RHEO-OPTICAL TRANSIENTS IN ERYTHROCYTE SUSPENSIONS

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When a dilute suspension of disc-shaped erythrocytes (normal or hardened, human or rabbit) is sheared, the optical transmittance oscillates. Random fluctuations (scintillations) are superimposed on the damping oscillations, but disappear instantaneously on cessation of shear, in contrast to the slower relaxation of the decreasing transmittance. Electro-optical experiments establish limiting orientations for erythrocytes corresponding to maximum (face-on to the optic axis) and minimum (edge-on) transmittance. This is the first observation of a general mode of behavior of orientation-dependent properties predicted for non-spherical particles of uniform shape, leading to simplified measurements of (bio)particle geometry and two-body interactions.

INTRODUCTION

Size distributions, conformational changes, and aggregation of bioparticles have been widely studied by the techniques of light transmission (1-7) and scattering (7-9). In the case of the anisometric blood cells, such as erythrocytes and blood platelets, these properties have generally been studied by tedious microscopy techniques of suspensions at rest (10,11) or sheared in tubes (12), and by light transmission studies of suspensions at rest (5-7) or sheared in viscometers (2-4,13) or aggregometers (suspensions are stirred) (7,9). Recent studies with the aggregometer of blood cells structure-function changes have pointed to the need for a similar study under defined shear conditions, especially to explain the observed stirring-dependent light transmittance changes and scintillations or oscillation amplitudes (1,7). In this communication, we report

the experimental observations of light transmission changes of dilute suspensions of erythrocytes under defined shear flow. Experimental results are interpreted in accord with statistical theory on orientation distributions of particles in sheared suspensions and with light scattering theory.

MATERIALS AND METHODS

The rheo-optical measurements were made by passing white light vertically through a microcouette (12) consisting of two parallel horizontal circular glass plates mounted, in lieu of a stage, on a microscope. The upper plate was rotated to shear the suspension contained in the space, usually 1.35 mm wide, between the plates. The velocity gradient $G = 2\pi Rf/h$ (f is the rotational frequency of the upper plate, R the distance of the transmitted light beam from the axis of rotation and h the gap) varied between 1 and 10 sec^{-1}. The transmitted light beam emerging from the upper plate passed through an 18x objective to a photodetector mounted on the ocular lens and connected to a power meter (Coherent Radiation Laboratories Model 212) whose output was displayed on an oscilloscope. The microscope was focussed between the plates. The diameter of the light beam sensed by the photometer was 1.5 mm.

Human and rabbit blood was collected and processed for cells as previously described (1), except that the residue from the platelet-rich plasma was spun at 300xg to yield loosely-packed erythrocytes, which were hardened by mixing with four volumes of fresh glutaraldehyde (1.3% in Tyrodes (1), v/v) at 37°C, incubating at room temperature for one-half hour, followed by washings in Tyrodes solution. Microscopic analyses indicated no significant changes in geometry (7). Spherical hardened erythrocytes were prepared by mixing sodium lauryl sulfate (0.1 v. of 1% SLS in Tyrodes) with packed erythrocytes (0.9 v.) and hardening the sheared erythrocytes as above. To reduce sedimentation, the cells were suspended in 2 v. glycerol/1 v. water solution. Particle concentrations N were

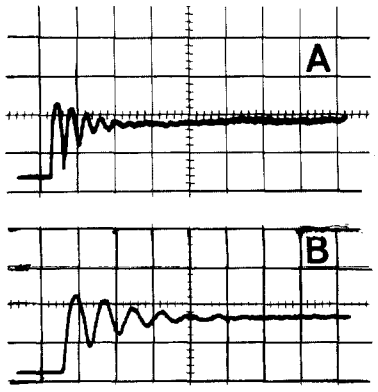

Fig. 1: Oscilloscope traces for hardened rabbit erythrocytes for h = .135 cm, $N = 55 \times 10^6 ml^{-1}$, and G = 13.3 and 6.1 sec^{-1} (A and B respectively). The horizontal time scale is 2 sec/div.

determined with a haemacytometer (1). No changes in geometry or dispersity occurred with shearing.

RESULTS AND DISCUSSION

Typical rheo-optical results are shown in Figure 1 for hardened rabbit erythrocytes: the transmittance increased with time from an initial value to a maximum, after which it oscillated 4 or more times until it reached a steady value. There were superimposed scintillations of about 5% of the transmittance change, which became most evident when a steady value was reached. On stopping flow, the scintillations ceased and a smooth monotonic decay occurred until the initial value was restored (not shown in Fig. 1). Similar results were observed for unfixed rabbit erythrocytes, and human erythrocytes (both hardened and unhardened), while no changes were observed for "sphered" erythrocytes. It should be noted that the amplitude of oscillations decreased nearly exponentially while the period of oscillation was constant at a given G, but increased with lower G.

