

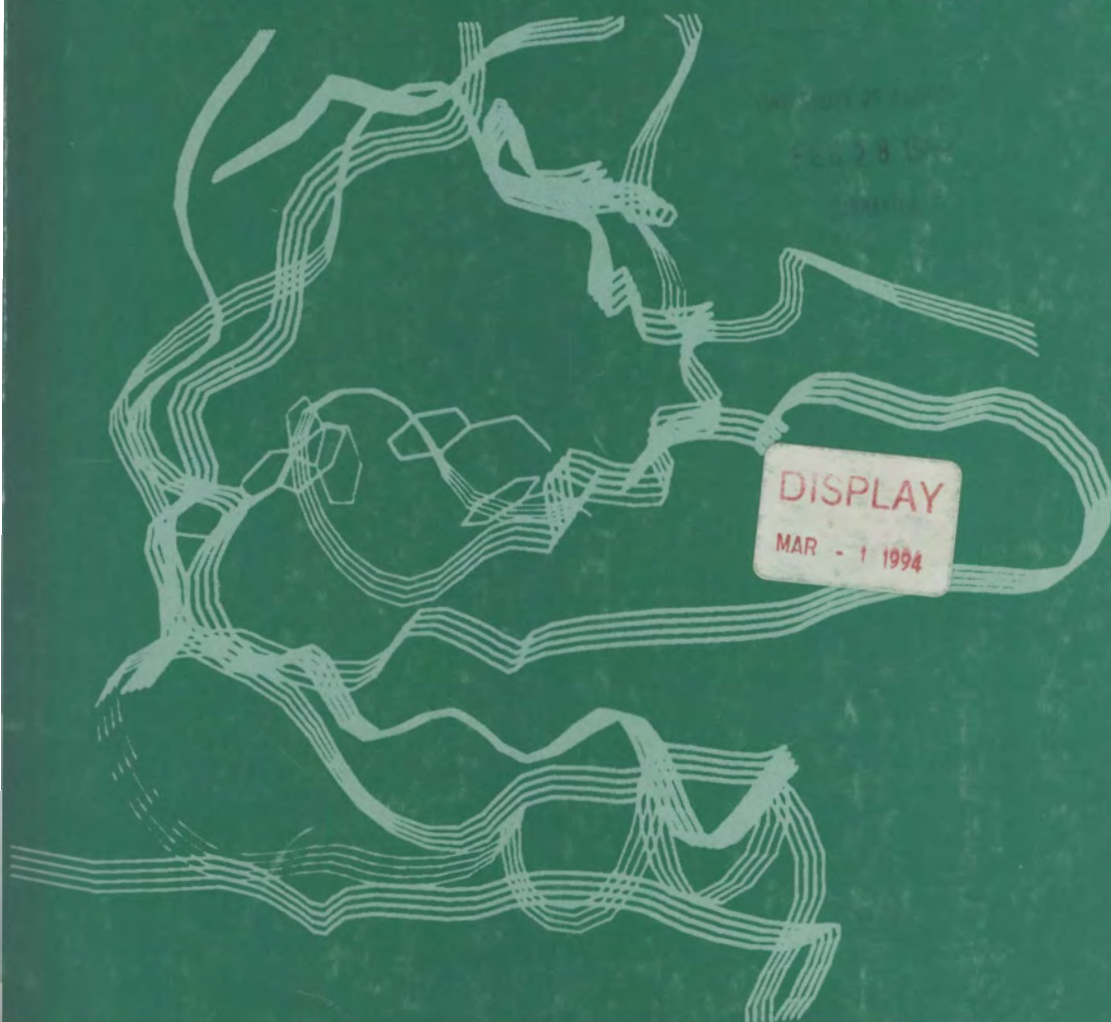
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STRUCTURE, FUNCTION AND INTERACTIONS



