CHAPTER - III

VISCOSITY OF PROTEIN SOLUTIONS

3.1 INTRODUCTION

Viscometric studies have been extensively used to characterise proteins as regards their size, shape and solvation and to investigate conformational transitions, denaturation, association-dissociation phenomena and chemical modifications (1). As the key parameter in such studies is the intrinsic viscosity, one finds that viscosity measurements have mostly been confined to very dilute solutions. However, viscosities of concentrated protein solutions are also important, since high concentrations of proteins are encountered in food processes and biological systems.

Proteins contain a large number of amino acid side chains with both negative and positive charges. The conformation of the proteins is determined by electrostatic interactions between these charges, intramolecular disulfide linkages, hydrogen bonds and polar and hydrophobic interactions. The conformation may be altered by the solvent medium through its effect on intramolecular interactions.

In their native state, proteins consist of one or more polypeptide molecules folded into

compact structures which are more or less rigid. Denaturation may take place at high temperatures or in highly acidic or alkaline solvents or concentrated aqueous solutions such as that of urea or guanidine hydrochloride (2). Denatured proteins assume more extended and less ordered conformations.

Solvent molecules associated with the proteins may be of two types (i) those embedded within the free spaces of the molecule and (ii) those intrinsically solvated due to the electrostatic charges. The former type of association is more extensive for less extended conformations and hence it is affected by any factor that affects the conformation. The latter type of solvation is influenced by the pH, which determines the charge on the molecule, and the presence of added ions, which may neutralise these charges to some extent.

Theoretical treatments of viscosity of protein solutions are based on theories of dilute suspensions of hard spheres with appropriate modifications to account for assymetry and for the hydration shell which must be considered as part of the hydrodynamic volume of the particle.

It is the purpose of this chapter to examine as to whether the AFVS model that was developed for the viscosity of polymer solutions can describe the viscosity behaviour of protein solutions. We shall also examine as to whether the effect of parameters such as pH and ionic strength can be accounted for.

3.2 THEORETICAL CONSIDERATIONS

3.2.1 Effect of Concentration

(1) Random Coil Proteins

When proteins are completely denatured, as for example in concentrated aqueous solutions of guanidine hydrochloride, they assume a random coil conformation. The viscosity behaviour in these conditions should be analogous to that of polymer solutions and hence should be described by the AFVS model [see eqn. (2.2.6) in chapter II].

$$\frac{1}{\ln \eta_r} = \frac{f_s}{B_\eta \left(1 + \frac{\beta - f_p}{f_s}\right) \phi_p} - \frac{f_s}{B_\eta} \qquad (3.2.1)$$

with ϕ_p defined as

$$\phi_p = \frac{1}{2.5} \frac{[\eta]c}{1 + 0.765[\eta]c - \frac{1.91c}{\rho}} \qquad (3.2.2)$$

One may expect transitions from dilute to semidilute concentration regime at ϕ^* and from semidilute to concentrated regime at ϕ^+. The value of B_η should decrease and that of β should increase with each transition for reasons discussed in chapter II.

(2) Proteins in the Native State

It is generally agreed that in their native state proteins have a compact and essentially rigid structure. Thus Tanford and Buzzel (3) concluded from their study of dilute solutions of bovine serum albumin that the protein is very compactly folded and its shape is nearly spherical. The degree of solvation was also determined. Changes in pH and ionic strength influenced both the conformation and the degree of solvation. The effect of the concentration of the protein on its conformation could not be ascertained, since the measurements were confined to dilute solutions.

Ross and Minton (4) modified the Mooney equation for concentrated suspension of hard particles

$$\eta = \eta_s \exp\left(\frac{2.5\,\phi}{1 - k\phi}\right) \qquad (3.2.3)$$

to account for assymetry of particles. They also considered the effect of including the hydration shell in the hydrodynamic volume of the particle. This is achieved through the relationship

$$\nu\phi = [\eta]c \qquad (3.2.4)$$

where ν has a value of 2.5 for spherical particles. Equation (3.2.4) is strictly valid for very dilute solutions. However, it can be assumed that it is applicable at higher concentrations if it is assumed that the volume of protein and that of the hydration shell do not change with concentration. Equation (3.2.3) is then modified to

$$\eta = \eta_s \exp\left(\frac{[\eta]c}{1 - \frac{k}{\nu}[\eta]c} \right) \qquad (3.2.5)$$

Equation (3.2.5) was shown to describe the viscosity data for a number of proteins upto a concentration of 0.04 g/cc. This implies that the above assumption regarding the volume of the protein is justified.

It would be interesting to verify this assumption at higher concentrations. We shall

tentatively apply eqn. (3.2.1) to viscosity of proteins in their native state and see whether the transitions from one concentration regime to another, which are expected in the case of random coil proteins, are also observed here.

3.2.2 Effect of Solvent

The influence of the solvent on the conformation of the protein is of three types. (i) The solvent acts as the dielectric medium and influences the strength of electrostatic interaction between charges on the protein molecule, (ii) specific interactions between solvent and polar groups of the protein may change the strength of hydrogen bonds and (iii) hydrophobic interaction may be influenced by structural changes in the solvent (5).

