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II. Rheology of Weakly Flocculated Suspensions of Viscoelastic Particles

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Abstract. — A microrheological model is proposed to estimate the steady state shear viscosity of concentrated suspensions of viscoelastic particles. We first present a Kelvin Voigt model to describe the deformation and stable orientation of a viscoelastic particle in a simple shear field. We then use a viscosity law for concentrated suspensions of hard particles in purely hydrodynamic interactions and we relate the maximum packing concentration to the component of the particle deformation tensor in the direction of the flow. We analyse the steady state viscometric behavior of red cell suspensions in saline solution and we show the influence of the nonlinear viscoelastic properties of the cell membrane. In a second part, we consider a flocculation of deformable particles and we deduce a viscosity law taking into account both the aggregation phenomena and the deformation-orientation of particles in the shear field. The rheological law describes the viscosity behavior of aggregated deformable red cells in dextran saline solution only for negligible shear induced restructuration of the aggregates.

Résumé. — Nous proposons un modèle microrhéologique pour estimer la viscosité de cisaillement des suspensions concentrées de particules viscoélastiques. Dans un premier temps, nous présentons un modèle de Kelvin Voigt tournant afin de décrire la déformation et l’orientation stable d’une particule viscoélastique dans un écoulement de cisaillement simple. Nous utilisons alors une loi de viscosité valable pour des suspensions concentrées de sphères dures en interaction purement hydrodynamique et nous retons la concentration maximale d’empilement à la composante du tenseur de déformation des particules dans la direction de l’écoulement. Nous analysons ensuite le comportement rheologique des suspensions de globules rouges et nous montrons le rôle des propriétés viscoélastiques non linéaires de la membrane cellulaire. Dans une seconde partie, nous introduisons une floculation des particules viscoélastiques et déduisons une loi de viscosité prenant en compte les phénomènes de floculation et de déformation des particules dans le champ de cisaillement. La loi rhéologique proposée décrit la rhéologie des suspensions de globules rouges aggrégés en présence de dextrane dans la mesure où nous pouvons négliger les phénomènes de restructuration des agrégats sous l’action des contraintes hydrodynamiques.

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1. Introduction

The viscoelastic properties of particles strongly influence the rheology of many concentrated industrial or biological dispersions (emulsions, paints, yoghurts or blood). The deformation-orientation and the aggregation of interacting units under flow induces a nonlinear rheological behavior [1-3]. The microrheological models based on understanding the relationships between bulk suspension properties and suspension microstructure are often limited to the diluted regime [2,3]. Our purpose in this paper is to propose a simple but general analysis of the non-Newtonian properties of weakly aggregated suspensions of viscoelastic particles in concentrated systems assuming reversible flocculation.

In the following, we consider particles larger than one micron in diameter so that the hydrodynamic effects dominate over Brownian motion. Reynolds number for flow around immersed particles is further assumed small enough compared to unity for neglecting inertial effects relative to viscous forces. The time dependent deformation of different types of particles such as liquid drops, macromolecules, microcapsules or biological cells is relevant from either pure fluid mechanics, statistical physics or the viscoelastic constitutive equations for composite materials [4-6]. However, the viscoelastic behavior of such particles emphasizes the particular role of a common relaxation time related to the properties of the material (particle diameter, interfacial tension, elastic modulus, internal viscosity...) [7,8]. The first order deformation of a spherical particle in a shear flow thus may be described by a rotating Kelvin-Voigt model [9] representative from a linear viscoelastic behavior. The nonlinear rheological properties of concentrated suspensions mainly result from stresses exerted by the surrounding medium on clusters or deformable particles.

In the first part of the paper, we derive the first order deformation of isolated liquid drops and complex bodies in a shear flow from a differential equation describing the time dependent deformation of a Kelvin Voigt element in a shear flow. We then propose a self consistent model based on an effective medium approximation for estimating the shear viscosity of a concentrated suspension of viscoelastic particles. For this purpose, we use a reference law for hard spheres in purely hydrodynamic interaction [10-13] and a first order development of the maximum packing fraction as a function of the strain gradient in the direction of the flow. Rigorous expressions from Barthes-Biesel and Chim [14] and Lhuillier [3] of the shear viscosity of diluted dispersions of liquid drops are compared to the predictions of the model. We further analyse the viscoelastic properties of the red cell considered as a solid-liquid composite with shear dependent relaxation times and we deduce the shear viscosity of concentrated unaggregated red cell suspensions.

In the next section, we predict the viscosity of suspensions consisting of fractal clusters of deformable particles from both the models outlined above and proposed in the first paper. We further present viscosity experiments for weakly aggregated red cells in a polymer solution (neutral dextran) or in human plasma. The break-up of red cell aggregates in Couette flow was investigated by a laser light reflectometry technique which provides a way for estimating the critical disaggregation shear stress of the suspension. From the rheological model and from estimates of the disaggregation shear stress, we then describe the shear-thinning behavior of deformable red cells in dextran solution.

In the last section, we discuss the shear-induced restructuration of aggregates in relation with the particle deformability, the intensity of the flow and the rheological history of the suspension.
Fig. 1. — Deformation and orientation of a spherical viscoelastic particle in a simple shear flow.

2. Deformation of a Viscoelastic Particle in a Shear Flow

Solid spheres in a shear flow undergo a periodic rotary motion with a mean angular velocity \( \omega = \gamma / 2 \) where \( \gamma \) denotes the local shear rate [2]. Liquid drops behave rather differently from a solid particle and undergo both deformation into spheroid and steady state orientation in the shear field after a transient regime because of the transmission of velocity gradient across the interface and the flow of the internal fluid [15, 16]. Microcapsules and biological cells such as red cells represent an intermediate case between solid particles and fluid drops, behaving as a solid like body in the low shear regime and attaining a steady state orientation with a tank-tread motion of the membrane only at high shear rates [17].

