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Diffraction measurement of erythrocyte sedimentation rate

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We propose a new method to measure the erythrocyte sedimentation rate (ESR). In clinical practice, the ESR is determined by visual inspection of the fall of blood/plasma boundary. The test applied to the blood of patients with polycythemia vera gives low or zero sedimentation rate. It is shown that in this case the upper boundary of the container and the frontier of the red blood form a slit filled with plasma. Diffraction of laser light by the slit was investigated. Intensity distributions of the diffraction pattern were measured during the sedimentation process. The ESR was determined from the temporal dependence of the position of successive intensity maxima of the diffraction pattern. The sedimentation curves signalise the existence of two phases of the blood formed in the process.

1. Introduction

Blood has been extensively studied by using biomedical and physical methods. In particular optical investigations deal with light scattering by whole blood or red blood cells in suspensions [1]. Optical methods allow us to obtain information about the size, shape, orientation, haemoglobin concentration and oxygenation degree of red blood [2], [3]. Theoretical models are proposed to describe the light scattering by single and adjacent red blood cells [4]. Another area of blood examination is Doppler flowmetry based on coherent light scattering [5]. However, up to now the optical studies of blood sedimentation are not numerous.

Sedimentation of erythrocytes in the earth's gravitational field is manifested by a fraction of transparent plasma. Since Biernacki discovered in 1894 the clinical meaning of blood sedimentation, the ESR test has commonly been adopted in clinical diagnosis [6]. Especially this test is useful in diagnosing and monitoring temporal arthritis, polymyalgia rheumatica and possibly other rheumatic diseases, in monitoring patients with Hodgkin's disease. High values of ESR observed in anaemia arise from iron deficiency and erythrocytes deficiency. In contrast, in the case of polycythemia vera, the ESR is close to zero [7], [8].

Blood is an aqueous bio-colloid system containing solutes from proteins to cells of widely different dimensions [9]. Attractive interaction between erythrocytes causes aggregation. Erythrocytes coalesce and rouleaux are formed. With the time

passing the rouleaux form a 3-dimensional network [9]. Another feature of blood is its high specific viscosity. All of these properties make difficulties in formulae of the theory of blood sedimentation [8], [9]. The sedimentation curve (the temporal dependence of the position of blood/plasma boundary) is commonly used in the study of the blood sedimentation. The erythrocyte aggregation is one of the reasons for which the Stokes model is inadequate in the explanation of the curve [8], [9]. HUNG *et al.* [10] proposed a model of this curve. Three characteristic segments of this curve were separately analysed. They argued that every segment of the curve manifests different process arising in the blood sediment. In the previous study the first segment of the sedimentation curve, observed in the early stages of the process, was omitted. High-resolution measurements show that accelerations and decelerations in the movement of the blood/plasma boundary take place [7].

In this paper, we report on ESR study of the blood obtained from the donor with polycythemia vera and from a healthy volunteer. The ordinary clinical test for the case of polycythemia vera gives zero value of the ESR, therefore the optical investigation is adopted. We present a description of diffraction of light passing through the container with blood. Temporal changes of the diffraction pattern are shown. The formula responsible for diffraction caused by a slit is adopted to measure the temporal dependence of the location of the interface between the plasma and red blood fraction. Finally, the existing two phases of the blood formed in the course of the process revealed by the sedimentation curve are discussed.

2. Material and method

Blood was obtained by venous puncture from the donor with polycythemia vera and from a healthy volunteer. The blood was prepared by addition of sodium citrate. After four hours the blood was gently mixed to obtain the uniform distribution of erythrocytes and placed between two glass plates distanced by teflon spacer. In this way, we have obtained a container of the following dimensions: depth 0.2 mm, height 10 mm and length 20 mm. Thus, in this procedure, a volume of 40 mm^3 is suitable for the experimental study. The measurements were performed at room temperature.

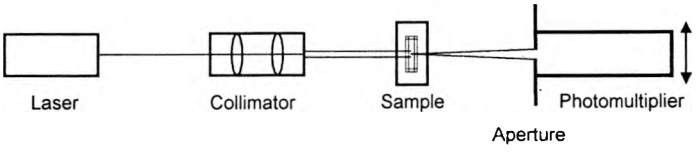

Fig. 1. Scheme of experimental setup.