In the neighbourhood of the charged groups the solvent is rich in counterions forming a diffuse double layer. As a result of strong electrostatic forces, the solvent molecules orient themselves, forming a more ordered structure. Thus there is an electroviscous contribution to β in addition to the usual immobilisation of

solvent molecules observed in polymer solutions. The degree of ionisation therefore affects the parameter β through its influence on the electroviscous effect.

Effect of pH

Many amino acid residues are charged at a given pH. Even at the isoelectric point where the net charge on the molecule is zero, there are a large number of positive and negative charges. The conformation depends on the number and distribution of these charges. At extreme values of pH there are a large number of charges of the same sign, and electrostatic repulsion causes the molecule to assume a more extended conformation.

Effect of Ionic Strength

When ionic salts are added to the protein solution, the ions tend to cluster around the charged groups of the protein molecule. This results in a shielding of the charges from each other, enabling the molecule to assume a more compact conformation, and reducing the thickness of the hydration shell. The presence of ions would also

affect the electroviscous contribution to the viscosity.

3.3 COMPARISON WITH EXPERIMENTAL DATA

In this section the data from the published literature sources are analysed in terms of AFVS model. The parameters of eqn. (3.2.1) are determined, and are summarized in table 3.1. Since f_s, the free volume fraction of the solvent, could not be estimated under different conditions of pH and ionic strength, B_η / f_s and β / f_s values are listed in table 3.1 instead of B_η and β.

3.3.1 Effect of Concentration

(1) Random Coil Proteins

Crombrugghe et al. (6) reported viscosity data for dilute solutions of thyroglobulin in aqueous guanidine hydrochloride. A plot of $\frac{1}{\ln \eta_r}$ vs $-\frac{1}{\phi_p}$ for this system (Fig. 3.1) as well as the regression coefficient (table 3.1) demonstrates that a straight line fits the data very well. Similar results are obtained for myosin in the same solvent (7).

Recently viscosity data for casein, lysozme, bovine serum albumin and ovalbumin in 6 M guanidine

Table 3.1

Summary of systems analysed

System	Concentration range (g/cc)	pH	Ionic strength	Concentration regime	$\frac{B\eta}{f_s}$	$\frac{\beta}{f_s}$	Regression coefficient R^2	No. of data points analysed	Reference
1. Thyroglobulin - 5M GuHCl	0-0.0061	7.0	0.1 M	I	1.4594	0.7325	0.9999	5	6
2. Myosin - 5M GuHCl	0-0.0174			I	1.5357	0.6374	0.9999	7	7
3. α-Casein - 6 M GuHCl	0-0.2700			I	1.6920	0.5610	0.9915	8	8
				II	1.4859	0.6969	0.9973	24	
				III	1.3512	0.7136	0.9993	3	
4. Ovalbumin - 6 M GuHCl	0-0.2900			I	2.2331	0.2090	0.9917	10	8
				II	2.0116	0.6793	0.9992	19	
5. BSA - 6 M GuHCl	0-0.2300			I	1.6706	0.5361	0.9994	7	8
				II	1.5746	0.5858	0.9979	17	
				III	1.1414	0.7321	0.9977	7	
6. Lysozyme - 6 M GuHCl	0-0.2800			I	2.6371	0.0724	0.9923	14	8
				II	1.5158	0.6727	0.9994	30	
7. Bovine serum albumin-water	0-0.0420	5.0	0.01 M	I	0.1183	1.1307	0.9999	5	9

Table 3.1 (contd.)

System	Concentration range (g/cc)	pH	Ionic strength	Concentration regime	$\frac{B_\eta}{f_s}$	$\frac{\beta}{f_s}$	Regression coefficient R^2	No. of data points analysed	Reference
8. Lysozyme - acetate buffer	0-0.3770	4.8		I	1.6932	0.5591	0.9946	12	8
				II	1.4522	1.4795	0.9956	10	
9. Ribonuclease - water	0-0.0303	4.4	0.02 M	I	0.2336	9.1115	0.9990	5	10
	0-0.0410	5.9	0.02 M	I	0.2777	6.6737	0.9999	5	
10. Bovine serum albumin - water	0-0.0347	5.0	0.001 M	I	0.4035	5.3778	0.9995	6	3
	0-0.0420	5.0	0.002 M	I	0.5209	3.9487	0.9994	9	
11. Histone - 0.1 M K_2SO_4	0-0.0154	4.2	0.1 M	I	0.3466	5.3453	0.9979	5	11
-0.2 M Na_3PO_4	0-0.0164	3.7	0.1 M	I	0.6560	2.9168	0.9998	5	
-0.01 M NaCl	0-0.0182	6.6	0.1 M	I	1.1482	0.7050	0.9999	4	
-0.02 N HCl	0-0.0163	2.3	0.02 M	I	2.5214	0.0141	0.9999	5	
12. Pectinic acid - 0.155 M NaCl	0.0005	6.0	0.155 M	I	1.2070	0.9835	0.9985	9	12
	0.0010			I	1.2812	0.8668	0.9961	7	
	0.0020			I	1.3697	0.7404	0.9691	6	