The first order deformation-orientation of a viscoelastic sphere in a shear flow can be described by a rotating Kelvin Voigt model early proposed by one of the authors [9]. We consider an initially spherical particle of radius \( a \) centered at the origin \( O \) of a plane \( xy \) parallel to the velocity vector \( \mathbf{V}(y\gamma, 0, 0) \) (Fig. 1). The position vector \( \mathbf{r} \) of an element of particle area rotates with an angular velocity \( \omega = \gamma / 2 \) and is assumed to behave as a Kelvin Voigt element with a characteristic relaxation time \( \theta \). An element of the external surface bound to the end of the rotating vector further experiences a normal stress \( \sigma_n \) depending on the time varying angular position \( \psi(t) \) [9]:

\[
\sigma_n = \mu_0 \gamma \sin 2\psi \quad \text{with} \quad \psi(t) = \int \omega dt = \frac{\gamma t}{2} + \psi_0
\]

where \( \mu_0 \) is the viscosity of the suspending fluid and \( \psi_0 \) the initial angular position of the rotating vector. It follows that the time dependence of \( r = |\mathbf{r}| \) obeys the first order differential equation:

\[
\frac{dr}{dt} = \frac{\mu_0}{\mu_p} a \gamma \sin(\gamma t + 2\psi_0) - \frac{r - a}{\theta}
\]

where \( \mu_p \) is an effective viscosity accounting for the overall dissipative phenomena in the particle. We can write the equation (2) in the dimensionless form:

\[
\frac{d\varepsilon}{dT} + \varepsilon = \frac{\Omega}{\Lambda} \sin(\Omega T + 2\psi_0)
\]
with \( \varepsilon = \frac{r - a}{a}, T = \frac{t}{\theta}, \Lambda = \frac{\mu_r}{\mu_0} \) and \( \Omega = \gamma \theta \).

The time dependent deformation \( \varepsilon(T) \) then obeys the general solution:

\[
\varepsilon(T) = \varepsilon_M \cos(\Omega T + 2\psi_0 - 2\psi_M) = \varepsilon_M \cos(2\psi - 2\psi_M)
\]

(4)

with \( \psi_M = \frac{1}{2} \tan^{-1} \frac{1}{\Omega} \) and \( \varepsilon_M = \frac{\Omega}{\Lambda (1 + \Omega^2)^{1/2}} \) where \( \varepsilon_M \) is the maximal relative deformation and \( \psi_M \) the angle of maximal deformation. In the low shear regime \( \gamma < \theta^{-1} \), the maximal relative particle deformation \( \varepsilon_M \) thus scales as the shear stress \( \mu_0 \gamma \) and decreases with the effective particle viscosity.

We then deduce the components \( \varepsilon_{xx} \) and \( \varepsilon_{yy} \) of the shear strain tensor representative of the particle deformation gradient along the directions \( \psi = 0 \) and \( \psi = \pi/4 \):

\[
\varepsilon_{xx} = \varepsilon(\psi = 0) = \frac{\Omega^2}{\Lambda (1 + \Omega^2)} \quad \varepsilon_{yy} = \varepsilon(\psi = \pi/4) = \frac{\Omega}{\Lambda (1 + \Omega^2)}
\]

(5)

The steady state deformation of a liquid drop (viscosity \( \mu_i \) and interfacial tension \( \sigma \)) in a shear flow is reached with a relaxation time \( \theta \approx \lambda Ca / \gamma \approx \mu_0 \alpha / \sigma \) depending on the viscosity ratio \( \lambda = \mu_i / \mu_0 \) and the capillary number \( Ca = \mu_0 \gamma \alpha / \sigma \) [2]. When applied to the case of liquid drops with \( \Omega = \gamma \theta = 19 \lambda Ca / 20 \) and \( \Lambda = 76 \lambda (\lambda + 1) / (95 \lambda + 80) \), the Kelvin Voigt model exactly gives the steady state orientation \( \psi_M \) and the maximal relative drop deformation \( \varepsilon_M \) obtained by Cox for small departure from the spherical shape (\( Ca \ll 1 \)) [7]. The frequency \( f \) of the periodic motion of the fluid particle further scales as the shear rate [18]. A Kelvin Voigt model thus gives a reasonable account for the drop motion in the range of small deformations.

3. Shear Viscosity of Suspensions of Viscoelastic Particles

The shear viscosity of a suspension of non-Brownian particles in purely hydrodynamic interactions depends on the pair distribution function and may exhibit non-Newtonian behavior. A mean field theory based on the estimation of the viscous dissipation in the fluid phase gives an expression of the relative shear viscosity \( \mu_r(\phi, \phi^*) \) of the suspension [10–13]:

\[
\mu_r(\phi, \phi^*) = \frac{\mu(\phi, \phi^*)}{\mu_0} = \frac{1 - \phi}{(1 - \phi/\phi^*)^2}
\]

(6)

where \( \phi^* \) is the maximum packing volume fraction relative to the transition threshold between the fluid state and the solid state in a shear flow. The maximum packing volume fraction \( \phi^* \) closely depends on the microstructure, the particle size particle and the flow type [19, 20].

3.1. Diluted Suspensions of Viscoelastic Particles. — The orientation of deformable particles along the main strain axes induces the formation of transient anisotropic microstructures with a higher maximum packing volume fraction. The flow induced deformation of particles thus results in a non-Newtonian behavior.

For weak particle deformations, we may estimate the shear rate dependence of the viscosity by expanding the maximum packing volume fraction \( \phi^*(\gamma) \) as a function of the shear strain gradient \( \varepsilon_{xx}(\gamma) \) in the direction of the flow:

\[
\phi^*(\gamma) = \phi_0^* \left[ 1 + \beta \varepsilon_{xx}(\gamma) + O \left( \varepsilon_{xx}^2 \right) \right]
\]

(7)
where \( \phi_0^* \) is the maximum packing concentration around the zero shear rate limit and \( \beta \) a constant related to the type of flow.