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Biological Macromolecules

STRUCTURE, FUNCTION AND INTERACTIONS

- 3 Close mutual contacts of the amino groups in DNA
J. Šponer and J. Kypr
- 7 Onset of the fully extended conformation in (α Me)Leu derivatives and short peptides
C. Toniolo, M. Pantano, F. Formaggio, M. Crisma, G. M. Bonora, A. Aubry, D. Bayeul, A. Dautant, W. H. J. Boesten, H. E. Schoemaker and J. Kamphuis
- 15 A study of enzymic degradation of a macromolecular substrate, poly[N^5 -(2-hydroxyethyl)-L-glutamine], by gel permeation chromatography and kinetic modelling
J. Pytela, J. Jakeš and F. Rypáček
- 21 Heparin binding to monodisperse plasma fibronectin induces aggregation without large-scale changes in conformation in solution
L. Vuillard, D. J. S. Hulmes, I. F. Purdom and A. Miller
- 27 Collagen organization in an oriented fibrous capsule
B. Brodsky and J. A. M. Ramshaw
- 31 Viscometric study of human, bovine, equine and ovine haemoglobin in aqueous solution
K. Monkos
- 37 Limited proteolysis of β -lactoglobulin using thermolysin. Effects of calcium on the outcome of proteolysis
E. Dufour, M. Dalgalarondo and T. Haertlé
- 43 Enzymatic degradation of chitins and partially deacetylated chitins
Y. Shigemasa, K. Saito, H. Sashiwa and H. Saimoto
- 51 Book review
- 52 Calendar
- 55 Guidelines for disk submission
- 56 Disk specification
- IBC Notes for authors



Int. J. Biol. Macromol., 1994 Volume 16 Number 1 1

Viscometric study of human, bovine, equine and ovine haemoglobin in aqueous solution

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This paper presents the results of viscosity determinations on aqueous solutions of several mammalian haemoglobins at an extremely wide range of concentrations. Rheological quantities such as the intrinsic viscosity and Huggins coefficient were calculated on the basis of the modified Mooney's formula. Using the dimensionless parameter $c[\eta]$, the existence of three characteristic ranges of concentrations was shown. By applying Lefebvre's formula for the relative viscosity in the semi-dilute regime, the Mark-Houwink exponent was evaluated.

Keywords: viscosity; Huggins coefficient; Mark-Houwink exponent

Viscometric measurements, as a convenient experimental tool, are still extensively used in many investigations of both synthetic polymers and biological macromolecules in solution¹⁻⁷. In biological systems, proteins (when present as solutions) are found in most cases at rather high concentrations. This is especially the case for haemoglobin (Hb), which is present in erythrocytes at the extremely high concentration of 5.4 mmol l^{-1} (Ref. 8). Our knowledge of the properties of proteins in concentrated solutions is still limited, and one of the reasons for this is that the choice of methods available is much more limited than in the case of dilute solutions. Only viscometry, which is a simple and convenient method, allows the study of molecular sizes and shapes as well as interactions of proteins in concentrated media. Viscometric measurements alone, or in conjunction with other methods such as dielectric or electron paramagnetic resonance spectroscopy, have been used in investigations of human haemoglobin in solutions⁹⁻¹⁴. However, as far as we know, very little attention has been paid to the viscometric study of other mammalian haemoglobins. In the present work, we report results on the viscosity of solutions of human, bovine, equine and ovine haemoglobin over a large range of concentrations extending from the dilute regime to concentrations higher than in physiological conditions. The dependence of viscosity on concentration in terms of the modified Mooney's equation is discussed. The Huggins coefficient and the exponent of the Mark-Houwink equation were determined for all investigated haemoglobins.

Experimental

Materials

Human blood was obtained from healthy, haemato-

logically normal adult volunteers via venepuncture into heparin. Bovine, equine and ovine blood samples were taken in the same way. The fresh erythrocytes were washed several times with 0.9% NaCl solution. Membrane-free Hb solutions were prepared by haemolysis with water, followed by high-speed centrifugation. The pH values of such prepared samples were as follows: human Hb, pH 7; bovine Hb, pH 7.3; ovine Hb, pH 7.4; equine Hb, pH 7.7. These values changed insignificantly during the dilution of the solutions. The samples were stored at 4°C until just prior to viscometry measurements, when they were warmed to 25°C.

