RHEOLOGICAL PROPERTIES OF PROTEIN IN SOLUTION

by

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B.Sc., Chulalongkorn University
(1975)

SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE
DEGREE OF

MASTER OF SCIENCE

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

FEBRUARY, 1977

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February 5, 1977

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ABSTRACT

The viscosity of the globular protein, β-lactoglobulin (Sigma Chemical Co.) as a function of shear rate was studied using a cone and plate viscometer (Ferranti-Shirley Viscometer System). An aqueous buffer solution (pH 7, ionic strength 0.04) containing up to 40% protein was subjected to a rate of shear between 800 and 17,000 sec^{-1}. At a protein concentration 10% or higher, the viscosity of the protein solution decreased asymptotically with the increasing rate of shear.

Under a constant rate of shear, the viscosity of 10 to 30% β-lactoglobulin solutions increased with shearing time. However, this rheopectic property was not consistently observed but rather was dependent on the rate of shear at concentrations lower than 20%. A hysteresis effect was also observed to be rheopectic for 10 to 30% protein solution while that of a 40% solution was found to be thixotropic. The rheopectic nature

appeared to be the result of the permanent denaturation of protein characterized by UV absorption and gel filtration. At a protein concentration of 5% and lower, the viscosity was independent of the rate of shear or the time of shearing.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Professor ChoKyun Rha, for her encouragement, guidance and patience during the course of these investigations.

I would like to thank Professor Nicholas Catsimpoolas and Dean S.T. Hsieh for their help and interpretation of gel filtration chromatography and UV absorption studies.

I am grateful to Dr. Amnuay Thithapandha, Arunsri Thithapandha and Dr. Tanit Kusamrarn who made my coming to the States possible.

I would like to thank Janet Sussman, Marie Ludwig, Sylvia Reed and Suvit Viranuwat for their help in finishing this Thesis.

I give my appreciation to all of my friends and especially to Dr. Kemarasami Banchuin.

Finally, very special thanks to my parents, Pol. Maj. Gen. Phasna and Yuwadee Pradipasena, my grandmother, Poon Pradipasena, and my sister, Yuwapa Pradipasena, who have done so much to make my continuing education possible.

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OBJECTIVES

The objective of this study is to determine flow behavior and effect of shear on the globular protein in solution. The shear stress-shear rate relationship for a range of shear rate, viscosity-shearing time relationship at constant shear rate and the effect of shear on the molecular denaturation of a globular protein in solution are investigated.

Protein is subjected to shearing force in many food processing operations such as extrusion, spinning, whipping, mixing, churning, centrifugation and flowing through the pipe lines. These shearing processes may change the protein molecules (Taylor, 1934; Edsall et al., 1965; Polson, 1939). Such change may affect both operating processes and final products. In food fabrication processes, such as extrusion and spinning, it is necessary to modify the conformation of protein to obtain the maximum effect of the processes. At present this is accomplished by enzymatic or chemical pretreatment or by concurrent heat treatment with shear. However, enzymatic, chemical and thermal modification of protein often accompanies off flavor and loss of nutrituional value. In addition, these modification processes are difficult to control. Therefore, possibility of the mechanical shearing as the alternative for the present method of modification of protein is investigated since the shearing force may also cause changes in the protein molecule.

When molecules of coiled protein are deformed or uncoiled,

the viscosity of the protein in solution subjected to shearing force should increase (Ram, 1967; Tanford et al., 1967; Van Holde, 1971). The viscosity is related to the molecular properties. For instance as the molecular axis or charge increases, the intrinsic viscosity as expressed by the ratio of viscosity to concentration extrapolated to zero concentration, increases (Tanford and Buzzell, 1956).

This study is aimed at elucidating what changes might be caused in protein during these processes by measuring viscosity under shearing force. A model protein, β-lactoglobulin, crystalized and lyophilized (Sigma Chemical Co.) globular protein, was made into solution with phosphate buffer of pH 7.0 and of a ionic strength of 0.04. The solutions of the different concentrations of protein (3%, 5%, 10%, 20%, 30% and 40% weight) were subjected to shear rate between 800 to 17,000 sec-1, and shear stress was determined. The protein solutions were then examined for conformational change by U.V. absorption and gel filtration.

1. LITERATURE REVIEW

Rheological properties of substances can be related to molecular properties and behaviors. Knowledge of rheological properties has wide application in various fields especially in polymer synthesis and processing. The relationship between molecular structure and viscosity and the effects of structural changes, temperature and pressure on viscosity are important in material synthesis and processing. The viscosity of various systems has been investigated and attempts were made to relate viscosity to a mathematical expression (Kruyt, 1952; Mill, 1959; and Sherman, 1963).

When shear stress (τ) acts on a homogeneous system parallel to the X-Y plane, as shown in Fig. 1.1, a shear strain is produced.

Newton noted that the velocity of deformation is related simply to shear stress as follows:

$$\tau = \eta D$$

when τ = shear stress

η = viscosity coefficient

D = velocity of deformation

A liquid system obeying this law is called Newtonian. The unit of viscosity is poise (named after Poiseuille). Viscosity of water at 20°C is 0.01005 poise. The effect of temperature on viscosity for an ideal liquid can be expressed by:

$$\eta = Ae^{B/T} \qquad \text{(Ia)}$$

$$\text{or } \log \eta = A + \frac{B}{T} \qquad \text{(Ib)}$$

when η = viscosity coefficient

A,B = constant

T = absolute temperature

These two equations indicate that viscosity decreases with increase in temperature and that a linear relation exists between log η and $\frac{1}{T}$.

For laminar flow of liquid in a tube or capillary, viscosity can be calculated by means of the following equation (Poiseuille, 1947).

$$\eta = \frac{hg\rho\pi r^4}{2l} \frac{t}{v}$$

when η = viscosity coefficient

h = height of liquid in tank

ρ = density of liquid

l = capillary length

v = volume of a liquid

r = radius of capillary

t = time

g = acceleration due to gravity.

Therefore, the viscosity of dispersion is higher than that of the pure liquid, since particles dispersed in the system will disturb the flow of the liquid. The degree of this disturbance was calculated using the change in energy dissipation of the flowing liquid caused by the presence of a rigid spherical particle (Einstein, 1906 and 1911), and the result was expressed as:

$$\eta_s = \eta_o (1 + \nu\psi) \qquad \text{(III)}$$

when η_s = viscosity coefficient of the dispersed system

η_o = viscosity coefficient of the surrounding liquid

ψ = volume fraction occupied by the dispersed particle

ν = constant for small spherical rigid particle which carries no electrical charge - 2.5.

Einstein's equation has been extended for dispersed particles which carry an electrical charge (Smoluchowski, 1921)

$$\frac{\eta_s - \eta_o}{\eta_o} = \nu\psi \left[1 + \left[\frac{1}{\lambda\eta_o r^2}\left(\frac{\zeta\varepsilon}{2\pi}\right)^2\right]\right] \qquad \text{(IV)}$$

when λ = specific conductivity

ε = dielectric constant of the system

r = radius of the particles

ζ = difference of potential in the double layer

η_s = viscosity coefficient of the dispersed system

η_o = viscosity coefficient of the surrounding liquid

ν = shape factor constant

When the dispersed particles are liquid, rather than

solid as in Einstein's equation, the viscosity of the system (η^*) depends on the viscosity of the dispersed liquid (η'), as well as on that of the continuous liquid (η_o) (Taylor, 1932).

$$\eta^* = \eta_o \left[1 + \frac{(2.5\eta' + \eta_o)}{(\eta' + \eta_o)} \psi\right] \quad \text{(V)}$$

when ψ = volume fraction

The shear deformation of a cycloHexanolphthalate (CHP) drop in corn syrup is shown in Fig. 1.3 (Bartok, 1958), and obeys Taylor's Equation (1934)

$$\frac{L-B}{L+B} = \frac{Da\eta_o}{2\sigma} \frac{(19p-16)}{(19p+16)} \quad \text{(VI)}$$

when L = length

B = breadth of the ellipsoid

σ = interfacial tension

D = shear rate

p = viscosity ratio = η'/η_o

η_o = viscosity of continuous phase

η' = viscosity of dispersed phase.

An empirical relation between the relative viscosity and the effective dispersed volume was proposed by Mooney (1946) as

$$\eta_{rel} = \frac{1+0.5\psi}{1-\psi} \exp \frac{1.25\psi}{1-\psi} \quad \text{(VII)}$$

where η_{rel} = relative viscosity

ψ = volume fraction

This relationship of log η_{rel} and ψ is given in Fig. 1.4.

For a dilute suspension of uniform small Hookean elastic solid spheres in Newtonian viscous liquid, the relationship of the elastico-viscous properties of shear stress (τ), shear rate (D) and viscosity of the system (η_o) is expressed by (Frohlich and Sack, 1946)

$$\tau + \lambda_1\dot{\tau} + \nu\ddot{\tau} + \ldots = \eta_o(D+\lambda_2\dot{D}+\nu_2\ddot{D}+\ldots) \qquad \text{(VIII)}$$

when λ_1, ν_1 = relaxation time

λ_2, ν_2 = retardation time

The theory of viscosity for liquids was developed by evaluation of the pressure tensor which gave an approximate solution for viscosity (Born and Green, 1947). Theories of the flow of liquids attempt to relate viscosity to the intermolecular potential. Starting from a very similar concept and by using calculations of the viscosity of liquid argon, an exact solution for the viscosity of simple fluids was derived (Kirkwood et. al., 1949).

In protein chemistry, as in other fields, the relationship between chemical structure and rheological properties has been investigated (Tanford, 1956, 1961 and 1967; Mancuso, 1973; Hamed, 1975 and Robinson, 1975). Viscosity and/or intrinsic viscosity of protein solutions have often been studied, and the size and shape of proteins in solution are rarely characterized without including a set of viscosity data

(Bull, 1940; Buzzell, 1956; Yang, 1958; Dokić, 1975 and Puri, 1975). The study of viscosity would aid in understanding the structure and chemistry of proteins since viscometeric theory was developed from both thermodynamic and hydrodynamic conceptions of the problem, (Debye, 1948; Kirkwood & Riseman, 1948; Rao, 1976). Lately, the interpretations of viscosity and intrinsic viscosity of protein solutions have undergone a careful reevaluation (Palit, 1955; Tanford, 1967; Van Holde, 1971). Consideration of intrinsic viscosity, the limit of the ratio of specific viscosity to concentration as concentration approaches zero, eliminates the effects of interactions, since it depends only on properties such as shape and specific volume of the isolated macromolecules (Van Holde, 1971). In addition, the development of theories of non-Newtonian viscosity has contributed more information concerning the shape of polymers and resulted in a simplification of the velocity gradient dependency of viscosity.

Shape factors of ellipsoidal molecules and different proteins were calculated (Polson, 1939; Simha, 1940) (Table 1.1, Table 1.2) by employing Jeffery's formula. The theoretical treatment of this problem is an extension of Einstein (1906), and was done using the rotary motion of ellipsoidal particles subjected to a velocity gradient (Jeffery, 1922-23).

In this theory, the dissymmetry of protein molecules is considered as an ellipsoid of revolution, characterized by

Table 1.1

Relationship between shape factor (ν) and the axial ratio of the ellipsoid

Axial Ratio	ν elongated	ν flattened	Axial Ratio	ν elongated	ν flattened
1.0	2.60	2.50	20.0	38.6	14.80
1.5	2.63	2.62	25.0	55.2	18.19
2.0	2.91	2.85	30.0	74.5	21.60
3.0	3.68	3.43	40.0	120.8	28.30
4.0	4.66	4.06	50.0	176.5	35.00
5.0	5.81	4.71	60.0	242.0	41.70
6.0	7.10	5.36	80.0	400.0	55.10
8.0	10.10	6.70	100.0	593.0	68.60
10.0	13.63	8.04	150.0	1,222.0	102.30
12.0	17.76	9.39	200.0	2,051.0	136.20
15.0	21.80	11.42	300.0	4,278.0	204.10

(Mehl, J.W., Oncley, J.L., and Simha, R., 1940)

Table 1.2

Relationship between shape factor and axial ratio of various protein studied by diffusion and viscosity experiments

Protein	f/fo	ν	a/b, elongated		b/a, flattened	
			Diffusion	Viscosity	Diffusion	Viscosity
Egg Albumin.....	1.17	5.7	3.8	5.0	4.0	6.7
Serum albumin.....	1.25	6.5	5.0	5.6	5.4	7.7
Hemoglobin.....	1.16	5.3	3.7	4.6	3.9	6.0
Amandin.....	1.28	7.0	5.4	6.0	6.0	8.5
Octopus Hemocyanin.....	1.38	9.0	7.2	7.3	8.2	11.4
Gliadin.....	1.60	14.6	10.9	10.5	13.6	21.0
Homatus hemocyanin.....	1.27	6.4	5.2	5.5	5.8	7.5
Helix pom. hemocyanin...	1.24	6.4	4.8	5.5	5.2	7.5
Serum globulin.....	1.41	9.0	7.6	7.3	8.9	11.4
Thyroglobulin.....	1.43	9.9	7.8	7.9	9.2	12.7
Lactoglobulin.....	1.26	6.0	5.2	5.1	5.7	6.9
Pepsin.....	1.08	5.2	2.5	4.5	2.6	5.8
Helix hemocyanin pH 8.6.	1.89	18.0	16.6	12.0	23.9	26.0

The values of ν in this table have been taken from A. Polson, Kolliod. A. 88, 51 (1939). Other values have been reported for some of these proteins. For hemoglobin, ν = 4.7 [E.J. Cohn and A.M. Prentiss, J. Gen. Physiol., 8, 619 (1927)]/ for serum albumin, ν = 8.25 [K.R. Fahey and A.A. Green. J. Am. Chem. Soc. CS, 3039 (1939)]. See also other serum protein fraction value in this paper. The viscosity increments of certain amino acids, peptides and related molecules have been reported by J. Daniel and E.J. Cohn, J. Am. Chem. Soc., 58. 415 (1936).

[From Mehl, J.W., Oncley, J.L., and Simha, R., Science, 92, 132 (1940)]

by the relative lengths of the semi-axis of revolution (a) and the quatorial semi-axis (b). The values of a/b so obtained are subject to some uncertainty, since the degree of hydration of the protein is unknown and the adequacy of the simplified ellipsoidal model to represent a protein is not established. The measurements involve the rotation and partial orientation of protein molecules in an external field of force. In viscous flow, rotation is produced by shearing forces arising from velocity gradients in the streaming liquid. The orientation achieved is only partial, since it is opposed by the disorienting action of Brownian movement of the molecule. The intensity of the orienting forces relative to that of the rotary Brownian movement may be characterized in terms of a rotary diffusion constant (measuring the mobility of the particle in its rotation about an axis perpendicular to the principle axis). The rotary diffusion constant is inversely proportional to the relaxation time (time required for the molecules to revert to a random distribution) when an orienting force has been applied to the system and is then suddenly removed. Rotary diffusion constants and relaxation time are a function of molecular size and shape, and of the temperature and viscosity of the solvent medium. From Jeffery (1922-23), an elongated ellipsoid (Fig. 1.5A) usually has the "a" axis parallel or nearly parallel to the stream lines. Conversely, a disc-shaped ellipsoid (Fig. 1.5B) usually has the "b" axis parallel to the stream lines.

