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Shear rate induced viscosity change of human blood samples and blood mimicking fluids

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Purpose: The aim of this study was to measure blood and blood mimicking fluids viscosity at different shear rates (on the interval of 0.1–5000 1/s and 0.1–10000 1/s) while taking into consideration the measuring device's capability and blood's characteristics. We also provided the measurement results of the most accurate measuring program. *Methods*: We measured blood samples from five donors, and four different blood mimicking fluid compositions. The measurements were done on an Anton Paar Physica MCR301 rotational rheometer with two measuring programs varying in the shear rate intervals, the number of measuring points and the measuring point durations. *Results*: The results confirmed the significant shear thinning and thixotropic effects of blood. Blood mimicking fluids also had these characteristics. The measured blood viscosity values are in agreement with those of the literature. *Conclusions*: It can be concluded that the step test program was able to give more stable results as the measured torque was over the nominal limit of 0.05 µNm over 0.1 1/s and over the selected torque limit of 0.5 µNm over 31.6 1/s. Blood mimicking fluid measurement results were different from that of the literature due to different measuring conditions. The sample consisting of water, glycerol and starch mimicked well blood's behaviour and viscosity values at 37 degrees Celsius.

Key words: *blood, viscosity, measurements, rheology, thixotropy, blood mimicking fluids*

1. Introduction

Cardiovascular diseases are the leading cause of death worldwide, according to the WHO [20]. To help prevent and cure these diseases, researchers worldwide have studied blood's behaviour for hundreds of years. The blood's composition is well known, however, its behaviour under shear stress still holds questions. Blood's behaviour is particularly important as shearing force serves as an endothelial regulator [11]. The study of blood flow is difficult because of the complex nature of blood. It consists of plasma, approximately 55%, and formed elements [15]. Plasma is an aqueous solution of molecules of 8–9% by weight. Plasma proteins' size and asymmetric shapes increase plasma viscosity, and fibrinogen plays a key role in red blood cell aggregation resulting in thixotropic – time dependent shear thinning – effect [8]. The importance of formed elements should be discussed in two aspects, regular flow [9], and microcirculation [7]. The viscosity of whole blood, therefore, the regular flow is mainly affected by red blood cells. Hematocrit, the percentage by volume of RBC-s ranges from 40 to 54% in men and from 36 to 48% in women [2] so its rheological effects are significant. RBC-s are approximately 7 µm in diameter and 2 µm in thickness [13] (Fig. 1b). They have a biconcave disc shape [18] (Fig. 1a), which is maintained by osmosis. They flexibly deform and extend under shear, and the highly concentrated hemoglobin solution in them affects the speed of deformation [14]. Other formed elements do not have a significant effect on regular flow. In microcirculation, the structure of the vessel walls changes, the elasticity is replaced by muscle contractions [19]. Endothelial regulators change the lumen as a function of shear rate for short term adaptation [19]. White blood cells (leukocytes) are the main cause of major properties of the

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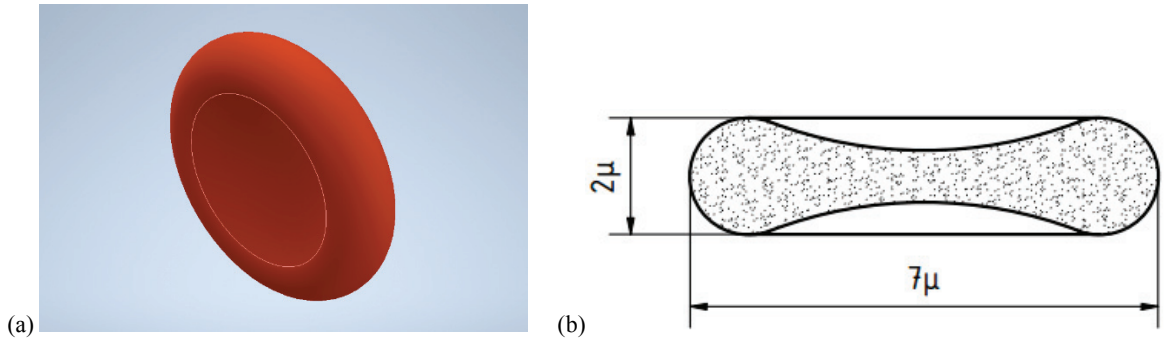


Fig. 1. (a) Schematic figure of the biconcave shape of red blood cells, (b) approximate dimensions of red blood cells

flow in microcirculation [10]. In blood vessels of magnitude of the cells, the cells' size and properties and the plasma viscosity determine the flow rather than the viscosity of whole blood. White blood cells are more viscoelastic and are larger than RBC-s [5], so even though there are magnitudes less white blood cells than red blood cells [14], they play an important role. The flow is promoted by the sliding of cells on the glycocalyx [17].