Viscometry

Capillary viscosity measurements were conducted using an Ubbelohde microviscometer placed in a waterbath controlled thermostatically at $25 \pm 0.1^\circ\text{C}$. The same viscometer was used for all measurements and was mounted so that it always occupied precisely the same position in the bath. Flow times were recorded to within 0.1 s. Solutions were temperature-equilibrated and passed once through the capillary viscometer before any measurements were made. Five to ten flow-time measurements were made on each concentration. The relative viscosity η_r was measured, where $\eta_r = \eta/\eta_0$ and η and η_0 are the viscosities of the solution and the solvent, respectively.

Solution densities and haemoglobin concentrations were measured by weighing, as described previously⁷. The relative viscosities of the haemoglobin solutions were measured for concentrations from several g l^{-1} up to $\sim 525 \text{ g l}^{-1}$ for human Hb, $\sim 500 \text{ g l}^{-1}$ for equine Hb and ovine Hb, and $\sim 490 \text{ g l}^{-1}$ for bovine Hb. The results are shown in Figure 1.

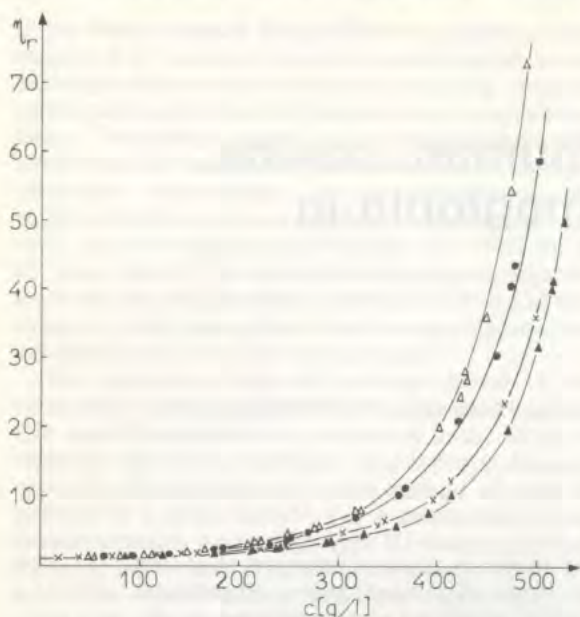


Figure 1 Plot of the relative viscosity η_r versus concentration c for human (▲), ovine (*), equine (●) and bovine haemoglobin (△); the curves show the fit obtained by using equation (3) with parameters A and B from Table 1

Results and discussion

Mooney's approximation

Despite substantial efforts¹⁵, a useful theory for the viscosity of moderately concentrated and concentrated solutions does not yet exist. Much effort has therefore been devoted to a search for empirical functional representations incorporating a wide concentration range^{12,16}. However, as has been shown in our earlier work¹⁷, in the case of aqueous solutions of globular proteins, the most useful functional form describing the dependence of relative viscosity on concentration is that of Mooney¹⁸:

$$\eta_r = \exp \left[\frac{S\phi}{1 - K\phi} \right] \quad (1)$$

where ϕ is the volume fraction of the dissolved particles, S denotes the shape parameter and K is a self-crowding factor. The volume fraction $\phi = N_A Vc/M$ where N_A , V and M are Avogadro's number, the volume of one dissolved particle and the molecular weight, respectively. The solute concentration c is in g l^{-1} . In his original work¹⁸, Mooney obtained equation (1) for spherical particles for which $S = 2.5$, so that, in the limit $\phi \rightarrow 0$, the equation yields the expression developed by Einstein: $\eta_r = 1 + 2.5\phi$. In the case of particles of arbitrary shape, S should exceed 2.5.