The relationship of apparent viscosity to a non-Newtonian suspension to the shape of particles or viscosity increment has also been investigated, (Edsall, 1965). At very low velocity gradients, the rod shaped molecules are oriented at random and their resistance to the viscous flow of the liquid is high. Therefore, under these conditions, the viscosity increment (ν_o) is high. But at higher gradients the molecules deviate appreciably from the state of purely random distribution, and at very high velocity gradients they are predominantly oriented parallel to the stream lines. In this position their influence to increase the viscosity of the solution is at a minimum, and the viscosity increment tends toward a lower limiting value for the infinite velocity gradient (ν_∞). The more the ellipsoidal molecule deviates from the spherical shape, the greater the difference between ν_o and ν_∞. The smaller the rotary diffusion constant of the molecule, the lower will be the velocity gradient at which the viscosity increment becomes effectively equal to ν_∞. If ν_∞ is plotted against the volume fraction and then extrapolated, the value of ν_∞ is obtained for zero concentration of myosin, (Edsall, 1940). The higher values of ν_∞ obtained at finite concentration are dued not only to the interaction of the flowing solvent with the individual myosin molecules, but also the interaction of the myosin molecules with one another, which involves one myosin molecule interfering with the rotation of the others.

Such concentration effects must be sharply distinguished from the effects which remain operative even at infinite di-

lution of the solute. The latter are a function of the shape and hydration of the solute molecule. The effects of concentration are likely to be particularly apparent in solutions of very long molecules, which may interfere or become entangled during flow. Such interference increases the measured viscosity, and also magnifies the change in viscosity with the velocity gradient. Neither of these concentration effects has yet been interpreted quantitatively in theoretical terms.

It is therefore important to make the measurement at low protein concentration, if viscosity measurements are to be used to determine shape factors. Since the viscosity increment ν_o for random distribution is more readily interpreted than ν_∞, it is also desirable to work at low velocity gradients.

The theoretical determination, as described above, can be applied to protein solution since a number of proteins in solution have been found to show a non-Newtonian type of flow such that viscosity decreases with an increasing velocity gradient. Protein molecules are very large in comparison with the molecules of the solvent. If very large solute molecules are introduced into the solvent, it is invariably found that the viscosity of the solution is greater than that of the pure solvent, and obeys Einstein's equation (Equation III).

A mathematical expression for flow behavior and its relation to size and shape of the molecule were determined (Einstein, 1906, and 1911; Born, 1947: Kirkwood, 1949; etc.). In addition, rheological properties relating to properties

such as molecular size, shape and weight have been studied (Tanford, 1967; Puri, 1974; Dokić, 1975; Huang, 1971; Tanford, 1958; Maruyama, 1974; Mancuso, 1973; Granato, Corigliano, 1973; Silverto, 1973 and Libondi, 1974).

Native protein molecules are known to be folded, and for the most protein the structure is compact and globular (Kendrew, 1961 and Blake, 1965). This structure is produced or maintained by hydrogen disulfide and ionic bonding, hydrophobic interaction, and electrostatic forces. The structural properties of the unfolded protein can be investigated by viscosity studies in the presence of reducing agents. For example, in a viscosity study of protein polypeptide chain (from beef insulin, myoglobulin, β-lactoglobulin, pepsinogen, serum albumin) the presence of β-mecaptoethanol, and ruptured disulfide bonds, the chains were found to be true random coils and the viscosity became higher than that of the native protein (Tanford, 1967).

The viscosity of bovine serum albumin in aqueous solution was measured as a function of concentration, and ionic strength and then the viscosity and molecular size and shape were determined (Tanford, 1956). The study was made with a capillary viscometer at 25°C. The protein molecule behaved like a sphere at a pH near isoelectric point (pH 4.5) and up to pH 10.5. This indicated that bovine serum albumin in aqueous solution is a compact molecule. As the molecular charge is increased, a small increase in intrinsic viscosity

is observed. Relative viscosity may be expressed as

$$\frac{\eta}{\eta_o} = 1 + c\,[\eta] + K\,[\eta]^2\,c^2 + \ldots$$

where

η = viscosity of solution

η_o = viscosity of solvent

c = concentration of solution

$[\eta]$ = intrinsic viscosity

K = constant = $K_o + K_1$

K_o = function of non-electrostatic interaction between dissolved particles which are independent of charge and ionic strength

K_1 = function of charge and ionic strength

(Gruth, 1936).

This expression shows that the effect of concentration involved the sum of two separate effects. One is independent of charge and ionic strength and is a constant universal to all protein (Tanford, 1956; Polson, 1939). The other is an electrostatic effect which for serum albumin is equal to $\frac{2 \times 10^{-5}\,(\text{charge})^2}{(\text{ionic strength})^{3/2}}$.

Colloidal characteristics of β-lipoprotein of pig blood that are of importance to viscous and rheological behavior were studied using both capillary and rotational viscometers at 5°C (Dokić, 1975). It was found that the viscosity and viscous behaviors of β-lipoprotein solution depended on its concentration. At concentration of 5gm/100cm^3, the shear stress shear rate relationship proved to be linear and

Newtonian in the range of shear rate 50-6,000 sec^{-1}. In the range of shear rate from 58.5 to 9480 sec^{-1} and 12.1 - 60.5 weight % concentration of protein, the solution is a thiotropic weak gel structure.

A viscosity study of 1·/. vegetable protein solution was made at 30°C for different pH values (Puri, 1974), in view of the fact that viscosity studies of protein in alkaline solutions can yield useful information regarding the degree of hydration, shape of molecules and critical micell concentration (Puri, 1972; Yang, 1961 and Mehl, 1940). The results show that viscosity of protein solution reaches a maximum at certain protein values.

These critical pH values vary with the type of vegetable protein. In this study, axial ratios of protein molecules were calculated from the slope of the plot of specific viscosity and volume fraction of the solute particles. Critical micelle concentration of those proteins was also determined from the plot of concentration of various protein fractions against viscosity at a given pH value. The concentration at which the slope of the curve changes is considered to be the critical micelle concentration.

It is well known that F-actin solution is thixotropic. A concentrated F-actin solution becomes a gel upon standing, particularly at low temperature, and turns to a solution when shaken. Thus, F-actin in solution is an interesting subject for a rheological study (Maruyama, 1974). The structural viscosity of an F-actin solution of very low protein concentra-

tion (0.015 - 0.033 mg/ml) was measured with a rotational viscometer at pH 7.2 and 20°C and at velocity gradients ranging from 0.0005 to 5 sec^{-1}. F-actin solution of 0.033 mg/ml showed a viscosity value of 44,000 c.p.s. at 0.0005 sec^{-1}. The viscosity decreased with an increase in shear rate, and the relationship between logarithms of viscosity and rate of shear. In viscosity-shearing time studies, viscosity increased rapidly and then decreased before levelling off at higher shear rate. However, at lower shear rate, viscosity increased more slowly and then leveled off during the observation period of up to 20 minutes. The viscosity of protein solution depended on concentration.

The effect of temperature on F-actin solution is that as temperature increases the viscosity decreases. Actually F-actin is not available as a pure component, but always contains tropomyosin and troponin. The effect of these two components on the rheological properties of F-actin in solution was investigated with a rotational viscometer (Maruyama, 1974). It was found that tropomyosin reduced the rate of gelation of F-actin, as measured in a very slowly rotating viscometer, al though the viscosity of F-actin-tropomysion complex was higher. The viscosity of the tropomyosin-F-actin complex was increased by the addition of troponin.

It is known that in solution collagen will form a gel, which depends on its concentration and temperature. There are

some interesting studies of the rheological properties of shear rate - shear stress and viscosity - shearing time (at constant shear rate) (Mancuso, 1973, Silvestro, 1973 and D'Ambrosio, 1972), but additional work would aid further in understanding the flow and structure properties of protein. Using a Ferranti-Shirley cone and plate viscometer, rheological properties of collagen in $NaHCO_3$ solution at 20°C were found to be thixotropic which is reversible during a resting period (D'Ambrosio, 1973). This was confirmed by a study of collagen in dimethyl-sulfoxide solution (Mancuso, 1973). But at 120°C, collagen in $NaHCO_3$ solution was found to be a plastic structure over 400-500 dyne cm^{-2} sec^{-1} rate of shear change (Silvestro, 1973).

On rheological analysis, it is found that myosin B of skeletal muscle is different from that of the uterus since uterus myosin B shows more plasticity and thixotropic phenomena (Granato, Corigliano, 1973).

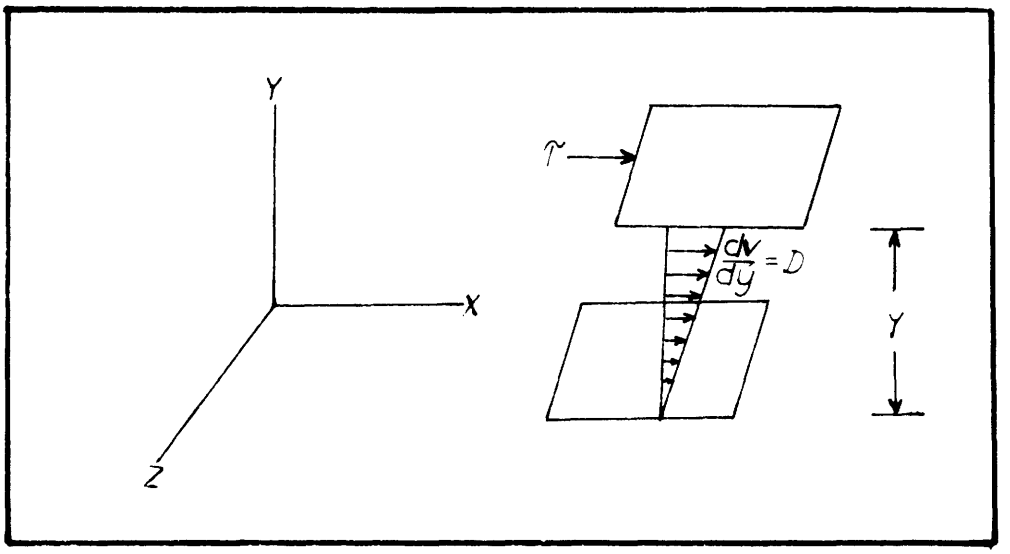

Fig. 1.1 A schematic representation of a Newtonian liquid between parallel lines.

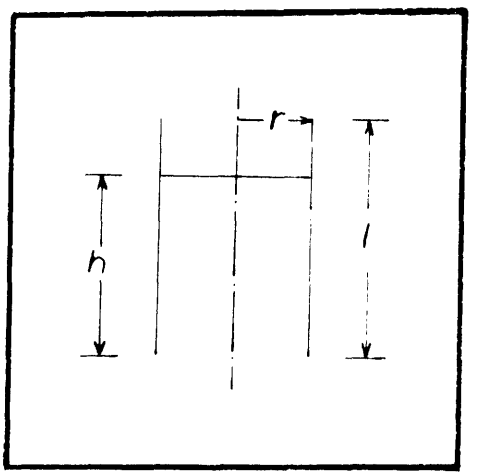

Fig. 1.2 Capillary Viscometer

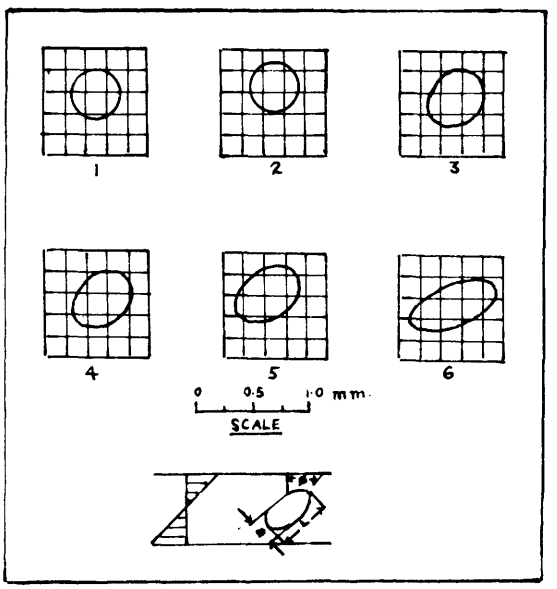

Fig. 1.3 Photomicrographs of the shear deformation of a cyclo Hexanolphthalate (CHP) drop in corn syrup.

1. Undeformed drop
2. D = 0.13 sec^{-1}
3. D = 0.83 sec^{-1}
4. D = 1.40 sec^{-1}
5. D = 1.84 sec^{-1}
6. D = 3.93 sec^{-1}

a = 574 μ
D = shear rate

From Bartok, W. et. al., J. Col. Sci.,13, 293(1958)

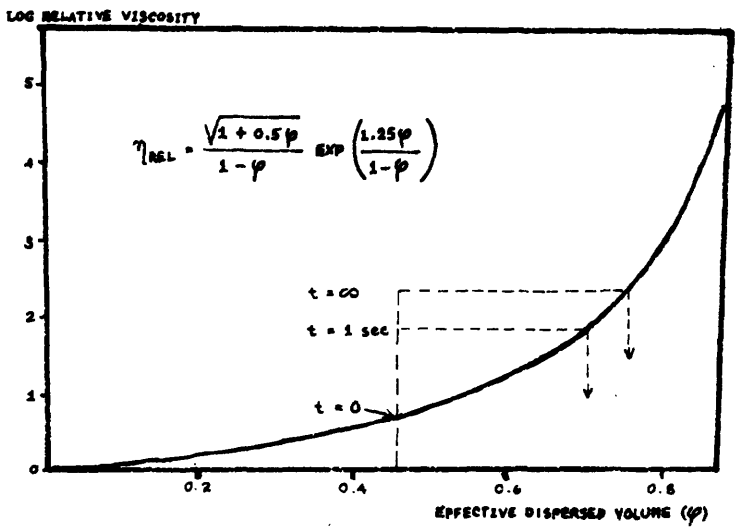

Fig. 1.4 Relationship between log of relative viscosity and effective dispersed volume

(Mooney, 1946)

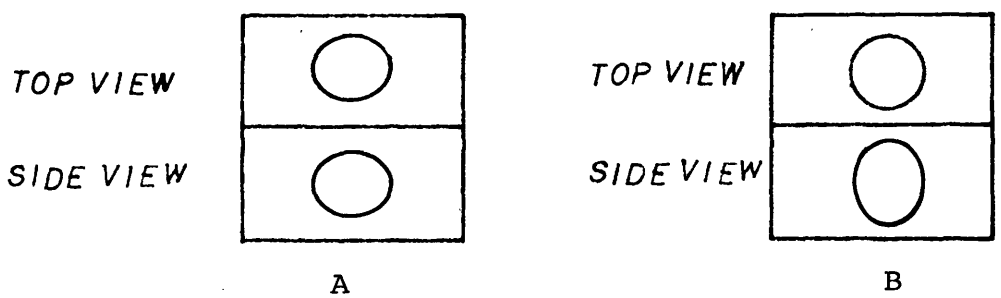

Fig. 1.5 The top view and side view of a. elongated ellipsoid, b. disc-shaped ellipsoid

2. MATERIALS AND METHODS

2.1 Viscometer

The apparent viscosity was measured with the Ferranti-Shirley Viscometer.

