Erythrocyte dynamics in flow affects blood rheology

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Abstract. Normal blood consists of highly deformable particles (red blood cells, RBC, or erythrocytes) suspended in a Newtonian fluid (blood plasma). As a rough physical model of erythrocytes, giant unilamellar vesicles (GUV) are successfully used to probe their membrane properties. In shear flows vesicles and red blood cells show rich variety of dynamical behaviours influencing the rheological properties of their suspensions. Here, we focus on new experimental aspects of the problem in the case, when a combination of an oscillatory shear rate and a basic constant shear rate is applied to suspensions. Experimental examples with concentrated RBC suspensions are presented together with a discussion on the importance of the superposition of a constant shear flow to the pure oscillation, which is usually used to extract the viscoelastic properties of a complex fluid.

1. Introduction

Representing a suspension of formed elements (red blood cells (RBC) or erythrocytes, white blood cells (leukocytes), and platelets) in aqueous solution of proteins and salts (plasma), blood is a prominent example of a complex fluid, whose physical properties have been in the scope of interest for researchers from diverse scientific fields [1-4]. The RBCs consist of a thin elastic membrane filled with a hemoglobin solution that is a Newtonian fluid with viscosity about 10 mPa.s [5, 6]. Besides the transport of oxygen and carbon dioxide, another important property of intracellular hemoglobin is its contribution to internal viscosity, which determines the rheological properties of erythrocytes [7]. The other particulate constituents of blood, white blood cells and platelets usually occupy only less than 1% of the total volume of blood cells in normal human blood, thus, the RBCs govern the flow properties of blood [3, 8]. RBCs are readily deformable and it allows them to pass through the smallest capillaries [2, 9]. Their ability to deform in flow is an essential feature [10] determined by the viscosity of the carrier fluid, its osmolarity (affecting the viscosity of the hemoglobin solution), the shear rate of the flow as well as of biological factors such as in-vivo aging for example [11].

Erythrocyte membranes as all biomembranes are characterized by a “fluid mosaic” structure [12] unifying in a perfectly functioning dynamical ensemble its lipidic and proteinic constituents with functionally important lipid asymmetry between the two membrane leaflets [13, 14]. The presence of a cytoskeleton attached to the inner surface of RBC membranes makes them resistant to shear.

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deformations, thus inducing additional dynamical regimes of cells in flow [15, 16]. Thanks to the biphilic nature of lipid molecules the lipid matrix of biomembranes is easily reproducible in laboratory conditions and closed structures, giant vesicles, are readily formed via elaborated experimental protocols with controlled physicochemical parameters [17-19].

So far, it has been revealed both theoretically and experimentally that in shear flows, vesicles and red blood cells show a rich variety of dynamical behaviours having a signature on the rheological properties of their suspensions [20, 21]. Several types of motion in linear hydrodynamic fields have been predicted and observed for vesicles and RBC, namely tank-treading (TT), tumbling (TB) (or flipping), vacillating-breathing (VB) (sometimes called also “trembling” or “swinging”) for vesicles and swinging, for RBC, and spinning or kayaking, where the main axis describes a cone about the perpendicular to the plane of the shear flow (see [22, 23] and the references therein).

The elasticity of the spectrin network leads to specific features of red cells’ dynamics in flow. As it has been predicted [15] (under the assumption of fixed cell shape) and observed [16], in very viscous solvent and depending on the applied shear stress, erythrocytes can execute several types of motion. At low shear stresses (less than 0.1 Pa [16]) cells tumble, at intermediate shear rates they are in swinging mode, while at higher shear stresses erythrocytes perform, similarly to vesicles, pure tank-treading.

The question about the intrinsic viscosity \( \eta \) of suspensions has intrigued the physicists for more than hundred years. Numerous theoretical works have been performed since the first study of Einstein, who derived the expression for the effective viscosity \( \eta = \eta_0 \left[ 1 + \eta \phi + O(\phi^2) \right] \) of dilute suspension of rigid spheres with volume fraction \( \phi \) in suspending medium with viscosity \( \eta_0 \) [24, 25]. Recently, expressions have been provided for the intrinsic viscosity of dilute suspensions of tank-treading nearly spherical vesicles as well as for suspensions of tumbling vesicles [26, 27].

The experimental parameters relevant to our study are as follows: (i) excess area \( \Delta = 4\pi \left( \nu^{-1/3} - 1 \right) \) (or reduced volume \( \nu = 3V / 4\pi (S/4\pi)^{3/2} \)) of a vesicle (or blood cell) with surface \( S \) and volume \( V \), quantifying its deflation from a sphere and thus determining its shape; (ii) viscosity ratio (or viscosity contrast) \( \lambda = \eta_\text{a} / \eta_0 \) between the viscosity \( \eta_\text{a} \) of the internal solution (hemoglobin solution for RBC) and the viscosity \( \eta_0 \) of the suspending medium; (iii) relative viscosity \( \eta_r = \eta / \eta_0 \) of suspensions; and (iv) capillary number \( Ca = \eta \dot{\gamma} (S/4\pi)^{1/2} / k_c \) accounting for the deformability of a vesicle with membrane bending modulus \( k_c \) in flow with shear rate \( \dot{\gamma} \).

