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Optical measurement of blood sediment formation at high haematocrit

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In this paper we have presented the results of optical measurements concerning blood sediment formation. The intensity of the laser light transmitted through a blood sample has been measured and analysed. It is shown that the mechanism of blood sedimentation at high haematocrit is different from the mechanism of sedimentation at normal haematocrit. In blood samples with normal haematocrit three blood phases are formed during the sedimentation process: the well-known supernatant plasma, the phase of *rouleaux* formation and the phase of the so-called demixed blood. However, at high haematocrit only two phases of blood can be distinguished: the phase of supernatant plasma and the phase of demixed blood. Our study shows that the slow aggregation process at high haematocrit leads to the reorganisation of the demixed blood structure, while at normal haematocrit this reorganisation is not observed. When the reorganisation process is finished, the squeezing of the demixed blood begins. The results of our research demonstrate that the optical method is very useful in understanding the mechanism of the blood sedimentation process.

1. Introduction

Blood sedimentation is the process of erythrocyte settling under the action of the gravitational field. Erythrocyte sedimentation rate (ESR) has been widely used for many years as a quick check of the patients' health. The clinical significance of ESR was pointed out by Biernacki in 1894 [1]. At present, ESR is a very common haematological test, but its clinical usefulness is hampered by the poor understanding of the basic phenomena lying beneath of whole-blood sedimentation.

Several theoretical models of blood sediment formation have been made, but they are insufficient to highlight the process even for the simplified system [2]–[4]. In contrast to mathematical modelling of erythrocyte sedimentation, semiempirical approaches are focused on the description of a sedimentation curve obtained from ESR measurements. These conventional investigations deal with a global effect of sedimentation process. Therefore, the analysis of a sedimentation curve can give a rather poor insight into the basic phenomena of whole-blood sedimentation.

Most models of an erythrocyte sedimentation curve are formulated as a sigmoid or S-shaped function, but they consider the process to be composed of three distinct phases [5], [6]. HUNG *et al.* [7] found the piecewise curve which gives a better fit than a sigmoid curve. From the shape of a sedimentation curve the authors hypothesized

the mechanism of blood sedimentation: the first two linear segments represent the sedimentation of individual cells and of aggregates of constant size, the last segment reflects asymptoting to packed cells.

As it was shown in many experiments, optical measurements are a good source of information on the blood sedimentation process. Our previous results [8] of diffraction measurements of ESR have shown that this method is very useful in estimating blood sedimentation in polycythemia vera, when an ordinary clinical test has given zero value of ESR. In our previous paper [9], we have carried out a series of measurements of blood sediment formation by three different optical techniques: the angular distribution of scattered light, the light transmission across the sediment and the intensity autocorrelation function of speckle pattern produced by red blood cells fraction. It has been shown that the measurement of the temporal dependence of transmitted light intensity is the best, the most convenient and powerful technique in continuous monitoring of blood sediment formation. Using this technique, we have shown that during the sedimentation process three blood phases are formed in the blood samples with normal haematocrit [10]. The well-known supernatant plasma layer appears at the top of a container. In the sediment of red blood cells we could distinguish the phase of *rouleaux* formation and the phase of the so-called demixed blood.

Most papers deal with the studies of blood sedimentation process at normal haematocrit [11], [12]. There are only a few papers concerning the measurements of concentrated blood samples [7], [8], [13]. HUNG *et al.* [7] suggested that the aggregation of erythrocytes into *rouleaux* was restricted by the lack of cells mobility in high concentrations. Additionally, DENG *et al.* [14] found that at high haematocrit the aggregation intensity tended to decrease and that haematocrit had a strong and nonlinear effect on erythrocyte aggregability. Red blood cells aggregation measurements by the light back scattering technique showed that the reflectivity ratio was independent on hematocrit up to 45%, but for hematocrits higher than 50% it decreased linearly up to 70%, which means that by chance packing of erythrocytes becomes more and more hindered [15]. This result indicates that in blood samples at hematocrit higher than 50% take place other aggregation mechanisms than in blood at normal and low hematocrits.