2.1.1 Cone Plate Viscometer

The essential elements of the Ferranti-Shirley Viscometer are shown in Figure 2.1 where (a) is a slightly conical disk, the apex of which just touches the surface of the flat plate (b). The sample fluid is contained in the narrow gap between the Cone and Plate (the cone angle Ψ in radians is exaggerated for clarity). The plate is rigidly constrained and the sample is sheared by rotating the Cone.

The rate of shear at any radius , r, is given by the ratio of the linear velocity ωr (ω = cone rotational velocity in radians per second) to the gap width C. Since both these quantities are proportioned to the radial distance, the shear rate is constant throughout the entire measured sample.

For small Cone-Plate angles, Ψ , the shear rate, D, is:

$$D = \frac{\omega r}{C} \text{ which reduces to } D = \frac{\omega}{\Psi} \text{ sec}^{-1} \qquad (1)$$

The shear stress, τ is given by

$$\tau = \frac{3G}{2\pi r^3} \text{ dynes/cm}^2 \qquad (2)$$

where G is the Cone torque in dyne·centimeters.

r is the Cone radius in centimeters

ψ is the Cone angle in radians.

ω is the Cone rotational velocity in radians

Apparent Viscosity, η_{app} is

$$\eta_{app} = \frac{\text{Shear Stress}}{\text{Shear rate}} \qquad (3)$$

Therefore

$$\eta_{app} = \frac{3G\ \psi}{2\pi r^3 \omega}$$

The Ferranti-Sherley viscometer provides meter readings which are proportional to the torque G and the readings are converted to shear stress units by using a shear stress multiplier constant K_T. Thus

Shear Stress = (K_T) (Meter divisions) dyne/cm^2

A shear rate multiplier constant K_d is used to convert cone rotational speed to shear rate units. Thus

Shear Rate = (K_d) (rpm) sec^{-1}

2.1.2 System Components

The Ferranti-Shirley Viscometer equipment has the following components which are illustrated in Fig. 2.2.

2.1.2.1 Measuring Unit

This unit consists of important parts as follow (Fig. 2.3)

1. Torque Dynamometer measures the viscous traction of the cone. Troque Dynamometer consists of torque spring (4 in. Fig. 213) and a potentiometer (6 in. Fig. 2.3)

2. Plate Height Adjustment (11 in. Fig. 2.3) allows the positioning of the apex of the cone to just touch the surface of the plate.

3. Plate Temperature Control (22 in. Fig. 2.3) is the means of controlling temperature of the sample.

4. Cone Speed Control The Cone speed range can be selected from 0 to 100 rmp or 1000 rpm by selecting a gear train (15 in. Fig. 2.3)

2.1.2.2 Amplifier Unit (b, Fig. 2.2)

This unit contains an amplifier which with the driving motor and the generator in the measuring unit make up the rotational velocity system used to control the cone rotational speed.

2.1.2.3 Program and Control Unit (e in Fig. 2.2)

This unit consists of an indicating meter with 100 unit divided scale used for indicating torque, cone speed or zero position of the torque dynamometer as selected by the scale reading selector switch, scale reading switch and cone speed RPM control. Switches for the scale reading consist of a multiplier for the reading of the shear stress range from x1 to x5; an indicator for contacting between cone and plate; and an indicator for the zero position of the torque potentiometer.

2.1.2.4 Cone and Plate Setting Unit (d in Fig. 2.2)

2.1.2.5 Set of Cones (e in Fig. 2.2)

A standard set consists of three cones: a 7 cm diameter with a 20' 57" cone angle, a 4 cm diameter with a 21' 8" cone angle, and a 2 cm diameter with a 23' 43" cone angle.

2.1.2.6 Temperature Controlled Circulating Bath, Thermocouple Potentiometer and X-Y Recorder

For this study, the X-Y recorder was not available. The Temperature Bath and Thermocouple Potentiometer used were from Neslab Instruments Inc. and Leeds & Northrup Co. (cat. No. 8692) respectively.

2.2 Calibration of the Viscometer

After changing the torque spring from 1200 gm-cm (standard torque spring) to 100 gm-cm spring in order to measure viscosity in the range of 1-300 cps for this study. Approximately every three months the viscometer was calibrated to insure the accuracy of the instrument. Whenever the torque spring was changed to accommodate the viscosity range, the instrument was calibrated. Standard viscosity solution (Cannon Instrument Co., State College, Pa.) was used for the calibration.

2.2.1 Determination of the Torque Spring Constant

The Torque spring constant was determined as follows:

1. The same standard solution (Cannon Instrument Co.) and the same cone size were used to get the shear stress reading from 1200 gm-cm spring which has a known torque spring constant and the spring to be calibrated for a range of shear rate selected.
2. The shear stress data was plotted against the shear rate and the slope was determined for both the unknown and standard spring.

3. From equation 4

$$\eta = \frac{3\ G\psi}{2\pi r^3 \omega}$$

and G = (T) (A)

where T = Torque spring constant in dyne cms/division on a 500 range.

A = Scale Reading

$$\eta = \frac{(3)\ (A)\ T\ \psi}{2\pi r^3 \omega}$$

$\frac{(3)\ (A)\ \psi}{2\pi r^3 \omega}$ is the constant, C_c, for a specific spring, cone size, and rotational speed.

Since the same cone size was used, the term is dependent on torque spring only. Therefore the subscripts std and u denote standard and unknown respectively,

for standard spring, $\eta = C_{std}\ T_{std}$

for unknown spring, $\eta = C_u\ T_u$

Since Viscosity is constant

$$C_{std}\ T_{std} = C_u\ T_u$$

$$\frac{T_u}{T_{std}} = \frac{C_{std}}{C_u}$$

Therefore $\frac{2\pi r^3}{3}\ C_c = \frac{A\ \psi}{\omega}$. The slope of the curve of scale reading versus shear rate will give $\frac{A\ \psi}{\omega}$. The slope can be found for both standard and unknown spring. Now

$$\frac{T_u}{T_{std}} = \frac{\text{Slope of standard spring curve}}{\text{Slope of unknown spring curve}}$$

and knowing T_{std} is 2649 dyne cms/Div, T_u can be calculated.

2.2.2 Determination of the Shear Stress Constant

The following method is used to determine the shear stress constant.

1. The shear stress scale for the known viscosity sample (standard solution) at the known shear rate is measured.

2. Shear stress was calculated from the known viscosity and shear rate.

$$T = \eta D$$

and $T = K_T$ x scale reading

K_T = shear stress constant

$$K_T = \frac{\eta D}{\text{scale reading}}$$

2.2.3 Shear Rate Constant

Shear rate constant was provided by the manufacturer of the equipment (Ferranti-Shirley Inc., Plainview, N.Y.), as follows:

Large Cone = 17.14

Medium Cone = 17.005

Small Cone = 15.14

Shear Rate = Shear Rate Constant x RPM sec^{-1}

2.3 Globular Protein Solution

β - Lactoglobulin (Sigma Chemical Co., St. Louis, MO), a globular protein with molecular weight 17,500 used as a medel protein. β - Lactogloulin was dissolved in a phosphate buffer (pH 7, ionic strength 0.04) to make 3%, 5%, 10%, 20%, 30% and 40% weight concentration.

Phosphate buffer (pH 7, ionic strength 0.04) was prepared from Potassium Monobasic, KH_2PO_4 (Baker Chemical Co., Phillipsburg, N. J.) and Pottassium Dibasic, K_2HPO_4 (Baker Chemical Co,, Phillipsburg, N. J.). The method followed the nonograms given in Appendix A (Boyd, 1965). Amount of KH_2PO_4 necessary was determined from desired pH and ionic strength in molar concentration. Amount of K_2HPO_4 was determined from

K_2HPO_4 = 1/3 (desired ionic strength - molar concentration of KH_2PO_4)

For the phosphate buffer at pH 7 and ionic strength 0.04, the amount of KH_2PO_4 and K_2HPO_4 needed is 0.009 M and 0.0103 M respectively. Then the spectrophotometric absorption of the solution was checked for the accordance of Lamberg-Beir's Law.

2.4 Shear Stress - Shear Rate Relationship and Hysteresis Effect

Protein solution (1.8 ml) was placed on the plate. The gear train was plaec at the 1000 gear, then the distance between the cone and the plate was adjusted such that the cone just touches the plate (Fig. 2.4). The cone speed control knob was turned to the desired shear rate. The shear stress reading was recorded manually. The temperature was set at 25° C ± 0.5.

For this study, the shear rate was increased and then

decreased continuously for the range of shear rate (Table 2.1).

Viscosity was plotted against the shear rate.

2.5 Time dependence of viscosity (viscosity and shearing time relationship)

The protein solution (1.8 ml) was placed on the plate. The plate was raised until the cone touched the plate as shown in Fig. 2.4. The experiment was carried out at 25° C ± 0.5 . The cone speed knob was turned to a desired R.P.M. and then stopped at that R.P.M. The shear stress reading was recorded against the shearing time. The viscosity or apparent viscosity was calculated from

$$\text{(Apparent) viscosity} = \frac{\text{shear stress}}{\text{shear rate}}$$

and then was plotted against shearing time.

Table 2.2 gives the ragnes of shear rates employed in the study of the viscosity and shearing time relationship for different concentrations of protein solutions.

2.6 The Effect of Evaporation

Since some of the measurements took up to 30 minutes and only a small amount of sample solution (1.8 ml) was used, there was a possibility that the solvent could evaporate causing an appreciable change in the viscosity. In order to verify this, a sucrose solution at 30% concentration was used. Since sucrose is Newtonian, the viscosity should not change with the shear time (Rha, 1975). The viscosity of the sucrose

Table 2.1

Determination of Experimental range of Shear Rate

% wt Concentration	Range of Shear Rate (sec^{-1})
3	6856 - 17140
5	6856 - 17140
10	1714 - 17140
20	857 - 17140
30	1700 - 17005
40	1514 - 15140

Table 2.2

Determination of experimental range of shear rate for time effect

% wt. Concentration	Shear rate (sec^{-1})
3	6856, 10284, 13712, 17140
5	6856, 10284, 13712, 17140
10	1714, 3428, 5142, 6856, 8570, 10284, 11998, 13712, 15426, 17140
20	1714, 3428, 5142, 6856, 8570, 10284, 11998, 13718, 15426, 17140
30	6856, 8570, 10284
40	3028, 4542, 6056

solution was determined against the shearing time at a constant shear rate. The method is the same as is described in Section 2.5, however the time of studying is extended to 60 minutes.

Another method used to investigate the evaporation of the sample was by attaching an outer jacket to the cover of the viscometer to prevent evaporation (Fig. 2.5). A container in the cover was designed to supply water vapor from the outer jacket to go into the cone and plate system, keeping the humidity in the system as high as possible. With this jacket, the 10% protein solution was studied to determine the relationship between the viscosity and the shearing time at constant shear rates, 10284 and 13712 sec^{-1} for 60 mins. The result was then compared with those from the experiment done without the outer jacket.

2.7 Determination of Reversibility of Shear Deformation

Sheared β-lactoglobulin solution was tested to determine in the change in protein resulting from shearing may be reversible. The method is the same as in Section 2.5 except that after 30 mins. of shearing the shearing was stopped for 30 minutes then the protein solution was sheared again.

2.8 Determination of the Conformational Change Due to the Shearing of Protein

This study was designed to confirm the conformational

change occurred in sheared β-Lactoglobulin. The experiments included UV absorbance and gel filtration chromatography. Sheared and unsheared control protein solution was freeze-dried for these experiments.

2.8.1 U.V. Absorbance

2 mg. of freeze-dried sample was dissolved in 3 ml phosphate buffer (pH 6.0, ionic strength 0.1), and the absorbance from UV 310 nm. to 240 nm. was measured with a Beckman Spectrophotometer (Model 25).

2.8.2 Gel Filtration Chromatography

5 mg of the sample was dissolved in 2 ml of phosphate buffer (pH 7.6) containing 0.05M NaCl and placed on the top of the Sephadex gel column (G-75) with a dimater of 2.5 m and 26 cm length. This column was calibrated by using blue dextran and N-2,4 Dinitrophenyl β-alamine. The void volume was 46 ml., and the terminal volume was 169 ml. The elution buffer is standard phosphate buffer (pH 7.6) containing 0.05 M NaCl. 4.2 ml per fraction was collected and UV absorption of the fraction at UV 220 nm was measured with a Beckman Spectrophotometer (Model 25).