Due to the small size and volume of platelets, their role is rheologically negligible.

Blood is not widely accessible for researchers, so there is a large demand for reliable blood mimicking fluids (BMF) to use for medical simulations. A promising fluid was developed by Perrira et al. [12]. It is a solution of xanthan gum and starch in glycerol and water mixture (60:40% w/v). According to their study, out of 10 measured samples of different percentages by weight, the one with 0.01% w/v Xanthan gum and 0.01% w/v starch was the closest to blood's behaviour. However, the study investigated the materials' behaviour at 25 degrees Celsius, whereas the *in vivo* temperature is 37 degrees Celsius.

In studies of blood flow, measurement results are rarely provided. The measuring process and conditions are unclear in most cases. The aim of this study was to measure blood viscosity respecting blood's unique characteristics. A further aim was to provide accurate results of blood's viscosity change induced by shearing force, and to compare blood's and blood mimicking fluids' viscosity behaviour.

2. Materials and methods

2.1. Measuring devices

Measurements were carried out on an Anton Paar Physica MCR 301 rotational rheometer (Serial number: SN80748717 FW3.40D090210) with a cone plate of diameter 24.978 mm (Serial number: SN80866510--80887454). The measuring gap should be set at 4–5 times the size of the measured particles, so it was set to 0.054 mm (Fig. 2).

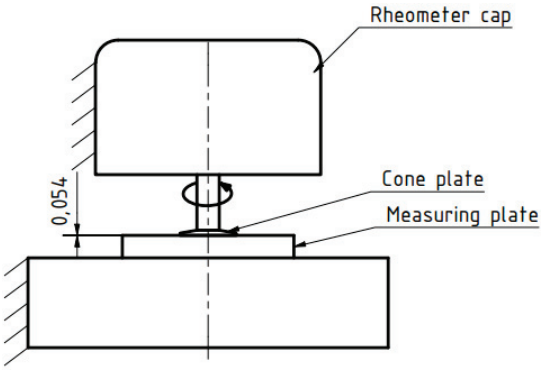


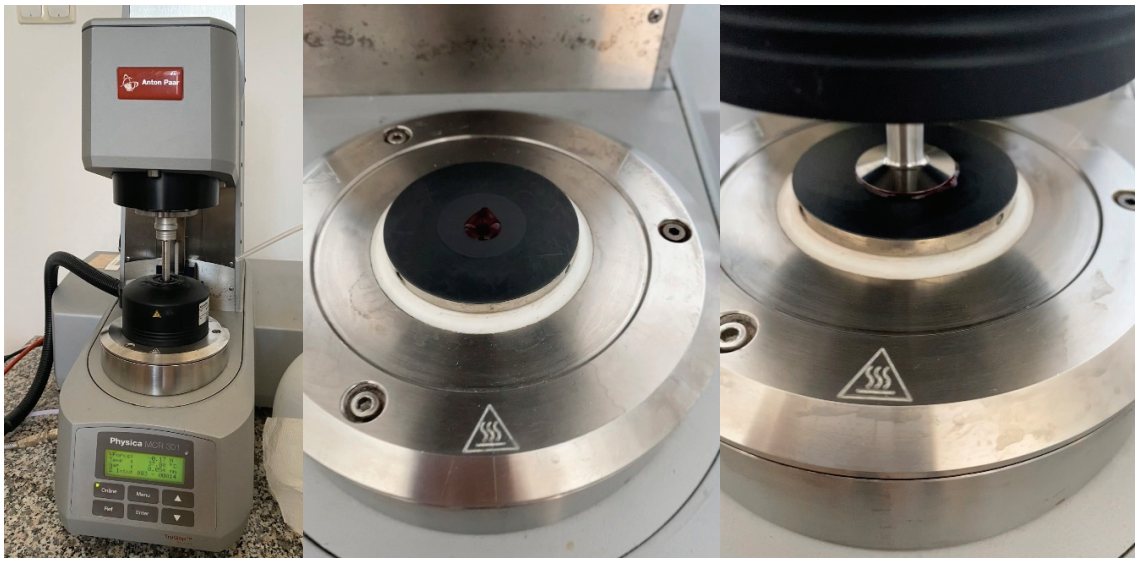
Fig. 2. Schematic figure of the measurement arrangement on an Anton Paar rheometer (dimensions are given in mm)

The measured data was processed with the Anton Paar Rheoplus software [1].