For light scattering particles, the transmittance relates to the scattering cross section K_{sca}, as follows (15):

$$I/I_o = \exp\ [-K_{sca} \cdot N \cdot h] \qquad [1]$$

where I and I_o are intensity of incident and transmitted lights respectively. This has been confirmed for erythrocytes (5,7). Assuming erythrocytes to be oblate spheroids, numerical evaluation of K_{sca} based on the Rayleigh-Debye scattering theory (15) shows that the K_{sca} of a single cell is determined by the orientation θ_2 (inset of Fig. 2) of the axis of symmetry of each cell to the optic axis X_2. The maximum transmittance occurred when $\theta_2 = 0$ (face-on to the optic axis) and the minimum when $\theta_2 = 90°$ (edge-on). These limiting orientations were confirmed experimentally by applying electric fields along the optic axis (G = 0), using transparent electrodes in a 1 mm cell (16), causing the erythrocytes to rotate until they could be seen in the microscope at $\theta_2 = 90°$; during this time, the transmittance decreased until a steady value was reached.

In shear flow the orientation distributions of dilute suspensions of nearly monodisperse anisometric particles such as rods and discs exhibit

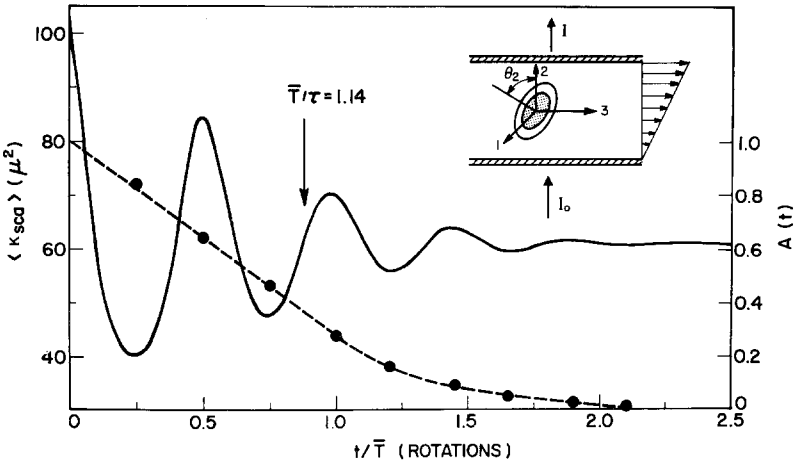

Fig. 2: Oscillations of $\langle K_{sca} \rangle$ for oblate spheroids with initially random orientations and $\bar{r}_e = 0.4$ and $\sigma_e = 0.07$ calculated from the shear orientation theory (14). At the relaxation time (shown on a dimensionless time scale) the relative amplitude A of the oscillations (broken line) has decreased to 1/e. In these calculations we assumed $\tau = \tau_1$. In the inset, the orientation θ_2 of cells is indicated relative to the incident light and flow field.

damped oscillations as a result of individual particle rotations (14). It should therefore follow that any macroscopic property (optical, rheological, electrical, etc.), whose instantaneous value depends upon particle orientations, will undergo corresponding damped oscillations. Therefore, from the shear orientation theory and light scattering theory, the mean scattering cross section $\langle K_{sca} \rangle$, for a dilute suspension of oblate spheroids with initially random orientations, is calculated to vary in a manner very similar to Fig. 1, as shown in Fig. 2. The period of oscillation T/2, where T is the period of rotation of a single particle about the vorticity axis (normal to the inset in Fig. 2) is given by

$$T = \frac{2\pi}{G} \left(r_e + \frac{1}{r_e} \right) \qquad [2]$$

where r_e (< 1), the equivalent ellipsoidal axis ratio of the particle, is somewhat greater than the actual axis ratio r_p (12,14). The oscillations damp out with a relaxation time τ, the time required for the amplitude to decay to 1/e of the initial value given by

$$\tau^{-1} = \tau_o^{-1} + \tau_1^{-1} + \tau_2^{-1} \qquad [3]$$

where (i) τ_o is the relaxation by rotary Brownian diffusion, (ii) τ_1 from rotational phase mixing (14,17) resulting from the dispersion about $\bar{r}_e$ when the particles are not exactly monodisperse and (iii) τ_2 is due to hydrodynamic interactions between particles. These quantities are

$$\frac{1}{\tau_o} \simeq 6D_r , \qquad \frac{\bar{T}}{\tau_1} = \frac{\sqrt{8}\,\pi\sigma_e(1 - \bar{r}_e^{\,2})}{\bar{r}_e(\bar{r}_e^{\,2} + 1)} , \qquad \frac{\bar{T}}{\tau_2} = \alpha N \qquad [4]$$

where D_r is the rotary Brownian diffusion coefficient, σ_e the standard deviation from $\bar{r}_e$ and α a constant (14). At sufficiently high G and low N, τ_o and τ_2 can be neglected in Eq. [3] as we have done in calculating the variation of $\langle K_{sca} \rangle$ shown in Fig. 2.

