Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713926090

Rheological study of blood cells containing haemoglobin S. Physical interpretation of viscosity laws

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To cite this Article Dejardin, J. -L.(1991) 'Rheological study of blood cells containing haemoglobin S. Physical interpretation of viscosity laws', Liquid Crystals, 9: 4, 599 — 605
To link to this Article: DOI: 10.1080/02678299108033156
URL: http://dx.doi.org/10.1080/02678299108033156

LIQUID CRYSTALS, 1991, VOL. 9, NO. 4, 599–605

Rheological study of blood cells containing haemoglobin S
Physical interpretation of viscosity laws

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(*Received 21 May 1990; accepted 16 November 1990*)

Starting from experimental results given in the literature, changes in viscosity with haematocrit on suspensions of HbS erythrocytes or mixtures of normal HbA and HbS cells, when deoxygenated, are obtained. Laws of an exponential type are derived and the notion of the haematocrit constant playing an important part in the cell–cell interaction is pointed out. Its physical significance corresponds to a variation in the activation energy between the solution and the solvent just equalling the thermal energy.

1. Introduction

The main feature of haemoglobin S (HbS) with respect to normal haemoglobin (HbA) consists in the substitution of a glutamic acid residue by a valine at the sixth position of each β chain. This genetic abnormality gives rise to deformation of red cells (sickling) for generally low oxygen tensions. The oxygen tension is, effectively, one of the most important factors leading to aggregation of haemoglobin S. So, the deoxygenated form (deoxy HbS) is apt to polymerize. It should be noted that intracellular polymerization of HbS occurs at blood oxygen tensions being able to exceed zero and even at arterial oxygen tension, while sickling which corresponds to a shape change of the blood cells happens at much lower oxygen tensions. To explain polymerization, it is now admitted that deoxyhaemoglobin S molecules, after a nucleation process [1, 2], associate with each other, forming long polymers whose final alignment is analogous to the nematic phase of paracrystalline gels [3]. From a rheological viewpoint, it is therefore necessary to distinguish oxyhaemoglobin S composed essentially of unsickled cells from deoxyhaemoglobin S where the cells are deformed.

It is this aspect which is developed in the present work in the form of viscosity laws as functions of haematocrit, applied to suspensions of red cells and established from experimental results obtained by Murphy *et al.* [4]. The full detailed description of materials and methods may be found in their article, and so, we shall recall some indications about the experimental procedure useful to the consideration of the problem with which we deal. The viscosity measurements were relative to the whole population of erythrocytes and made at 37°C with a Wells–Brookfield cone-plane viscometer (model LVT 1/2). With such rheological data, we might object that reversibly sickled cells are not differentiated from permanent sickled cells, but our objective is to give a physical significance to the argument in the exponential describing the general relation between viscosity and haematocrit rather than obtaining appropriate functions for either type of cells which are exponential-type, as well.

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Viscosity–volume fraction relationships are derived keeping a constant value of shear rate equal to 5·8 s^{-1}. That particular value of 5·8 s^{-1} has been chosen in order to answer and illustrate our purpose, bearing in mind that the viscosity of blood decreases at increasing shear rates (non-newtonian character). Haematocrits of suspensions were determined in triplicate with 10 min centrifugation (11500 rpm) and read in a Microreader (Model 72, International Centrifuge Co.). Finally, we note that this paper gives the finishing touches to a report presented as an oral communication at the fifth European Conference on Clinical Haemorheology held in Bordeaux [5].

2. Effect of haematocrit on the viscosity coefficient

Models of blood viscosity, η, against haematocrit, H, have received considerable attention from numerous authors. Among them, we recall the work of Chien *et al.* [6], using a polynomial equation ($\ln \eta = \Sigma a_j H^j$), which is well verified on very concentrated suspensions of red cells and given shear rate. Quemada [7] has attempted a general review about standardization in clinical haemorheology by putting in opposition *models for description* to *models for knowledge*, the latter being best adapted to a physical understanding. Moreover, it is convenient to stress the fundamental features in blood rheology, such as the amount of plasmatic molecules as well as erythrocyte deformability and aggregation [8, 9]. On taking into account these preliminary remarks, the experimental conditions used by Murphy *et al.* [4] correspond to a mean shear rate (5·8 s^{-1}) where blood thixotropy prevails. Subsequently, according to the definition of the limiting viscosity b' defined by Stoltz [10], changes in the viscosity coefficient of a solution of normal red cells in serum (suspending fluid) as a function of haematocrit H are well described by an exponential law