The experimental set-up used for the study of the diffraction is shown in Fig. 1. A laser diode module operating at the wavelength $\lambda = 650$ nm was used as the light source. The laser may emit up to 3 mW continuously. The container with blood was illuminated by collimated beam with uniform brightness. In this way, light with power level lower than 0.3 mW could be absorbed in the sample. This light cannot

disturb the structure of the cells [11]. A vertically moving photomultiplier is used in conjunction with a horizontally oriented slit aperture. This aperture was at a distance of 0.27 m away from the sample. The intensity distributions of the resultant pattern were registered. The ESR of the healthy volunteer blood was measured with the use of an optical imaging system.

3. Experimental results

In the initial stages of sedimentation light passing through the blood from the donor with polycythemia vera produces a random speckle pattern. The pattern characteristic of diffraction arises among the speckles after 80 min. Distances between successive diffraction maxima decrease with time. After 150 min a single maximum only is observed. The plasma supernatant can be visually observed from that moment. The diffraction pattern registered after 115 min is shown in Fig. 2.

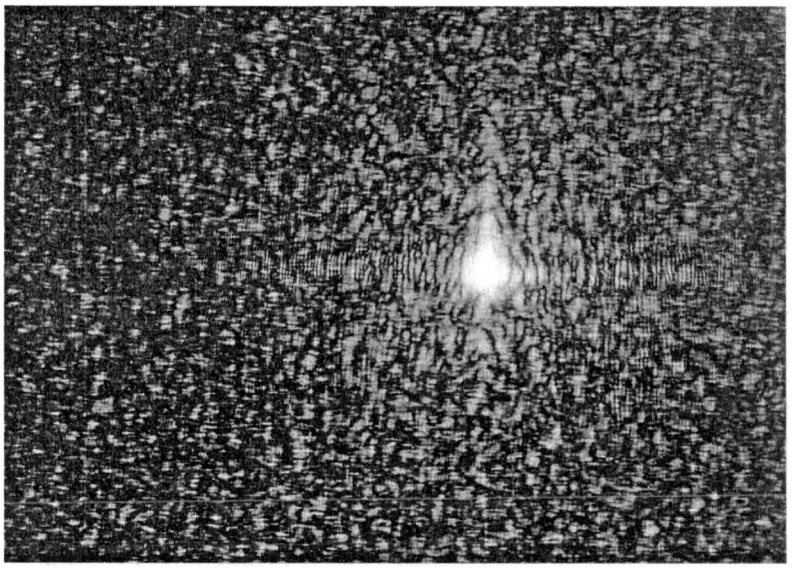

Fig. 2. Pattern produced by laser light emanating from the sample.

Although speckles blur this diffraction pattern the maxima are clearly seen. The intensity distribution corresponding to this pattern is shown in Fig. 3. This intensity distribution consists of a component characteristic of a slit and an incoherent background. The intensity distribution of this background demonstrates the asymmetry. From Fig. 3 one can see that in this case two diffraction maxima are well resolved. The early diffraction patterns show the first maxima only. Finally, we have obtained three well-resolved maxima. The width between the successive maxima as a function of time is shown in Fig. 4. From this figure one can see characteristic jumps of the widths. In general, the widths decrease with time.

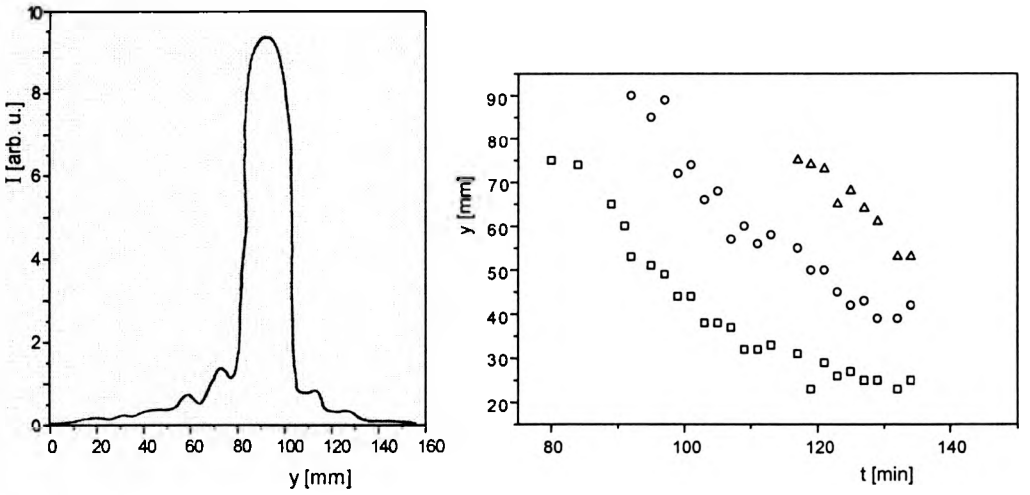

Fig. 3. Intensity distribution of the pattern obtained after 115 min from the beginning of the sedimentation.