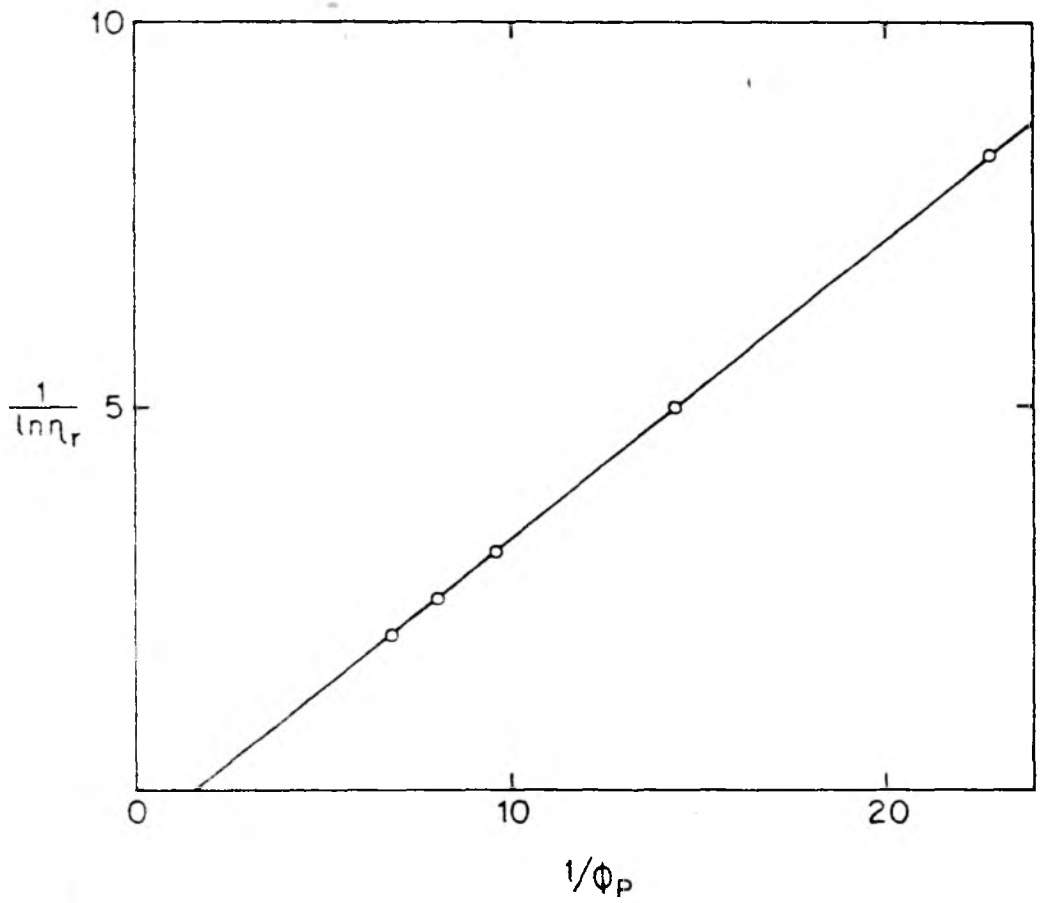

FIG. 3·1 : VISCOSITY - CONCENTRATION RELATIONSHIP FOR RANDOM COIL THYROGLOBULIN SOLUTIONS (REF. 6)

hydrochloride in the high concentration range have been reported (8). The data for α-casein are shown in Fig. 3.2. The AFVS plot consists of three intersecting straight lines. The points of intersection correspond to transitions from dilute to semidilute and from semidilute to concentrated regimes. The value of B_η / f_s decreases with each transition, while the value of β/f_s increases, in accordance with trends predicted for random coil polymers. Similar behaviour is observed for the other three proteins, but in the case of ovalbumin and lysozyme there are too few data points in the concentrated regime to permit evaluation of the model parameters. Lefebvre (8) also interpreted his data by considering the effect of concentration on molecular domain volume, and observed two transitions. The verification provided in this work supports the application of the AFVS model to viscosity of random coil proteins.

It may be mentioned here that in the dilute concentration regime the predictions of the AFVS model closely follow the equations commonly used for correlation of viscosity data in this range, such as the Huggins equation,