High haematocrit is a symptom of some diseases (polycythemia vera, hyperviscosity syndrom) and a risk factor leading to myocardial infarction [16] and a predictor of coronary heart disease and thrombosis [17]. Therefore, the studies of blood properties at high haematocrit are very important. It is well known that in patients with high haematocrit, the standard ESR measurements provide no data for clinical diagnostics, because in such cases ESR values are close to zero.

The purpose of this research is, first, to study the process of blood phases formation during the sedimentation of blood samples at high haematocrit. Second, it is to show the effectiveness of the light transmitted technique in the study of high concentrated blood properties during the sedimentation process. As it was shown earlier [10], the light transmitted profiles can provide a better picture of aggregation and sedimentation process.

2. Material and method

Blood of healthy volunteers was obtained by venous puncture and stabilized with sodium citrate. The high haematocrit in the investigated samples was adjusted by the change of the amount of plasma from the original blood. Prepared blood was placed between two glass plates distanced by a teflon spacer. The thickness of a container was fixed to be 0.2 mm and the height was 20 mm. The measurements were performed at room temperature. Blood was well mixed before putting it in an experimental set up and we obtained the homogeneous system of uniformly distributed erythrocytes.

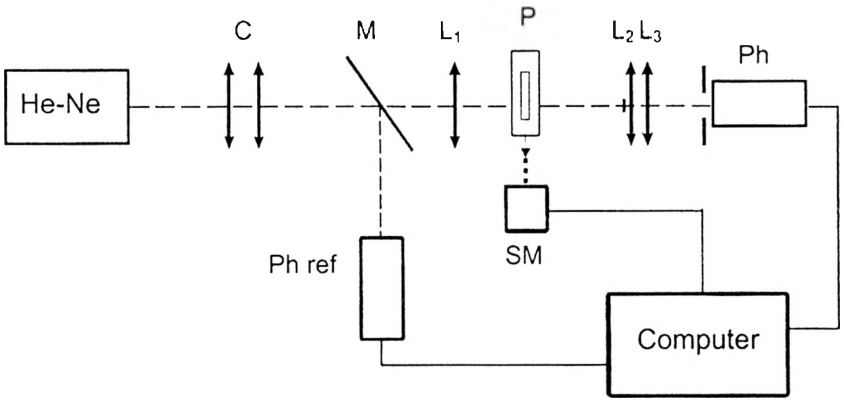

Fig. 1. Schematic diagram of an experimental set up: He-Ne – laser, C – collimator, M – beam splitter, L1, L2, L3 – lenses, P – blood sample, Ph, Ph ref – photomultipliers, SM – step motor.

The experimental set up used for measurements is shown in Fig 1. The laser (He-Ne) light beam passed through the collimator (C) and was focused by the lens (L1). The sample was placed at the beam waist position. The step motor (SM) moved the sample vertically at constant velocity of 2 mm/min. The light emerging from the sample was collimated by the lens (L2) and focussed by the lens (L3) in the plane (P). A photomultiplier (Ph) connected with the aperture detected the transmitted intensity. In front of the lens (L2) a circular stop filter was placed to reduce the height intensity specular component from plasma. The half-mirror (M) was used to obtain the reference intensity. The time varying signals were stored in a computer. The blood samples were measured within two hours.

3. Experimental results

Figure 2 shows typical profiles of the laser light intensity transmitted through the blood samples at normal (46.5%) and high haematocrit (56.4%). The dimensionless variable x/l is the x coordinate in the units of the height l of the container; $x/l = 0$ represents the top of the container and $x/l = 1$ indicates the bottom position. The vertical axis corresponds to the transmitted light intensity. Each profile represents the intensity as

