

A viscoelastic model of shear-induced hemolysis in laminar flow

G. Arwatz* and A.J. Smits

Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ, USA

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Abstract. We present measurements of human blood hemolysis caused by laminar shear stresses ranging from 50 to 500 Pa for exposure times extending from 60 to 300 s using a Taylor–Couette device. A viscoelastic model is proposed that captures the response of the red blood cells to shear stress. The model is based on well-established mechanical properties of the red blood cell membrane, and shows good agreement with data from the experiments presented here, as well as data from the existing literature. Two characteristic time scales are identified: a fast time scale corresponding to the relaxation time of the red blood cell membrane and a slow time scale that represents the onset of plasticity and is related to hemoglobin release from a damaged cell. The model proposed here collapses the available data over almost five orders of magnitude in exposure time and shear stresses up to 500 Pa.

Keywords: Hemolysis, Kelvin–Voigt, Couette flow, blood experiment, erythrocyte

1. Introduction

Mechanical damage of red blood cells (hemolysis) imposes a high risk during hemodialysis and is a key concern in the design of medical devices such as the extracorporeal heart-lung machine, dialysis machines and implantable vascular prostheses. Often, it is a limiting factor in the duration of a critical procedure. The mechanisms responsible for blood damage have been studied since the mid-1960s and it is now widely accepted that both shear stress and time of exposure play important roles [9,10]. High shear stresses can result in premature breakdown of the red blood cells, and since hemoglobin is released into the surrounding plasma during the breakdown, the most common measure of red blood cell damage due to shear stress is the hemolysis percent ($\Delta Hb/Hb$), where ΔHb is the change in plasma hemoglobin, and Hb is the hemoglobin of the whole blood.

It is sometimes held that hemolysis can be described as a power law of shear stress and exposure time. For example, Blackshear et al. [2] proposed that $\tau_t \sqrt{\Delta t} = \text{const}$, where τ_t is the threshold shear stress for hemolysis and Δt is the exposure time, while Giersiepen et al. [9] suggested that for short exposure times:

$$\frac{\Delta Hb}{Hb} = 3.62 \times 10^{-7} \tau^{2.416} \Delta t^{0.785}. \quad (1)$$

*Address for correspondence: Gilad Arwatz, Mechanical and Aerospace Engineering, Engineering Quad Room D214, Princeton, NJ 08544, USA. Fax: +1 609 258 6109; E-mail: garwatz@princeton.edu.

Earlier, Heuser et al. [10] had suggested a similar relationship, where:

$$\frac{\Delta Hb}{Hb} = 1.8 \times 10^{-6} \tau^{1.991} \Delta t^{0.765}. \quad (2)$$

However, these power laws are limited to narrow ranges of shear stress and exposure time. In addition, since they are purely empirical, they do not take into account the mechanical properties of the red blood cell and lack any physical basis.

The current study proposes a new model that agrees well with a wide range of experimental data and relates hemolysis to the physical properties of red blood cells.

To develop a more physical model, we note that the cell membrane consists of an outer lipid bilayer, a spectrin network and transmembrane proteins. The area expansion modulus of the cell membrane is controlled mostly by the lipid bilayer, while the shear modulus is governed by the elastic properties of the cytoskeleton. The viscous properties of the cell are due to glycoproteins in the lipid bilayer [5,23]. The cell membrane is conventionally treated as a two-dimensional incompressible viscoelastic thin layer in which the tension is defined in terms of the mechanical properties. It is known that the RBC membrane can support an estimated surface strain of a few percent before rupture [1].