$$\eta = \eta_0 \exp(\alpha H), \tag{1}$$

where η_0 is the serum viscosity and α is a typical constant of the suspension. Equation (1) has been tested on suspensions of oxygenated and deoxygenated HbS erythrocytes by looking for a linear regression between $\ln \eta$ and H, where H varies from 20 per cent to 60 per cent. Owing to few points which were available, interpolations were necessary for a statistical treatment of the experimental data, taking into account the general behaviour of the plot. Figure 1 shows, within some uncertainty due to this method, a close enough correlation between these two physical parameters. The main feature of both suspensions of HbS erythrocytes is shown by the slope of the regression straight line which is steeper for deoxyhaemoglobin S cells than for oxy-HbS cells. This last result can be well explained by the sickled and aggregative nature of deoxygenated HbS erythrocytes (steric hindrance increasing during polymerization) which involves important cell–cell interactions. Thus, the knowledge of the constant α seems useful to provide one with the criteria in estimating the degree of interaction for the molecular species. We can add that for normal red cells, equation (1) also applies and the plot of the viscosity against haematocrit is similar to that obtained with oxygenated HbS erythrocytes (see figure 1). Furthermore, it should be noted that the inverse of α has the dimensions of H. As the haematocrit H of the suspension specifies a relative volume of red cells in terms of percentage, it is possible to work with fluidity $\phi (\phi = 1/\eta)$ instead of viscosity, as already indicated in previous papers [11, 12]. On setting $H_0 = 1/\alpha$, the expression

$$\phi = \phi_0 \exp(-H/H_0). \tag{2}$$

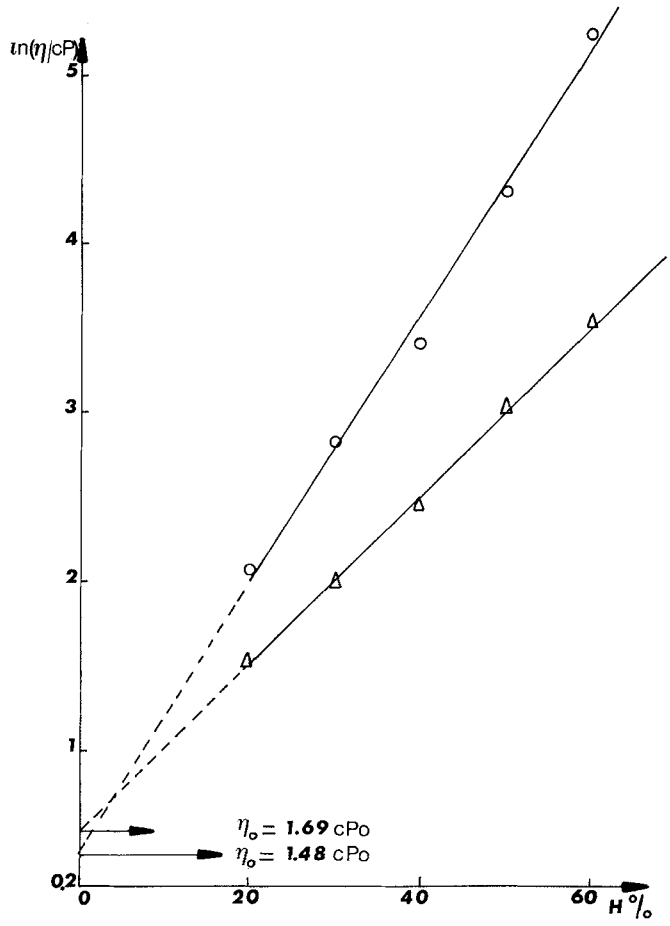

Figure 1. Plots of regression straight lines between $\ln\eta$ and H. Correlation coefficients: $r=0{\cdot}996$ for deoxy HbS; $r=0{\cdot}999$ for oxy HbS. Haematocrit constants: $H_0=12{\cdot}6$ per cent for deoxy HbS; $H_0=12{\cdot}6$ per cent for oxy HbS. Experimental conditions: $T=37°C$, pH = 7·4; Shear rate = $5{\cdot}8\,s^{-1}$. ○, deoxy HbS; △, oxy HbS.

for ϕ is deduced. The initial slope of $\phi(H)$ plots goes through H_0 and for $H=H_0$, the amplitude is divided by e ($\ln e=1$). Equation (2) is only written to show the analogy with equivalent formulae encountered in electricity. H_0 appears, therefore, as a haematocrit constant in the same way as time constants found in the discharge of capacitors, for instance. That physical significance is developed in further detail in § 5.