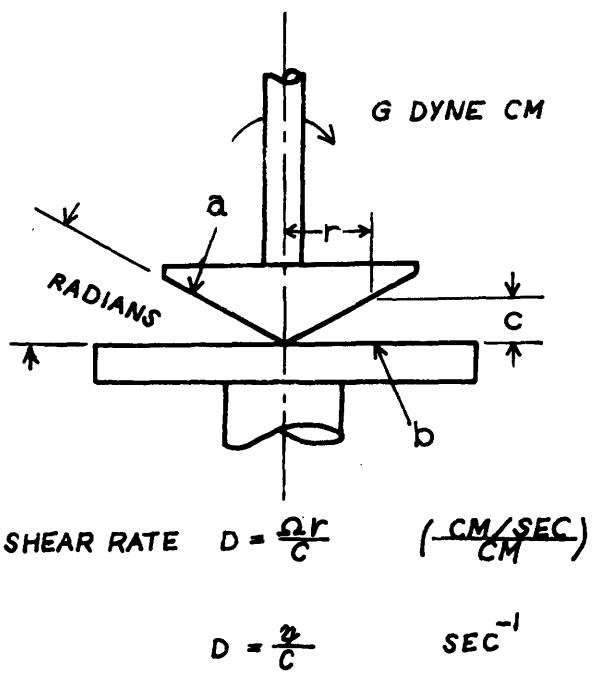

Fig. 2.1 Cone-Plate Viscometer

Fig. 2.2. Ferranti-Shirley Viscometer System

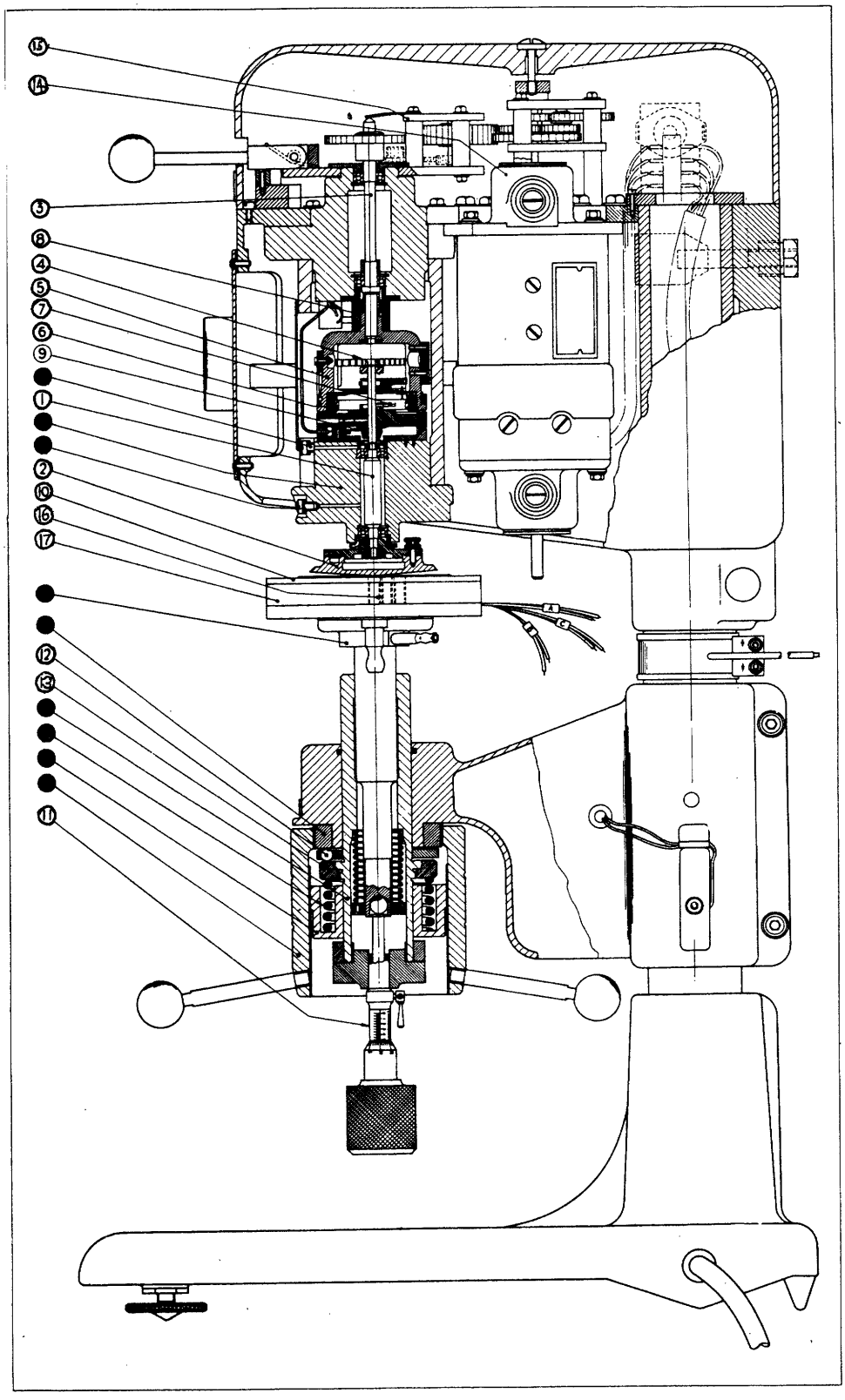

Fig. 2.3 Measuring Unit

Fig. 2.3

1	Cone Spindle
2	Cone
3	The driving spindle
4	The flat spiral torque spring
5	The bridge housing
6	A precision wound linear potentiometer
7	The wiper arm
8-9	The slip rings
10	The plate
11	Micrometer Screw
12	Three matched steel balls
13	A hardened flange
14	A DC motor
15	A gear train
16	Three thermocouples
17	A cooling jacket

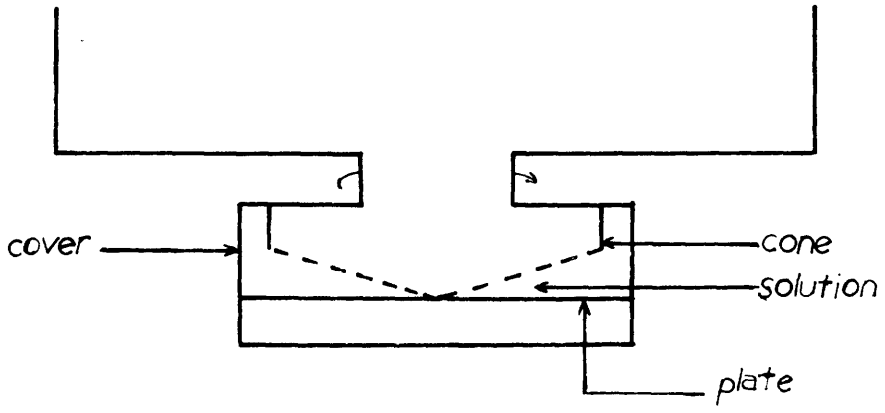

Fig. 2.4 Cone-Plate Viscometer when operated

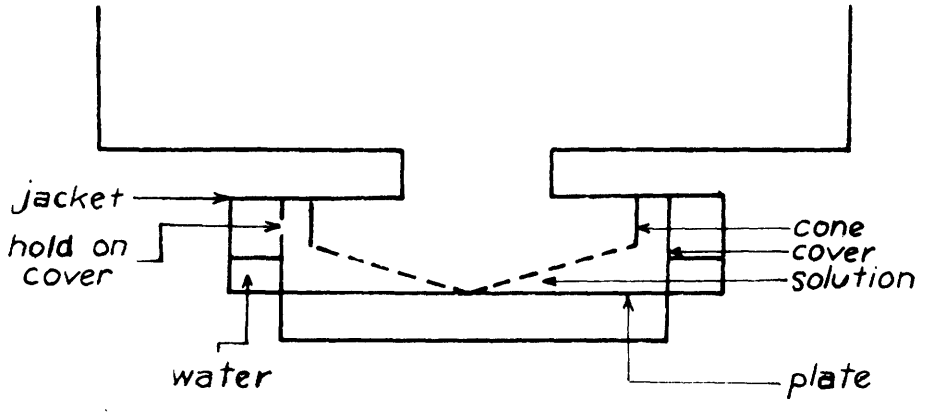

Fig. 2.5 Cone-Plate Viscometer when operated with outer jacket to avoid evaporation effect.

3. RESULTS

3.1 Determination of Torque Spring Constant.

Torque spring constant for 100gm-cm spring as determined from the slope of scale reading and rate of shear is 230.6 dyne-cms/Division.

3.2 Apparent Viscosity as Affected by Protein Concentration.

In Fig. 3.1, average apparent viscosity was plotted as a function of concentration for the range of shear rate between 6,500 to 17,000 sec^{-1}. At low concentration (up to 10 weight %) the relationship between apparent viscosity and concentration is linear. The constant equivalent to Einstein's constant, 2.5, shape factor, used with a volume fraction was found to be 0.8 for weight % concentration of β-lactoglobulin solution. Thus the apparent viscosity of β-lactoglobulin in terms of weight % concentration can be calculated by

$$\eta_s = \eta_o(1+0.8C)$$

where η_s = apparent viscosity of solution

η_o = viscosity of phosphate buffer

= 1.0 c.p.s.

C = concentration in weight %

However above 10 weight % concentration apparent viscosity increases more rapidly with concentration (Fig. 3.1). In addition the apparent viscosity-shear rate relationship was also affected by concentration (Fig. 3.2). Effects of shear rate and shearing time are observed at concentration above 5% and become more prominent at higher protein concentration.

For higher concentration (20 to 40 weight % concentration), the relation between apparent viscosity and weight % concentration of β-lactoglobulin ion phosphate buffer solution deviates from Einstein's Equation.

3.3 Apparent Viscosity as Affected by Shear Rate.

The result of this study is presented in Fig. 3.2. In the range of 6,850 to 17,000 sec^{-1}, apparent viscosity of 3% and 5% solution is independent of shear rate. At higher concentration apparent viscosity decreases as shear rate increases. The decrease is rapid at lower shear rate and higher concentration, in the range of 850 to 17,000 sec^{-1}, as shown in Table 3.1. This experiment was made in duplicate with deviation in the range of ±0.05 C.P. or 3 - 10%, ±0.1 C.P. for 20% and ±0.5 C.P. for 30 - 40%.

3.4 Hysteresis Effect

Hysteresis loop was observed at β-lactoglobulin concentration higher than 5% weight. Hysteresis loop showed that the solution of 10 to 30 weight % concentration behaves as a rheopectic solution while that of 40 weight % concentration is thixotropic (Fig. 3.2). Time required for complete hysteresis effect is 8 ± 0.5 min. By considering each step (as in Fig. 3.7) and the average time spent in each step, the hysteresis effect can be determined as in Table 3.2. This experiment was duplicated, and results were reproducible within the range of

±0.05C.P. for 3-10%, ±0.1 C.P. for 20% and ±0.5 C.P. for 30-40%.

3.5 Effect of Shearing Time on Apparent Viscosity.

Protein solutions (3 ∿ 40%) were subjected to constant rate of shear for up to 30 minutes. The results of this experiment confirmed the hysteresis effect shown in the preceeding experiment.

For 3% and 5% weight concentration, apparent viscosity remained constant, showing no time effect over the shear rate range of 6,850 - 17,000 sec^{-1}. For 10% weight concentration, apparent viscosity is a function of shearing time at shear rate higher than 5,142 sec^{-1}. For 6,850 - 17,140 sec^{-1}, apparent viscosity increases with shearing time with all cases being similar (Fig. 3.3). For 20% and 30% weight concentration, apparent viscosity increases with shearing time for all shear rates. The effect of shearing time is higher at higher shear rate (Fig. 3.3 and Fig. 3.4). As distinct from the rheopectic properties determined for 10, 20 and 30% solutions, 40% weight concentration β-lactoglobulin solution shows thixotropic behavior, with a decrease in apparent viscosity with shearing time at constant shear rate,(Fig. 3.4). The rate of increase in apparent viscosity is presented in Table 3.3. This experiment was reproducible within the range of ±0.05 C.P. for 3 - 10%, ±0.1 C.P. for 20 - 30 % and ±0.3 C.P. for 40%.

3.6 Evaporation Effect.

Evaporation effect was negligible for the duration of the experiments.

3.7 Reversibility of Protein Deformation.

Observation of apparent viscosity made after resting for 30 minutes indicated that protein deformation caused by shearing is irreversible (Table 6.9 and 6.12).

3.8 Conformation Change Study.

Gel filtration chromatography showed that the elution volume of sheared β-lactoglobulin is 72±0.5 ml. while the elution volume of native β-lactoglobulin is 80±1 ml. (Fig. 3.5). (The experiment was made in triplicate.) This indicates that the size of sheared β-lactoglobulin is larger than that of the native one.

U.V. absorption of sheared protein using the native protein as reference is given in Fig. 3.6. The result shows the peaks at 287-288 nm and 293 nm. The results of four experiments were within the range of ±0.001 of absorbancy.

This indicates that shear β-lactoglobulin is different from the native β-lactoglobulin.

Table 3.1

Rate of decreasing in apparent Viscosity with Shear Rate

Concentration (%)	Shear Rate Range (sec^{-1})	Rate of Decrease in Apparent Viscosity (C.P.S.-sec)
3	6,850-17,000	0.0
5	6,850 - 17,000	0.0
10	1,700 - 6,850	$8.0 \pm 0.05 \times 10^{-5}$
	6,850 - 17,000	0.0
20	850 - 1,700	$47.0 \pm 0.05 \times 10^{-5}$
	1,700 - 8,500	$0.1 \pm 0.05 \times 10^{-5}$
	8,500 - 17,000	0.0
30	1,700 - 3,400	$59.8 \pm 0.05 \times 10^{-5}$
	3,400 - 8,500	$7.8 \pm 0.05 \times 10^{-5}$
	8,500 - 17,000	0.0
40	1,700 - 5,200	$817.1 \pm 0.05 \times 10^{-5}$
	5,200 - 15,000	$422.4 \pm 0.05 \times 10^{-5}$

Table 3.2

Hysteresis Effects

Time (sec.)	10%		20%		30%		40%	
	Shear Rate (sec^{-1})	η_{app} Change (c.p.s.)	Shear Rate (sec^{-1})	η_{app} Change (c.p.s.)	Shear Rate (sec^{-1})	η_{app} Change (c.p.s.)	Shear Rate (sec^{-1})	η_{app} Change (c.p.s.)
0	17,140	0	17,140	0	17,005	0	15,140	0
48	15,426	0	-	-	15,304	0.4	13,626	- 1.2
72	-	-	12,855	0	-	-	-	-
96	13,712	0	-	-	13,604	0.5	12,112	- 2.8
140	11,998	0	8,570	0.3	11,903	0.7	10,598	- 3.2
192	10,284	0	-	-	10,203	0.9	9,084	- 9.7
216	-	-	4,285	0.4	-	-	-	-
240	8,570	0	-	-	8,502	1.1	7,570	-21.9
288	6,856	0.1	1,714	0.4	6,802	1.2	6,050	-23.6
336	5,142	0.3	-	-	5,101	1.1	4,542	-26.7
360	-	-	1,288	0.8	-	-	-	-
384	3,428	0.3	-	-	3,401	1.4	3,028	-32.6
432	1,714	0.4	857	0.8	1,700	1.6	1,514	-36.4