It is known that the volume of hydrodynamic particles may include a shell of water of hydration. Because the shell may change with concentration, it is difficult to evaluate accurately the value of ϕ as a function of protein concentration. This problem was circumvented by Ross and Minton⁹. They generalized Mooney's equation to the form:

$$\eta_r = \exp \left[\frac{[\eta]c}{1 - \frac{K}{S}[\eta]c} \right] \quad (2)$$

where

$$[\eta] = \lim_{c \rightarrow 0} \frac{\eta_{sp}}{c}$$

is the intrinsic viscosity and $\eta_{sp} = \eta_r - 1$ is the specific viscosity. There is only one adjustable parameter (K/S) in equation (2). For two sets of data for human haemoglobin, the best fit of the above formula to the experimental points was obtained for $K/S = 0.4$ and 0.42 . However, as seen in Figure 1 in Ref. 9, the fit is not the best one, especially in the moderately concentrated region.

The problem can be treated in another way. The volume fraction ϕ can be rewritten as $\phi = \alpha c$, where $\alpha = N_A V/M$, and then equation (1) takes the form:

$$\eta_r = \exp \left[\frac{Ac}{1 - Bc} \right] \quad (3)$$

where $A = \alpha S$ and $B = \alpha K$ are two adjustable parameters and the ratio $B/A = K/S$. Mooney's relation given in the above form has two merits: (i) it is not necessary to know the intrinsic viscosity, and (ii) by fitting of the two parameters A and B , the equation gives good approximation to experimental values over the whole range of concentrations and the ratio K/S can be obtained as well. Mooney's equation has been fitted in this way to the experimental values for all haemoglobin solutions investigated. As seen in Figure 1, a good fit over the whole range of concentrations was obtained. The adjustable parameters for all samples are shown in Table 1. The value of $K/S = 0.432$ obtained for human haemoglobin is in good agreement with that of Ross and Minton. However, the most interesting parameters are the absolute values of S and K in equation (1). Some indications about these parameters can be obtained in the following way. As is known from crystallographic studies, human haemoglobin is a spheroid with main axes $64 \times 55 \times 50 \text{ \AA}$ ¹⁹ and molecular weight 68 000. Let us suppose that there is no hydration shell around the haemoglobin molecules. In this case, the volume fraction ϕ can be calculated for all concentrations, and equation (1) can be fitted to the experimental points with two parameters S and K . Such a procedure gives $S = 3.45$ and $K = 1.491$ for our experimental data. Tanford²⁰ has calculated that the value of S for human haemoglobin should lie between 2.5 and 4.8. This allows indirect calculation of the self-crowding factor K , using the experimental values of K/S . The value of $K/S = 0.4$ obtained by Ross and Minton⁹ gives, in this case, a value of K in the range between 1 and 1.92. For our value of $K/S = 0.432$, K is in the range of 1.08 to 2.07. Our values

Table 1 Parameters of the haemoglobin samples obtained from the fit of Mooney's relation to the experimental points (Figure 1) and from equations (6) and (7)

	Haemoglobin			
	Human	Bovine	Equine	Ovine
$A \text{ (ml g}^{-1}\text{)}$	2.77	4.37	4.22	3.4
$B \text{ (ml g}^{-1}\text{)}$	1.2	1.03	0.96	1.06
$\frac{B}{A} = \frac{K}{S}$	0.432	0.236	0.228	0.312
k_1	0.932	0.736	0.728	0.812
k_2	0.786	0.458	0.447	0.576
k_2/k_1^2	0.904	0.846	0.843	0.874

of S and K lie nearly in the middle of these ranges and they probably are very close to true values for human haemoglobin. However, such evaluation is only possible in the case of molecules of known sizes. It is worth noting that Tanford²⁰, using independent measures of the amount of water of hydration, has also estimated that the most probable value of S in this case lies between 3.7 and 3.9. Because the ratio of K/S should not depend on the amount of water of hydration, one can use it to calculate K . For $K/S = 0.4$, it gives K in the range of 1.48 to 1.56, and for $K/S = 0.432$, K is in the range of 1.598 to 1.68.

