# HARD QUASISPHERICAL MODEL FOR THE VISCOSITY OF HEMOGLOBIN SOLUTIONS

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Summary: The viscosity of concentrated hemoglobin solutions of moderate ionic strength at pH values near the isoelectric point may be quantitatively described by the generalized form of a relation commonly applied to suspensions of hard spherical particles. This finding is consistent with the hard quasispherical model previously proposed to account for the thermodynamic properties of concentrated hemoglobin solutions under comparable conditions (1).

We have recently shown that under conditions of moderate ionic strength, the osmotic pressure and sedimentation equilibrium of concentrated solutions of hemoglobin at pH values near the isoelectric point may be quantitatively accounted for by a simple model, in which the hemoglobin molecules are represented by hard quasispherical particles with no long-range interparticle interactions (1). It was found that the model particles which best account for the experimental data closely resemble the hemoglobin molecule in their size, shape, and mass. This result suggests that the model may provide a realistic picture of the hemoglobin solution at low resolution. If this is in fact the case, one would expect the hard quasispherical particle model to furnish a conceptual basis for the understanding of the hydrodynamic as well as the thermodynamic properties of concentrated hemoglobin solutions.

The viscosity $\eta$ of concentrated suspensions of hard





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spherical particles is commonly represented by the semiempirical equation of Mooney (2):

$$\eta = \eta_o \exp\left[\frac{2.5\phi}{1 - k\phi}\right] \tag{1}$$

where $\eta_o$ is the viscosity of the suspending medium, $\phi$ is the volume fraction of the suspended particles, and k is a "crowding factor", estimated by Mooney to be between 1.35 and 1.91 for hard spheres. Experimental measurements on suspensions of spherical latex particles in salt solution yield a value of 1.56 for k (3).

Equation 1 is strictly applicable to suspensions of spherical particles, and must be generalized to allow for particles which deviate from sphericity. In the limit of infinite dilution, equation 1 reduces to the Einstein relation for hard spheres, which is generalized to the case of particles of arbitrary shape by replacement of the coefficient 2.5 by a parameter $\nu$, whose value exceeds 2.5 for non-spherical particles (4).

$$\lim_{\phi \to 0} \frac{\eta - \eta_o}{\eta_o} = \nu\phi \tag{2}$$

A second difficulty in applying equation 1 to protein solutions is that the value of $\phi$ cannot be accurately evaluated as a function of protein concentration. The volume of the hydrodynamic particle may include a shell of water of hydration which moves with the protein core. If the density of the hydration shell is equal to that of bulk water, the shell will not contribute to the specific volume of the protein, and the volume of the hydrodynamic particle will then exceed the specific volume of the protein by an undetermined amount (5). This problem may be circumvented

by use of the intrinsic viscosity, an experimentally measured property of the solution:

$$[\eta] \equiv \lim_{c \to 0} \frac{\eta - \eta_o}{\eta_o c} \tag{3}$$

where c is the weight concentration of the protein. Combining equations 2 and 3, we obtain

$$\nu \phi = [\eta] c \tag{4}$$

This relation strictly holds only in the limit of infinite dilution. However, it has been found experimentally that the specific volume of hemoglobin varies only slightly (<1%) on increasing the weight concentration to over 40 g/dl (6). If we assume that the volume of the hydration shell is similarly independent of protein concentration, then equation 4 will be valid independent of the value of c. The generalized Mooney equation which we use to analyze the viscosity of hemoglobin solutions is thus

$$\eta = \eta_o \exp\left[\frac{\nu\phi}{1 - k\phi}\right]$$

$$= \eta_o \exp\left[\frac{[\eta] c}{1-(k/\nu)\,[\eta] c}\right] \tag{5}$$

Measurements of the viscosity of concentrated solutions (stroma-free hemolysates) of oxyhemoglobins A, S, C, and mixtures of A and S have been reported by Charache et al (7), Ham et al (8) and Chien et al (9). These solutions were adjusted to osmolarities of 0.2-0.3 by the addition of Ringer's solution or Tris buffer as necessary. It was found that the concentration dependence of the viscosity is the same (to within experimental error) for solutions of hemoglobins A and S under the same conditions, and for solutions of hemoglobin C as well, so long as the abnormally

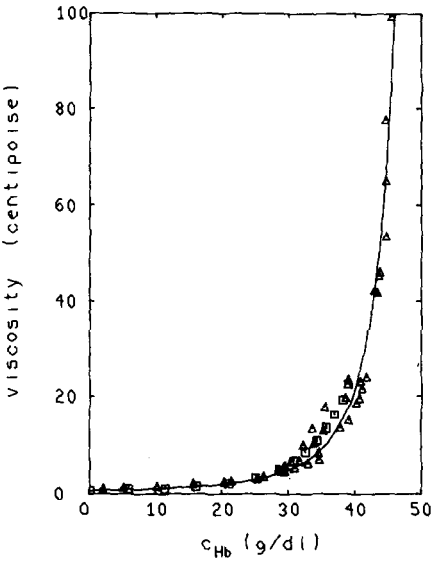


Figure 1. Solution viscosity as a function of hemoglobin concentration. Temperature 37 ° C, pH 7.0 ± 0.2. Curve is calculated using equation 5 with parameter values given in the text. Triangles, data of ref. 7; squares, data of ref. 9.

low solubility of hemoglobin C is not exceeded. The data of Charache et al (7) and Chien et al (9) are plotted in Figure 1. The data of Ham et al (8) are very similar to, but less extensive than, the data of the other two groups. It may be observed that there is a slight but significant difference between the results of Charache et al (7) and Chien et al (9) for normal hemoglobin, which may be due either to differences in the composition of the hemolysate or in the technique of measurement.

In order to reduce the number of independently variable parameters, the value of $\eta_o$ is fixed equal to 0.70 centipoise, which is the viscosity of comparable salt solutions at 37° C (10). Variations of ±0.05 in the value of $\eta_o$ were found to have only a very minor effect upon the results of curve-fitting described below. The value of [η]

was set equal to 0.036 dl/g (11). The value of $k/\nu$ was then allowed to vary in order to obtain a least-squares fit of equation 5 to the data of Charache et al (7) and Chien et al (9). It was found that a best fit was obtained to the combined sets of data for $k/\nu = 0.40$, and that a best fit to the data of Chien et al (9) alone was obtained for $k/\nu = 0.42$. The dependence of $\eta$ upon c calculated using equation 5 with $k/\nu = 0.40$ is plotted in Figure 1 together with the data. It may be seen that the agreement between the calculated value and experimental data is satisfactory, particularly so in view of the fact that the fit was obtained with only one independently variable parameter.

Tanford (11) has calculated that the value of $\nu$ for hemoglobin lies between 2.5 and 4.8, and using independent measures of the amount of water of hydration, has estimated that the most probable value of $\nu$ lies between 3.7 and 3.9. According to this estimate and the best fit value of $k/\nu$, the value of the "crowding factor" k lies between 1.48 and 1.62, which is close to the experimentally determined value characterizing suspensions of latex particles in salt solution (3).

In summary, we find that a semiempirical equation used to describe the viscosity of suspensions of hard particles may be used with minor modification to quantitatively describe the concentration dependence of viscosity of hemoglobin solutions over a wide range of concentration and viscosity. This result provides additional evidence that in solutions of moderate ionic strength at pH values near the isoelectric point, long range electrostatic interactions between hemoglobin molecules are essentially completely damped out, and that the concentration dependence of the thermodynamic and hydrodynamic properties is determined

essentially entirely by the mutual impenetrability of the hemoglobin molecules.

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