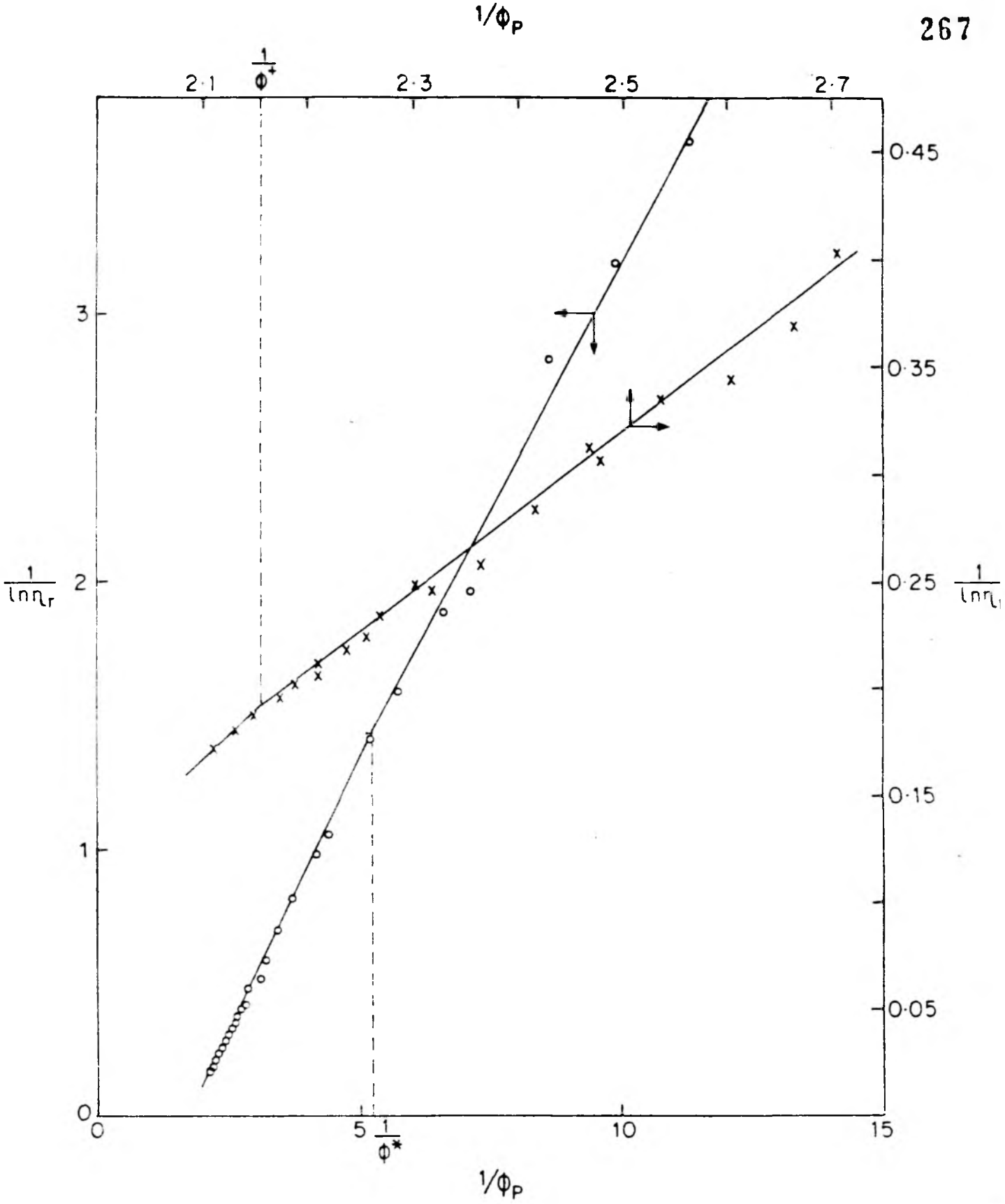

FIG.3·2: REGIMES OF VISCOSITY BEHAVIOUR OF RANDOM COIL α-CASEIN SOLUTIONS (REF. 8)

$$\eta_r = 1 + c[\eta] + k_H(c[\eta])^2 \qquad (3.3.1)$$

The Shultz-Blaschke equation,

$$\frac{\eta_{sp}}{c} = [\eta] + k_{sB}[\eta]\eta_{sp} \qquad (3.3.2)$$

and the Baker equation,

$$\eta_r = \left(1 + \frac{c[\eta]}{n}\right)^n \qquad (3.3.3)$$

(2) Proteins in the Native State

To determine the applicability of eqn. (3.2.1) for correlation of viscosity of proteins in their native state, the data provided by Tanford (9) for bovine serum albumin in aqueous solution at a pH of 5.0 (isoionic) and ionic strength 0.01 M were analysed (Fig. 3.3). The high value of the regression coefficient shows that the equation describes the data accurately. The value of B_η / f_s is very low compared to the corresponding value for the denatured protein. This is consistent with the compact conformation of the former. The value