Fig. 4. Width between the first (□), second (○) and third (Δ) diffraction maxima as a function of time.

The formula responsible for the intensity of the diffraction pattern of a slit with Kirchhoff boundary conditions was adopted to the experimental data [12]. In the case the aperture is normally illuminated by a monochromatic plane wave the intensity can be expressed by

$$I(y) \propto \frac{\sin^2(dy/\lambda z)}{(dy/\lambda z)^2}$$

where d denotes the width of the slit, λ is the wavelength of the incident radiation and z is the distance between the sample and the aperture connected with the photomultiplier. Thus the distance between respective maxima or minima is a simple function of the width d.

Figure 5 shows the temporal dependence of the width of the slit filled with the plasma supernatant. After 80 min as a result of sedimentation the position of the blood/plasma boundary is 80 μm from the top of the container. After the next 60 min the boundary reaches the distance 280 μm. Taking into account the experimental accuracy we can see that the results obtained from the analysis of the three maxima are met. In this case, the linear function can be representative for the sedimentation process. In the time interval of 80 – 140 min the velocity of the blood/plasma boundary is 3.9 μm/min. Detailed analysis of the boundary displacements reveals some non-linear effects of the kinetics. In the time interval of 80 – 140 min, excluding a single case, the position $d(t)$ of the boundary fulfils the following relation: if $t_2 > t_1$ then $d(t_2) \geqslant d(t_1)$, where t denotes time. This indicates that accelerations of the blood/plasma boundary take place. Although we are unable to determine the position of the blood/plasma boundary at the early stages of the

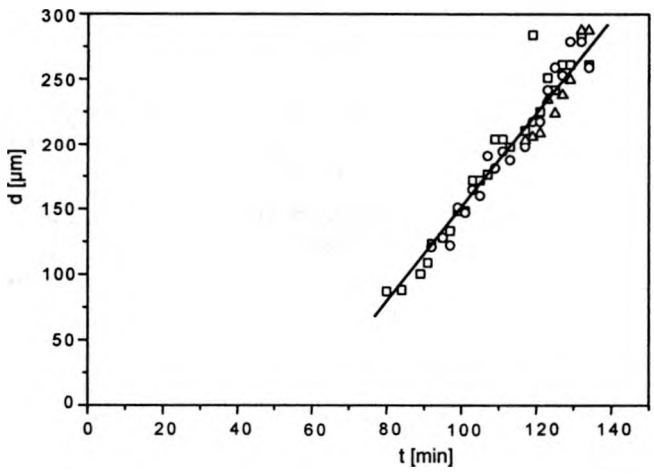

Fig. 5. Blood sedimentation from the donor with polycythemia vera. The blood/plasma boundary positions as a function of time. The data were calculated from the width between the first (□), second (○) and third (△) diffraction maxima. The solid line reptesents linear regression.

process it is clear from Fig. 5 that the velocity of the blood/plasma boundary during the first hour is very slow or zero. Thus the results show that the sedimentation curve consists of two segments. By means of an extrapolation one could admit that formation of the second segment starts after $\sim$60 min.

We have carried out five measurements of the sedimentation rate for the blood of the donor with polycythemia vera. The every successive sample was obtained after two months from the previous investigation. In three cases we have observed the two