We may use the reference viscosity law (6) only for negligible viscous dissipation in the particulate phase of effective viscosity \( \eta_p \). Considering the continuity of the shear stress across the interface, the average shear rate \( \gamma_i \) in the particle volume scales as \( \mu_0 \gamma^*/\mu_p \approx \gamma^* \Lambda^{-1} \) (\( \gamma^* \) is the average shear rate in the fluid phase). The condition for a dissipation energy \( d_t D_p \) per unit volume in the viscoelastic phase much lower than the viscous dissipation \( d_t D \) in the fluid fraction \( 1 - \phi \) then takes the form:

\[
d_t D_p \approx \mu_p \left( \gamma^* \Lambda^{-1} \right)^2 \phi \ll d_t D \approx \mu_0 \gamma^{-2}(1 - \phi)
\]  

(8)

We deduce the condition \( \Lambda^2 \gg \frac{\phi}{1 - \phi} \).

For a viscosity ratio \( \Lambda = \mu_p / \mu_0 \gg 1 \), then we may neglect the viscous dissipation in the solid phase excepted in highly concentrated suspensions (\( \phi \approx \phi^* \)).

The reference rheological law (6) together with the expression (7) for the maximum packing volume fraction \( \phi^*(\gamma) \) then gives the relative shear viscosity of a diluted suspension of weakly deformed viscoelastic particles:

\[
\mu_r(\phi, \varepsilon_{xx}) = \frac{1 - \phi}{\left( 1 - \frac{\phi}{\phi_0^* (1 + \beta \varepsilon_{xx})} \right)^2}
\]  

(9)

with \( \varepsilon_{xx} = \frac{\Omega^2}{\Lambda (1 + \Omega^2)} \) and \( \Omega = \gamma \theta \).

In the range of small deformations and diluted suspensions (\( \phi \ll \phi^* \)), equation (9) yields a viscosity law similar to the relation obtained by Lhuillier in 1987 [3]:

\[
\mu_r(\phi, \varepsilon_{xx}) = \frac{1 - \phi}{(1 - \phi / \phi_0^*)^2} \left( 1 - \frac{2\phi / \phi_0^*}{1 - \phi / \phi_0^*} \beta \varepsilon_{xx} \right).
\]  

(10)

Considering a maximum packing concentration at zero shear rate close to the random isostatic packing fraction \( \phi_0^* = 4/7 \) of solid spheres, a first order expansion of the equation (10) with \( \phi_0^* = 4/7 \) further leads to the rheological law obtained by Barthes-Biesel and Chim in 1981 [17]:

\[
\mu_r(\phi,\Gamma) = 1 + 2.5\phi - \frac{7 \beta \Lambda^{-1} \Omega^2}{2} \frac{1}{1 + \Omega^2} \phi.
\]  

(11)

3.2. CONCENTRATED SUSPENSIONS OF VISCOELASTIC PARTICLES. — In concentrated suspensions, hydrodynamic interactions enhances the shear gradients and the deformation of particles but the flow induced anisotropic microstructures lead to a decrease in viscous dissipation and suspension viscosity. Therefore, the non-Newtonian behavior of deformable particles is essentially due to the nonlinear relation which exists between the suspension viscosity and the particle deformation. Some works introduce a phenomenological expression of the structure parameter \( \phi^*(\gamma) \) for describing the nonlinear rheological properties of concentrated suspensions [21].

Here, we propose a self consistent theory based on an effective medium approximation in agreement with data from rheo-optical experiments presented in the first paper [13, 22]. We consider that viscoelastic units behave like isolated particles in a fluid of viscosity equal to the viscosity of the suspension and thus experience an effective shear stress \( \tau = \mu(\phi, \gamma) \gamma \). From
Fig. 2. — Theoretical variations of the relative shear viscosity $\mu_r(\phi, \gamma)$ of concentrated suspensions of viscoelastic spheres with the Deborah number $\Omega = \gamma \theta$ and the particle volume fraction $\phi$. Solid curves are obtained from equation (13) with $\Lambda/\beta = 10$ and $\phi_0^* = 4/7$ ((○) $\phi = 0.2$, (□) $\phi = 0.3$, (●) $\phi = 0.4$, (■) $\phi = 0.5$).

By introducing the shear rate dependence of the maximum packing concentration in the reference rheological law (5–7), a nonlinear equation for the relative shear viscosity $\mu_r(\phi, \Omega)$ is obtained:

$$\mu_r(\phi, \Omega) = \frac{1 - \phi}{\left(1 - \frac{\phi}{\phi_0^*} \left(1 + \mu_r(\phi, \Omega) \frac{\beta \Lambda^{-1} \Omega^2}{1 + \Omega^2} \right)^{-1}\right)^2}, \quad (13)$$

Taking $\beta \Lambda^{-1} = 0.1$ and $\phi_0^* = 4/7$, the viscosity was determined numerically as a function of the dimensionless shear rate $\Omega$. Figure 2 clearly shows the shear thinning behavior of the suspension associated with the shear induced deformation of viscoelastic spheres. At high reduced shear rates $\Omega \gg 1$, the relative viscosity reaches a constant value $\mu_{\infty}(\phi, \phi_0^*, \beta/\Lambda)$ which is a decreasing function of the maximal particle elongation $\varepsilon_{xx}(\Omega \to \infty) = \Lambda^{-1}$ in the direction of the flow.

4. Viscosity of Concentrated Red Cell Suspensions

4.1. Viscoelastic Properties of Red Cells. — Red cells or erythrocytes are deformable disc-shaped cells of 8 µm in diameter and 2 µm thickness which mainly consist in an external biological membrane enclosing a Newtonian fluid of viscosity $\mu_i \simeq 6 \times 10^{-3}$ N s m$^{-2}$ containing hemoglobin and salts. Microscopic observations and mechanical experiments demonstrate that the red cell membrane is easily deformed at constant area but greatly resists area dilation because of the strong hydrophobic interactions of the membrane lipids and the adjacent aqueous media [23, 24].
Mechanical extension of the red cell membrane at constant area in a micropipette yields an elastic shear modulus \( G \approx 6 \times 10^{-6} \text{ N m}^{-1} \) without dependence on the degree of cell deformation [25]. The swollen network of spectrin molecules weakly bounded to the internal lipid bilayer mainly accounts for the elasticity of the membrane since the lipid bilayer is in a fluid state. Experimental results from Stokke et al. [26] indicate that spectrin molecules free in solution or crosslinked into three dimensional networks behave essentially like purely entropic springs and constitute a reversible ionic gel with nonlinear viscoelastic properties [27]. A simple elastomer theory further yields an elastic modulus \( G \approx 3 \times 10^{-6} \text{ N m}^{-1} \) for the human erythrocyte spectrin network [27] in good agreement with experimental values.