In the present work, experimental results about the effective viscosity and viscoelasticity of concentrated RBC suspensions are presented and discussed. The focus is put on new aspects of the problem in the case, when a combination of an oscillatory shear rate and a basic constant shear rate is applied to the suspension [28, 29].

2. Materials and methods

Fresh whole blood was supplied by Etablissement Français du Sang (EFS) and Centre Hospitalier Universitaire (CHU) in Grenoble, France from hematologically healthy donors. Phosphate buffer saline (PBS) with pH 7.4 and osmolarity \( 290 \pm 10 \) mOsm/kg and dextran, a biocompatible polymer of anhydroglucose, with three different average molecular weights (15000-30000, \( \sim 70000 \) and \( \sim 500000 \)) were purchased from Sigma-Aldrich Chemie (Saint-Quentin Fallavier, France). Due to the high hygroscopicity of the polymer, all dextran solutions in PBS were prepared from fresh, newly open flasks.

2.1. Preparation of RBC suspensions

Blood washing by gentle centrifugation in PBS buffer solution assured the removal of plasma proteins responsible for the cell-cell adhesion. The volume fractions of RBCs (hematocrits) in all experimental samples were measured by cell counting as described in detail in [20]. In order to obtain the desired
viscosity ratio $\lambda$ in each sample and taking into account the final hematocrit needed, erythrocytes were dispersed in an external solution, containing PBS and/or a given concentration of dextran. Dextran type and concentrations were adapted to cover all the studied range of viscosity ratios $\lambda \in [0.05, 20]$ taking care to avoid any dextran-induced aggregation of cells in suspensions [30]. The cytoplasmic viscosity of RBCs was determined on the basis of the mean corpuscular hemoglobin concentration [5]. For each RBC suspension, a control sample with the same dextran concentration in the PBS was prepared separately for the independent measurement of the external viscosity $\eta_e$.

2.2. Rheological measurements
Viscosities of RBC suspensions were measured as a function of the viscosity ratio $\lambda$ between the inner and outer fluids. Viscoelasticity measurements were made with two instruments: a stress-controlled Bohlin Gemini 150 rheometer (Malvern Instruments, Orsay, France) with a cone-plate geometry (60 mm diameter, 2° angle), and a stress- or shear rate-controlled Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria) with a cone-plate (50 mm diameter, 0.5° angle) and Couette (2 mm gap, 40 mm length) geometries. All measurements were performed at constant temperature of 22°C and in conditions preventing the undesirable drying of the sample.

3. Results and discussion
The effective viscosity $\eta$ of concentrated RBC suspensions was measured for a wide range of viscosity ratios $\lambda$ (figure 1). Various models for the viscosity of concentrated suspensions of non-aggregated erythrocytes have been developed so far, based on the differential medium approach [31]. In figure 1, in order to avoid any model-dependent artefacts, instead of the intrinsic viscosity, the relative viscosity of RBC suspensions $\eta_r$ is represented as a function of the viscosity contrast.

![Figure 1](image-url)  
**Figure 1.** Relative viscosity of suspensions with RBC volume fractions $\phi = 0.2$ and $\phi = 0.5$ as a function of the viscosity contrast.

In our previous study [20], a clearly distinguishable transition between the $TT$ and $TB$ dynamical regimes with a minimum of the intrinsic viscosity around the transition point ($\lambda \approx 2$ [32, 33]) was obtained experimentally for dilute ($\phi = 0.05$) erythrocyte suspensions. For concentrated suspensions ($\phi > 0.1$), the hydrodynamic interactions between cells are considerable and alter the individual cell...
dynamics in flow. Consequently, pure tumbling motion is expected to be hindered unlike for erythrocytes in dilute suspensions above a critical value of the viscosity contrast. Nevertheless, our rheological measurements of suspensions with \( \phi = 0.2 \) and \( \phi = 0.5 \) showed \( \lambda \)-dependent behaviour of their relative viscosity, which remains practically constant for small \( \lambda \), while a steep increase of \( \eta \) is obtained for higher values of \( \lambda \). These results lead to the conclusion that rheology of concentrated suspensions is also sensitive to the cell dynamics similarly to the dilute limit. Future experimental and theoretical advances on hydrodynamic interactions of vesicles and red cells in flow could shed light on the origins of such increase in the relative viscosity of suspensions for small external viscosities (high values of \( \lambda \)).