Transferpette digital pipette with disposable tips was used to load the samples in the rheometer to eliminate the opportunity of contamination and to measure the right amount of blood. 120 µl of sample was used per test (Fig. 3b, c).

Temperature was set at 37 °C (Fig. 3 a) as per the temperature *in vivo*, while the temperature of the measuring environment was kept at around 24 °C and was regularly monitored.

The independent variable of the measurements was the shear rate, and the measured quantity was the viscosity. Two measuring programs were set up. With the quasi-analogue program, data from 45 measuring points was collected, where the scale ranged from 0.1 1/s to 5000 1/s. Each point was measured for 5 s. The step program measured the interval of 0.1 to 10000 1/s. The shear rate was increased logarithmically, where 11 points were measured for 30 s each.



(a) (b) (c)

Fig. 3. (a) Rheometer, (b) loaded sample, (c) measurement arrangement

2.2. Blood samples and blood mimicking fluids

Five donor's blood samples were collected. Blood was taken on the same day of the measurements. Anticoagulant 9NC Coagulation (3.2% sodium-citrate) was added to the samples. Three samples of 3.5 ml were taken per donor.

Measurements were done on two samples alternately, while the third sample of each donor was kept as a control group and separation was analysed on them later. The quasi-analogue program was used to measure each of the five donor's blood samples five times, within four hours from the extraction of blood. With the step test program, all five samples were measured twice, within five hours from the extraction of blood.

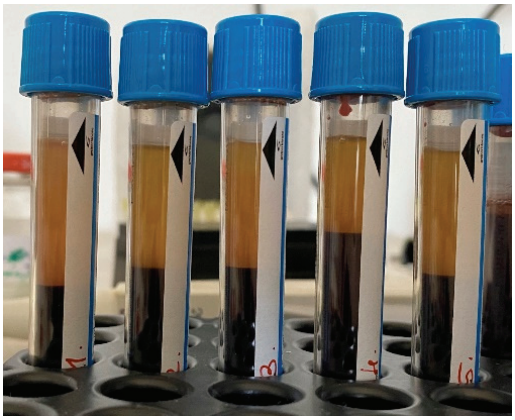


Fig. 4. Blood samples after separation. Donor 1, 2, 3, 4, 5, respectively

The samples were tested 7 hours after extraction to leave enough time for the two phases to develop. Then each phase was measured separately. The top phase contained little amount of red blood cells and mostly plasma. The bottom phase was a concentrated solution of red blood cells (Fig. 4).

The differences between hematocrit values can be influenced by the gender of the donors. Males generally have higher hematocrit than females. A difference between the hematocrit of age groups was studied by the US Department of Health, Education and Welfare [4]. The study found that the hematocrit of men decreases with age, while it increases for women until the age of 65, after which it starts to decrease. For privacy reasons, the gender, race and age of the donors are not available, however, these differences should still be noted.

It was suspected that the blood samples' viscosity changes over time after extraction. To prove this, a blood sample of Donor 1 was measured eight hours from extraction with the quasi-analogue measuring program.

Table 1. Blood mimicking fluid composition

Blood mimicking fluid composition (w/v %)				
	glycerol	water	starch	xanthan gum
1.	60	40	–	–
2.	59.98	40	0.02	–
3.	59.98	40	–	0.02
4.	59.98	40	0.01	0.01

Four BMF samples consisting of the materials recommended by Perrira et al. [12] were tested with the

quasi-analogue measuring program. Their composition is given in Table 1.

3. Results

The rheometer uses the physical parameter of the torque and measures the angular displacement to calculate the shear rate and the viscosity. The nominal minimum limit for torque is 0.05 μNm, below that, the device error is not negligible. The selected torque limit was defined to be 10 times the nominal limit, 0.5 μNm.

The step test results (Table 2) are accurate when considering the nominal limit of torque with one exception (highlighted with red). Data highlighted with orange is under the selected torque limit. Over a shear rate of 31.6 1/s, the measured torque was higher than the selected limit for all samples (Table 3).