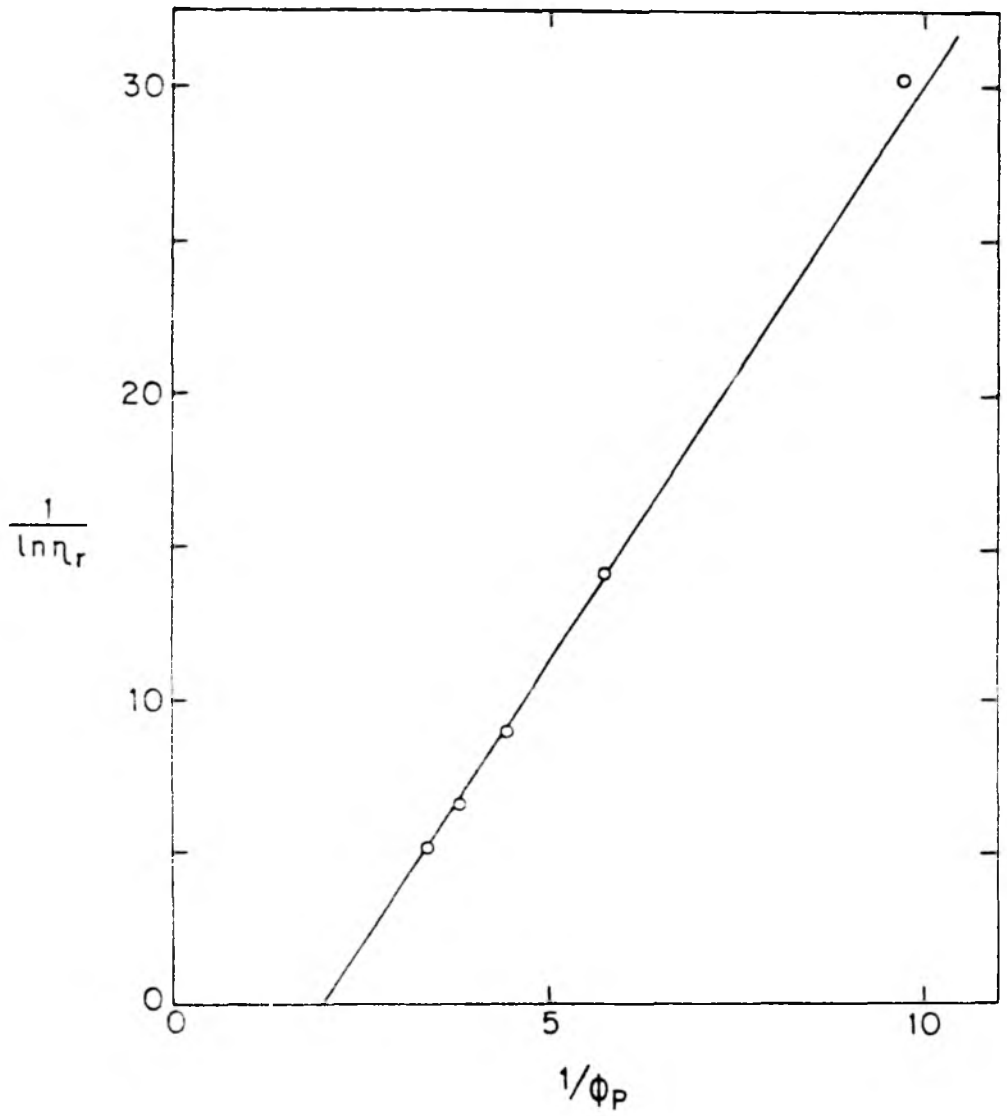

FIG. 3·3 : VISCOSITY - CONCENTRATION RELATIONSHIP FOR ISOIONIC SOLUTIONS OF BOVINE SERUM ALBUMIN (REF. 9)

of β/f_s is much higher, indicating that the second type of solvation, i.e. specific solvation at the charged amino acid residues perhaps plays a major role.

Lefebvre (8) has provided data for lysozyme in its native state at high concentrations. This is plotted in Fig. 3.4. Although there is considerable scatter in the data, a transition from the dilute to the semidilute regime is evident, with an accompanying decrease in B_η/f_s and an increase in β/f_s. Comparison with corresponding values for the same protein in its denatured state indicates that in the dilute range B_η/f_s in the native state has a much smaller value, while in the semidilute regime they are comparable. This indicates that the random coil protein has an extended conformation in the dilute regime but in the semidilute regime it contracts to a conformation only slightly more extended than that of the native protein. The values of β/f_s for the native protein are much higher in both concentration regimes.

Unfortunately there is a lack of an extensive data base on the viscosity of proteins in the high