Rand [15] suggested that hemolysis occurs at a critical strain of the cell membrane rather than a critical stress. He also proposed a rheological mechanical model that fitted his data, where he used a Burger model. His model consists of a series arrangement of a purely elastic element represented by a spring, a purely viscous component represented by a damper (dashpot) and a parallel arrangement of each, as shown in Fig. 1. According to this model, the strain in the membrane for a constant applied shear stress is expressed by:

$$S = \tau \left[\frac{1}{G_2} + \frac{1}{G_1} (1 - e^{-(G_1/\eta_1)t}) + \frac{1}{\eta_2} t \right], \quad (3)$$

where τ is the applied shear stress, G is an elastic modulus (originally, Rand used the notation Y as the Young modulus instead of G) and η is a viscosity [15].

The mechanical properties of the red blood cell membrane have been investigated in depth and values for the shear modulus, area expansion modulus, and membrane viscosity are available (see, for example, Wan et al. [23]). However, the relationship between these properties and hemolysis is not well established and the mechanism by which the hemoglobin is released under stress has not yet been determined. Some studies suggest that hemolysis is linked to a change in the permeability of the cell membrane as a function of intensity and duration of loading, as well as the mechanical properties of the membrane [12].

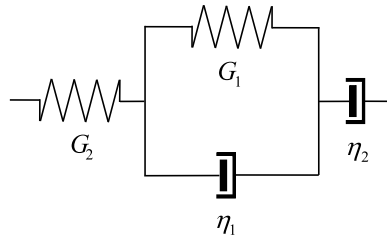


Fig. 1. Mechanical model of the cell membrane used to describe the membrane strain used by Rand et al. [15]. The model consists of a series arrangement of a spring, a damper and a parallel arrangement of each, also known as Burger model.

Others assume hemolysis occurs as a result of the membrane rupture under stress, and attribute the monotonic dependence on time to age distribution among the cells [24] (red blood cells normally live for 110–120 days, but as the cells age their membranes become brittle and eventually break down).

These inconsistencies and general lack of understanding emphasize the need for a new approach that will provide a more physical understanding of hemolysis. Here, we present measurements of the hemolysis in human blood caused by laminar shear stress ranging from 50 Pa to 500 Pa and exposure times extending from 60 s to 300 s. The experiments are then used to develop a general viscoelastic model based on an extension of the work by Rand [15].

2. Materials and methods

Fresh blood with the anticoagulant heparin (16.1 USP/ml) was obtained from two healthy adult human males. Blood was purchased through Lampire Biological Laboratories where the subjects gave permission for their blood to be drawn and used for research purposes. This work was approved by the Princeton University Institutional Biosafety Committee.

All studies were completed within 72 hours of phlebotomy. The blood was transported in thermo-insulated cases and stored at 4°C in sealed air tight bags and brought to room temperature before the experiment. Hematocrit of the two subjects was 48% and 46% respectively and pH levels were 7.3 and 7.4 respectively. The degree of hemolysis was measured at the beginning of every experiment to ensure that blood was not damaged during storage and handling.

Prior to the hemolysis experiments, the blood viscosity was measured using an Anton Paar MCR rheometer. Figure 2(a) presents data of shear rate versus shear stress for shear rates greater than 100 s⁻¹. The slopes of the graph represent the viscosity of each blood sample, and as expected for these high shear rates the curves are linear (Newtonian fluid behavior).

Since the desired levels of shear stress cannot be generated by an ordinary rheometer, a Taylor–Couette apparatus was designed and built. The inner cylinder of radius R_1 was rotated at a constant angular velocity ω while the outer cylinder was held fixed. The experiments were confined to shear rates greater than 1000 s⁻¹ so that the blood behaves as a Newtonian fluid with a viscosity μ and density ρ . When the gap δ is small compared to R_1 , as is the case in all the experiments presented here, the velocity profile in the gap is linear and the shear stress is given by $\tau = \mu R_1 \omega_1 / \delta$. Laminar flow was maintained by keeping the Taylor number below 1706 [22].