As seen from Table 1, substantial differences exist in the values of K/S for the investigated samples. This indicates that different mammalian haemoglobins do not have the same shape in solution and/or that they interact with the solvent in a somewhat different manner.

Intrinsic viscosity and the Huggins coefficient

At low concentrations, the relation between the solution viscosity and the concentration may be expressed by the polynomial¹⁶:

$$\frac{\eta_{sp}}{c} = [\eta] + k_1[\eta]^2c + k_2[\eta]^3c^2 + \dots \quad (4)$$

where the dimensionless parameter k_1 is the Huggins coefficient. The simplest procedure for treating viscosity data consists of plotting the η_{sp}/c against concentration, extrapolating it to the intercept (equal to $[\eta]$) and obtaining the coefficient k_1 from the corresponding slope. However, as was pointed out in Ref. 16, even if $\eta_{sp} < 0.7$, the concentration dependence of η_{sp}/c is curved, so that linear extrapolation gives a serious error in $[\eta]$ and k_1 .

The problem can be solved for solutions for which the conditions of Mooney's formula are fulfilled. Mooney's equations (1) or (3) can be expanded in the power series of concentration. Limiting to the second-order term, an identical expansion to that in equation (4) can be obtained from:

$$[\eta] = \alpha S = A \quad (5)$$

$$k_1 = \frac{1}{2} \left(2 \frac{K}{S} + 1 \right) \quad (6)$$

and

$$k_2 = \frac{1}{6} \left(6 \frac{K^2}{S^2} + 6 \frac{K}{S} + 1 \right) \quad (7)$$

The intrinsic viscosity and the Huggins coefficient obtained on the basis of equations (5) and (6), for all our haemoglobin samples, are shown in Table 1. The results of theoretical calculations for rigid, non-interpenetrating spheres have given a range of numerical values of k_1 ¹⁶. It seems that our results for equine, bovine and ovine haemoglobin are quite consistent with results of Birnbaum²¹ ($k_1 = 0.76$) and with the precise results obtained for the Gaussian random coil chain by Freed and Edwards²² ($k_1 = 0.7574$). Surprisingly enough, the Huggins coefficient value for human haemoglobin is close to the value $k_1 = 0.894$ obtained by Peterson and Fixman²³ in a model of penetrable spheres.

There are no theoretical estimations of the second coefficient k_2 in equation (4). However, the theory²⁴ predicts that, for rigid, non-interpenetrating spheres, the ratio k_2/k_1^2 should equal 1. The values of k_2 calculated on the basis of equation (7), as well as the ratios k_2/k_1^2 ,

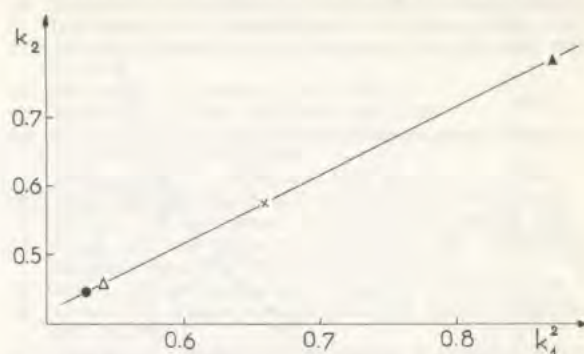


Figure 2 Correlation of coefficients k_1 and k_2 . Experimental data: human (▲), ovine (×), equine (●) and bovine haemoglobin (△); the straight line is plotted according to equation (8)

are given in Table 1. For all investigated haemoglobins, $k_2/k_1^2 \neq 1$. However, as is shown in Figure 2, the plot of k_2 versus k_1^2 is linear and the following analytical relation is fulfilled (with correlation coefficient 0.999):

$$k_2 = k_1^2 - 0.0834 \quad (8)$$

It is worth noting that Maron and Reznik²⁵, on the basis of experiments with polystyrene and poly(methylmethacrylate), obtained a similar relation with a numerical value of 0.09. This suggests that equation (8) has quite a general character and is correct for different sorts of molecules.