In a shear flow, isolated red cells rotate like a rigid ellipsoid at low shear stresses and behave in a manner analogous to suspended fluid drops when subjected to shear stresses \( \tau > 0.1 \text{ N m}^{-2} \) [16,28,29]. Cells deformed into ellipsoids then align at a constant angle to the direction of the flow with a tank-treading motion of the membrane about the cell interior scaling as the shear rate (tank-tread frequency \( f \approx \gamma/20 \) [30]). Red cell deformation further increases with the shear stress and reaches a maximum elongation at high shear rates [31].

The backscattering diagram of sheared red cell suspensions probed by a laser light indeed displays an angular asymmetry indicating a cell orientation at angles \( 20^\circ - 35^\circ \) with respect to the flow direction [32]. For hardened red cells, the scattering diagram remains symmetric because of the flip flop motion and the random orientation state of rigid particles in a shear flow [32].

A Kelvin Voigt model thus may be used for describing the deformation-orientation of red cells in a shear flow when suspended in a low viscosity fluid. However, the discoid shape and the viscoelastic properties of the cell membrane result in differences between the periodic motion of red cells and liquid droplets in a shear flow [30].

The nonlinear viscoelastic properties of the spectrin network influences the relaxation time of the membrane. The deformation of a red cell aspirated into a micropipette indeed exhibits a time dependent behavior [33]. The relaxation time ranging from 0.1 s to 0.01 s decreases when increasing the deformation rate which indicates a change in membrane surface viscosity \( \mu_m \) during a rapid deformation and a shear thinning behavior of the spectrin network. Since the tank-tread motion frequency of the membrane scales as the shear rate [30], the relaxation time \( \theta(\gamma) \) of the red cell in a shear flow then may be written as:

\[
\theta(\gamma) = \frac{\mu_m(\gamma)}{G} = \theta_0 \left( \frac{\gamma_0}{\gamma} \right)^n \quad \text{with} \quad \mu_m = \mu_c \left( \frac{\gamma_0}{\gamma} \right)^n
\]  

(14)

where \( \theta_0 = \mu_c/G \) is a characteristic relaxation time for the spectrin network, \( \mu_c \) the characteristic surface viscosity of the cell membrane close from the resting configuration and \( n \) a scaling exponent. The relaxation time \( \theta_0 \) further scales as the characteristic shear rate \( \gamma_0 \). The shape recovery of a red cell expelled from a micropipette exhibits a single relaxation time \( \theta_r \approx \theta_0 \approx 0.1 \text{ s} \) since the deformation rate is slow and the membrane structure remains closer from the resting configuration of characteristic surface viscosity \( \mu_c \approx \theta_0 G \approx 6 \times 10^{-7} \text{ N s m}^{-1} \) (with \( G \approx 6 \times 10^{-6} \text{ N m}^{-1} \)) [25,34]. In the following we take \( n = 3/4 \) since the dependence of the viscosity upon the shear rate obeys a power law with \( 0.7 < n < 0.8 \) for many physical colloidal gels [35].

From equations (12) and (14), the Deborah number \( \Omega = \gamma \theta \) and the shear strain \( \varepsilon_{xx} \) of red cells are then given by:

\[
\Omega = \gamma \theta(\gamma) = (\theta_0 \gamma)^{1-n}
\]

and

\[
\varepsilon_{xx} = \mu_c \left( \phi \varepsilon_{xx} \right) \Lambda^{-1} \frac{\left( \theta_0 \gamma \right)^p}{1 + \left( \theta_0 \gamma \right)^p}
\]  

(15)
with \( p = 2 - 2n \).

Taking \( n = 3/4 \), red cell deformation in a shear flow then scales as \( \mu_0 \gamma^{0.5} \) in good agreement with data from rheo-optical experiments [36, 37]. When plotted as a function of the variable \( \mu_0 \gamma^{0.5} \), the changes in diffuse reflectivity resulting from cell deformation-orientation in a shear flow indeed lie on a single curve without significant dependence upon the viscosity of the suspending fluid [36, 37].

A semi-empirical law proposed by Quemada [38] and often used to describe the blood rheology further involves a structure parameter depending on the variable \( (\gamma / \gamma_c)^{0.5} \) in good agreement with a scaling exponent \( p = 0.5 \) (\( \gamma_c \) is a characteristic shear rate related to shear induced changes in the microstructure of the suspension).

The parameter \( \Lambda \) involves an effective viscosity \( \mu_p \) accounting for the overall viscous dissipation in the sheared red cell. The average shear rate \( \gamma_i \) in the cell interior is higher than the deformation rate \( \gamma_m \) of the membrane due to the continuity of shear stress across the membrane. As shown by Fisher [39], the viscous dissipation rate \( d_\gamma E_\gamma \) in the cell interior then dominates over the viscous dissipation rate \( d_\gamma E_m \) in the cell membrane:

\[
\frac{d_\gamma E_m}{d_\gamma E_\gamma} \approx \frac{\mu_m \gamma_m^2 S}{\mu_i \gamma_i^2 V} \approx \frac{\gamma_m S \delta}{\gamma_i V} < 1 \quad \text{with} \quad \frac{\mu_m}{\delta} \gamma_m \approx \mu_i \gamma_i
\]

where, \( S \) is the cell area, \( V \) the red cell volume, \( \delta \) the thickness of the cell membrane and \( \mu_m \) the surface viscosity of the membrane. The effective viscosity \( \mu_p \) is then close from the viscosity \( \mu_i \) of the intracellular fluid and we may consider the approximation \( \Lambda \approx \lambda = \mu_i / \mu_0 \).