The mean period of shear rotation $\bar{T}$ and the relaxation time τ can be evaluated from oscillatory traces like Fig. 1. In this way, we estimated

for 16 different combinations of f, N, R and h (Fig. 3) an average $\bar{T}$ G = 17.0 (S.D. = 1.0) from which, with the aid of Eq. [2], we calculate $\bar{r}_e$ = 0.4 to 0.5. This agrees reasonably well with measurements for normal human cells (12), indicating the similarity of $\bar{r}_e$ and σ_e for rabbit hardened cells and the latter. In fact, we have found by microscopy for normal or hardened rabbit erythrocytes, that $\bar{r}_p$ = 0.29 (S.D. = 0.04), the same as for normal human cells (12).

The damping of the oscillations in I/I_o calculated in the same way as in Fig. 2 yielded $\bar{T}/\tau$ = 1.0 approx. and increased slowly with increasing N as expected from Eqs. [3] and [4]. The value for τ_o calculated from $D_r(1)$ is > 10^3 sec. i.e. too large to affect τ in the shear experiments. We conclude that most of the damping was caused by the spread in r_e which from Eq. [4] we calculate to have the plausible value σ_e = 0.06 (12). It should be noted that when $\bar{T}/\tau >> 1$, the oscillations are overdamped, i.e. both $\langle K_{sca} \rangle$ and I/I_o change monotonically (14). This will occur on increasing D_r, σ_e or N.

We believe that the random fluctuations (scintillations) superimposed

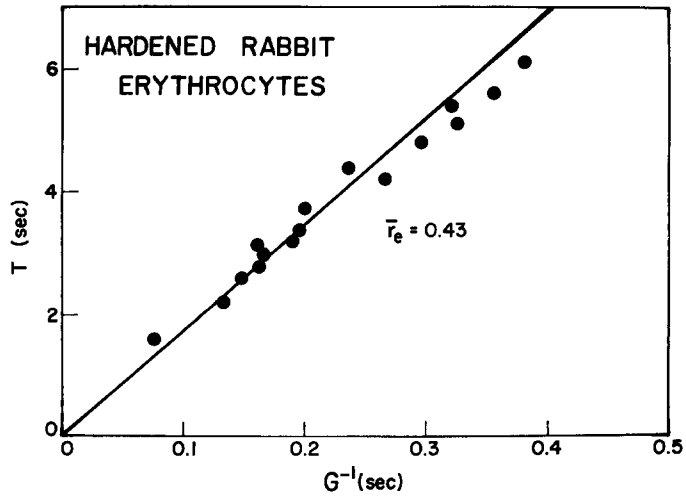

Fig. 3: The period of particle rotation $\bar{T}$, determined from 2 x the observed period of damped oscillations (as in Figs. 1,2), is plotted against the inverse of shear rate for hardened rabbit erythrocytes, for various f(1-2 rpm), N((15-150) x $10^6 ml^{-1}$), R(4.2 - 4.4 cm) and h(1.30 - 1.35 cm).

on the shear-induced damping oscillations, observed only with non-spherical particles and in the presence of shear, were caused by statistical fluctuations in the number of erythrocytes sensed by the beam which have orientations where they have the minimum transmittance, i.e. at $\theta_2 = 90° \pm \delta\theta_2$ where, incidentally, they are also rotating most rapidly (14). For $\delta\theta_2 = 2°$ the fraction is calculated to be .016; since the total number of cells in the beam was about 10^5, the S.D. of fluctuations in this number was $100/\sqrt{0.016 \times 10^5} = 2.5\%$, the same magnitude as estimated from the fluctuations in transmittance at steady state.

In addition to confirming the oscillatory behavior of orientation-dependent properties of suspensions in shear flow, these experiments show that with improvements in the light scattering theory and in the geometry and precision of the measurements, the optical determination of $\bar{T}$ and τ may provide a rapid method for measuring $\bar{r}_e$, σ_e and two-body interaction effects in erythrocyte and other suspensions of nearly monodisperse and non-spherical particles. The analysis of the size and duration of scintillations may prove useful as a simple method of assessing sphericity when τ is too small to yield oscillations. Rheo-optical measurements with erythrocytes and platelets (assymetric bioparticles in general), should prove extremely useful in physiological structure-function studies.

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