3. Viscosity law for deoxy-HbS–HbA mixture

Addition of normal HbA cells to a suspension of deoxygenated HbS erythrocytes implies the delay in fashioning critical nuclei, initiation of aggregation, but without modification of their size in the nucleation process (unaltered aggregating rate) [13, 14] and the decrease of the viscosity of that solution [4, 15]. For these reasons, it is interesting to study changes in viscosity of a deoxygenated A + S mixture as a function of the relative proportion of A or S erythrocytes, at a constant haematocrit. With the

aid of the experimental values obtained by Murphy *et al.* [4], these variations have been found to be in excellent agreement with the relationship

$$\ln \eta_{A+S} = ap + b, \tag{3}$$

where η_{A+S} is the viscosity coefficient of the A + S mixture, p is the percentage of S erythrocytes with respect to the whole number A + S, a and b are two real constants. A linear regression applied to equation (3) between $\ln \eta_{A+S}$ and p gives a good correlation coefficient of 0·999. Limiting conditions enable the determination of a and b. If η_A and η_S specify respectively the viscosities of suspensions of HbA and HbS red cells taken separately, we have

$$\text{for} \quad p=0, \quad b=\ln \eta_A \quad \text{and} \quad \text{for } p=1, \quad a=\ln(\eta_S/\eta_A).$$

Finally, equation (3) may be written as

$$\eta_{A+S} = \eta_A^{1-p}\eta_S^p. \tag{4}$$

This law, established for a deoxygenated mixture of A + S erythrocytes, is of the Eyring-type [16], which is well verified for liquid mixtures having molecular weights of the same order. However, a difference in the exponent of viscosities should be noted, representing here a number percentage instead of a mole fraction. Equation (4) is illustrated in figure 2, for $H = 40$ per cent. As haematocrits are very difficult to measure accurately for sickled cells, the haematocrit of the deoxygenated blood was determined previous to deoxygenation [4].

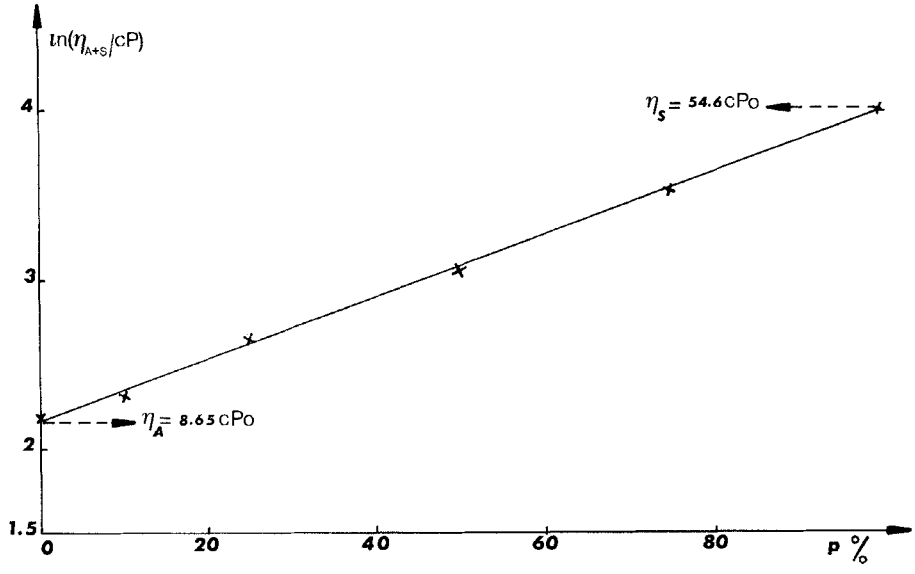

Figure 2. Plot of the regression straight line between $\ln \eta$ and p for a deoxygenated mixture of A and S erythrocytes. Experimental conditions: $T = 37°C$; pH = 7·4; shear rate = 5·8 s^{-1}; $H = 40$ per cent.

4. Influence of haematocrit on the viscosity of HbA–HbS mixture

From the results derived in previous sections, it is possible to seek the relationship between the viscosity of the A+S mixture against haematocrit, for a constant shear rate. According to equation (1),

$$\left.\begin{aligned} \eta_A &= \eta_0 \exp(\alpha_A H) \quad \text{for A suspension,} \\ \eta_S &= \eta_0 \exp(\alpha_S H) \quad \text{for S suspension.} \end{aligned}\right\} \tag{5}$$

By substitution of these relations in equation (4) we find that the relative viscosity η_r of the mixture (η_{A+S}/η_0) is

$$\eta_r = \exp(p'\alpha_A + p\alpha_S)H, \tag{6}$$

where $p' = 1 - p$ is related to the percentage of A erythrocytes. In passing, we note that in equation (6) the plasma viscosity η_0 in the A and S suspensions is identical to that of the A+S erythrocyte suspensions, but there is no fundamental change if it is not so.

On introducing haematocrit constants defined in the second paragraph, namely $H_{0A} = 1/\alpha_A$ and $H_{0S} = 1/\alpha_S$, everything occurs as if η_r was characterized by only one constant H'_0 such as

$$\eta_r = \exp(H/H'_0), \tag{7}$$

where H'_0 is an equivalent haematocrit constant given by

$$1/H'_0 = p'(1/H_{0A}) + p(1/H_{0S}). \tag{8}$$

From an analogous viewpoint, Equation (8) is to be compared to the association of shunt resistors; this is portrayed in figure 3. For an indicative information and after calculation, typical values extracted from figure 2 lead to $H_{0A} = 22{\cdot}8$ per cent, $H_{0S} = 12{\cdot}6$ per cent and $H'_0 = 16{\cdot}2$ per cent.