Table 3.3

Shearing Time Effect in Apparent Viscosity of β-Lactoglobulin Solution

Time Range (min)	Concentration	Shear Rate (sec^{-1})	Rate of Change Apparent Viscosity (c.p.s./sec)
1-30	3%	6,856-17,140	0
1-30	5%	6,856-17,140	0
1-30	10%	1,714-5,142	0
1-30	10%	6,856-17,140	$2.0\pm0.5 \times 10^{-2}$
1-30	20%	1,714	$5.0\pm0.5 \times 10^{-2}$
1-30	20%	3,428	$7.0\pm0.5 \times 10^{-2}$
0- 6	20%	5,142	$7.0\pm0.5 \times 10^{-2}$
6-15			$18.0\pm0.5 \times 10^{-2}$
15-25			$21.0\pm0.5 \times 10^{-2}$
25-30			$43.0\pm0.5 \times 10^{-2}$
0- 5	20%	6,856	$6.0\pm0.5 \times 10^{-2}$
5-10			$14.0\pm0.5 \times 10^{-2}$
10-15			$34.0\pm0.5 \times 10^{-2}$
15-20			$46.0\pm0.5 \times 10^{-2}$
20-30			$57.0\pm0.5 \times 10^{-2}$
0- 5	20%	8,540	$18.0\pm0.5 \times 10^{-2}$
5-10			$30.0\pm0.5 \times 10^{-2}$
10-15			$42.0\pm0.5 \times 10^{-2}$
15-20			$54.0\pm0.5 \times 10^{-2}$
20-25			$60.0\pm0.5 \times 10^{-2}$
0- 5	20%	10,284	$22.0\pm0.5 \times 10^{-2}$
5- 7			$25.0\pm0.5 \times 10^{-2}$
7-10			$37.0\pm0.5 \times 10^{-2}$
10-15			$46.0\pm0.5 \times 10^{-2}$
15-20			$66.0\pm0.5 \times 10^{-2}$
0-10	20%	11,998	$14.0\pm0.5 \times 10^{-2}$
10-30			$7.0\pm0.5 \times 10^{-2}$

Table 3.3 (continued)

Time Range (min)	Concentration	Shear Rate (sec^{-1})	Rate of Change Apparent Viscosity (c.p.s./sec.)
0- 5	20%	13,712	$24.0\pm0.5 \times 10^{-2}$
5-10			$34.0\pm0.5 \times 10^{-2}$
10-15			$48.0\pm0.5 \times 10^{-2}$
0- 5	20%	15,426	$24.0\pm0.5 \times 10^{-2}$
5- 7			$35.0\pm0.5 \times 10^{-2}$
7-10			$37.0\pm0.5 \times 10^{-2}$
10-12			$110.0\pm0.5 \times 10^{-2}$
0- 5	20%	17,140	$26.0\pm0.5 \times 10^{-2}$
5- 7			$36.0\pm0.5 \times 10^{-2}$
7- 9			$55.0\pm0.5 \times 10^{-2}$
0-10	30%	6,856	$8.0\pm0.5 \times 10^{-2}$
10-25			$13.0\pm0.5 \times 10^{-2}$
25-30			$20.0\pm0.5 \times 10^{-2}$
0- 4	30%	8,570	$50.0\pm0.5 \times 10^{-2}$
4- 6			$80.0\pm0.5 \times 10^{-2}$
6- 8.5			$96.0\pm0.5 \times 10^{-2}$
8.5-17			$137.0\pm0.5 \times 10^{-2}$
0- 3	30%	10,284	$67.0\pm0.5 \times 10^{-2}$
3- 6			$120.0\pm0.5 \times 10^{-2}$
0- 2	40%	3,028	$-550.0\pm0.5 \times 10^{-2}$
2- 5			$-333.0\pm0.5 \times 10^{-2}$
5-15			$-50.0\pm0.5 \times 10^{-2}$
15-25			$-10.0\pm0.5 \times 10^{-2}$
0- 5	40%	4,542	$-260.0\pm0.5 \times 10^{-2}$
5-15			$-90.0\pm0.5 \times 10^{-2}$
15-30			$-27.0\pm0.5 \times 10^{-2}$
0- 5	40%	6,052	$-120.0\pm0.5 \times 10^{-2}$
5-15			$-90.0\pm0.5 \times 10^{-2}$
15-25			$-30.0\pm0.5 \times 10^{-2}$