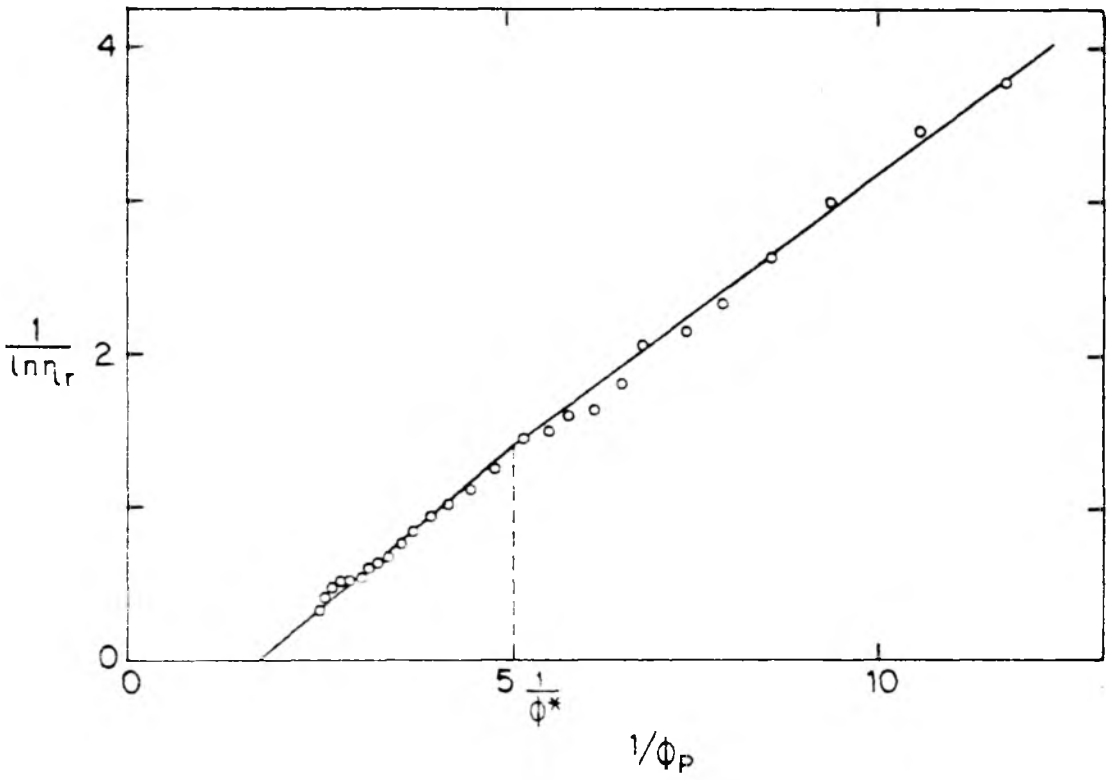

FIG.3·4 : REGIMES OF VISCOSITY BEHAVIOUR OF SOLUTIONS OF LYSOZYME IN ITS NATIVE STATE (REF. 8)

concentration range. We therefore have to conclude that although the validity of the theoretical concepts inherent in the derivation of eqn. (3.2.1) is doubtful in this case, the equation is nevertheless useful for correlative purposes.

3.3.2 Effect of pH

As described earlier, the degree of ionisation and hence the conformation of protein molecules is a function of the pH. Many proteins undergo denaturation at a characteristic pH. The parameter B_η can serve as a measur of change in the size due to denaturation or due to a change in pH, while β serves as a measure of change in the extent to which counterions cluster around charged groups or the orientation of water molecules. Thus B_η represents the geometric contribution and β the electroviscous contribution to the solution viscosity. A similar separation of the two effects was achieved in the case of aqueous polymer solutions (section 2.5) and polymer latices (chapter V).

An example is shown in Fig. 3.5 which represents data for solutions of ribonuclease at an ionic strength of 0.02 M and pH values of 4.0 and 5.9 (10). With the

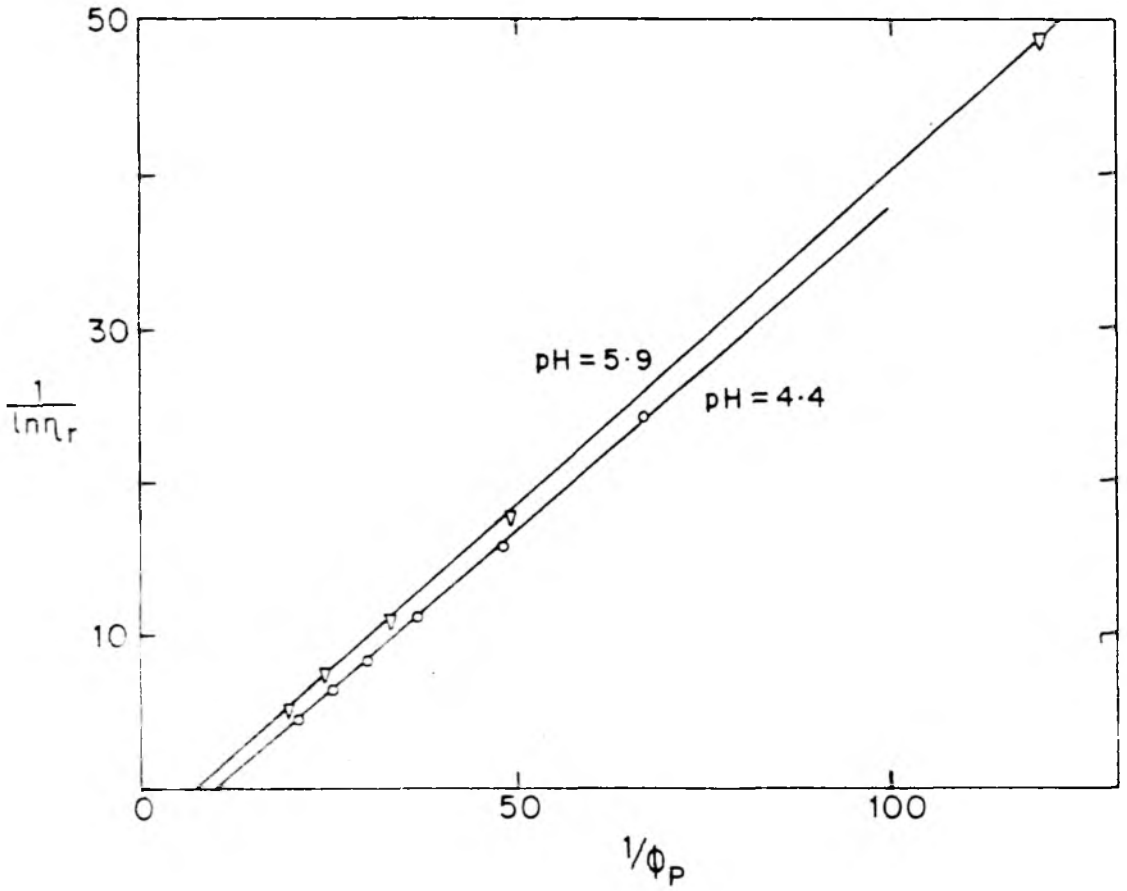

FIG.3·5: AFVS PLOTS OF VISCOSITY OF RIBONUCLEASE SOLUTIONS (REF. 10)

increase in pH, $\frac{B_\eta}{f_s}$ changes from 0.2336 to 0.2777, i.e., an increase of 19%, while $\frac{\beta}{f_s}$ changes from 9.1115 to 6.6737, i.e., a decrease of 15%. An increase in B_η is expected to lead to an increase in viscosity, while a decrease in β is expected to lead to a decrease in viscosity. As the viscosity of the solution in this case decreases with an increase in pH, we may conclude that the electroviscous effect plays the predominant role in determining the solution viscosity.