### 4.2. Viscosity of Non-Aggregated Red Cell Suspensions

Equations (6–7) and (15) together with the condition \( \Lambda = \lambda \) give a nonlinear expression for the relative shear viscosity of deformable red cell suspensions:

\[
\mu_r(\phi, \gamma) = \frac{1 - \phi}{\left[ 1 - \frac{\phi}{\phi_0^*} \left( 1 + \mu_r(\phi, \gamma) \frac{\beta \lambda^{-1} (\theta_0 \gamma)^p}{1 + (\theta_0 \gamma)^p} \right) \right]^{1/2}}
\]

The relative viscosity \( \mu_{r\infty}(\phi, \phi_0^*, \beta/\lambda) \) of red cell suspensions in the high shear rate limit reduces to:

\[
\mu_{r\infty} = \frac{1 - \phi}{\left[ 1 - \frac{\phi}{\phi_0^*} (1 + \mu_{r\infty} \beta \lambda^{-1})^{-1} \right]^{1/2}}
\]

The maximum packing concentration \( \phi_0^*(\phi) \) is estimated from the viscosity data at low shear rates and the reference equation (6) for a suspension of dispersed particles. At low volume fraction, particles are randomly distributed and then the maximum packing fraction is close to the random isostatic packing \( \phi_c^* \approx 4/7 \) (Fig. 3). The maximum packing fraction increases above a critical volume fraction \( \phi_c \approx 0.2 \) (Fig. 3) due to the interpenetration of the virtual spheres encircling the non-spherical particles and the formation of anisotropic structures [13]. Cell deformability and membrane bending favor the motion of near contact particle and the structuration of the suspension which remains fluid at high particle concentration.

Shear induced deformation and orientation of red cells influence the microstructure and result in a viscosity decrease. However, the shear viscosity reaches an asymptotic value at high shear rates [40] (Fig. 4) as predicted by the rheological model. For a red cell volume fraction \( \phi = 0.45 \), the nonlinear equation (17) with \( \phi_0^* = 0.65, \beta = 0.3 \) and \( \lambda = \mu_i / \mu_0 \) shows a
considerable decrease of the high shear relative viscosity when increasing the viscosity of the suspending fluid in very good agreement with the experimental data from Chien [40] (Fig. 4).

We have measured the apparent shear viscosity of non-aggregated normal red cell suspensions in a Couette geometry (Contraves LS30 low shear viscometer, temperature 20 °C). The whole blood of healthy donors was centrifuged three times (10 min at 3000 rpm) and then washed each time in Physiological Buffer Solution (PBS). The particle volume fraction ranging from 0.15 to 0.5 was adjusted by microhematocrit technique. The sample was previously dispersed at a high shear rate (130 s⁻¹) before each measurement and then allowed to relax for 3 minutes. Steady shear experiments were performed by increasing the shear step by step and waiting...
Fig. 5. — Relative shear viscosity $\mu_r(\gamma, \phi) = \mu_A(\gamma, \phi)/\mu_0$ against the shear rate for normal red cells suspended in saline solution (●) ($\mu_0 = 0.9$ cP) with a particle volume fraction $\phi = 0.15, 0.25, 0.35, 0.45$ or $\phi = 0.55$. The solid curves are calculated from the relation (16) with $p = 1/2, \theta_0 = 5 \times 10^{-2}$ s, $\beta = 0.3, \lambda = \mu_1/\mu_0$ and $\mu_1 = 6$ cP. The maximum packing fraction $\phi^N_0(\phi)$ is derived from the shear viscosity of unaggregated particles in the low shear regime (Fig. 3).

for steady state before the measurement. In repetitive experiments with a fresh sample, the procedure gives reproducible data with an error of about 5% for the relative shear viscosity.

Figure 5 shows the shear rate dependence of the relative viscosity for non-aggregated red cells suspended in PBS – saline solution. The full rheological equation (16) with $n = 3/4$ ($p = 2 - 2n = 1/2$), $\beta = 0.3$ and $\theta_0 = 0.05$ s predicts the experimentally observed shear thinning behavior (Fig. 5). The inflection point in the curves occurs when $\gamma \approx \gamma_0 \approx 20$ s$^{-1}$ in relation with the characteristic relaxation time $\theta_0 \approx 1/\gamma_0 \approx 0.05$ s of the spectrin network.

4.3. Properties of Normal Red Cell Aggregates. — Reversible red cell aggregation requires the presence of macromolecules in the suspending fluid such as fibrinogen or high molecular weight dextrins [12,41,42]. It has long been known that fibrinogen and some globulin fractions present in the normal blood plasma induce red cell aggregation and play an important role in many pathological states [40]. After the pioneering work of Farhaeus [43] about the stability and the sedimentation velocity of the blood suspension, viscometric studies at low shear rates are now widely used to determine a red cell aggregation index [44,45].

Red cell adhesion by the neutral dextran polymer in Physiological Buffer Solution (PBS) provides an excellent way for controlling the surface adhesive energy $\Gamma$ between aggregated red cells. Dextran polymer can induce red cell aggregation above a molecular weight of about $4 \times 10^4$ [12–43]. The cell adhesive energy increases with polymer concentration and molecular weight to reach a disaggregation phase at high dextran concentrations [22,42]. The maximum aggregation occurs at a dextran concentration of about 4 g%. Red cells adhere to each other reversibly in dextran-PBS solution to form stable linear aggregates or rouleaux (Fig. 8b in [13]) with a large contact area between adjacent cells since the adhesion energy is changed in membrane strain energy. At very high adhesive energies, cell doublets present a spherical shape and then red cells form porous spherical clumps (Fig. 8c in [13]). Above the percolation threshold $\phi_g$, a spanning network may appear which consists in interconnected cylindrical rouleaux or spherical clumps.
By increasing the shear stress, aggregates and rouleaux are broken up. The effective volume fraction of the aggregates decreases and so does the relative viscosity of the suspension. In the first paper [13], we have proposed a microrheological model for weakly aggregated suspensions using the concept of fractal clusters. The rheological law assumes unpermeable aggregates and further involves a scaling exponent varying from \( m = 1/3 \) for rigid clusters to \( m = 1/2 \) for soft clusters.