Three ranges of concentrations and determination of the Mark-Houwink exponent

The usual method of generalization of experimental results for different polymer systems consists of using reduced variables. In the case of the viscosity-concentration relation, this parameter is a dimensionless quantity $[\eta]c^{26}$. The dependence of the specific viscosity on $[\eta]c$ in a log-log plot exhibits classical behaviour for all our samples, with transitions from dilute to semi-dilute solution at concentration c^* , and from semi-dilute to concentrated solution at concentration c^{**} . Such behaviour has been observed for cellulose derivatives²⁷, citrus pectins²⁸ and some globular proteins in random coil conformation²⁹. In Figure 3, the master curve for bovine haemoglobin is shown. The master curves have the same form for the other haemoglobins. The parameters describing the curves are shown in Table 2. The boundary concentrations c^* and c^{**} are nearly superimposed, especially for equine, bovine and ovine haemoglobins.

In the dilute region ($c[\eta] < c^*[\eta]$), the molecular dimension is not perturbed by the other molecules and the average hydrodynamic volume of the molecule is the same as for infinite dilution. As is seen in Table 1, the slopes for all investigated samples are nearly identical in this range. It is worth noting that the slopes in the dilute domain are in the range of 1.1–1.4 for quite different sorts of molecules^{1,27,28}.

As was shown by Lefebvre²⁹, in the semi-dilute region, the following equation for the relative viscosity is fulfilled:

$$\ln \eta_r = 2a[\eta]c^* \left(\frac{c}{c^*} \right)^{1/2a} - (2a - 1)c^*[\eta] \quad (9)$$

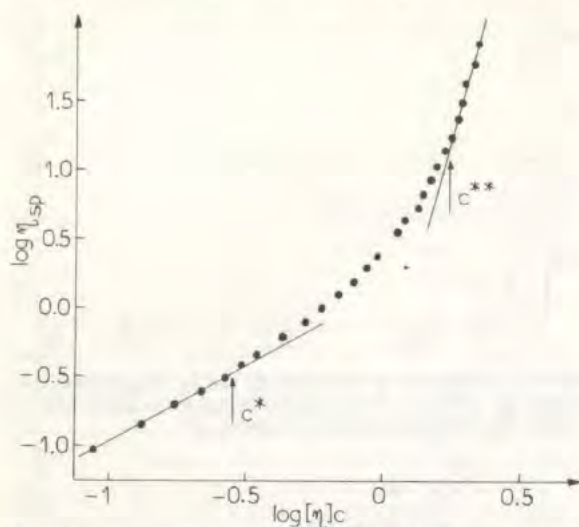


Figure 3 Specific viscosity as a function of $c[\eta]$ in a log-log plot for bovine haemoglobin; straight lines show different slopes in dilute ($c < c^*$) and concentrated ($c > c^{**}$) regions

where a is the Mark-Houwink exponent. Figure 4 shows the experimental points for bovine haemoglobin and the curve resulting from the fit, taking c^* and a as adjustable parameters. The values of c^* obtained by this procedure are in good agreement with the values determined from the master curve of Figure 3. The values of a (Table 2) are nearly the same, except for human haemoglobin. The Mark-Houwink exponent for flexible polymers is in the range of 0.5–1 (Ref. 30). However, $a = 0$ in the case of hard spherical particles, and $a = 1.7$ for hard long rods. The Mark-Houwink exponent values listed in Table 2 indicate that all haemoglobins studied here behave as hard quasi-spherical particles, in agreement with the model proposed for human haemoglobin by Ross and Minton^{9,13,31}. It is important to add that the Lefebvre equation was originally applied to zero shear rate data. In our case, for concentrations close to c^{**} ,

