was formed, showed reversible sol-gel transformation with "melting" range within 1°C, when the surface temperature was raised or lowered repeatedly by the intermittent exposure of infra-red lamp. If the film was newly formed under the same condition, however, the observed transition temperature was scattered considerably. The extent of the fluctuation was about ± 3°C. This may be due to some uncontrollable factors of the experimental conditions.

The films, when they were spread on 5% ammonium sulfate solution at pH 5.5, showed the instantaneous elasticity at the area above 15 sq.m. per mg., about three times as much area as on 0.1 N hydrochloric acid. In this case, "melting" of the film could not be observed at room temperature.

When the spreading solution of ovalbumin containing 10% formaldehyde was applied on 5% ammonium sulfate solution, at any temperature, only simple retarded elastic response was exhibited, without any instantaneous one. Although the reactions between formaldehyde and protein are complicated, in view of the fact that the amino or imino groups of protein molecules take a great part in this reaction, it seems probable that these groups are responsible for the instantaneous elasticity of the film.

Horse Serum Albumin and Hemoglobin—The proteins were spread under the same conditions with ovalbumin, but the mechanical behavior was found to be strikingly different from the case of ovalbumin. It was found that serum albumin did not exhibit any perceptible resistance against the motion of the ring, until the film was compressed to a considerably reduced area at the temperature from 5°C to 23°C. Below 1.0 sq. m. per mg., the film showed appreciable viscosity whose values increased gradually with the decrease of area, showing Newtonian flow (crosses in Fig. 6). Below 0.8 sq. m. per mg., the retarded elasticity appeared, which follows a simple viscous flow, but no sign of instantaneous elasticity was perceptible (inset of Fig. 6). This rheological behavior was referred to the compressibility-area relation obtained from the F-A curve. The compressibility curve is shown in Fig. 6 together with the illustration of the rheological behavior. It is noted that the minimum point of the compressibility curve just corresponds to the largest area

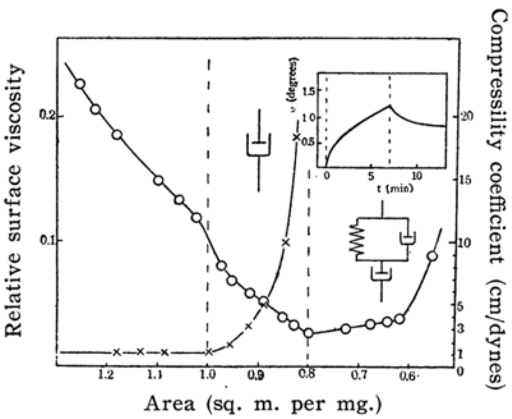


Fig. 6.—Compressibility-area curve (circles) and surface viscosity-area curve (crosses) for serum albumin film, together with the illustration of mechanical model corresponding to the film states. Relative surface viscosity is defined as the ratio of surface viscosity of the film to that of clean water. Temperature, 16°C. Inset gives the ω-t curve at the area of 0.78 sq. m. per mg. The region of (X) represents dead time, (Y), the time, stress is imposed, and (Z), the time, stress is removed.

where the film exhibited the retarded elasticity under stress, and the kink point of the compressibility at the area of 1.0 sq. m. per mg., is consistent with the largest area where the ring caught appreciable viscosity of surface film. Even though the film was extremely compressed below 0.3 sq. m. per mg., it failed to show instantaneous elasticity, exhibiting only the flow accompanied by the retarded elasticity.

Sasaki and Kimizuka[13] reported that an adsorbed layer of serum albumin aqueous solution exhibited an instantaneous elasticity, and the present author also observed this phenomenon with the solution of 10 to 50mg. per 100 cc. containing 0.1 N hydrochloric acid. It is noteworthy that the adsorbed layer exhibits an instantaneous elasticity, whereas the monolayer, as well as the polylayer made from the compression of a monolayer, fails to show any sign of instantaneous elasticity. Concerning the structural difference between a monolayer and an adsorbed layer, no work has been done, waiting for further research. Our rheological method of approach will also be promising for the solution of this problem.

The rheological behavior of horse hemoglobin was nearly similar to that of serum albumin. When hemoglobin was spread on 0.1 N hydrochloric acid solution at 14°C, it showed no elasticity at large areas, while below about 0.85 sq. m. per mg. the retarded elasticity appeared, accompanied by viscous flow. The correlation of rheological behavior with compressibility of the films was also similar to the case of serum albumin.

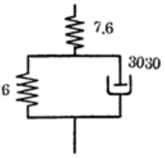
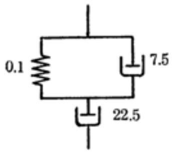
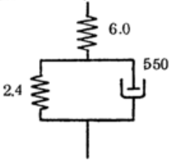
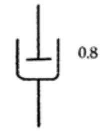
Discussion

The rheological method mentioned above enables the film properties to be quantitatively described in mechanical terms by the way of assigning absolute values of viscosity and elasticity to each element of corresponding mechanical model. Table II shows an example of the result obtained near the collapsing pressure of the film, i.e., at 6 dynes per cm. and 9 dynes per cm. For simplicity of computation, three element model was adopted as the mechanical model for ovalbumin film. The numbers in the table indicate the values of surface shear modulus and surface viscosity coefficient in c. g. s. unit. These values were computed approximately from the observed values corresponding to the instantaneous elastic deformation, the

TABLE II

COMPARISON OF MECHANICAL PROPERTIES OF OVALBUMIN AND HORSE SERUM ALBUMIN MONOLAYERS.