The significant shear thinning characteristic of blood is well visible at the low shear region on the logarithmic shear rate – viscosity diagram. At higher shear rates, the viscosity converges to an equilibrium

Table 2. Step test results (Shear rates 0.1–31.6 1/s). Red-coloured values correspond to torque values under the nominal, and yellow-coloured values are under the chosen torque limit

Shear rate [1/s]	0.1	0.316	1	3.16	10	31.6
Sample 1, test 2						
viscosity [Pa·s]	0.101	0.0564	0.0203	0.00528	0.00376	0.00342
torque [μNm]	0.0415	0.073	0.0831	0.0683	0.154	0.442
Sample 2, test 2						
viscosity [Pa·s]	0.733	0.448	0.083	0.0227	0.0085	0.00604
torque [μNm]	0.3	0.579	0.339	0.293	0.348	0.782
Sample 3, test 2						
viscosity [Pa·s]	0.443	0.236	0.0452	0.0112	0.00646	0.00506
torque [μNm]	0.181	0.305	0.185	0.145	0.264	0.655
Sample 4, test 2						
viscosity [Pa·s]	0.422	0.2	0.0518	0.012	0.00936	0.00613
torque [μNm]	0.173	0.258	0.212	0.156	0.383	0.793
Sample 5, test 2						
viscosity [Pa·s]	1.12	0.603	0.113	0.0188	0.00894	0.00626
torque [μNm]	0.456	0.78	0.462	0.243	0.366	0.81

Table 3. Step test results (shear rates 100–10000 1/s)

Shear rate [1/s]	100	316	1000	3160	10000
Sample 1, test 2					
viscosity [Pa·s]	0.00316	0.00288	0.00272	0.00269	0.00289
torque [μNm]	1.29	3.72	11.1	34.8	118
Sample 2, test 2					
viscosity [Pa·s]	0.00468	0.00382	0.00345	0.00333	0.00348
Torque [μNm]	1.92	4.94	14.1	43.1	142
Sample 3, test 2					
viscosity [Pa·s]	0.00413	0.00345	0.00319	0.00303	0.00323
torque [μNm]	1.69	4.46	13	39.2	132
Sample 4, test 2					
viscosity [Pa·s]	0.00448	0.00373	0.00342	0.00328	0.00342
torque [μNm]	1.83	4.82	14	42.4	140
Sample 5, test 2					
viscosity [Pa·s]	0.00432	0.00358	0.00332	0.00318	0.0033
torque [μNm]	1.77	4.63	13.6	41.2	135

value and blood behaves as a Newtonian material (Fig. 5).

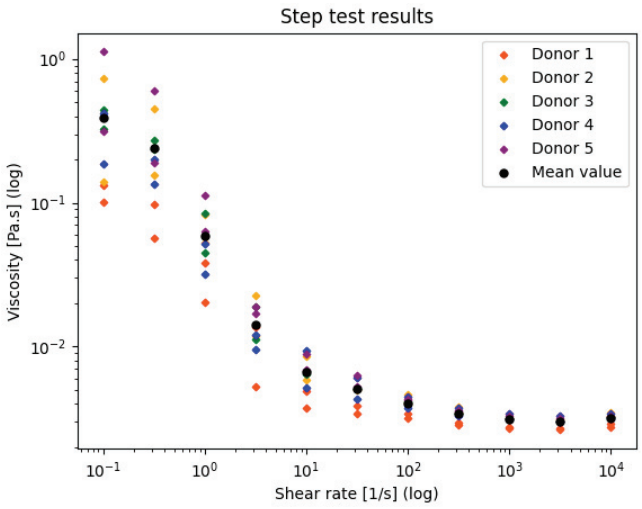


Fig. 5. Step test results of blood samples

Using the quasi-analogue program, for shear rates higher than 25.1 1/s, the measured torque values are over the nominal minimum value. From the shear rate of 39.8 1/s, the torque is within the chosen measuring range. In Figures 6 and 7, the results of the quasi--analogue test at shear rates 0.1–1000 and 1000–5000 1/s, respectively are shown. Blood shows similar characteristics for the quasi-analogue test as for the step test.

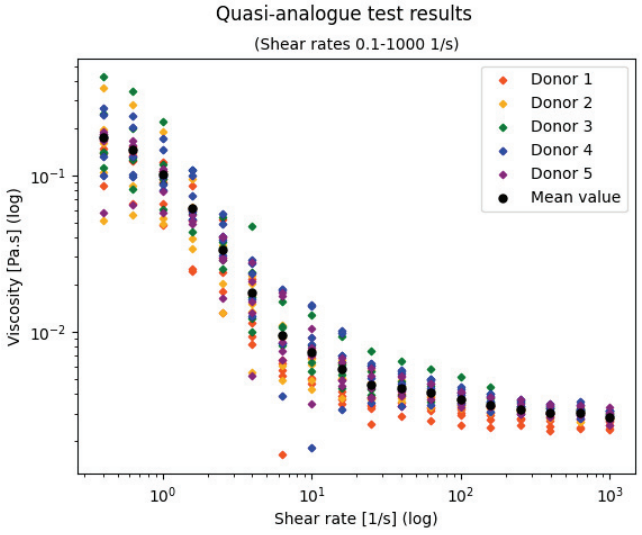


Fig. 6. Quasi-analogue test results of blood samples (shear rates 0.1–1000 1/s)

Over 1000 1/s shear rate, blood shows shear-thickening characteristics (Fig. 7). This could possibly be because of the destruction of red blood cells at high shear rates, which results in reduced elasticity. Another possible cause can be the drying of the samples.