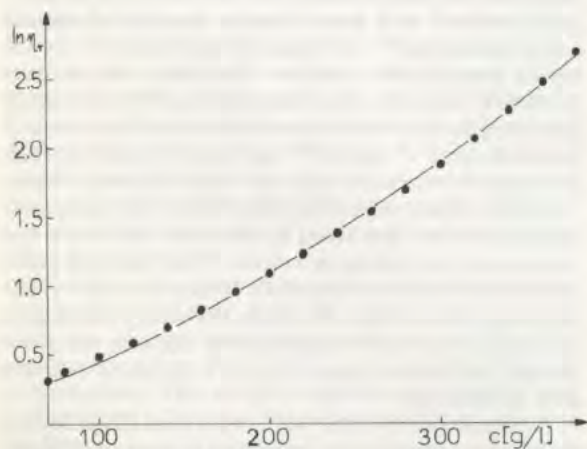


Figure 4 Plot of the relative viscosity versus concentration in a log-normal plot in a semi-dilute region. (●) experimental points for bovine haemoglobin; the curves show the fit obtained by using equation (9)

Table 2 Parameters of the haemoglobin samples obtained from the fit of the curves in Figures 3 and 4 and from equation (9)

	Haemoglobin			
	Human	Bovine	Equine	Ovine
a	0.3	0.338	0.348	0.333
c^* (g l^{-1})	76.3	66.5	64.9	67.2
c^{**} (g l^{-1})	394	375	376	375
c^* $[\eta]$	0.21	0.29	0.27	0.23
c^{**} $[\eta]$	1.09	1.64	1.59	1.28
<i>Slopes</i>				
$c < c^*$	1.1	1.13	1.12	1.1
$c > c^{**}$	7.57	7.02	6.47	6.05

the shear rate was about 100 s^{-1} . However, as was quite recently shown by Müller *et al.*¹⁴ for shear rates ranges from 1 to 200 s^{-1} , human haemoglobin solutions exhibit Newtonian behaviour up to a concentration of at least 450 g l^{-1} . This indicates that application of the Lefebvre equation in our case is justified and that the Mark-Houwink exponent has its usual meaning.

In the concentrated region ($c[\eta] > c^{**}[\eta]$), the effects of entanglements become important. As was shown by Axelos *et al.*²⁸ for citrus pectins, which are relatively flexible polymers, the slope in this region is 3.4. A higher value (about 5) was obtained by Castelain *et al.*²⁷ for a non-rigid molecule of hydroxyethylcellulose. The values listed in Table 2 suggest that the highest value of slope in this region occurs for stiff molecules. This is in agreement with the results of Ref. 32, where the authors showed that the slope should be approximately 8 for stiff chained molecules. It is interesting to note that the second critical concentration c^{**} for all investigated haemoglobins is only slightly higher than the concentration of the haemoglobin in erythrocytes, which is about 5.4 mmol l^{-1} or 367 g l^{-1} . This may explain why the concentration of haemoglobin in erythrocytes is not higher, i.e. the haemoglobin concentration achieves the highest value at which entanglements are not yet present. In other words, haemoglobin molecules in erythrocytes achieve the highest concentration at which they can move relatively freely with minimal frictional interaction.

Conclusions

The viscosity of mammalian haemoglobin solutions over a wide range of concentrations at pH values near the isoelectric point may be quantitatively described by the modified Mooney's equation (equation (3)). On the basis of Mooney's asymptotic form for small concentrations, the intrinsic viscosity and the Huggins coefficient may be calculated. The Huggins coefficient k_1 and the second coefficient of expansion k_2 are connected by equation (8), which seems to have quite a general form. The values for the Mark-Houwink exponent confirm that all investigated haemoglobins behave as hard quasi-spherical particles. The values for the second critical concentration c^{**} suggest that mammalian haemoglobin concentration in erythrocytes achieves the optimum value. Despite the similarities, substantial differences exist between species, especially for K/S and k_1 values. This indicates that each mammalian haemoglobin in solution should be studied in detail separately.