Based on these considerations, the gap was chosen to be 0.1 mm, R_1 was set to 40 mm, and the height of the cylinder was fixed at 80 mm. A direct drive DC motor was used to apply the required angular torque. Two bearings were used to prevent vibration and to ensure a concentric rotation. One bearing was placed at the bottom of the cup and the other on top above the blood level. Consequently, the radial tolerance of the gap was less than 6 μ m. The lower bearing was exposed to the blood, but its effect on blood damage was minimized by using a sealed bearing filled with high-speed grease that prevented the blood from flowing through the bearing. By measuring blood damage at different heights along the cylinder for different exposure times, it was verified that the effect of the bearing was negligible.

A substantial increase in temperature may occur at high shear rates, and increasing temperature will increase hemolysis. Prior studies have shown that temperatures below 37°C will not cause hemolysis, temperature above 37°C will cause slow hemolysis (less than 0.2% per hour), while temperatures above 45°C will cause fast and significant hemolysis and fragmentation of red blood cells [8]. To avoid these effects, the apparatus was equipped with a water based cooling system such that the temperature was

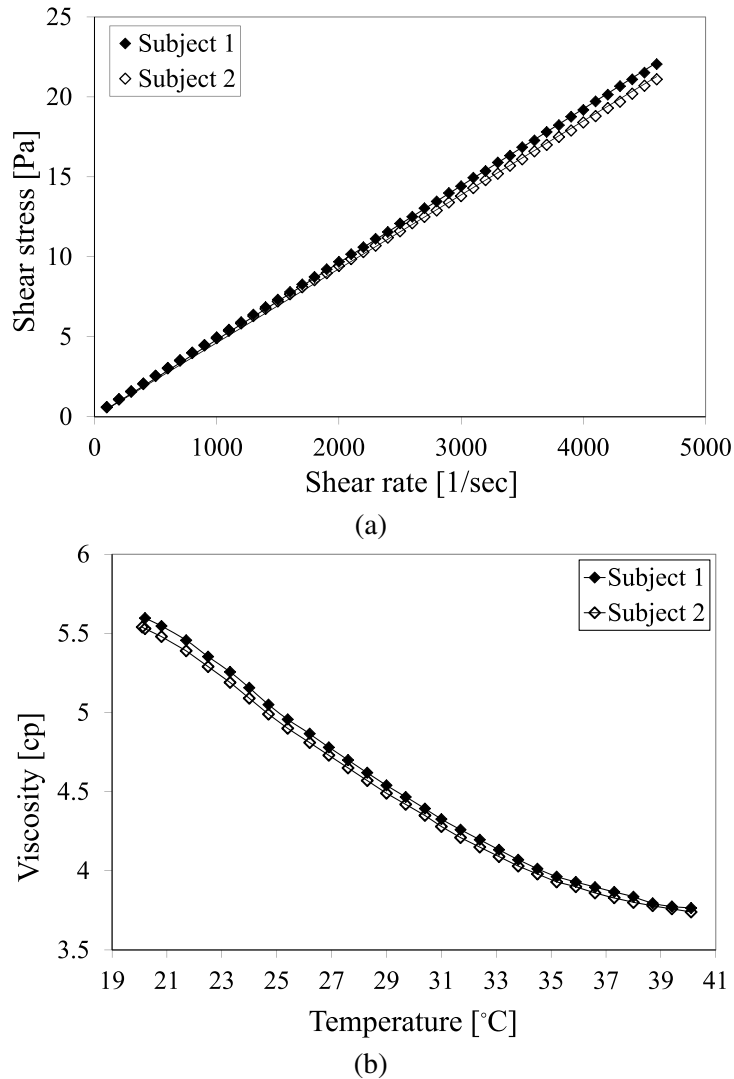


Fig. 2. (a) Shear stress versus shear rate for the two subjects with linear trend lines, $T = 25^{\circ}\text{C}$. (b) Viscosity versus temperature at shear rate of 3000 s^{-1} . This data was used to maintain constant shear stress by compensating for changes in viscosity due to small temperature fluctuations.

controlled within $25 \pm 2^{\circ}\text{C}$. However, even a small temperature increase due to shear will affect the viscosity and hence the applied shear stress. Therefore, the temperature was monitored during the experiment and the shear stress was controlled within $\pm 5\text{ Pa}$ by compensating for the change in blood viscosity due to changes in temperature by using the data presented in Fig. 2(b).