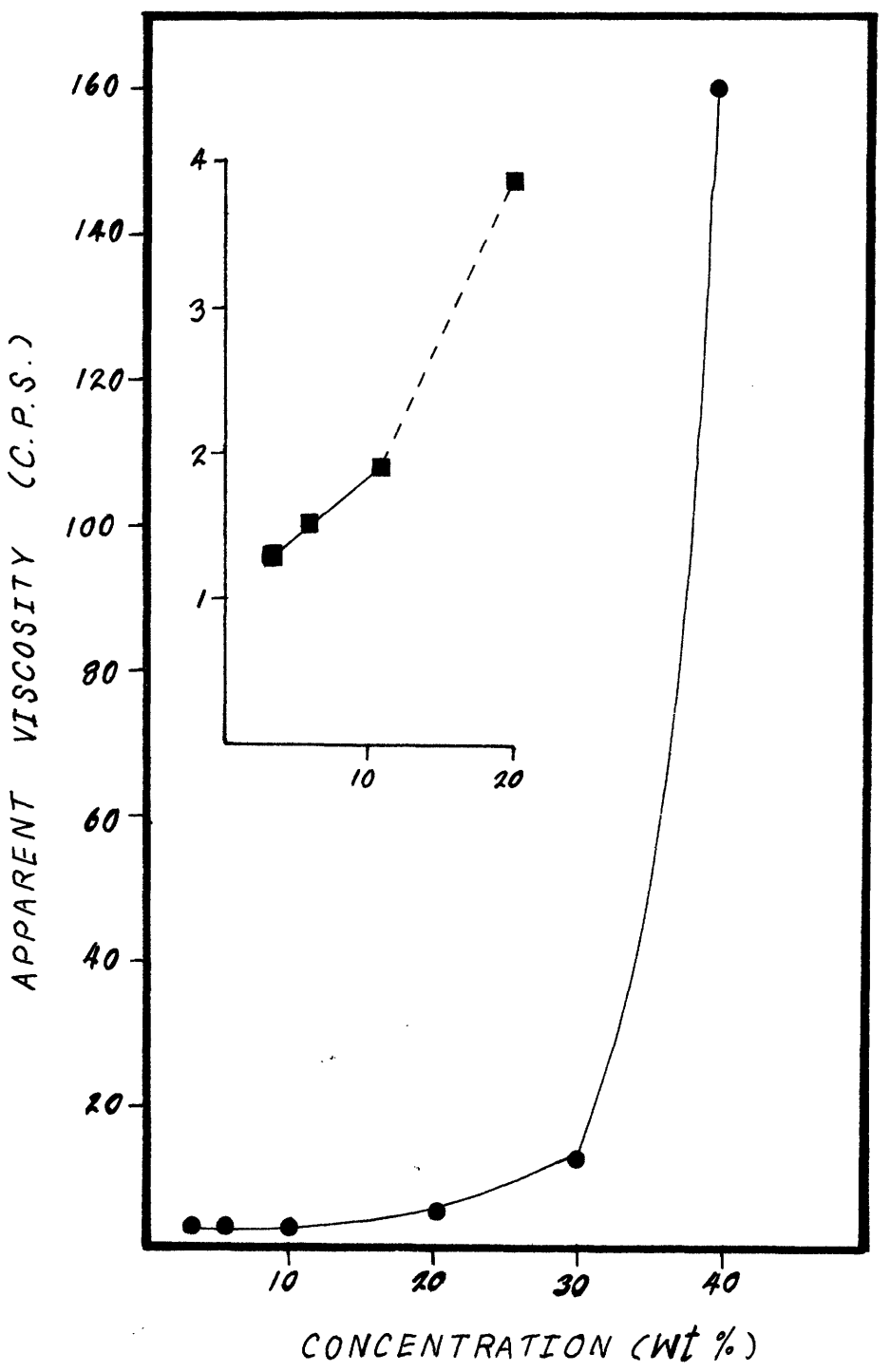

Fig. 3.1 Effect of Concentration on Apparent Viscosity

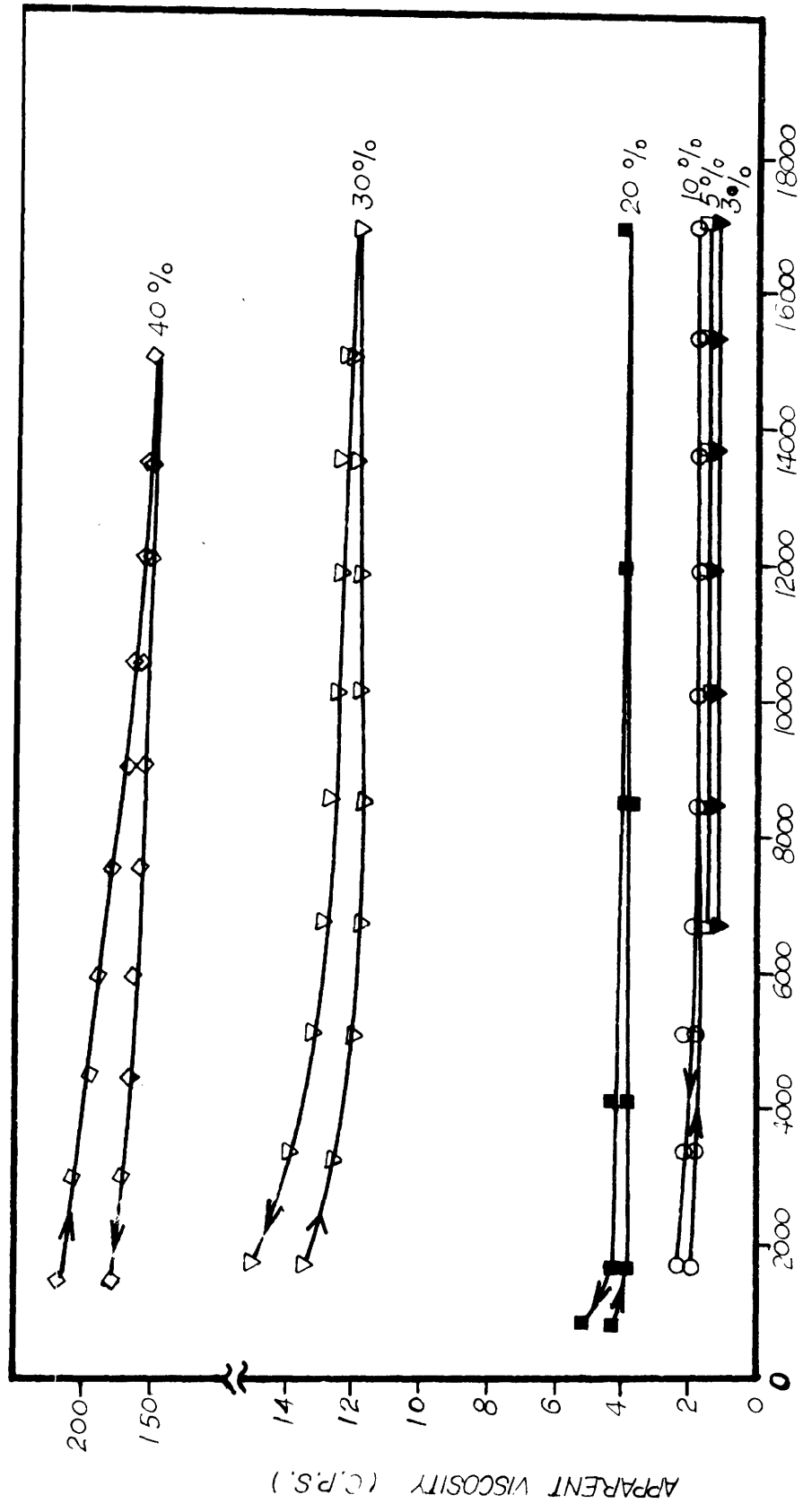

Fig. 3.2 Effect of Shear Rate on Apparent Viscosity

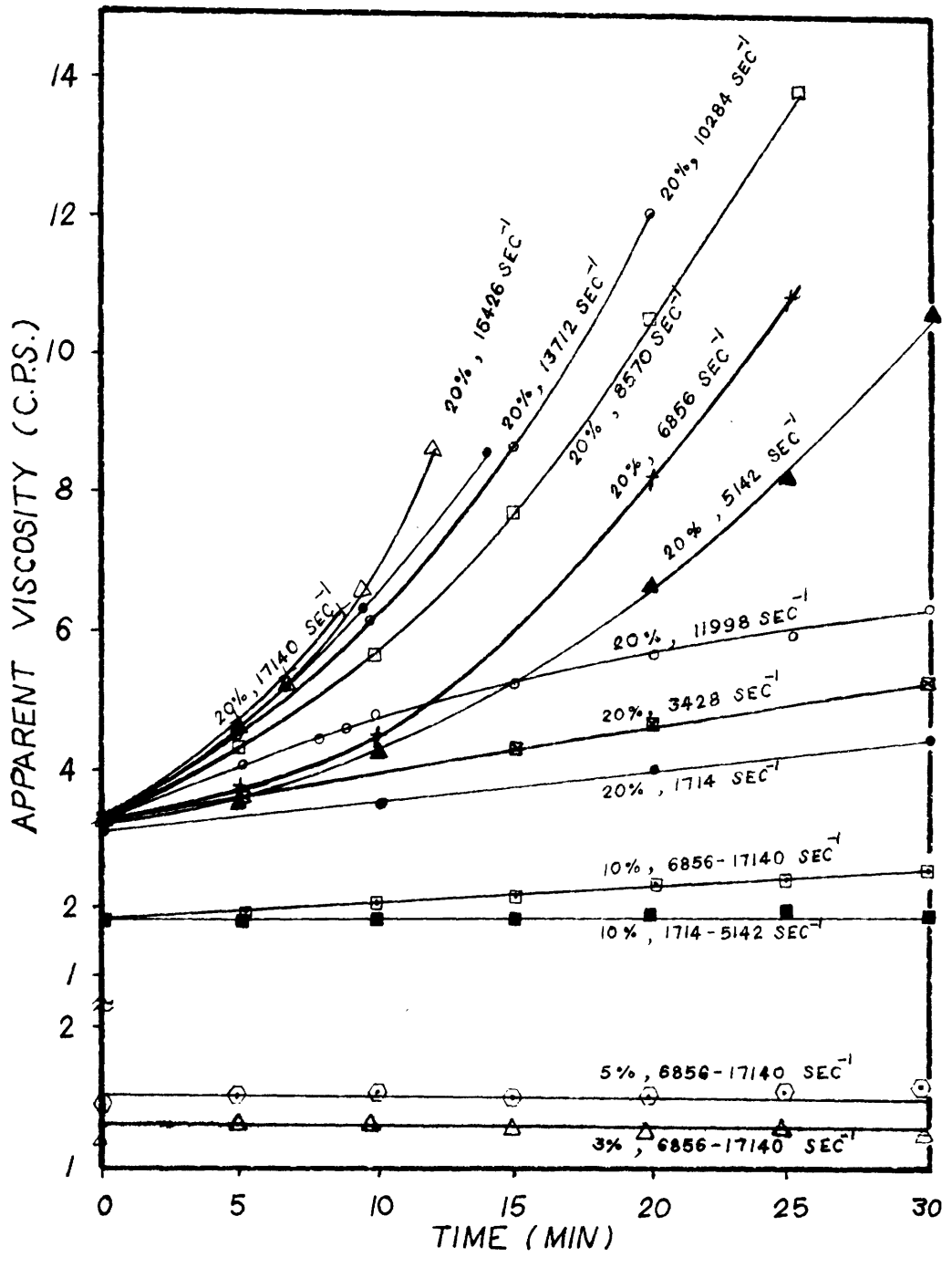

Fig. 3.3 Effect of Shearing Time on Apparent Viscosity

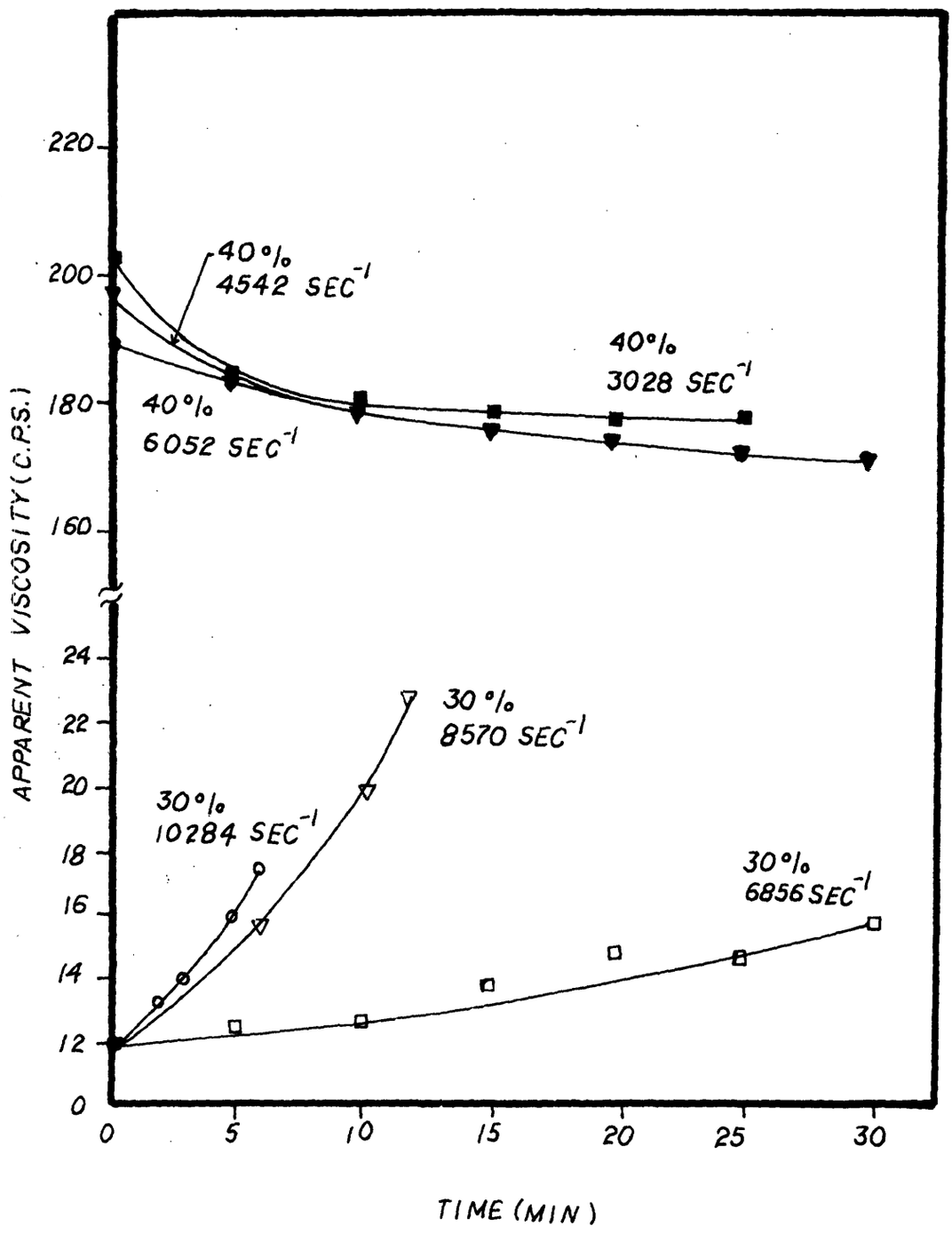

Fig. 3.4 Effect of Shearing Time on Apparent Viscosity

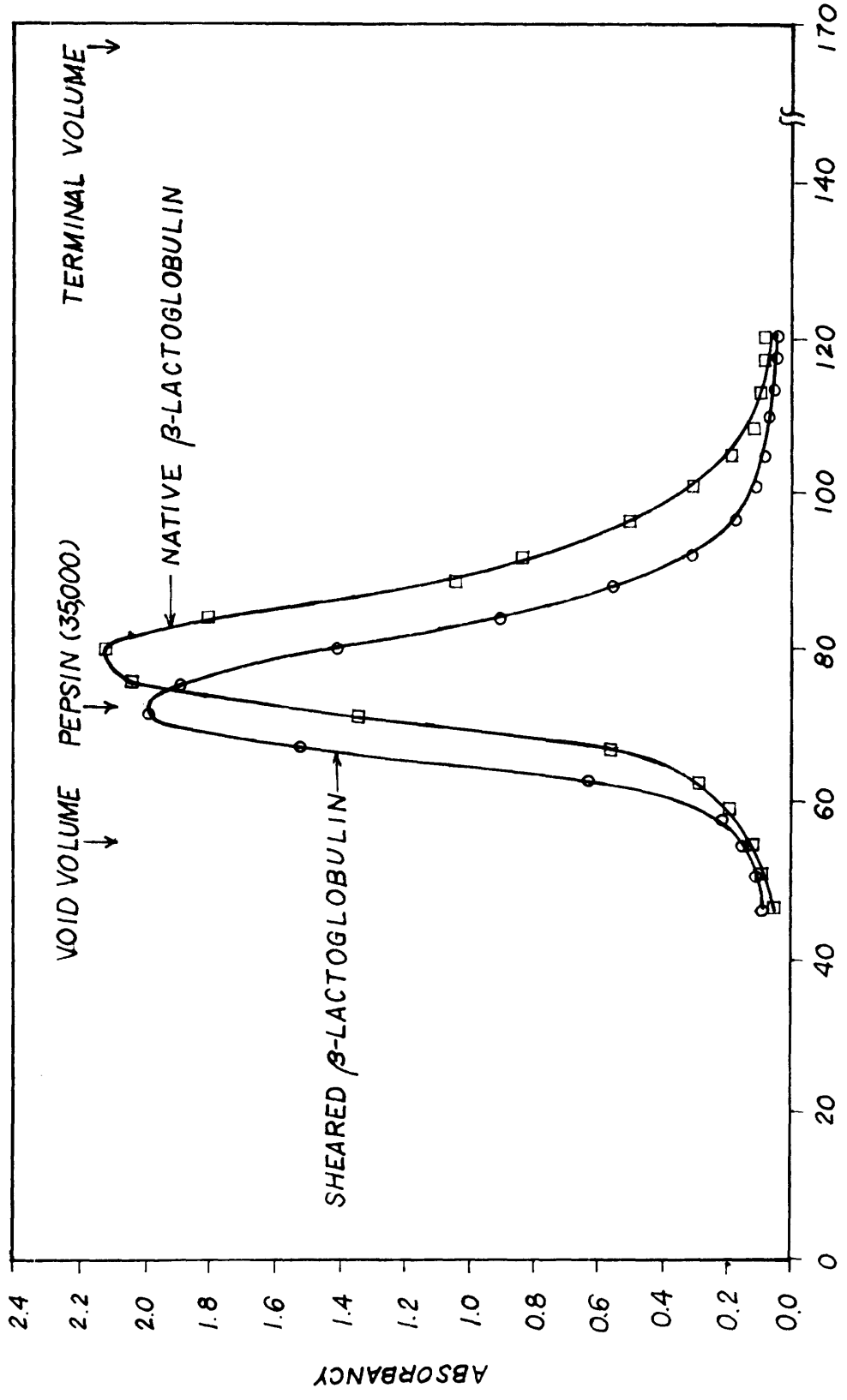

Fig. 3.5 Gel Filtration Chromatography

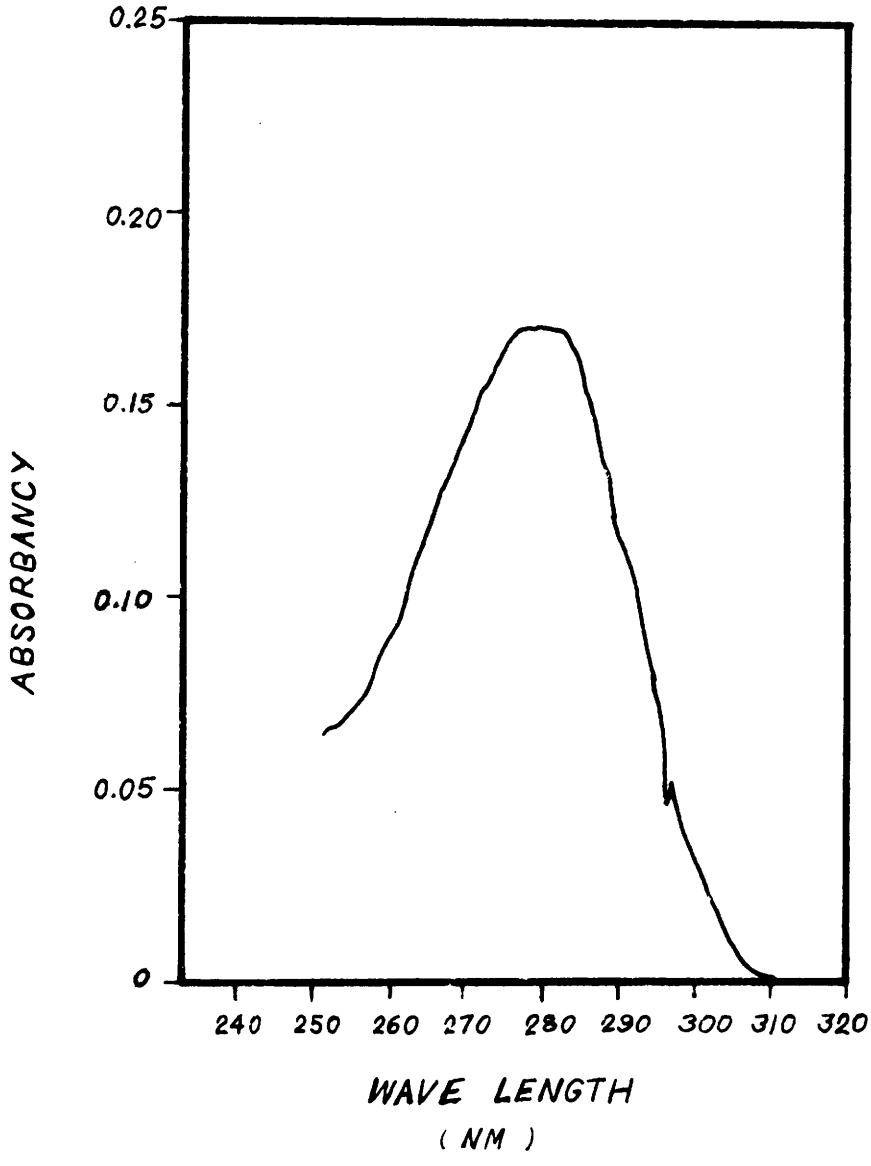

Fig. 3.6 UV Different Spectra Absorption

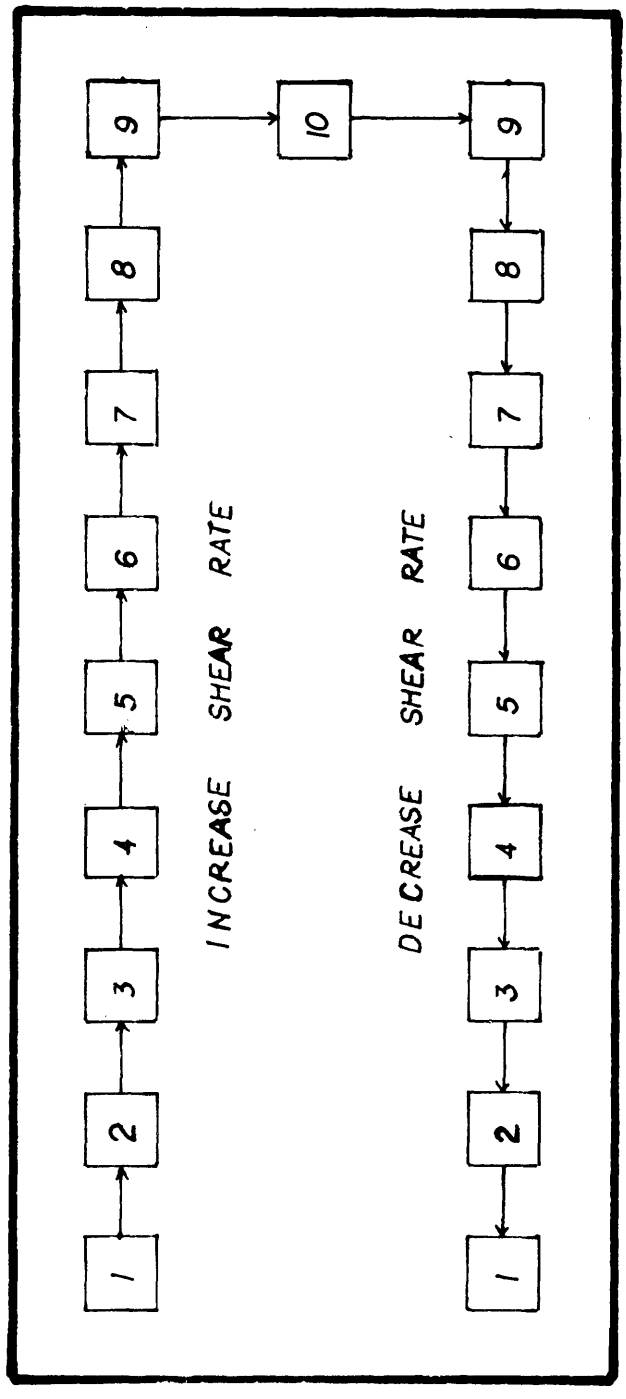

Fig. 3.7 Determination of Hysteresis Effect

4. DISCUSSION AND CONCLUSIONS

In view of the fact that the concentration of protein used for each process is different, and that the viscosity of a suspension is affected by concentration (Philppoff, 1942), viscosity of protein at different concentrations was studied. The result of this study confirms the concentration dependence of the viscosity of protein solution. The effect of concentration may be represented by three separate regions (Frisch, 1956). In the first region, at extremely low concentration (up to 10 weight %), the solution obeys Einstein's equation (III). In the second regions, at higher concentration, a deviation from Einstein's equation is observed. In this region, a non-linear viscosity dependency of the particle concentration must be considered. This non-linearity may arise from the action of mutual hydrodynamic forces between the solute particles. The third region represents the concentration at which the mutual hydrodynamic interactions of the suspended particles reaches a maximum. The viscosity also reaches a maximum in this region. After this point if concentration continues to increase, viscosity will decrease.