References

- Hwang, J. and Kokini, J.L. *Carbohydr. Polym.* 1992, **19**, 41
- Hwang, J. and Kokini, J.L. *J. Texture Studies* 1991, **22**, 123
- Ansari, A., Jones, C.M., Henry, E.R., Hofrichter, J. and Eaton, W.A. *Science* 1992, **256**, 1796
- Wang, D. and Mauritz, K.A. *J. Am. Chem. Soc.* 1992, **114**, 6785
- Hayakawa, E., Furuya, K., Kuroda, T., Moriyama, M. and Kondo, A. *Chem. Pharm. Bull.* 1991, **39**, 1282
- Guaita, M. and Chiantore, O. *J. Liquid Chromatogr.* 1993, **16**, 633
- Monkos, K. and Turczynski, B. *Int. J. Biol. Macromol.* 1991, **13**, 341
- Jung, F. *Naturwissenschaften* 1950, **37**, 254
- Ross, P.D. and Minton, P. *Biochem. Biophys. Res. Commun.* 1977, **76**, 971
- Monkos, K. and Turczynski, B. *Studia Biophysica* 1985, **107**, 231
- Ebert, B., Schwarz, D. and Lassmann, G. *Studia Biophysica* 1981, **82**, 105
- Endre, Z.H. and Kuchel, P.W. *Biophys. Chem.* 1986, **24**, 337
- Minton, A.P. and Ross, P.D. *J. Phys. Chem.* 1978, **82**, 1934
- Müller, G.H., Schmid-Schönbein, H. and Meiselman, H.J. *Biorheology* 1992, **29**, 203
- Freed, K.F. and Edwards, S.F. *J. Chem. Phys.* 1974, **61**, 3626
- Bohdanecky, M. and Kovar, J. in 'Viscosity of Polymer Solutions' (Ed. Jenkins, D.A.), Elsevier Scientific, Amsterdam, 1982, p 168
- Monkos, K., Monkos, J. and Turczynski, B. *Post. Fiz. Med.* 1988, **23**, 215
- Mooney, M. *J. Colloid Sci.* 1951, **6**, 162
- Huisman, T.H.J. *Adv. Clin. Chem.* 1963, **6**, 236
- Tanford, C. in 'Physical Chemistry of Macromolecules', Wiley and Sons, New York, 1961, p 394
- Birnbaum, H.C. *J. Chem. Phys.* 1952, **20**, 571
- Freed, K.F. and Edwards, S.F. *J. Chem. Phys.* 1975, **62**, 4032
- Peterson, J.M. and Fixman, M. *J. Chem. Phys.* 1963, **39**, 2516
- Frisch, L.H. and Simha, R. in 'Rheology' (Ed. Eirich, F.R.), Academic Press, New York, 1956, Vol. 1, p 525
- Maron, S.H. and Reznik, R.B. *J. Polym. Sci. A-2* 1969, **7**, 309
- Dreval, V.E., Malkin, A.Ya. and Botvinnik, G.O. *J. Polym. Sci.* 1973, **11**, 1055
- Castelain, C., Doublier, J.L. and Lefebvre, J. *Carbohydr. Polym.* 1987, **7**, 1
- Axelos, M.A.V., Thibault, J.F. and Lefebvre, J. *Int. J. Biol. Macromol.* 1989, **11**, 186
- Lefebvre, J. *Rheol. Acta* 1982, **21**, 620
- Wolkenshtein, M.W. in 'Molekularnaja Biofizika', Nauka, Moscow, 1975, p 149
- Ross, P.D. and Minton, A.P. *J. Mol. Biol.* 1977, **112**, 437
- Baird, D.G. and Ballman, R.L. *J. Rheol.* 1979, **23**, 505