Concentrated RBC suspensions were subjected to oscillatory shear stresses \( \tau(t) = \eta^*(\omega)\dot{\gamma}(t) \) in order to extract their complex viscosity \( \eta^* = \eta' + i\eta'' \) and complex modulus \( G^* = G' + iG'' = \eta^*/\omega - i\eta''/\omega \), where \( \omega \) is the angular velocity of oscillations. In the cases, when \( G' > G'' \), the elastic response of the suspension is higher than the viscous dissipation. Such an example is given in figure 2 for two volume fractions of erythrocytes \( \phi = 0.2 \) and \( \phi = 0.33 \) and for viscosity ratio \( \lambda = 1.2 \), which is still in the \( TT \)-branch of the curve \( \eta_\rho(\lambda) \) but close to the transition point. This signature of a viscoelastic medium has been previously observed for RBC suspensions [34], with high volume fractions \( \phi > 0.2 \), and is believed to be a consequence of the elasticity of the cells, packed more densely at high concentrations.

Blood flow in aorta and large arteries is pulsatile, which motivates the investigation of RBC rheology in conditions similar to the real ones, i.e. when a combination of an oscillatory flow at constant frequency and a steady flow is applied to the suspension:

\[
\tau(t) = \tau_0(1 + \alpha \cos \omega t)
\]  

(1)

where \( \alpha \tau_0 \) is small enough in order to expect linear response from the suspension.

Such experimental studies on whole blood have been previously conducted and some features of blood viscoelasticity were revealed and attributed to the RBC disaggregation and deformation in combined steady and oscillatory shear flows [29]. Very recently, it was shown theoretically that the complex viscosity of dilute vesicle suspensions subjected to superimposed steady and oscillatory shear stress reveals much more insightful properties of the suspension than its complex viscosity in the case when only oscillating shear stress is applied [28]. It has been obtained that in weak flows vesicles relax to \( TT \) motion monotonously, while for strong flows and high enough viscosity contrasts the relaxation is achieved after a transient of damped oscillations. It was shown that different relaxation regimes result in qualitatively distinct dependencies \( \eta^*(\omega) \) and that the relaxation times of vesicle dynamics govern the quantitative behaviour of \( \eta^*(\omega) \) [28].

Due to instrumental limitations we were able to perform reproducible measurements only with concentrated RBC suspensions with \( \phi \geq 0.2 \). Results for the complex viscosity of RBC suspensions with \( \phi = 0.2 \) and \( \phi = 0.3 \) and with \( \lambda = 0.2 \) are presented in figure 3. The applied shear stress according to Eq. (1) had constant part \( \tau_0 = 0.1 \text{ Pa} \) (swinging motion expected [16]) with oscillation amplitude \( \alpha \tau_0 = 0.01 \text{ Pa} \). Fitting with three parameters \( (\eta_0, A, \nu) \) was performed on the experimental data points for the real part of \( \eta^*(\omega) \) using the real part of the complex equation \( \eta = \eta_0 + A/(v - i\omega) \), namely \( \text{Re}(\eta) = \eta'_0 + A\nu/(\nu^2 + \omega^2) \). The same values of parameters were used to trace the theoretical curve for the imaginary part of \( \eta^*(\omega) \), \( \text{Im}(\eta) = \eta''_0 + A\nu/(\nu^2 + \omega^2) \). For all studied ranges of \( \lambda \) and \( \phi \), the values of \( \eta_0 \), obtained by the fit, are in excellent agreement with the values of the dynamic viscosity, experimentally determined via independent measurements in steady flow. In all studied cases, the values of the fitting parameter \( \nu \) seem to be independent of the applied
shear rate. The model \cite{28} suggests that $1/\nu$ is a relaxation time of the RBC dynamics under constant shear flow. To probe the origins of the characteristic time related to $\nu$, further experiments are needed in a wide range of shear rates and for various $\lambda$.

![Figure 2](image-url)

**Figure 2.** Complex modulus of suspensions with volume fractions of RBC $\phi = 0.2$ and $\phi = 0.33$ and viscosity ratio $\lambda = 1.2$.

![Figure 3](image-url)

**Figure 3.** Complex viscosity of suspensions with RBC volume fractions $\phi = 0.2$ and $\phi = 0.3$ with viscosity ratio $\lambda = 0.2$. The applied shear stress is $\tau_0 = 0.1$ Pa with oscillation amplitudes $\alpha \tau_0 = 0.01$ Pa.

4. Conclusion
In the present work, the rheological properties of concentrated RBC suspensions were studied in order to reveal the correlation between the individual dynamics of cells and the suspension rheology. Blood disorders strongly affect the deformability of erythrocytes as well as their dynamics in the blood
stream leading in some cases to dramatic physiological consequences for the organism [35]. Numerous examples with strong sociological impact can be given, such as sickle cell anemia, iron deficiency anemia, thalassemia, chronic alcoholism, etc, affecting the morphology and/or mechanical properties of erythrocytes. Since the rheological measurements presented here are sensitive to the physical properties of the cells, they can be used to capture pathological deviations in the shape, the reduced volume, and/or rigidity of cells by such macroscopic assay of suspension rheology. Future efforts must be focused on the relation between the rheological properties of suspensions and the contents of pathological cells in them.

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