For each data point, blood was allowed to come to room temperature before it was injected into the bottom of the Taylor–Couette apparatus so as to exclude the formation of air bubbles. Blood was injected using a 14 gauge syringe (nominal inner diameter = 1.6 mm) at a flow rate of 60 ml/min. At this flow rate and syringe size, hemolysis from the injection process is negligible [19]. The inner cylinder was then accelerated to the required angular velocity (calculated from the desired shear stress) and maintained for the specified time period. The acceleration and deceleration times were 5 s. At the end of the experiment,

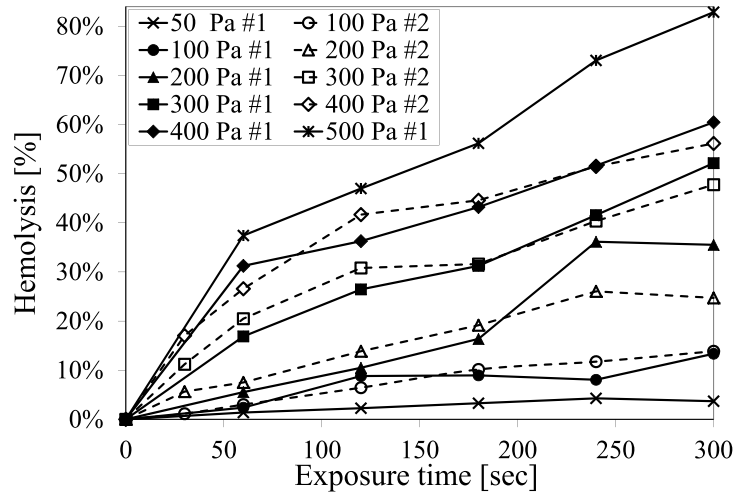


Fig. 3. Hemolysis percent vs. exposure time for different shear stresses for both subjects. Each data point is an average of three measurements with standard deviation smaller than 20%.

samples of blood were withdrawn through a 14 gauge syringe at the mid-height of the inner cylinder to avoid any possible end effects.

The hemoglobin concentration of each sample was measured using spectrophotometry. The total hemoglobin concentration was measured by mixing the blood with Drabkin's reagent and Brij 35 solution. Due to the difference in osmotic pressure, the red blood cells lyse and release their hemoglobin to the plasma. Measuring the hemoglobin concentration of the plasma then gives the whole blood hemoglobin concentration. The amount of hemoglobin released to the plasma during the experiment was calculated by subtracting the initial hemoglobin concentration in the plasma from the hemoglobin concentration that was measured after exposure to shear stress. Hemolysis was then determined by normalizing the change in plasma hemoglobin with the whole blood hemoglobin concentration.

All hemolysis experiments were performed at shear rates $> 10,000 \text{ s}^{-1}$ (shear stresses $> 50 \text{ Pa}$). At these shear rates, the amplitude of inclination oscillations of the red blood cells is constant and small (around 1 degree) [20]. In addition, red blood cell aggregation and cell tumbling do not affect the cell behavior [20]. In fact, at these shear stresses, the cell reaches an equilibrium ellipsoidal shape with its major axis aligned in the direction of flow while the membrane undergoes a tank-treading motion around the cell body [7].

The results are shown in Fig. 3. As expected, hemolysis generally increases with exposure time and shear stress. Also, some hemolysis occurs even at a shear stress as low as 50 Pa, increasing slowly with exposure time. This observation is in contrast to some previous studies where no damage to red blood cells was found to occur below a certain threshold shear stress. This point is further discussed in Section 4.