Numbers indicate the values of surface shear modulus and surface viscosity coefficient of each element of the mechanical model in c. g. s. unit. At the bottom of the table, the Joly's data of surface viscosity are given, in which R indicates the surface viscosity coefficient in c. g. s. unit at the surface pressure of 6 dynes per cm., P_n, the critical surface pressure at which the nature of flow transforms from Newtonian to non-Newtonian by compression.

		Ovalbumin	Serum albumin
Surface Pressure in dynes/cm	9		
	6		
Jolys data	R	0.038	0.076
	P_n (dynes/cm)	4	10

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[13] T. Sasaki and H. Kimizuka, This Bulletin, **25**, 318 (1952).

ultimate strain at infinite time, the rate of initial flow, and the rate of the stationary flow.[14] In this table, characteristics of ovalbumin and serum albumin films are quite clear at a glance. At the bottom of this table, Joly's[7] data on surface viscosity are tabulated; these date were obtained by the method of damped oscillation of ring. It is to be noted that Joly's measurement failed to show any difference in mechanical properties of these proteins. In addition, he reported the critical pressure (P_n), at which the nature of flow is transformed from Newtonian to non-Newtonian by compression, to be 4 dynes per cm for ovalbumin, 10 dynes per cm. for serum albumin. Such values, especially for ovalbumin, are hardly expected from the present method, since the films of these proteins exhibit distinct elasticity at such high surface pressures. Such large discrepancy may presumably be due to the too large shear stress employed by the oscillation ring method. The reason becomes apparent in view of the fact that ovalbumin film shows thixotropic behavior, that is, the film structure undergoes destruction and the film shows only simple flow under too large stress. It must be emphasized that sufficiently small stress should be applied to the film if the detailed information is wanted for the film structure.

From the view point of structural theory, the occurrence of the instantaneous elasticity is to be attributed to the exsistence of intermolecular bondings. The conclusion could, therefore, be drawn that even in a large area such as 5 sq. m. per mg., ovalbumin molecules are linked together with each other, making a two dimensional network covering the whole area of spread film.* It had been suggested that the protein molecule exists on surface in an unfolded structure, probably in the form of straightened β-configuration, and at low pressure, the side chains are lying flat on the surface.[15] The calculation based on the molecular model leads to the results that the area occupied by a polypeptide chain with the side chains flat on water surface is about 1.7 sq. m. per mg. In addition, according to Bull's[16] data the area of ovalbumin film available for the close packing state is not over 2 sq. m. per mg. under the best spreading condition. Our experimental result, therefore, shows that ovalbumin film forms a two-dimensional network before its spread molecules are closely packed, i.e., spread ovalbumin molecules have a marked tendency to combine with each other.

On the other hand, with serum albumin, it is to be noted that the film did not manifest any elastic behavior until it was compressed down to the area about 0.8 sq. m. per mg., and that the critical area is just consistent with the area of minimum point of film compressibility. It is commonly said that when the compressed film exists in a close packed state of molecules, whose side chains are oriented normal to the surfacee, compressibility-area curve exhibits a minimum point. Therefore, with serum albumin, it is considered that the closest packing state of film molecules just rendered the film viscoelastic and that the looser packing no longer brings about any elastic behavior. This shows that the interaction between the film molecules is very weak. Moreover, our experiments show that no perceptible viscosity of the film was observed until it was compressed to the area about 1. sq. m. per mg. The case is similar with hemoglobin. These facts may suggest that the film molecules of serum albumin exist with the side chains oriented normal to the surface even at the uncompressed film state as well as the compressed one. In these respects, the film structure of serum albumin and hemoglobin is considered to be in striking contrast to that of ovalbumin film, whose side chains lie flat on the surface in a large area forming the interchain bonds between the film molecules.

Summary

A static method of measuring the viscoelasticity of spread monolayers is presented, and the rheological study was carried out with the monolayers of ovalbumin, horse serum albumin, and hemoglobin. The deformation-time relations, obtained under the shear stress, were found to give very detailed information about the structure of films.

With ovalbumin films spread on 0.1 N hydrochloric acid, it was found that an instantaneous elasticity, accompanying retarded elasticity, appeared in an unexpectedly large area, i.e., in the area above 5 sq. m. per mg. Surface shear modulus of instantaneous elasticity increased linearly with the decrease of surface area, exhibiting several breaks in the slope.

The films of serum albumin and hemoglobin behaved differently from ovalbumin film, as they did not exhibit any viscoelasticity until they were compressed to a considerably small area. Compression down to the area

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[14] cf. T. Nakagawa, "Kagaku" (Science) **21**, 138 (1951).

* Complete spreading of the film was checked by the talcum test.

[15] H. Neurath and H. B. Bull, *Chem. Revs.* **23**, 391 (1938).

[16] H. B. Bull, *J. Biol. Chem.*, **185**, 27 (1950).

below 1.0 sq. m. per mg., however, makes a simple viscous film and further compression down to the area below 0.8 sq. m. per mg., just rendered the film viscoelastic. Such changes of film state were found to be consistent with the breaks in the compressibility-area curve obtained from the forcearea curve. Discussions of the characterization of these protein films were briefly made in terms of molecules.

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