We may consider red cell aggregates as soft clusters undergoing reversible deformation under the action of external stresses. Red cell clusters further may be described as scale invariant objects. The fractal dimension \( 1.5 < D < 1.6 \) of two dimensional red cell aggregates (Fig. 9 in [10]) is coherent with the predictions of the reaction limited aggregation model giving a fractal dimension \( D = 2 \) for three dimensional clusters.

4.4. Viscosity of Aggregated Red Cell Suspensions. — For reversible soft clusters \( (m = 1/2) \) of fractal dimension \( D = 2 \), the rheological law proposed in [13] then leads to a Casson like behavior:

\[
\sqrt{\tau} = \sqrt{\tau_0} + \sqrt{\mu_D \gamma} \left[ 1 - \frac{\phi}{1 - \phi} \left( \frac{\tau^*}{\tau} \right)^{1/2} \right]^{1/2}
\]

with

\[
\tau_0 = \tau^* \left( \frac{\phi_0^*}{\phi} - 1 \right)^{-2} \quad \text{for} \quad \phi > \phi_s
\]

where \( \mu_D \) is the viscosity of the disaggregated suspension, \( \tau_0 \) the shear yield stress and \( \tau^* \approx \Gamma/a \) a characteristic shear stress for cluster break-up.

At high shear stresses, red cells become oriented and aligned in the flow and then the viscosity behavior is governed by the viscoelastic properties of the particles. However, the transition between the flow regimes, either dominated by aggregation phenomena or particle deformation, is smooth since clusters and single particles may coexist at intermediate shear rates. We may nevertheless account for red cell deformation in (18) by considering the relative viscosity \( \mu_D/\mu_0 \) of a disaggregated suspension of viscoelastic particles given by (16):

\[
\frac{\mu_D(\phi, \gamma)}{\mu_0} = \frac{1 - \phi}{\left[ 1 - \frac{\phi}{\phi_0} \left( 1 + \frac{\mu_D(\phi, \gamma)}{\mu_0} \frac{\beta \lambda^{-1} (\theta_0 \gamma)^p}{1 + (\theta_0 \gamma)^p} \right)^{-1/2} \right]^2}
\]

Measurements of the diffuse reflectivity \( r_A \) of the red cell suspension in a shear flow provides a way for estimating the cell adhesive energy and the characteristic shear stress \( \tau^* \) for cluster break-up [12,22]. Cell aggregation results in a lower relative diffuse reflectivity \( r_A/\tau \) since the light is not scattered by the contact cell area \( (\tau \) is the diffuse reflectivity of the unaggregated suspension). The aggregation index \( G = 1 - r_A/\tau \) scales as the ratio \( \sigma_A/\sigma \) of the bridging area \( \sigma_A \) to the total scattering area \( \sigma \) per unit volume [22]. The progressive shear induced dispersion of red cell clusters into smaller ones then results in a decrease of the reflectometric index \( G(\tau) \) when increasing the effective shear stress \( \tau = \mu(\phi, \gamma) \gamma \) (Fig. 6). The critical disaggregation shear stress \( \tau_c \) defined in terms of extrapolated intercept was shown to be representative of the surface adhesive energy \( \Gamma \) without significant dependence upon particle volume fraction or cell deformability [22]:

\[
\tau_c \approx \frac{\Gamma}{a}
\]
For a dextran concentration of 2 g%, the critical disaggregation shear stress increases with dextran molecular weight (Fig. 6) indicating a higher surface adhesive energy.

We have measured the shear viscosity of normal red cells suspended in human plasma (Fig. 7) or in dextran saline solutions when varying the particle volume fraction (Fig. 8) and the polymer molecular weight (Fig. 9). The cells were previously dispersed by shearing at 130 s\(^{-1}\) and then allowed to relax for 3 minutes. Steady shear experiments were performed by increasing the shear step by step and waiting for steady state before the measurement.

The maximum packing fraction was derived from the reference law (6) and the viscosity around the zero shear rate limit of normal red cells in saline solution (Fig. 3). The critical disaggregation shear stress \(\tau_c\) was further determined from the reflectometric curves \(G(\tau)\) (Fig. 6). Taking \(\tau^* = \tau_c/600\), the nonlinear relations (18) and (20) well describe the shear rate dependence of the viscosity (Figs. 7-9).

However, the model overestimates the shear viscosity in the low shear rate regime (\(\gamma < 1\) s\(^{-1}\)) for high particle volume fractions (Figs. 7-8) and high adhesive energies (red cells in 2 g% dextran 80 - PBS, Fig. 9). The observed reduced viscosity may be explained by a shear induced restructuring of the aggregates or by the formation of a marginal layer free of particles near the viscometer walls. Both of these processes may occur for deformable particles and strong particle aggregation in agreement with the experimental observations. Cell deformability results in large contact area and bond energy between aggregated particles. Particle crowding and strong aggregation then favors a restructuring of the suspension because of a significant increase of the suspension internal energy. In the next section, we discuss the influence of restructuring processes upon the suspension rheology.