3. Viscoelastic model

The response of a viscoelastic material to stress is characterized by a combination of elastic and viscous behaviors. The observed time dependence is conventionally replicated through mechanical models,

and although these models can reproduce the phenomenological behavior of a viscoelastic material, they do not usually connect to the mechanical properties underlying this behavior. Here we aim to develop a mechanical model for the hemolysis behavior shown in Fig. 3, which also incorporates the mechanical properties of red blood cells.

A simple model that exhibits a viscoelastic behavior consists of a purely elastic spring in parallel with a damper. This combination is called the Kelvin–Voigt model. By applying Hooke’s law of elasticity and Newton’s law of viscosity, the stress is then given by

$$\tau_{ij} = GS_{ij}(t) + \eta \frac{dS_{ij}(t)}{dt}, \quad (4)$$

where S is the strain, G corresponds to the elastic constant of the spring and η is a viscosity corresponding to the damper. Equation (4) applies to the shear stress and the normal stress.

If the Kelvin–Voigt model is subjected to a constant shear stress τ , the solution to Eq. (4) is

$$S = \frac{\tau}{G}(1 - e^{-t/\tau}), \quad (5)$$

where, in this case, G represents the shear modulus (Pa). The time constant $T = \eta/G$ observed in the exponential is known as the relaxation or retardation time and it represents the time needed for the strain to fall to $1/e$ of its original value if the stress was suddenly removed. It is important to note that this exponential form is rarely observed and pertains only to very simple cases. In order to extend this model into a more realistic representation of viscoelastic behavior, a series arrangement of Kelvin–Voigt models is often considered.

In his experiment, Rand measured the time for hemolysis to happen in a micropipette device, therefore capturing a single cell breakdown, by assuming that hemolysis is the rupture of the membrane which occurs at a critical strain [15]. He then used a Burger model (Fig. 1) where the Kelvin–Voigt unit was used to mimic the viscoelastic behavior of the membrane, and the damper element in series was used to capture the purely viscous behavior at large exposure times. The addition of a purely elastic element in series was necessary to fit the data, adding the undesirable effect that the strain is not zero at $t = 0$. This model can predict the time for rupture under a constant stress for a given critical strain, and therefore it does not give measure of hemolysis in the form of $\Delta Hb/Hb$.

Based on these considerations, we expect that hemolysis is closely related to strain, and so we will follow Rand’s approach to model hemolysis as a function of stress and time, implementing the changes that are required to satisfy certain physical constraints. One important observation is the presence of two time scales in some experiments. For example, Chien et al. [4] measured deformation of red blood cells under tension and observed a fast response of the order of a hundred milliseconds, followed by a slower response on the order of a few seconds. Evans and Hochmuth [5] also found similar time scales and associated them with elastic and plastic deformations. This feature can also be observed in hemolysis by comparing different experiments with different time scales. For instance, Giersiepen et al. [9] reported 2.5% hemolysis for an exposure time of 0.1 s at 255 Pa, while our data show that at 60 s and 200 Pa hemolysis is around 10%. Therefore, it appears that a substantial part of the damage happens during the first 100 ms, perhaps by as much as a quarter of the damage found after 60 s exposure to shear. In addition, a physical model of hemolysis should capture the fact at $t = 0$ hemolysis is zero. Hence, we incorporate a damper in parallel with the purely elastic element in Rand’s model (Fig. 1), and therefore

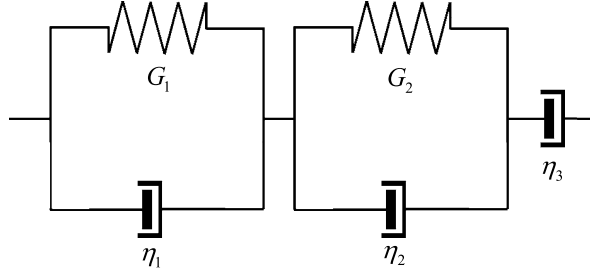


Fig. 4. Mechanical model of the cell membrane suggested in this study and used to describe hemolysis. The model consists of two Kelvin–Voigt units and a damper in a series arrangement. This particular arrangement satisfies the necessary physical considerations of hemolysis.