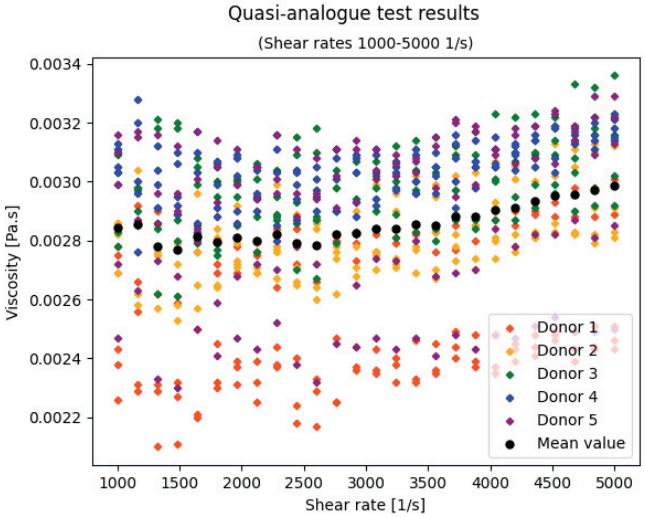


Fig. 7. Quasi-analogue test results of blood samples (shear rates 1000–5000 1/s)

The results of the plasma test show values within the range of normal plasma viscosity (Fig. 8), so the amount of red blood cells left in this phase is negligible at higher shear rates.

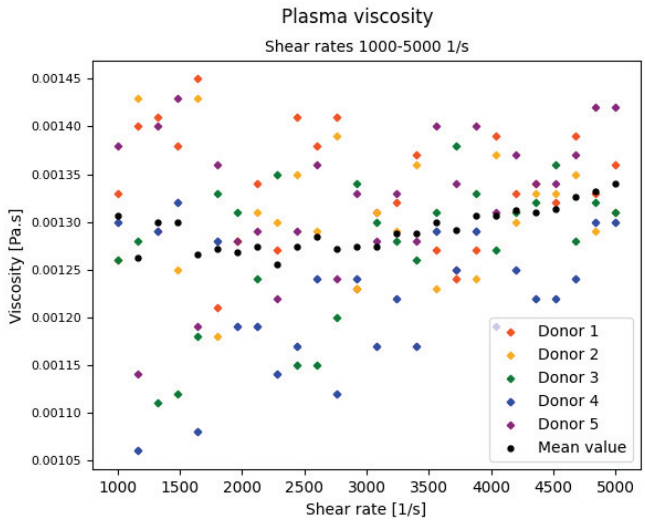


Fig. 8. Blood plasma viscosity (shear rates 1000–5000 1/s)

The viscosity of the concentrated red blood cell solution follows the characteristics of whole blood, with orders of magnitude higher values both at the low, the medium (Fig. 9) and the high shear region (Fig. 10).

There are visible differences between the viscosity values of each plasma and each concentrated RBC solution. Sample 1 has the highest viscosity in both cases, however, even though sample 3 has the lowest viscosity when testing the concentrated RBC solution, the

plasma viscosity isn't the lowest. The differences between the values can be a result of several factors. The two main factors are the rate of separation, and the hematocrit percentage. The rate of separation and the volume of the phases were different for each sample.

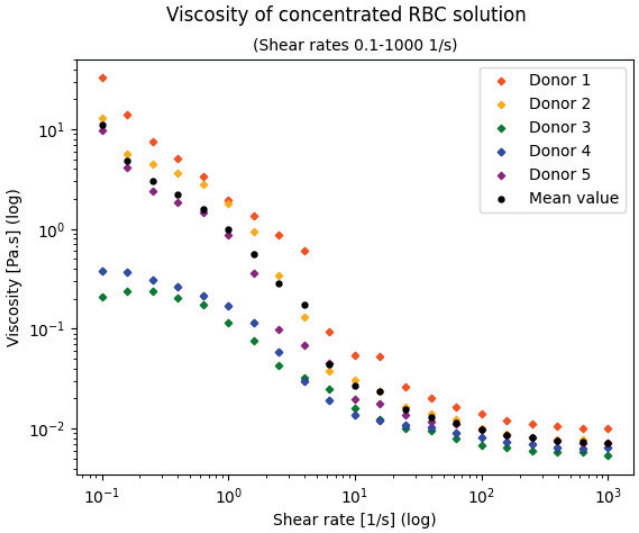


Fig. 9. Viscosity of concentrated RBC solution (shear rates 0.1–1000 1/s)