In contrast with the predictions of the model, the shear viscosity further keeps a nearly constant value in the low shear rate regime for weakly aggregated red cells in 2 g% dextran 40-PBS (Fig. 9). As discussed in the first paper [13], the disappearance of the yield stress may arise from the weak aggregation of particles and the finite lifetime of reversible clusters unable to reach the maximum size and to form an infinite spanning cluster. At high shear rates, the mean size of clusters is controlled by shear forces and then the rheological model describes the viscosity data (Fig. 9).
Fig. 7. — Relative shear viscosity \( \mu_r(\gamma) = \mu_A(\gamma)/\mu_0 \) against the shear rate for normal red cells suspended in human plasma \( (\mu_0 = 1.6 \text{ cP}) \) with a particle volume fraction \( \phi = 0.45 \). The solid curve is calculated from the relations (18) and (20) for soft clusters of fractal dimension \( D = 2 \) with \( \theta_0 = 5 \times 10^{-2} \text{ s} \), \( \phi_0^* = 0.605 \), \( \beta = 0.3 \), \( \lambda = \mu_1/\mu_0 \), \( \mu_i = 6 \text{ cP} \) and \( \tau^* = \tau_c/600 \). The critical disaggregation shear stress is determined from the reflectometric curves \( G(\tau) \) \( (\tau_c = 0.4 \text{ N m}^{-2}, \text{Fig. 6}) \) and the maximum packing fraction \( \phi_0^* \) is derived from the shear viscosity of unaggregated particles in the low shear rate regime (Fig. 3).

Fig. 8. — Relative shear viscosity \( \mu_r(\gamma, \phi) = \mu_A(\gamma, \phi)/\mu_0 \) against the shear rate for normal red cells suspended in saline solution \( (\bullet) \) \( (\mu_0 = 0.9 \text{ cP}) \) or in 1.2 g\% dextran70-PBS \( (\bigcirc) \) \( (\mu_0 = 1.2 \text{ cP}) \). The solid curves are calculated from the relation (18) and (20) for soft clusters of fractal dimension \( D = 2 \) with \( \theta_0 = 5 \times 10^{-2} \text{ s}, \beta = 0.3, \lambda = \mu_1/\mu_0, \mu_i = 6 \text{ cP} \) and \( \tau^* = \tau_c/600 \). The critical disaggregation shear is determined from the reflectometric curves \( G(\tau) \) \( (\tau_c = 0.2 \text{ N m}^{-2}, \text{Fig. 6}) \) and the maximum packing fraction \( \phi_0^*(\phi) \) is derived from the shear viscosity of unaggregated particles in the low shear rate regime (Fig. 3).
Fig. 9. — Relative shear viscosity $\mu_r(\gamma) = \mu_A(\gamma)/\mu_0$ against the shear rate for normal red cells suspended in saline solution (○) ($\mu_0 = 0.9$ cP), 2 g% dextran 40-PBS (●) ($\mu_0 = 1.2$ cP), 1.2 g% dextran 70-PBS (□) ($\mu_0 = 1.2$ cP) and 2 g% dextran 80-PBS (■) ($\mu_0 = 1.49$ cP) with a particle volume fraction $\phi = 0.45$. The solid curves are calculated from the relation (18) and (20) for soft clusters of fractal dimension $D = 2$ with $\theta_0 = 5 \times 10^{-2} s$, $\phi^*_0 = 0.605$, $\beta = 0.3$, $\lambda = \mu_r/\mu_0$, $\mu_r = 6$ cP and $r^* = \tau_c/600$. The critical disaggregation shear stress $\tau_c$ is determined from the reflectometric curves $G(\tau)$ ($\tau_c = 0.04$ N m$^{-2}$ for 2 g% dextran 40-PBS, $\tau_c = 0.2$ N m$^{-2}$ for 1.2 g% dextran 70-PBS, $\tau_c = 0.4$ N m$^{-2}$ for human plasma $\tau_c = 0.55$ N m$^{-2}$ and for 2 g% dextran 80-PBS). The maximum packing fraction $\phi^*_0$ is derived from the shear viscosity of unaggregated particles in the low shear rate regime.

In the case of highly concentrated suspensions ($\phi \approx \phi^*_0$), we may no longer neglect the viscous dissipation in the viscoelastic particles and the model becomes inadequate. The difficulty to predict the viscosity also arises from the quasi-crystal lattice structure of very concentrated suspensions. The rheological behavior is then dominated by the viscoelastic properties of the particles and the shear viscosity depends in a less extent on the aggregation phenomena (Fig. 10) since the characteristic size of the clusters is close from the particle radius.

4.5. SHEAR INDUCED RESTRUCTURATION OF RED CELL AGGREGATES. — The diffuse reflectivity of red cell suspension mainly depends on the scattering area $\sigma$ per unit volume and may give some insight about the structure of aggregates in a shear flow. The reflectometric index $G = 1 - r_A/r \approx \sigma_A/\sigma$ scales as the mean number $z_A$ of contacts around each particle (mean coordination number) and directly reflects the extent of red cell aggregation [13]. The aggregation index $G$ increases both with the size and the compactness of clusters.

Figure 11 shows the shear rate dependence of the equilibrium aggregation index $G(\gamma)$ for concentrated red cell suspended in dextran saline solutions. Samples were homogenised by shearing at 130 s$^{-1}$ before imposing a constant shear rate. For moderate and strong red cell aggregation (2 g% dextran 110-PBS and 2 g% dextran 500-PBS), we observe a significant increase of the equilibrium aggregation index $G(\gamma)$ in the intermediate shear regime $5 \times 10^{-4} < \gamma/\gamma_c < 10^{-2}$ which indicates a shear compaction of the aggregates ($\gamma_c$ is the characteristic shear rate for near complete cell dispersion). Hydrodynamic stresses may cause a particle rearrangement in the growing soft fragments and lead to the formation of aggregates of lesser porosity [46,47]. Bending and sliding of deformable cells over each other as well as particle adhesiveness are promoting factors in the restructuration of clusters.
The existence of such restructuration processes leads to time dependent and memory effects in the rheological behavior of strongly aggregated suspensions. The viscosity behavior indeed becomes strongly dependent upon the rheological history of the suspension. When decreasing step by step the shear rate, the flow induced compact aggregates persist at low shear stresses (Figs. 12a-13a) and then result in a low apparent viscosity (Figs. 12b-13b). For a strong adhesive energy (2 g% dextran 150-PBS), memory effects can reduce dramatically the apparent viscosity of the suspension (Fig. 13b). On the other hand, the viscosity at low shear rates is higher when the suspension is first dispersed in a rapid flow since a preshearing period eliminates any memory effects (Figs. 12-13).
Fig. 12. — Shear rate dependence of the relative viscosity $\mu_r(\gamma)$ and the equilibrium reflectometric index $G(\gamma)$ for red cells suspended in 2 g% dextran 80-PBS with a particle volume fraction $\phi = 0.45$. The suspension is allowed to relax for 5 minutes before imposing the flow and then the shear rate is either increased (○) or decreased (●) step by step, waiting 15 s before each measurement.