3.3.3 Effect of Ionic Strength

Addition of ionic salts affects the viscosity by neutralising to some extent the charged groups of the molecule. This may result in a change in conformation and/or the degree of solvation due to the influence of the electroviscous contribution. The two effects will be reflected in the values of B_η and β, respectively. For example, the effect of ionic strength on the viscosity of histone solutions was studied by Jirgensons et al. (11). Histone is a basic, positively charged protein which has an extended conformation when no salts are present, but it assumes a more compact form in the presence

of salts. The increasing compactness of the molecule is reflected in the decreasing value of $\frac{B_\eta}{f_s}$. The value of $\frac{\beta}{f_s}$ increases with increasing amount of added anions, which is as should be expected.

Histone however, is a special case. In most cases the effect of increasing ionic strength is in the opposite direction. This is illustrated in Fig. 3.6 which shows the viscosity of bovine serum albumin at a pH of 5.0 and ionic strengths of 0.001 M and 0.002 M (3). The increased number of ions present causes an expansion of the protein molecules, as a result of which the effective size increases. This is accompanied by a decrease in the degree of solvation due to the shielding of charged groups by ions from the solvent molecules.

3.3.4 Molecular Weight-Concentration Superposition

It was shown in chapter II that ϕ_p is a useful scaling parameter which leads to a concentration-molecular weight superposition. A change in ϕ_p may be brought about by a change in either the concentration or the molecular weight. The AFVS plots for the molecular weight dependence of viscosity at different fixed concentration superimpose.

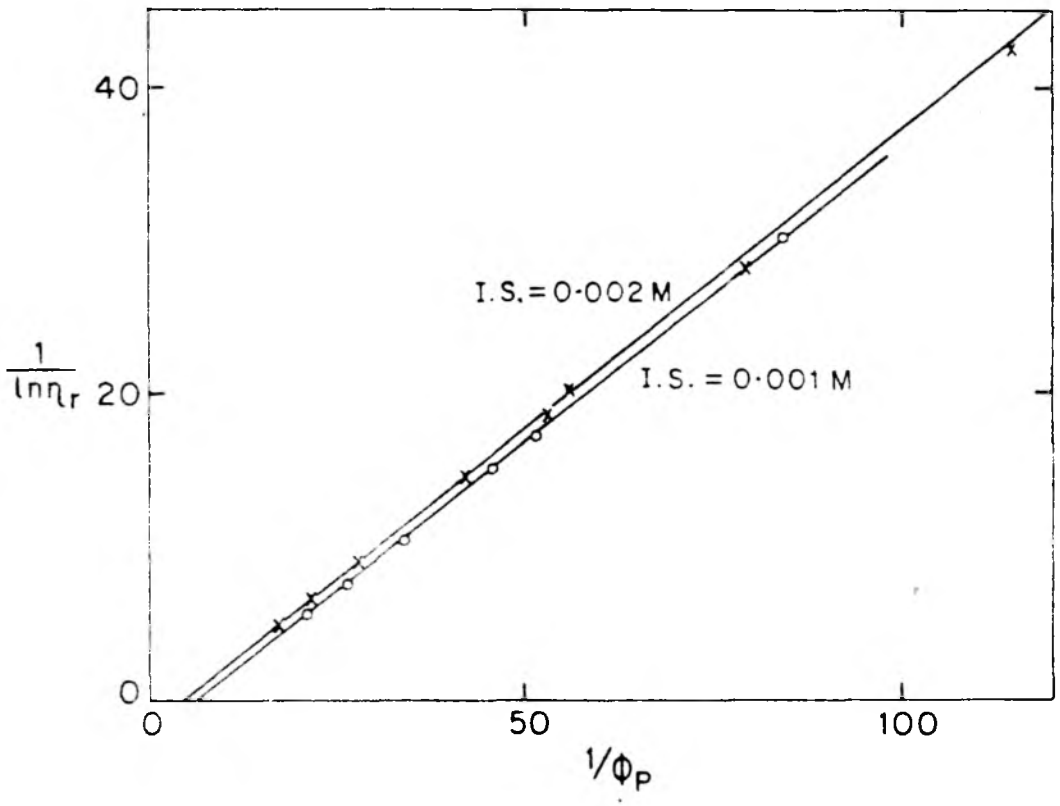

FIG.3·6 : AFVS PLOTS OF VISCOSITY OF BOVINE SERUM ALBUMIN SOLUTIONS (REF. 3)

There is only one set of data in the literature which may be used to examine as to whether a similar superposition can be achieved in the case of biopolymers. Owen et al. (12) measured the viscosities of solutions of pectinic acid in 0.155 M NaCl as a function of molecular weight at different fixed concentrations. Pectinic acid is a cellulose-like polymer for which the AFVS model may be assumed to be valid. The data lie in the dilute solution regime. The AFVS plots (Fig. 3.7) appear to superpose but the value of B_η / f_s increases slightly with increasing concentration while the value of β/f_s decreases. This implies that there are certain unknown factors governing the viscosity behaviour which have not been entirely taken care of by the model.