we suggest a model for hemolysis that consists of two Kelvin–Voigt models in series with a purely viscous element (Fig. 4). When subjected to a constant stress, the strain for this model is given by

$$S = \tau \left[\frac{1}{G_1} (1 - e^{-t/T_1}) + \frac{1}{G_2} (1 - e^{-t/T_2}) + \frac{t}{\eta_3} \right], \quad (6)$$

where $T_1 = \eta_1/G_1$ and $T_2 = \eta_2/G_2$.

Our data cover exposure times starting at 60 s, the data of Giersiepen et al. [9] are used for short exposure times (these data were used to derive the well-established power law for short exposure times given by Eq. (1)), and the data of Paul et al. [14] are used for slightly longer exposure times. This combined data set closely follows the trend given by Eq. (6). Therefore, we propose:

$$\frac{\Delta Hb}{Hb} = A\tau \left[\frac{1}{G_1} (1 - e^{-t/T_1}) + \frac{1}{G_2} (1 - e^{-t/T_2}) + \frac{t}{\eta_3} \right]. \quad (7)$$

By fitting the combined data to the theoretical model, we find T_1 is remarkably close to the reported values of the red blood cell relaxation time, 0.1 s. We suggest, therefore, that the first Kelvin–Voigt model incorporates the viscoelastic properties of the membrane, and consequently we use values reported in the literature for the characteristic time and shear modulus $G_s = 10^{-6}$ N/m [23], where $G_1 = G_s t_m$ and t_m is the membrane thickness taken to be 100 Å. As to the value of G_2 , previous studies showed that the shear modulus does not significantly change during deformation, and therefore the viscosity is the main contributor to the difference in time scales. Therefore, we assume $G_1 = G_2$. As to the second time constant, previous investigations have reported values of 5–20 s [4,5,15,17]. By choosing $T_2 = 20$ s, the data collapse well with $\eta_2 = \eta_3 = 2 \times 10^4$ Pa · s, which is equivalent to a surface viscosity $\eta_s = \eta t_m$ of 2×10^{-4} N · s/m. Hence our model for hemolysis reduces to

$$\frac{\Delta Hb}{Hb} = \frac{A\tau}{G} \left[2 - (e^{-t/T_1} + e^{-t/T_2}) + \frac{t}{T_2} \right]. \quad (8)$$

Evans and Hochmuth [5] found that for large deformation of red blood cells the surface viscosity η_s increases from 10^{-6} to 10^{-5} N · s/m for $t_m = 100$ Å. Our model shows the same trend with η_2 being one order of magnitude larger. The proportional constant A is equal to 0.1 for the best fit.

The model as presented originally in Eq. (7) had six unknown parameters: A , G_1 , G_2 , T_1 , T_2 and η_3 . As we saw, the parameters G_1 and G_2 can be obtained from the literature, and the two time constants

T_1 and T_2 can be related to the physical mechanisms of the cell rupture, as will be discussed further. In addition, it was shown that $\eta_2 = \eta_3$, so that the final model presented in Eq. (8) contains only one empirical parameter, A .

The comparison of the model with our data is given in Fig. 5, where the average hemolysis deviation is shown to be about 3.5%. To see the comparison at short exposure times, the same graph is given in log-log form in Fig. 6 including both our data and data from the literature. Heuser et al. [10] measured hemolysis using a Taylor–Couette device with axial flow for different shear rates, and the results for a viscosity of 4.5 cP also show good agreement with our model. In both Figs 5 and 6, the ordinate