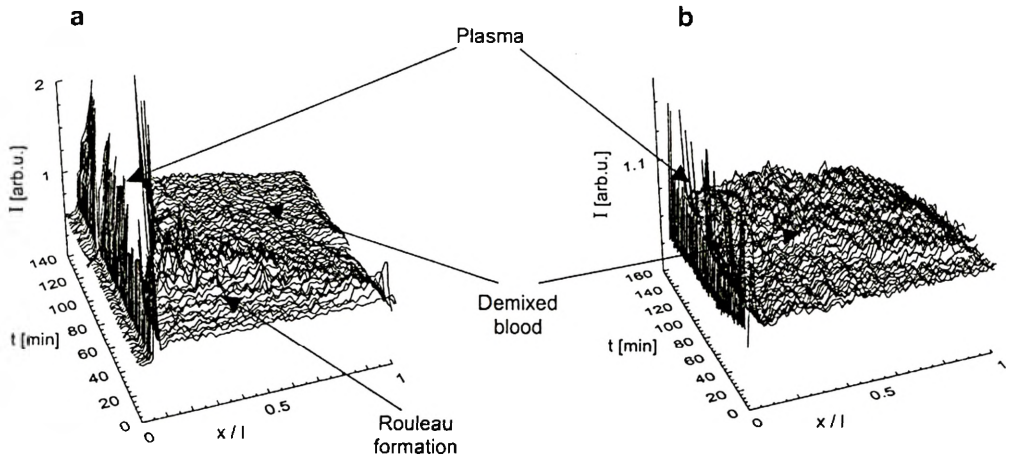

Fig. 2. Intensity profiles of light transmitted through the blood samples as a function of time at haematocrit: 46.5% (**a**) and 56.4% (**b**), respectively.

a function of the distance from the top of the container. Profiles were sampled every two minutes. The set of profiles constitutes the 3D plot. Figure 2**a** shows that at normal haematocrit three phases of the blood can be distinguished. The high values of the transmitted light intensity registered at the top of the container are optical representations of the supernatant plasma. Random intensity fluctuations represent the phase of the blood, where formation of small and one-dimensional *rouleaux* takes place. This phase is called the phase of *rouleaux* formation. The existence of the next phase is manifested by quasi-periodic intensity fluctuations. These fluctuations reflect the density fluctuations of the blood. During the procedure of sample preparation the blood was mixed to reach the uniform erythrocyte distribution, therefore the density fluctuations result from a demixing process. For this reason this phase is called the phase of demixed blood. From Fig. 2**b** one can see that at high haematocrit the whole blood was already demixed at the beginning of measurements. At high haematocrit the phase of rouleaux formation is not observed. As previously, in this case the high values of the intensity represent the supernatant plasma.

Temporal dependences of the position of boundaries between the various phases of blood are shown in Fig. 3. The temporal dependence of the position of the red blood/plasma boundary is represented by a classical sedimentation curve. For the blood with haematocrit 46.5%, the position of the boundary between the phase of *rouleaux* formation and the phase of demixed blood is shown. The results shown in Fig. 3**a** permit to find regions occupied by various phases of blood at a given moment of time. From this figure one can see that the phase of *rouleaux* formation disappears after 87 minutes from the beginning of the experiment. After that time only plasma and the demixed blood remain in the container. Figure 3**b** shows that at haematocrit 56.4% the sedimentation of demixed blood is very slow.

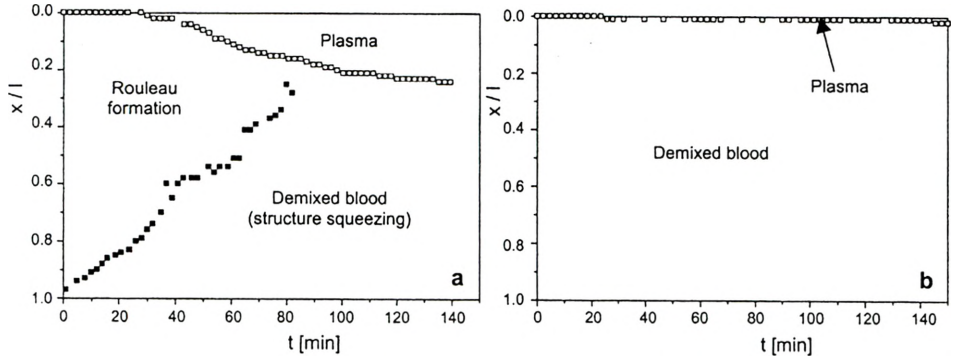

Fig. 3. Kinetics of the boundaries between blood phases.