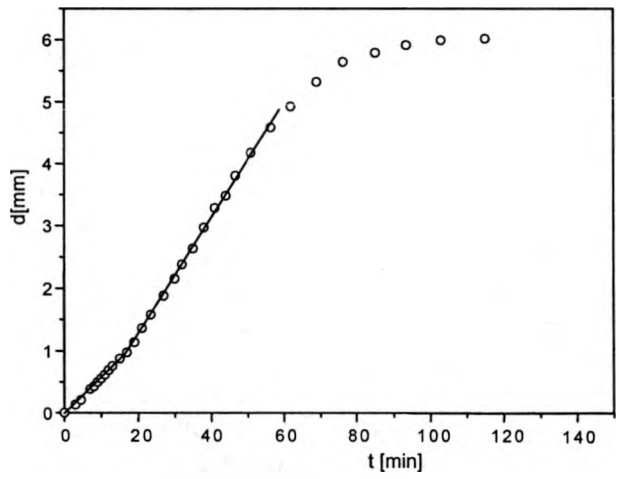

Fig. 6. Sedimentation of a healthy donor blood. The blood/plasma boundary position as a function of time. The lines represent the first and second segment of the sedimentation curve.

segments of the sedimentation curve. We have found that the second segment of the curves starts to form after 17, 27 and 60 min while the velocity of the blood/plasma boundary is 6.3, 16.4 and 3.9 μm/min, respectively. In two cases the fall of the blood/plasma boundary started immediately after the sample preparation. Thus the first segment of the two sedimentation curves was not observed. The velocity of the blood/plasma boundary in the two cases was 6.3 and 9.3 μm/min.

We have also observed diffraction patterns produced during sedimentation of the healthy donor blood. The diffraction effects were observed just during the first 10 s of the sedimentation. Therefore, for the healthy donor blood a classical method of ESR measurement was used. Figure 6 shows the temporal dependence of the boundary position of the blood. The sedimentation curve was obtained with the use of the imaging system. From Fig. 6 one can see three segments of the sedimentation curve. The beginning of the second segment can be determined with relatively high accuracy from the data shown in this figure. The second segment of the curve starts to form after 18 min. The velocity of the blood/plasma boundary corresponding to the first segment of this curve is 56 μm/min, while the velocity corresponding to the second segment is 90 μm/min.

4. Discussion

The sedimentation process causes nontrivial boundary conditions for the diffraction problem. In contrast to the Kirchhoff boundary conditions, the field amplitude is non-zero outside the plasma. In fact, one of the boards is defined by the teflon spacer, the other one is the fraction containing erythrocytes. Another problem arises from the fact that the plasma contains scattering units. Due to the lack of a theoretical description of such a system we have adopted the formula responsible for intensity of the diffraction pattern of a slit to find the ESR. Although the formula cannot entirely represent the experimental data, the agreement between the ESR results obtained by measurements of the position of the successive maxima is remarkable.

For the blood of healthy donors usually three segments of the sedimentation curve can be distinguished. According to the hypothesis of HUNG *et al.* [10] in the early stages of the process the sedimentation occurs via individual cells falling. The first segment of the sedimentation curve represents this falling. We were unable to estimate the sedimentation curve for the blood of the donor with polycythemia vera in the early stages of the process, however, it is clear that the velocity of the blood/plasma boundary in the stages is very low or zero. We have shown that the second segment starts to form after about 1 hour. Characteristic times of erythrocyte aggregation are about 1 min, thus it is rather impossible that the erythrocytes could be free during 1 hour [9]. It seems that the first segment of the sedimentation curve for the blood of the donor with polycythemia vera cannot be ascribed to the individual cells falling. Note that in two cases the first segment of the sedimentation curve was not observed.

According to the hypothesis of Hung *et al.*, the second segment of the sedimentation curve corresponds to aggregates falling and the final part reflects the formation of the deposit of packed cells. The second segment was not observed when blood haematocrit was higher than 55% [10]. The haematocrit of the blood of the donor with polycythemia vera was higher than 55%. Thus the second segment described in this paper can be a representation of the formation of the deposit of packed cells. We have found accelerations of the blood/plasma boundary. The existence of accelerations was a base of the hypothesis that in the course of sedimentation a self-organisation of blood takes place [9]. Thus the accelerations observed in the case of polycythemia vera suggest that the deposit formation is due to the shrinking of a network formed by rouleaux.

5. Conclusions

In this paper, diffraction patterns observed during blood sedimentation were analysed. The experimental measurements demonstrate that diffraction data permit one to achieve the ESR. The sensitivity of the method can be helpful when currently used diagnostic methods are insufficient to evaluate the ESR. Especially, the method can be very useful in studying the kinetics of the blood/plasma boundary. This diffraction measurements can be a base for clinical monitoring of polycythemia vera.

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