In general there are two types of concentration dependent on viscosity of solution (Frisch, 1956). One is the ideal solution in which viscosity continously and monotonically increases with concentration. The other is anomalous solution which exhibits inflection points in viscosity as functions of the concentration. This study showed the β-lactoglobulin

solution to increase in apparent viscosity with the increase in concentration. The first region, in which Einstein's Equation applied, was at concentration 3 - 10% for the rate of shear range of 6,500 - 17,000 sec^{-1} (Fig. 3.1). In this region, the relationship of concentration and apparent viscosity of β-lactoglobulin can be expressed as

$$\eta_s = \eta_o (1 + 0.8C)$$

where the concentration is in weight % concentration. Based on diffusion study, the Einstein's constant for β-lactoglobulin is 6.0, where concentration is expressed in fractional volume (Mehl, 1940). For concentrations higher than 10%, the deviation from Einstein's equation was observed as expected. For the dilute concentration, the interaction between solute particles is negligible. As concentration increases, the volume of the dispersed phase and the interaction of solute particles increases. This causes the deviation from Einstein's equation. Based on this study, 10% weight concentration seems to be a critical concentration, since concentrations below it obey Einstein's equation, but concentrations above it will deviate from Einstein's equation. However the third region of concentration was not observed for the range of concentration used in this study. It should be noticed that viscosity at 20% weight concentration is equal to double of that of 10%

weight concentration. The effect of concentration increased more rapidly as concentration increased above 10% weight as shown in Fig. 3.1.

In addition to this, shear rate effect on apparent viscosity is also affected by concentration effect (Balmaceda and Rha, 1973). For 3% and 5% concentration over 6,850 - 17,140 sec^{-1}, viscosity is not a function of shear rate. For concentration above 5% over 850 - 17,140 sec^{-1}, viscosity decreases asymptotically with increasing shear rate and the effect of shear rate on viscosity increases as concentration increases (Table 3.1). This may be caused by:

a. molecules of β-lactoglobulin changing under shearing force,

b. the solute particle forming aggregates at higher concentration. Under shearing, aggregates may break down (Rha, 1975).

As in the case of other proteins such as myosin, whey protein concentrate and single cell protein (Edsall, 1940; Hermanson, 1975; Huang and Rha, 1971), the apparent viscosity at β-lactoglobulin solution is not found to be a function of shear rate at low concentration (3% and 5% weight concentration) and to be a pseudoplastic solution above 5% weight concentration (Fig. 3.2). The decrease in viscosity with shear rate can be caused by:

a. molecules of protein changing under shearing,

b. solute molecules being distributed at random with

their resistance to flow being higher at lower shear rate, whereas at higher shear rate they are more oriented and parallel to the stream line. So viscosity of solution at higher shear rate is lower than that at lower shear rate.

c. aggregates breaking apart under shearing.

Considering that the flow properties of many viscous solutions can change with shearing time, and that shearing force can deform a protein molecule (Taylor, 1934; Edsall et. al., 1965; Polson, 1939; Rha, 1975), the study of the viscosity-shearing time relationship is the main point of interest in this study. Like concentration and shear rate effect, effect of shearing time appears above 5 weight % concentration. The apparent viscosity of 10 weight % concentration starts to increase with shearing time at 6,856 sec^{-1}. Thereafter this effect of time remains the same even if the shear rate is increased (Fig. 3.3 and Table 3.3). Apparent viscosity of 20 and 30 weight % concentration generally increases with shearing time and the increase is more rapid as shear rate increases (Fig. 3.3, Fig. 3.4, Table 3.3). The increase in apparent viscosity with shearing time may be affected by the following:

a. aggregation during shear,

b. shear deformation of β-lactoglobulin molecules.

Under shearing, protein molecules can be uncoiled. The uncoiled protein will change and increase the effective volume of the solute in solution, so that apparent viscosity

increases (Van Holde, 1971; Tanford, 1967). In addition changes in size and shape of protein molecules affects apparent viscosity, which increases with increase in size and shape of the molecules (Edsall, 1965; Mehl, 1940). In order to determine the cause of the change in flow behavior, gel filtration chromatography and U.V. difference spectra absorption were used. The gel filtration and U.V. difference absorbancy indicate that sheared β-lactoglobulin is different from native β-lactoglobulin (Fig. 3.5 and Fig. 3.6). Gel filtration chromatography also shows that sheared β-lactoglobulin is larger than the native one, since the elution volume of sheared β-lactoglobulin is less than that of native β-lactoglobulin. U.V. difference spectra showed that chromophores, tryptophan and tryosine became exposed after the shearing by the increase in absorption near nm 287 ∿ 293 (Lehninger, 1975).

While 10 to 30% solution shows rheopectic properties, 40% solution has thixotropic properties (Fig. 3.5). Thixotropy of 40% solution may be caused by the breaking of aggregates, since when the concentration of solution is high enough aggregates are formed. Initially breaking of the aggregate by shear force would require higher energy, but subsequently only the energy for shear flow would be required (Charm, 1962; Rha, 1975).

The hesteresis roop of the solution confirms the effect of shearing time on apparent viscosity, since the rheopectic roops were observed for 10 to 30 weight % concentration, and thixotropic roop was observed for 40 weight % concentration,

while 3 and 5 weight % concentration solution shows no hysteresis effect (Table 3.2). Changes in rheological properties was observed to be irreversible both for rheopectic and thixotropic properties.

Since 10 weight % β-lactoglobulin solution obey Einstein's equation, changes in axial ration of β-lactoglobulin with the shearing time is determined by the method of Mehl et. al. (1940), while that of 20 - 40 weight % β-lactoglobulin solution cannot. The axial ratios determined for 10% β-lactoglobulin is given in Table 4.1. The increase in axial ratio of approximately 40% occured due to 30 minutes of shearing at constant shear rate in the range of 6,856 - 17,140 $sec.^{-1}$.

In summary β-lactoglobulin solution shows the effect of shear rate and time above 5 weight % concentration. β-lactoglobulin solution is a pseudoplastic solution for 10-40 weight concentration. β-lactoglobulin solution shows a rheopectic property at 10-30 weight % concentration while 40% concentration shows thixotropic property. Shearing force causes permanent change in β-lactoglobulin molecule. The denaturation is characterized to be the increase in size, increase in axial ratio and increase in the chromophore exposed.

Table 4.1

Changing in Axial Ration of 10 weight % β-Lactoglobulin Solution Shared at Constant Shear Rate in the Range 6,856-17,140 sec^{-1}

Time (min)	η_{app} (c.p.s.)	Viscosity Increment	Axial Ratio	
			Rod Shape	Disc Shape
0	1.7	6.0	5.0	7.2
5	1.8	6.3	5.2	7.4
10	1.9	6.7	5.3	7.5
15	2.0	7.4	6.0	8.5
20	2.1	7.8	6.2	9.0
25	2.2	8.1	6.4	9.8
30	2.4	8.9	7.0	10.9

5. SUMMARY

The results of the study of rheological properties of solution in phosphate buffer (ph 7, ionic strength 0.04) are summarized as follows.

1. Apparent viscosity of β-lactoglobulin solution is concentration, shear rate and time dependent.

2. Apparent viscosity of β-lactoglobulin solution can be calculated by

$$\eta_s = \eta_o(1 + 0.8C)$$ for concentrations less than 10%, where C is β-lactoglobulin concentration.

3. β-lactoglobulin solution is pseudoplastic at concentrations above 5%.

4. β-lactoglobulin solutions, at concentration of 10 to 30 weight %, showed rheopectic property while 40 weight % solution showed thixotropic property.

5. Mechanical shearing causes permanent deformation of β-lactoglobulin in solution.

6. The shear deformation increases the size of the molecule and the exposed tryptophan and tyrosine.

6. FUTURE RESEARCH RECOMMENDATION

1. In this study, the increase in viscosity with shearing time was not observed for concentrations of β-Lactoglobulin solution lower that 10%. Theoretically, the protein molecules would be effected similarly by shear, and would similarly be deformed even at lower concentrations. Therefore, change in protein conformation should be determined by other methods such as gel filtration chromatography and UV difference spectra absorbancy for sheared β-Lactoglobulin at low concentration.

2. Friction and interaction between the protein molecules can be minimized with the use of a lower concentration of β-Lactoglobulin. It is then possible to determine which is the more important cause of shear deformation: the drag between the protein molecules or the drag of the solvent.

3. The effect of shearing time on apparent viscosity at high shear rate (up to 171,400 $sec.^{-1}$) should be determined. Disassociation of the β-Lactoglobulin molecule may occur at a higher shear rate (above 171,400 $sec.^{-1}$), which would be observed as the thixotropic property.

4. Flow properties can often be expressed by a power law equation. The study of shear rate - shear stress relationship should be extended to lower shear rates to obtain the power law constants for β-Lactoglobulin solution. This was not feasible in this experiment since the readings at shear rates less than 800 $sec.^{-1}$ were unreliable. In order

to obtain readings at lower shear rates with the Cone-Plate Viscometer used in this study it is necessary to make the torque spring force lower than 100 gm-cm.

5. The rheological properties of protein solution are also dependent on pH, ionic strength, charge and temperature. The effect of these parameters on apparent viscosity of β-Lactoglobulin solution should be studied.

6. At present, proteins are manipulated by chemical and heat-treatment. This study showed that conformation change in protein can be induced by mechanical treatment and by simple shearing. The combined effect of chemical and mechanical treatment and/or heat and mechanical treatment should be determined.

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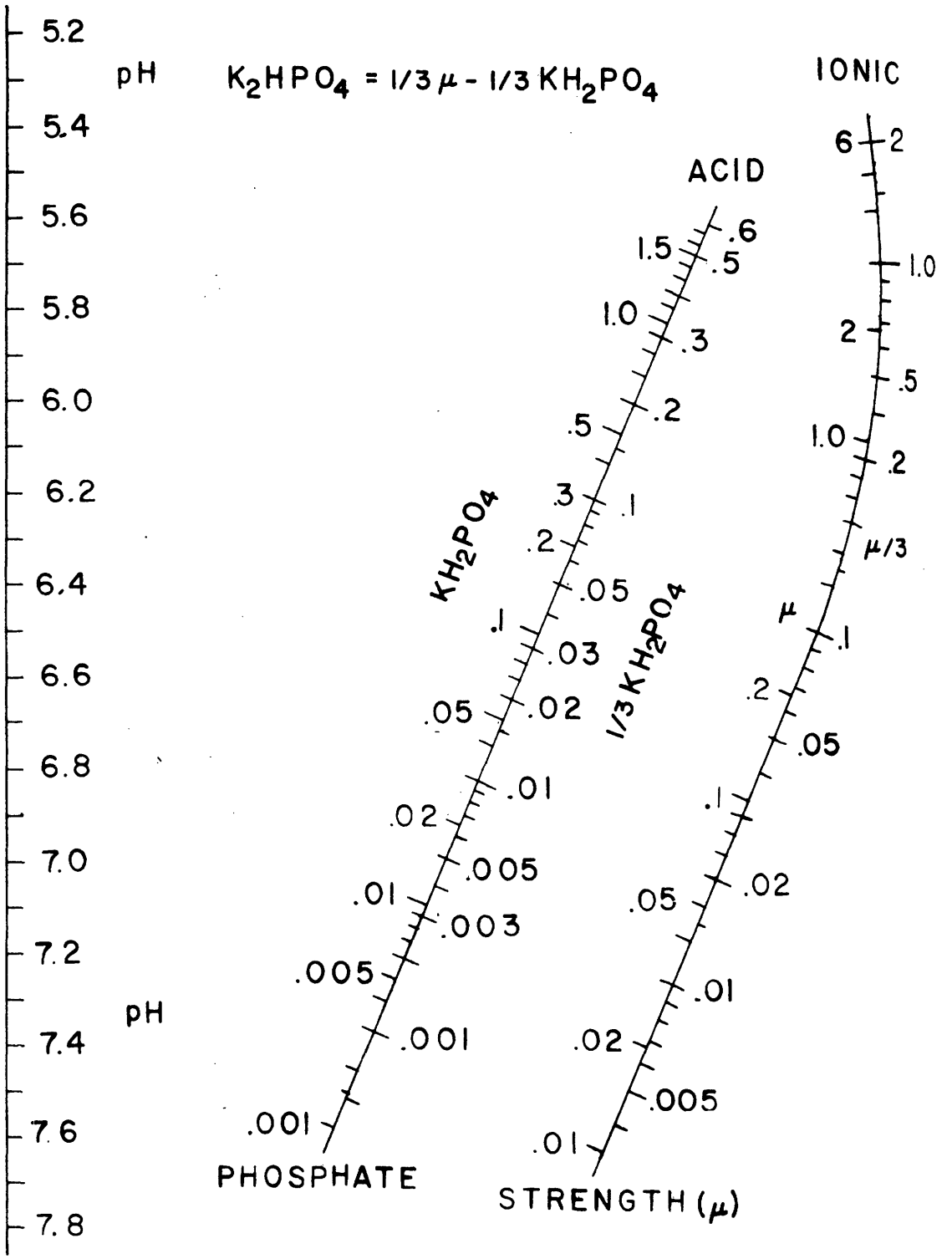
APPENDIX



Appendix A Nomogram For Phosphate Buffer

Appendix B

Table 6.1 Hysteresis Effect

Apparent Viscosity and Shear Stress Versus Shear Rate
3% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 7 cm. diameter, 20'57" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Shear Rate (sec^{-1})	Increasing Shear Rate		Decreasing Shear Rate	
	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)
6,856	80.0	1.2	7.8	1.1
8,570	104.4	1.3	102.4	1.2
10,284	129.7	1.3	128.8	1.3
11,998	152.2	1.3	150.2	1.3
13,712	180.4	1.3	177.5	1.3
15,426	204.8	1.4	201.9	1.4
17,140	231.1	1.4	226.2	1.4