In order to find the properties of the demixed blood we have analysed the temporal dependences of the transmitted light intensity. In the case of normal haematocrit, as it is shown in Fig. 4**a**, the intensity monotonically decreases with time at every place of the sample. It reflects a monotonic increase in the demixed blood density. On the other hand, the results reflect the squeezing of the structure formed by erythrocytes in the phase of demixed blood. Figure 4**b** shows the temporal dependence of the transmitted intensity for the blood sample with high haematocrit. In this case the intensity initially increases, then reaches a maximum and finally decreases. It is well known that an increase in the transmitted light intensity reflects the reorganisation of the structure formed by erythrocytes. Thus, this increase in the light intensity, shown in Fig. 4**b**, indicates that at high haematocrit the structure reorganisation of the demixed blood occurs. The time after which the light intensity transmitted through the sample at a given position reaches maximum reflects the duration of the reorganization process. After that time the intensity decreases, what means that density of the demixed blood increases. As was previously indicated, it reflects the squeezing of the structure formed by erythrocytes of the demixed blood.

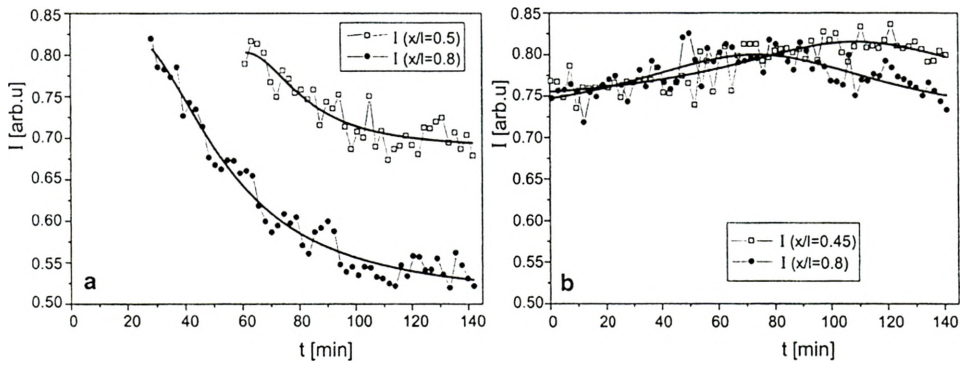

Fig. 4. Transmitted light intensity as a function of time at different distances from the top of the container for samples at haematocrit: 46.5% (**a**) and 60.2% (**b**), respectively.

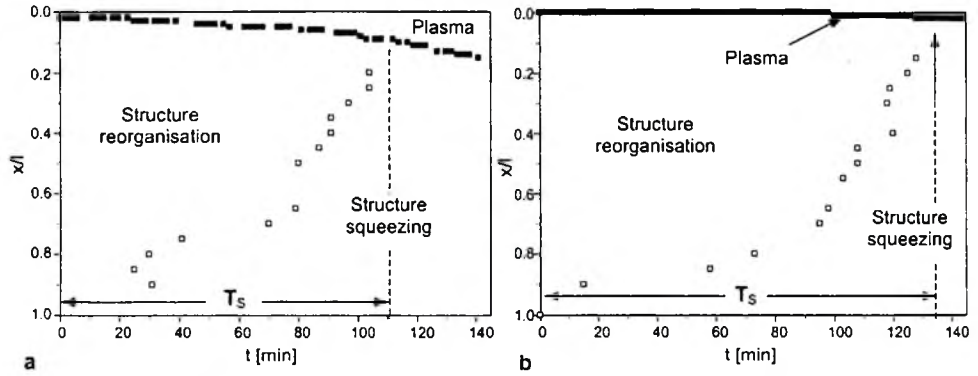

Fig. 5. Temporal dependence of the position of intensity maxima (□) and the sedimentation curve (■) for samples at haematocrit: 55.1% (**a**) and 60.2% (**b**), respectively.