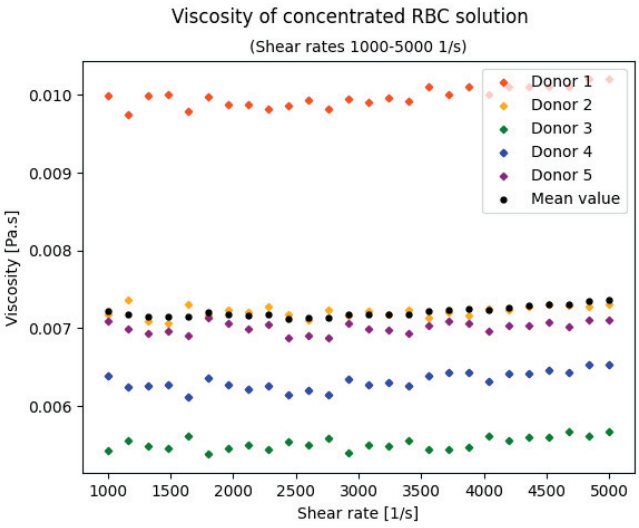


Fig. 10. Viscosity of concentrated RBC solution (shear rates 1000–5000 1/s)

The mean measured viscosity values of whole blood, plasma and a concentrated red blood cell solution are shown in Fig. 11. Plasma shows Newtonian behaviour, while the concentrated red blood cell solution shows shear thinning behaviour. The results support that whole blood's behaviour is mainly influenced by red blood cells.

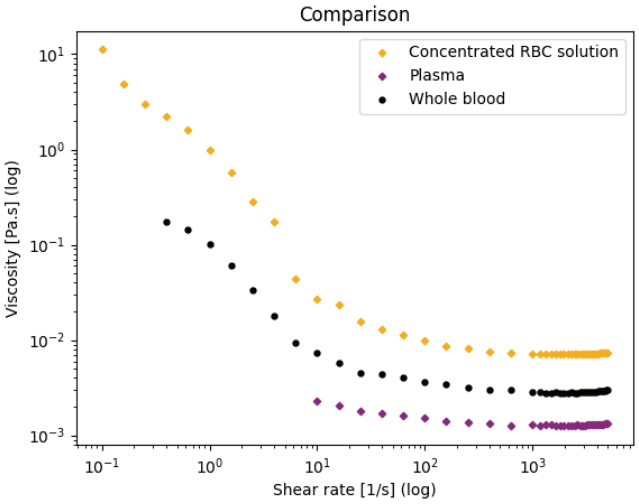


Fig. 11. Comparison of the viscosity of concentrated RBC solution, blood plasma and whole blood

The measured data supports that the viscosity changes over time. It can be clearly seen in Fig. 12 that the sample's viscosity increased over time. The few extremely high values can be due to clots forming.

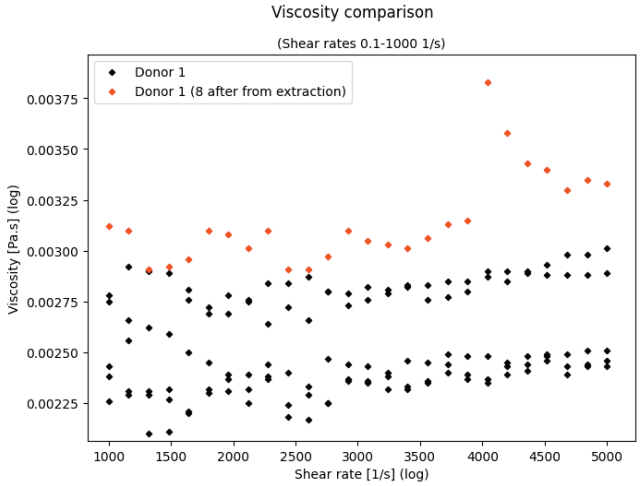


Fig. 12. Viscosity comparison of freshly taken blood and the sample after eight hours from extraction

Measurements done on BMF samples (Figs. 13 and 14) show promising results for the blood mimicking fluid 2, consisting of glycerol, water and starch, in comparison with human blood. Conversely to the result of the literature [12], the viscosity values of BMF 4, consisting of glycerol, water, starch and xanthan gum are significantly higher than that of blood.

The equilibrium viscosity (Fig. 14) is lower than that of blood for the first and second samples, and it is higher for the third and fourth samples.