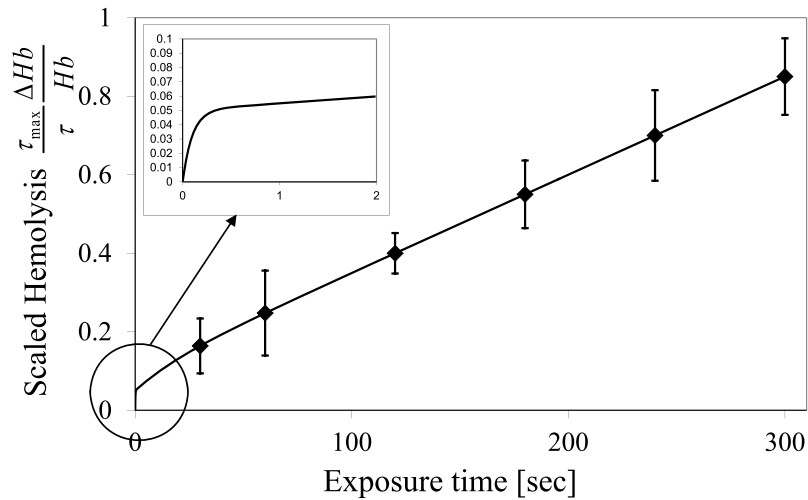


Fig. 5. Scaled hemolysis versus exposure time, where $\tau_{\max} = 500$ Pa, is the maximum shear stress used in our experiment. Symbols represent our data, and vertical lines are mean absolute deviation. Solid line is the theoretical model, Eq. (8). The inset presents the behavior of the model at short exposure times.

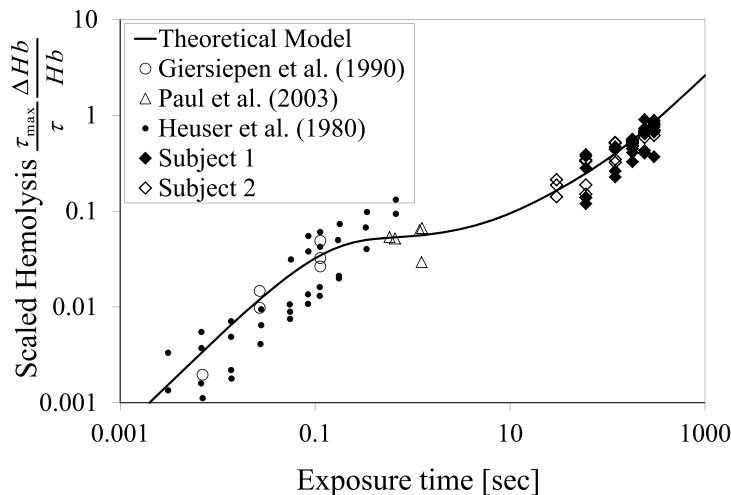


Fig. 6. Scaled hemolysis versus exposure time, where $\tau_{\max} = 500$ Pa, is the maximum shear stress used in our experiment. The figure shows good agreement between the model (solid line) and the experimental that includes our data and data from the literature [9,10,14].

represents hemolysis scaled with shear stress and therefore these figures show the collapse of our full data set (Fig. 3) in time and shear stress.

Note also that, since hemolysis $\Delta Hb/Hb$ cannot exceed 1.0, our model suggests the following relation for maximum hemolysis at a given shear stress and exposure time:

$$\tau = \frac{G}{A} \left[2 - (e^{-t/T_1} + e^{-t/T_2}) + \frac{t}{T_2} \right]^{-1}. \quad (9)$$

4. Discussion

Since our model was constructed to fit experimental data, we need to be careful in relating it to the physical mechanisms underlying hemolysis. As previously mentioned, some studies have suggested that hemolysis is the result of membrane rupture and the observed dependence on exposure time and shear stress can be explained by age distribution among the cells [24]. More specifically, as the red blood cells age, the mechanical properties change, for example Sutura et al. [21] found that the relaxation time increases by about 20% with age. However, this mechanism alone cannot explain the significant variation in time scales observed in our experiment.