3.4 CONCLUSIONS

In the case of random coil proteins the AFVS model has been proved to be applicable upto high protein concentrations. In the case of globular proteins in their native state, the theoretical validity of the model is doubtful but it is still useful for the correlation of data. The effect of pH and ionic strength can be satisfactorily explained. The parameters B_η and β are measures of the effective

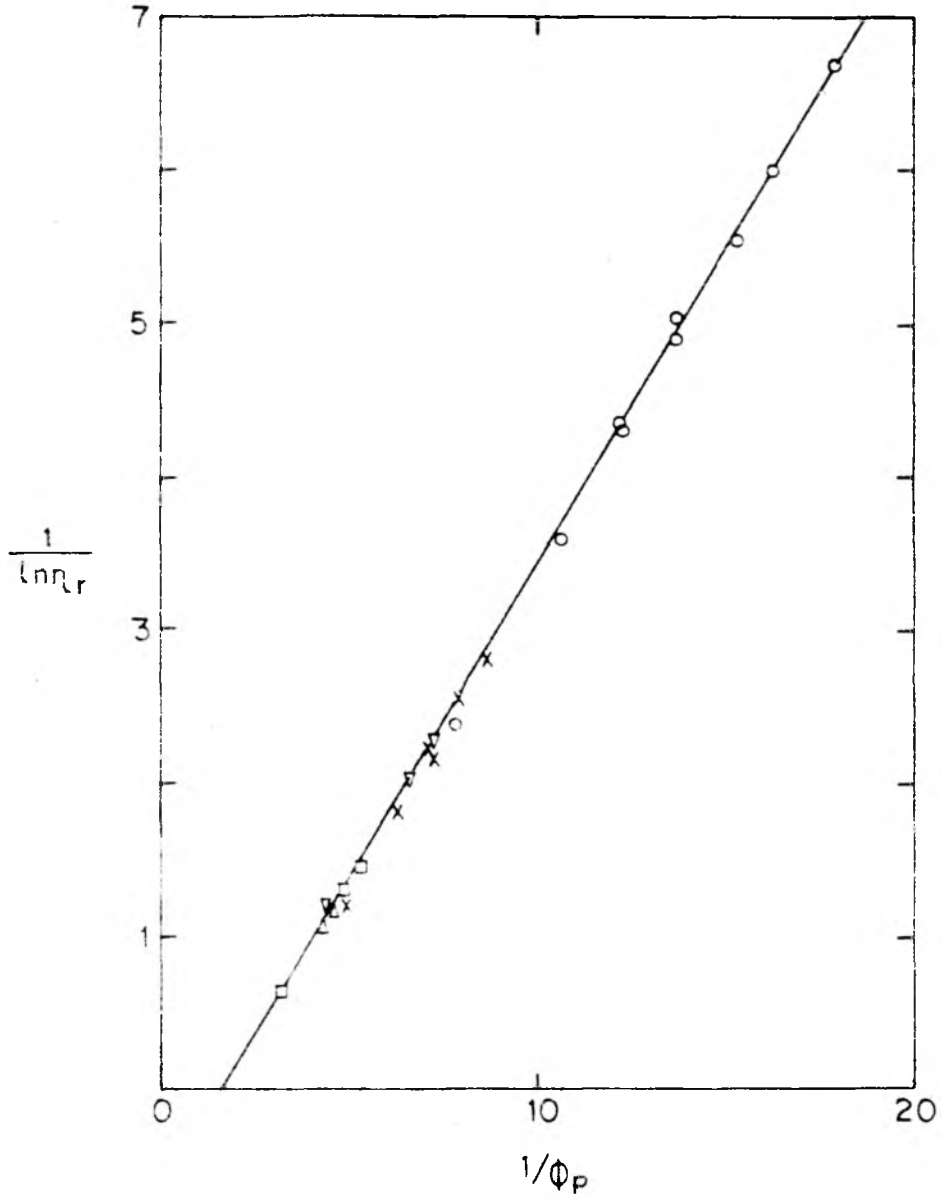

FIG.3·7 : MOLECULAR WEIGHT DEPENDENCE OF VISCOSITY OF PECTINIC ACID SOLUTIONS OF CONCENTRATION (o) 0·005 G/CC,(x) 0·0010 G/CC, (□) 0·0015 G/CC,(▽) 0·0020 G/CC, (△) 0·0030 G/CC (REF. 12)

hydrodynamic volume of the molecule and the degree of solvation, respectively. The limited availability of data makes it impossible to verify other features of the model for protein solutions, although there appears to be a strong indication that the molecular weight-concentration superposition is approximately valid.

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