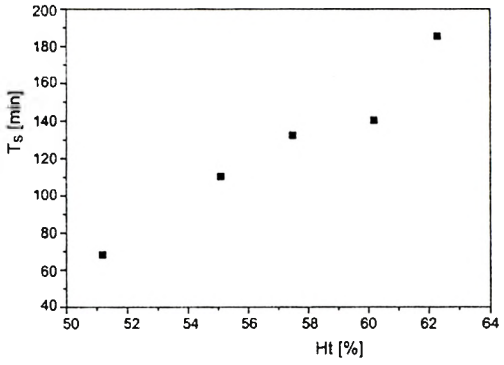

Fig. 6. Dependence of the parameter T_s on haematocrit.

Figure 5 shows the position of the intensity maximum as a function of time for the blood samples with haematocrit 55.1% and 60.2%, respectively. In the pictures the sedimentation curves are also shown. From these figures one can see that the position of the intensity maximum moves with time from the top to the bottom of the container. The results shown in Fig. 5 permit to find such a region in the container where the reorganisation of the blood takes place. The time at which the reorganisation of the structure formed by erythrocytes is finished we will denote as T_s. After that time, only plasma and the reorganised demixed blood remain in the container. Figure 6 shows the influence of the haematocrit on the parameter T_s. The rise of haematocrit increased the duration of the reorganisation of the structure formed by erythrocytes of the demixed blood.

4. Discussion and conclusion

In this paper we have presented our results of optical measurements concerning blood sediment formation. The results demonstrate that the optical method is very useful in

the study of the blood sedimentation process. It is shown that the mechanism of blood sedimentation at high haematocrit is different from the mechanism of sedimentation at normal haematocrit.

The blood sedimentation process is usually investigated by the analysis of a sedimentation curve. Using this technique, HUNG *et al.* [7] found that at normal haematocrit the sedimentation curve consists of three segments, while at high haematocrit only two segments of this curve can be observed. From the shape of sedimentation curves HUNG *et al.* [7] hypothesised that at high haematocrit the sedimentation occurs via asymptoting to packed cells. Our investigations show that this hypothesis can be treated as an approximate explanation of the process.

In our previous works [9], [10], using the same optical method, we have shown that in blood samples with normal haematocrit three distinct blood phases can be distinguished: the phase of supernatant plasma, the phase of *rouleaux* formation and the phase of demixed blood. It was shown especially that immediately after the sample preparation the *rouleaux* formation phase occupied the whole blood container. The phase of demixed blood started to form from the bottom of the container and propagated upwards, till the moment when the phase of *rouleaux* formation disappeared. After that time the whole blood fraction containing erythrocytes was in the phase of demixed blood. In this paper we have shown that the squeezing of the structure formed by erythrocytes of the demixed blood could be one of the mechanisms of the sedimentation process. The optical method does not permit to recognise that structure.

At high haematocrit only two phases of blood can be distinguished: the phase of supernatant plasma and the phase of demixed blood. It was shown that immediately after the sample preparation the phase of demixed blood occupied the whole container with blood. On the contrary, in comparison with the blood with normal haematocrit, at high haematocrit the structure formed by erythrocytes is initially reorganised and then squeezed. The reorganisation process starts to develop from the bottom and propagates upwards. When the reorganisation process is finished, the squeezing of the demixed blood begins.

Aggregation of erythrocytes affects the blood sedimentation process. Erythrocyte aggregation is the subject of intensive investigations. DENG *et al.* [14] have shown that haematocrit exerts a strong and non-linear effect on erythrocyte aggregability. They have shown that high haematocrit slowed the aggregation process. BÄUMLER *et al.* [15] have shown that the mechanism of the aggregation changes when the haematocrit exceeds 45%. HUNG *et al.* [7] have suggested that at high haematocrit the behaviour of the sedimentation curve results from the fact that the erythrocyte aggregation is restricted by lack of mobility in high concentrations. Our study shows that the slow aggregation process causes the reorganisation of the structure of the demixed blood at high haematocrit, while at normal haematocrit this reorganisation is not observed.

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