We note that the model suggests that hemolysis is governed by two different time scales regimes. The first regime is related to the short time scale where the red blood cells deform under stress and release part of their hemoglobin content, which could be related to the development of a membrane rupture or a change in membrane permeability. The former can be supported by Lister [12] who describes a model for membrane failure by the creation of a hole in an incompressible membrane. The second regime, governed by the larger characteristic time scale and the viscous element in the model, represents the decay of the elastic behavior. The viscous element could represent the irreversibility (plasticity) of the process that has limited effect at small time scales. This response supports the notion that the membrane failure does not necessarily involve a complete breakdown of the cell but rather the formation of an opening through which hemoglobin escapes on a long time scale.

Although the approach presented in this paper for modeling viscoelastic material replicates its phenomenological behavior, it does not directly connect to the molecular structure underlying this behavior. In an attempt to relate our model to the structure of the red blood cell and specifically the red blood cell membrane, one can consider a simple two-layer model consisting of the lipid bilayer and the spectrin network. It is known that the lipid bilayer is associated with the viscous component while the elastic modulus is primarily dictated by the response of spectrin network to the applied load [3,6]. Therefore, the two time constants in our model reflect the change of the mechanical properties of the two layers under load. However, at this stage there is no justification to connect specific aspects of the model and the molecular structure of the cell.

We now turn to the concept of a shear threshold below which no hemoglobin release is observed. In the past, the relation between hemolysis and exposure time was not well established. Some early investigations performed their experiments for a single exposure time and therefore reported a critical shear stress without considering time dependence [11,16], while Blackshear et al. [2] reported the hemolysis threshold to be $\tau_t \sqrt{t} = \text{const}$. Later, it became apparent that hemolysis and hemolysis threshold are coupled with shear stress and time, and studies started to report critical shear stress accompanied with exposure time [13,18]. The use of the term threshold is therefore misleading. By definition, a threshold is an absolute value of shear stress for which no hemolysis is observed for any given exposure time. Since

we believe that hemolysis is related to strain, one would think that hemolysis should start at a critical strain. Although our experiments did not show any threshold, we propose that the onset of hemolysis may be described in a form similar to Eq. (6), which represents the strain of the mechanical model that fits hemolysis. However, further investigations are needed to support this suggestion.

5. Conclusions

An experimental investigation was conducted to determine the influence of increasing laminar shear stress and exposure time on red blood cell damage. Although hemolysis has been studied for four decades, the available data is often limited to a narrow range of shear stress and exposure time. This study provides data for hemolysis caused by laminar shear from 50 to 500 Pa and exposure time from 60 up to 300 s extending the existing data and previous models that were based on smaller exposure times.

A viscoelastic strain-based model was derived to satisfy necessary physical conditions and to fit this large range of data. In contrast to previous studies, our model is based on physical reasoning and well-established properties of the red blood cell. The model consists of two Kelvin–Voigt models in series with a purely viscous element and therefore exhibits two characteristic time scales. The first time scale (0.1 s) agrees well with the reported values of the relaxation time of the red blood cell membrane. Based on previous studies, the second time constant represents the onset of plasticity and is related to hemoglobin release from a damaged cell. This time constant was chosen to be 20 s, agreeing well with our data and previous studies. As suggested by prior investigations, the shear modulus does not significantly vary during deformation. This feature is supported by our model and agrees well with the data. As a result, our model [Eq. (8)] has only one empirical parameter, A . By fixing $A = 0.1$, the model collapses the available data over almost five orders of magnitude in exposure time and stress up to 500 Pa. In addition, the model offers a relation between shear stress and exposure time for maximum hemolysis [Eq. (9)].

Our experiment did not show any hemolysis threshold and hemolysis was observed for stress levels as low as 50 Pa, monotonically increasing with exposure time. Our model suggests that if it exists, a hemolysis threshold should be related to a critical strain rather than shear stress.

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