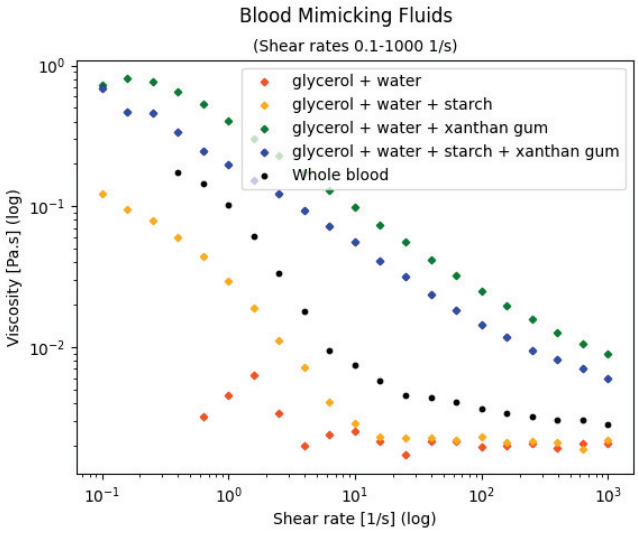


Fig. 13. Blood Mimicking Fluids (shear rates 0.1–1000 1/s)

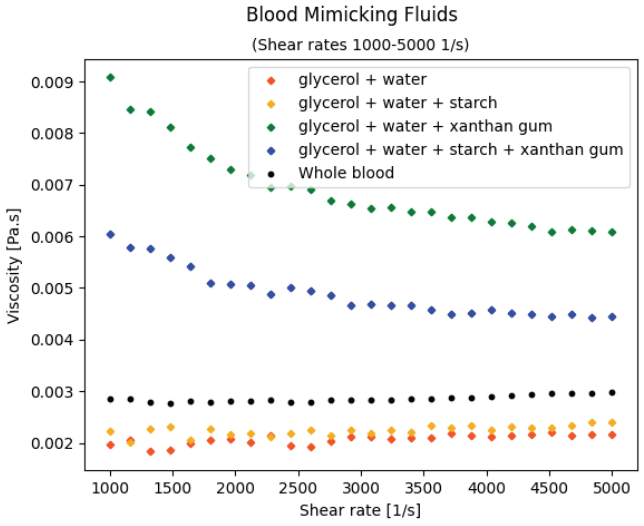


Fig. 14. Blood Mimicking Fluids (shear rates 1000–5000 1/s)

Statistical parameters mean value and dispersion were calculated for viscosity values corresponding to torque over 0.05 μNm for the results of the quasi--analogue test and the step test at each measured shear rate. Dispersion of both tests converges as blood reaches its equilibrium viscosity. The dispersion of the step test was slightly lower for viscosity values over 0.1 1/s.

From the dispersions, a confidence interval at a confidence level of ~95% was calculated for viscosity values at shear rates with Eq. (1):

$$\zeta = \overline{\mu}_l \pm 2s^*, \tag{1}$$

where $\overline{\mu}_l$ is the mean viscosity and s^* is the dispersion. The viscosity values of blood mimicking fluids were then compared with the calculated ζ values. The 2. blood mimicking fluid's (consisting of water, glycerol, starch, and xanthan gum) viscosity was within the confidence interval for shear rates 0.1–25.1. For higher shear rates, its viscosity values were slightly lower than expected.

4. Discussion

Several difficulties occurred when measuring blood's behaviour. Blood samples are hard to access and should be carefully handled for safety reasons. The viscosity of extracted samples increases with time, so the measurements shall be done shortly after withdrawal to obtain accurate results. The measurement equipment is not widely available as rheometers are rare and expensive. There are many different types of rheometers, but most of them are not suitable for measuring low viscosity materials at low shear rates, which is the most critical when it comes to blood rheology.

The advantage of measuring with an Anton Paar Physica MCR 301 rotational rheometer is that the measuring program can be directly set up to be a function of shear rate and the rheometer itself calculates the viscosity based on the measured torque values. It is possible to reliably measure the viscosity of the high shear region which gives information about the blood flow behaviour outside of the healthy range, at values that only occur in atherosclerotic patients.

With the used rheometer, the step test gave more stable results, because the steady state flow could develop over the given time interval, whereas turbulence influenced the results of the quasi-analogue test. Measured torque values were over the nominal torque limit of 0.05 μNm for shear rates over 0.1 and 25.1 1/s, and values were over the selected torque limit over 31.6 and 39.8 1/s shear rates for the step test program and the quasi-analogue program respectively. However, with the step test, it is not possible to measure as many points as with the quasi-analogue test, because over the measuring time, the samples would start to separate and dry in the rheometer, resulting in false values.