Table 6.2 Hysteresis Effect

Apparent Viscosity and Shear Stress Versus Shear Rate
5% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 7 cm. diameter, 20'57" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Shear Rate (sec^{-1})	Increasing Shear Rate		Decreasing Shear Rate	
	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)
6,856	93.6	1.4	101.4	1.5
8,570	122.9	1.5	128.7	1.5
10,284	151.2	1.5	156.0	1.5
11,998	181.4	1.5	185.3	1.6
13,712	208.7	1.5	212.6	1.6
15,426	240.9	1.6	239.9	1.6
17,140	271.1	1.6	271.1	1.6

Table 6.3 Hysteresis Effect

Apparent Viscosity and Shear Stress Versus Shear Rate
10% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 7 cm. diameter, 20'57" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Shear Rate (sec^{-1})	Increasing Shear Rate		Decreasing Shear Rate	
	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)
1,714	34.7	2.0	41.1	2.4
3,428	60.4	1.8	73.2	2.1
5,142	88.7	1.7	104.1	2.0
6,856	128.5	1.8	133.6	1.9
8,570	151.6	1.9	156.7	1.8
10,284	183.7	1.8	185.0	1.8
11,998	213.3	1.8	215.8	1.8
13,712	244.1	1.8	249.2	1.8
15,426	274.0	1.8	274.9	1.8
17,140	303.2	1.8	303.2	1.8

Table 6.4 Hysteresis Effect

Apparent Viscosity and Shear Stress Versus Shear Rate
20% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 7 cm. diameter, 20'57" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Shear Rate (sec^{-1})	Increasing Shear Rate		Decreasing Shear Rate	
	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)
857	39.8	4.4	44.6	5.2
1,285.5	52.7	4.0	64.3	5.0
1,714	66.8	3.9	72.7	4.3
4,285	165.7	3.8	180.0	4.2
8,570	334.0	3.9	359.7	4.2
12,855	524.2	4.0	524.2	4.0
17,140	678.3	3.9	678.3	4.0

Table 6.5 Hysteresis Effect

Apparent Viscosity and Shear Stress Versus Shear Rate
30% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 4 cm. diameter, 21'8" angle
Spring = 100 gm. cm.
Temperature = 25 ± 0.5°C

Shear Rate (sec^{-1})	Increasing Shear Rate		Decreasing Shear Rate	
	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)
1,700.5	234.6	13.5	262.2	15.1
3,401	434.7	12.5	483.0	13.9
5,101.5	634.8	12.2	690.0	13.3
6,802	825.2	11.9	910.8	13.1
8,502.5	1,021.2	11.7	1,104.0	12.8
10,203	1,242.0	11.9	1,324.8	12.8
11,903.5	1,435.2	11.8	1,518.0	12.5
13,604	1,642.2	11.8	1,711.2	12.3
15,304.5	1,849.2	11.8	1,904.4	12.2
17,005	2,070.0	11.9	2,070.0	11.9

Table 6.6 Hysteresis Effect

Apparent Viscosity and Shear Stress Versus Shear Rate
40% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 2 cm. diameter, 23'43" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Shear Rate (sec^{-1})	Increasing Shear Rate		Decreasing Shear Rate	
	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)	Shear Stress ($dyne/cm^2$)	η_{app} (c.p.s.)
1,514	3,309.5	218.6	2,757.9	182.2
3,028	6,232.9	205.8	5,240.0	173.2
4,542	8,880.4	195.5	7,667.0	168.8
6,056	11,472.9	189.4	10,038.8	165.8
7,570	13,789.5	182.2	12,134.8	160.3
9,084	15,223.6	167.6	14,341.1	157.9
10,598	16,878.4	159.3	16,547.4	156.1
12,112	18,864.0	155.8	18,533.1	153.0
13,626	20,684.3	151.8	20,518.9	150.6
15,140	22,504.5	148.6	22,504.5	148.6

Table 6.7 Effect of Shearing Time on Apparent Viscosity
Apparent Viscosity Versus Time at Constant Shear Rate
3% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 7 cm. diameter, 20'57" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Time (mins)	Shear Rate 6,856 sec^{-1}		Shear Rate 10,284 sec^{-1}		Shear Rate 13,712 sec^{-1}		Shear Rate 17,140 sec^{-1}	
	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)
0	86.8	1.3	134.6	1.4	196.0	1.5	239.9	1.4
5	89.7	1.4	135.6	1.4	184.3	1.4	249.6	1.5
10	88.7	1.3	136.6	1.4	190.2	1.4	243.8	1.4
15	88.7	1.3	138.9	1.4	189.2	1.4	251.6	1.5
20	87.8	1.3	139.5	1.4	190.2	1.4	250.5	1.5
25	93.6	1.4	140.5	1.4	190.2	1.4	246.9	1.5
30	86.8	1.3	139.5	1.4	191.1	1.4	249.6	1.5

Table 6.8 Effect of Shearing Time on Apparent Viscosity

Apparent Viscosity Versus Time at Constant Shear Rate
5% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 7 cm. diameter, 20"57" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Time (mins)	Shear Rate 6,856 sec^{-1}		Shear Rate 10,284 sec^{-1}		Shear Rate 13,712 sec^{-1}		Shear Rate 17,140 sec^{-1}	
	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)
0	101.4	1.5	156.0	1.5	224.3	1.6	296.4	1.7
5	97.5	1.4	158.0	1.5	220.4	1.6	292.5	1.7
10	97.5	1.4	154.1	1.5	230.1	1.7	280.8	1.6
15	97.5	1.4	152.1	1.5	222.3	1.6	298.4	1.7
20	95.6	1.4	156.0	1.5	222.3	1.6	308.1	1.8
25	93.6	1.4	159.9	1.6	234.0	1.7	288.6	1.7
30	97.5	1.4	159.9	1.6	216.5	1.6	296.4	1.7

Table 6.9 Effect of Shearing Time on Apparent Viscosity
Apparent Viscosity Versus Time at Constant Shear Rate
10% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 7 cm. diameter, 20'57" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Time (mins)	Shear Rate 1,714 sec^{-1}		Shear Rate 3,428 sec^{-1}		Shear Rate 5,142 sec^{-1}		Shear Rate 6,856 sec^{-1}	
	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)
0	30.8	1.8	60.4	1.8	82.2	1.6	114.3	1.7
5	30.8	1.8	60.4	1.8	82.9	1.6	115.6	1.7
10	30.8	1.8	61.0	1.8	84.8	1.6	122.1	1.8
15	30.8	1.8	61.0	1.8	85.4	1.7	127.2	1.9
20	30.8	1.8	61.0	1.8	85.7	1.7	136.2	2.0
25	30.8	1.8	63.6	1.9	87.4	1.7	141.3	2.1
30	30.8	1.8	65.5	1.9	89.3	1.7	144.5	2.1
after stop 30 min	-	-	-	-	-	-	144.5	2.1

(continued)

Table 6.9 (continued)

Time (mins)	Shear Rate 8,570 sec^{-1}		Shear Rate 10,284 sec^{-1}		Shear Rate 11,998 sec^{-1}		Shear Rate 13,712 sec^{-1}	
	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)
0	138.8	1.6	174.8	1.7	195.3	1.6	237.9	1.7
5	149.0	1.7	190.1	1.8	218.4	1.8	253.5	1.8
10	154.2	1.8	195.3	1.9	231.3	1.9	263.3	1.9
15	167.0	1.9	205.6	2.0	236.4	2.0	273.0	2.0
20	185.0	2.2	215.8	2.1	249.3	2.1	284.7	2.1
25	196.6	2.3	231.3	2.2	269.8	2.2	290.6	2.1
30	205.6	2.4	251.8	2.4	292.9	2.4	296.4	2.2
after stop 30 min	205.6	2.4	251.8	2.4	292.9	2.4	296.4	2.2

(continued)

Table 6.9 (continued)

Time (mins)	Shear Rate 15,426 sec^{-1}		Shear Rate 17,140 sec^{-1}	
	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)
0	282.7	1.8	316.1	1.8
5	318.6	2.1	331.5	1.9
10	334.0	2.2	340.5	2.0
15	357.2	2.3	349.7	2.1
20	385.7	2.5	377.7	2.2
25	395.7	2.6	395.7	2.3
30	421.4	2.7	418.8	2.4
stop after 30 min	421.4	2.7	418.8	2.4

Table 6.10 Effect of Shearing Time on Apparent Viscosity
Apparent Viscosity Versus Time at Constant Shear Rate
20% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 7 cm. diameter, 20'57" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Time (mins)	Shear Rate 1,714 sec^{-1}		Shear Rate 3,428 sec^{-1}		Shear Rate 5,142 sec^{-1}		Shear Rate 6,856 sec^{-1}	
	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)
0	50.0	2.9	109.2	3.2	161.9	3.1	220.9	3.2
5	-	-	125.9	3.7	-	-	256.6	3.8
10	56.5	3.3	-	-	228.7	4.4	302.8	4.4
15	-	-	143.9	4.2	-	-	-	-
20	68.1	4.0	158.0	4.6	352.0	6.8	577.3	8.4
25	-	-	-	-	429.0	8.3	738.9	10.8
30	75.5	4.4	181.1	5.3	555.0	10.8	949.4	14.0

(continued)

Table 6.10 (continued)

Time (mins)	Shear Rate 8,570 sec^{-1}		Shear Rate 10,284 sec^{-1}		Shear Rate 11,998 sec^{-1}		Shear Rate 13,712 sec^{-1}	
	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)
0	292.9	3.4	349.5	3.4	385.4	3.2	477.9	3.5
5	364.6	4.3	439.7	4.3	470.2	3.9	624.4	4.6
8	-	-	-	-	531.9	4.4	-	-
9	-	-	-	-	555.0	4.6	-	-
10	488.2	5.7	657.1	6.4	570.4	4.8	868.5	6.3
14	-	-	-	-	-	-	1,182.0	8.6
15	678.3	7.9	904.5	8.8	626.9	5.2	-	-
20	935.3	10.9	1,252.6	12.2	668.1	5.6	-	-
25	1,175.5	13.7	-	-	719.5	6.0	-	-
30	-	-	-	-	729.7	6.1	-	-

(continued)

Table 6.10 (continued

Time (mins)	Shear Rate 15,426 sec^{-1}		Shear Rate 17,140 sec^{-1}	
	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)
0	524.2	3.4	562.4	3.3
5	697.6	4.5	781.1	4.6
7	-	-	894.2	5.2
9	-	-	1,040.6	6.1
10	945.6	6.1	1,104.9	6.4
12	1,207.7	7.8	-	-

Table 6.11 Effect of Shearing Time on Apparent Viscosity

Apparent Viscosity Versus Time at Constant Shear Rate
30% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 7 cm. diameter, 20"57" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Time (mins)	Shear Rate 6,856 sec^{-1}		Shear Rate 8,570 sec^{-1}		Shear Rate 10,284 sec^{-1}	
	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)
0	815.9	11.9	1,011.3	11.8	1,213.5	11.8
2	-	-	-	-	1,345.5	13.0
3	-	-	-	-	1,443.0	14.0
5	858.0	12.5	-	-	1,647.8	16.0
6	-	-	1,345.5	15.7	1,813.8	17.6
10	865.8	12.6	1,716.0	20.0	-	-
12	-	-	1,872.0	22.8	-	-
15	943.8	13.7	-	-	-	-
20	1,014.0	14.8	-	-	-	-
25	998.4	14.6	-	-	-	-
30	1,068.6	15.6	-	-	-	-

Table 6.12 Effect of Shearing Time on Apparent Viscosity

Apparent Viscosity Versus Time at Constant Shear Rate
40% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 2 cm. diameter, 23'43" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Time (mins)	Shear Rate 3,028 sec^{-1}		Shear Rate 4,542 sec^{-1}		Shear Rate 6,056 sec^{-1}	
	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Shear Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)
0	6,159.0	203.4	8,984.1	197.8	11,488.2	189.7
5	5,586.7	184.5	8,377.6	184.4	11,173.3	184.5
10	5,514.0	182.1	8,141.5	179.3	10,914.0	180.2
15	5,444.3	179.8	7,978.0	175.65	10,595.0	175.0
20	5,417.1	178.9	7,888.0	173.7	-	-
25	5,402.5	178.5	7,816.8	172.1	10,464.0	172.8
30	-	-	7,760.0	170.8	-	-
stop after 30 min	5,402.5	178.5	7,760.0	170.8	10,434.5	172.3

Table 6.13 Effect of Evaporation

Apparent Viscosity Versus Time at Constant Shear Rate 8,570 sec^{-1}
30% Sucrose Solution in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 7 cm. diameter, 20'57" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Time (mins)	Shear Stress ($dyne/cm^2$)	Apparent Viscosity (c.p.s.)
0	303.2	3.5
5	308.3	3.6
10	310.0	3.6
15	310.0	3.6
20	205.77	3.6
25	308.3	3.6
30	308.3	3.6
35	303.2	3.5
40	323.0	3.8
45	331.5	3.9
50	331.5	3.9
55	331.4	3.9
60	334.0	3.9

Table 6.14 Effect of Evaporation
Apparent Viscosity Versus Time at Constant Shear Rate
10% β-Lactoglobulin in Phosphate Buffer (pH 7, ionic strength 0.04)
Cone = 7 cm. diameter, 20'57" angle
Spring = 100 gm. cm.
Temperature = 25.0 ± 0.5°C

Time (mins)	Shear Rate 10,284 sec^{-1}				Shear Rate 13,712 sec^{-1}			
	Without Jacket		With Jacket		Without Jacket		With Jacket	
	Sheer Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Sheer Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Sheer Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)	Sheer Stress ($\frac{dyne}{cm^2}$)	η_{app} (c.p.s.)
0	182.4	1.8	187.2	1.8	237.9	1.7	241.8	1.8
5	190.4	1.8	195.4	1.9	253.4	1.8	253.5	1.8
10	195.3	1.9	187.2	1.8	263.3	1.9	263.3	1.9
15	205.6	2.0	216.0	2.1	273.0	2.0	273.0	2.0
20	215.8	2.1	216.0	2.1	284.7	2.1	284.7	2.1
25	231.3	2.2	226.2	2.2	290.6	2.1	288.6	2.1
30	251.3	2.4	236.5	2.4	296.4	2.2	296.4	2.2
35	257.0	2.4	246.8	2.4	300.3	2.2	296.4	2.2
40	280.1	2.7	253.3	2.4	308.2	2.2	304.2	2.2
45	313.5	3.0	269.1	2.6	326.3	2.4	320.0	2.3
50	353.3	3.4	273.0	2.6	359.7	2.6	323.7	2.4
55	389.3	3.8	273.0	2.6	406.0	3.0	343.2	2.5
60	416.3	4.0	284.7	2.7	457.4	3.3	362.7	2.6

BIOGRAPHICAL NOTE

Pasawadee Pradipasena was born on September 15, 1953, in Bangkok, Thailand. She attended elementary school at Rajinee School and then entered Chulalongkorn University to study Food Technology. In 1975 she received the B.Sc. degree.

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