However, the time dependent torque in the low shear regime $\gamma < 1 \text{ s}^{-1}$ of red cell suspensions initially dispersed in a rapid flow often exhibits a complex response depending on the viscometer geometry [49]. Therefore, we have performed simultaneous optical and rheological experiments in a special rotational viscometer consisting of two coaxial cylinders, the larger of which is transparent and can rotate. After initial dispersion of the red cell suspension in a rapid flow, we impose a constant shear rate at time $t = 0$ and we record both the time dependent apparent viscosity $\mu(\gamma, t)$ and the time dependent diffuse reflectivity $r_A(\gamma, t)$ of the aggregating red cell suspension (Figs. 14-15). In the low shear regime, the apparent viscosity rises to a peak value and then slowly decreases while the aggregation index continuously increases before the suspension reaches a dynamical equilibrium (Figs. 14-15). The peak value of the torque always arises for an aggregation index $G_N \approx 0.45$ and probably results from the appearance of an infinite spanning network. The aggregation index $G \approx S_A/S \approx 0.48$ of an infinite linear aggregate or red cell rouleau is indeed close from the critical index $G_N \approx 35 \text{ $\mu$m}^2$ is the adhesive area between aggregated cells and $S \approx 145 \text{ $\mu$m}^2$ the red cell area).

We can expect a synaeresis process under the action of shear forces (contraction of the
network) and a movement of particles away from the viscometer surfaces soon after the formation of an infinite network especially for deformable particles and strong adhesive energies. Both the restructuration phenomena (formation of less porous clusters) and the simultaneous appearance of a marginal layer clear of particles near the walls then result in the observed decrease of the apparent viscosity (the shear gradient mainly develops in the fluid layer with low viscosity). As evidenced by Thomas [48] in narrow capillary tubes or by Cokelet et al. [49] in a Couette viscometer, the apparent viscosity of aggregated red cell suspensions in the low shear regime ($\gamma < 1 \text{ s}^{-1}$) then may depend on geometric factors such as tube diameter or gap width.

We may nevertheless consider an homogeneous medium and use the rheological model for moderate red cell adhesiveness since the shear restructuration of clusters and the formation of a marginal layer by contraction of the spanning network remain negligible. For a strong aggregation of deformable particles which is the case of some pathological blood, low shear viscosity measurements are strongly dependent upon the rheological history of the suspension and the viscometer geometry.
Fig. 14. — Plot of the reflectometric index $G(t, \gamma)$ versus the apparent shear viscosity $\mu_r(t, \gamma)$ for red cells suspended in 2 g% dextran 80-PBS with a particle volume fraction $\phi = 0.45$. After initial dispersion of the suspension in a rapid flow (○), we impose a constant shear rate $\gamma$ and then we record the time dependence of both the viscosity and the diffuse reflectivity until a dynamical equilibrium is reached (●). a) $\gamma = 0.025 \text{ s}^{-1}$, b) $\gamma = 0.08 \text{ s}^{-1}$, c) $\gamma = 0.3 \text{ s}^{-1}$, d) $\gamma = 0.56 \text{ s}^{-1}$, e) $\gamma = 0.8 \text{ s}^{-1}$, f) $\gamma = 2 \text{ s}^{-1}$, g) $\gamma = 5 \text{ s}^{-1}$, h) $\gamma = 10 \text{ s}^{-1}$

Fig. 15. — Plot of the reflectometric index $G(t, \gamma)$ versus the apparent shear viscosity $\mu_r(t, \gamma)$ for red cells suspended in 5.5 g% dextran 500-PBS with a particle volume fraction $\phi = 0.45$. After initial dispersion of the suspension in a rapid flow (○), we impose a constant shear rate $\gamma$ and then we record the time dependence of both the viscosity and the diffuse reflectivity until a dynamical equilibrium is reached (●). a) $\gamma = 0.012 \text{ s}^{-1}$, b) $\gamma = 0.031 \text{ s}^{-1}$, c) $\gamma = 0.06 \text{ s}^{-1}$, d) $\gamma = 0.11 \text{ s}^{-1}$, e) $\gamma = 0.21 \text{ s}^{-1}$, f) $\gamma = 0.77 \text{ s}^{-1}$, g) $\gamma = 3.8 \text{ s}^{-1}$, h) $\gamma = 19 \text{ s}^{-1}$

On the other hand, no significant memory effects and restructuring processes are observed for hardened red cell clusters in the low shear regime [13]. Aggregated rigid particles only establish contact points during adhesion and the low internal energy variation of the suspension then prevents a restructuring process.
5. Conclusion

The motion of deformable particles in a shear flow was described by a Kelvin Voigt model. The shear viscosity of a collection of deformable particles was derived from the concept that the deformation-orientation of particles enhances the maximum packing fraction of particles. A self consistent theory using a reference rheological law and an effective medium approximation then predicts the viscosity of concentrated suspensions of viscoelastic particles such as red cells in saline solution and allows information about the nonlinear viscoelastic properties of the cell membrane. For weakly aggregated suspensions of deformable particles, aggregation phenomena dominate the rheological behavior in the low shear regime. A rheological law for fractal clusters taking into account the deformation-orientation of particles in the high shear regime well describes the viscosity data for aggregated red cells suspensions. However, particle deformability and adhesiveness strongly favors a shear induced restructuration of clusters and a contraction of the suspensions network in the low shear regime. The formation of less porous aggregates and the simultaneous appearance of a region free of particles near the viscometer walls then result in a marked decrease of the suspension apparent viscosity at low shear rates.

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