The measurement results showed that on the macroscopic level, the shearing rate significantly influenced blood's viscosity. Shear thinning behaviour was visible at the low shear region, whereas at high shear, the viscosity converged to an equilibrium value. The results are in agreement with those of the literature [3], [6], [16]. At very low shear rates, thixotropic behaviour is present. The comparison done on samples of plasma and concentrated red blood cell solution showed that higher red blood cell concentration elevates the viscosity. Thus, whole blood viscosity on the microscopic level is a function of the viscosity and ratio of plasma and red blood cells.

As expected, the measurements of BMF samples showed that they cannot represent blood perfectly. The results of this study are not in agreement with that of the literature [12], the inconsistency can be a result of the different measuring temperatures, as we measured the samples at 37 °C as per *in vivo*, whereas Perrira et al. measured at 25 °C. This study and the comparison with the literature shows that the flow properties of the materials consisting of water, glycerol, starch and xanthan gum are highly dependent on the temperature. However, the statistical analysis showed that the viscosity behaviour of BMF consisting of water, glycerol and starch is close enough to that of blood to be used for testing and experimenting with new measuring techniques. It should be noted that there are other factors that influence the flow properties of fluids, such as the density of the fluid, and the particle size. Therefore, further tests are needed to determine the significance of the differences caused by such factors.

5. Conclusions

In this study, viscosity measurements were carried out on an Anton Paar Physica MCR301 rotational rheometer with two measuring programs on the interval of 0.1–5000 (quasi-analogue program) and 0.1–10000 1/s (step test program) shear rates. Human blood samples and blood mimicking fluids were measured. The main novelty value of the study is that we paid special attention to set up the rheometer considering the particle size and the *in vivo* temperature, and we also paid attention to the *in vitro* viscosity change and separation of samples. Moreover, we compared the viscosity nature of blood and blood mimicking fluids. The viscosity measurements were done within 4 hours from extraction with the quasi-analogue program, and within 5 hours with the step test program. The rheometer's limits were also considered during the data evaluation process.

The blood samples' measurement results were accurate on a wide interval with the step test program. Over 0.1 1/s shear rate, measured data was over the nominal torque limit of the rheometer. Data measured over 31.6 1/s shear rate was over the selected torque limit, which was defined to be 10 times the nominal limit. Data on this interval is more precise and should be used for further research purposes.

The behaviour of the BMF sample consisting of water, glycerol and starch was close enough to blood. Thus, the proposed blood mimicking fluid can be used as substitute for human blood to aid the development of measuring devices and to be used for simulations, such as artificial blood vessel testing in the experimental stage. However, the temperature dependency of blood mimicking fluids consisting of water, glycerol, starch and xanthan gum must be considered in any case of application.

The data provided from these measurements can be used as a reference in future studies for hemorheological modelling aimed at disease prediction. The description of the measuring technique can be utilised in further hemorheological research.

Further tests

The Anton Paar Physica MCR 301 rotational rheometer has a wide measuring range and is capable of accurately measuring low viscosity at low shear rates, as the nominal torque limit is 0.05 μNm, however, this is still a significant limitation when it comes to blood measurements. The measurement technology can be improved by developing a measuring device equipped with finer sensors, specifically designed to measure low viscosity materials at low shear rates.

The thixotropic behaviour should be further tested by a harmonic measuring program, to evaluate the loading and unloading curves thus the hysteresis.

It should be noted that the focus of this study was blood's viscosity behaviour in general. The influence of certain diseases and other factors should be considered in future studies.

Abbreviations

RBC – Red blood cells
BMF – Blood mimicking fluids
w/v% – Weight by volume percentage

Nomenclature

$\dot{\gamma}$ – Shear rate, [1/s]
τ – Shear stress, [Pa]
μ – Viscosity, [Pa·s]
$\overline{\mu_i}$ – Mean viscosity value for the *i*-th shear rate value, [Pa·s]
ζ – Bound of confidence interval, [Pa·s]
s^* – Dispersion of the viscosity, [Pa·s]

Authors' contribution

A. The preparation of the research program: Eszter Szabó, Ernő Zsolt Baka, Kornél Tamás;
B. The execution of the research: Eszter Szabó, Ernő Zsolt Baka, Kornél Tamás;
C. The statistical analysis: Eszter Szabó;
D. The interpretation of the data: Eszter Szabó;

E. Preparation of the manuscript: Eszter Szabó, Kornél Tamás;
F. Obtain financing: Kornél Tamás.

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