5. Physical interpretation of the haematocrit constant

The problem now is to have a physical understanding of haematocrit constants (H_0 or H'_0) from the viscosity equations. From classical fluid dynamics applied to large

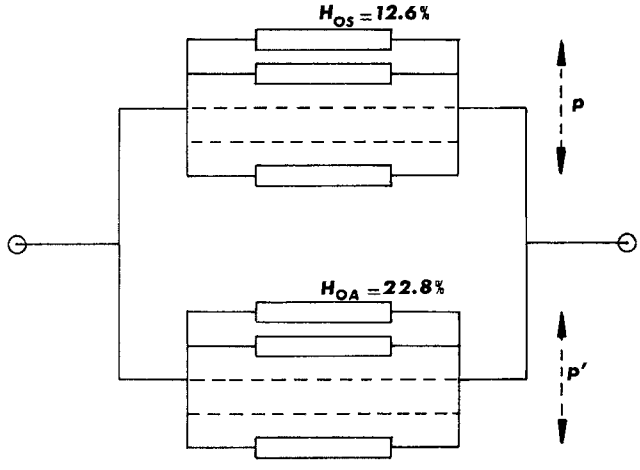

Figure 3. Analogous representation of the equivalent haematocrit contant by a double set of shunt resistors, illustrating equation (8). Values of H_{0S} and H_{0A} are extracted from figure 2, where $H = 40$ per cent.

particles in suspensions, we can assume that the neighbourhood of the red cell is a continuum because the molecules of the suspending medium (serum, in our case) are, in comparison, quite small. On considering such a medium acted on by an electric field of the impulse-type, the expression for the birefringence reduces to a single exponential [17], characterized by a relaxation time given by

$$\tau = V\eta/kT, \tag{9}$$

where V is the equivalent hydrodynamic volume of the suspended particle. Equation (9) is usually applied to a dilute suspension of monodisperse rigid particles. But, it was shown elsewhere [17] that it remains valid to a first and good approximation for a pure liquid or a concentrated suspension if the hydrodynamic volume is considered to be different from the real volume of the particle and generally smaller. This equation is particularly true for a particle possessing both optical and geometric symmetry about one of its axes, which is nearly the case for red cells, even when deformed [18]. It is customary to represent the variation of viscosity with temperature according to an Arrhenius equation [19], such that

$$\eta(T) = \mathrm{A}(T)\exp(W/kT), \tag{10}$$

where W designates the apparent activation energy of the viscous solution. $\mathrm{A}(T)$ is a characteristic function of the medium, slowly varying with T and thus, assimilated into a constant over a wide thermal range. Since the haematocrit is analogous to a concentration of erythrocytes in the whole blood, the thermodynamic model expressed by equation (10) may be applied, as previously described by Minton [20]. Taking a similar expression for the temperature dependence of the relaxation time [21], that is:

$$\tau = \tau_0 \exp(W/kT), \tag{11}$$

where τ_0 is the relaxation time characteristic of the solvent, it follows that

$$\eta(T) = (\tau_0 kT/V)\exp(W/kT). \tag{12}$$

On operating at a constant temperature, which is a condition fulfilled in the whole paper, it is possible therefore, to have the following formulation of the activation energy by equating equations (1) and (12):

$$W = W_0 + kT(H/H_0), \tag{13}$$

where $W_0 = kT\ln(\eta_0 V/kT)$ is the activation energy of the pure solvent, obtained for $H = 0$. Setting $H = H_0$ in the latter equation, we obtain

$$W - W_0 = \Delta W = kT. \tag{14}$$

So, the haematocrit constant H_0 (or H'_0) could physically represent a volume fraction of red cells for which the variation of the activation energy between the solute and the solvent should be equal to the thermal energy.

6. Conclusion

In spite of sickling of HbS erythrocytes, when deoxygenated, changes in viscosity, at a constant shear rate, against haematocrit, are of the same nature as for oxygenated S or A red cells (exponential pattern). An analogous conclusion may be applied to a mixture of S and A suspensions. The slope of plots $\ln\eta = f(H)$ is larger for the deoxygenated medium than for the oxygenated. The inverse of this slope, called here the haematocrit constant, is an intrinsic feature of the medium and seems to specify a limiting volume

fraction beyond which interaction energies arising from intermolecular contacts between haemoglobin tetramers become predominant with respect to thermal energy. This result is not surprising in character because activation energies which appear in all haemorheological phenomena are very sensitive to variations of physical parameters, such as temperature, pH and haematocrit.

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