Biorheology 36 (1999) 299–309
IOS Press

Distributions of rheological parameters in populations of human erythrocytes

I.L. Lisovskaya *, E.S. Shurkhina, E.E. Yakovenko, N.V. Tsvetaeva, S.V. Kolodei, S.P. Shcherbinina and F.I. Ataullakhanov
National Research Center for Hematology, Russian Academy of Medical Sciences, Moscow 125167, Russia

Received 25 September 1998
Revised 1 July 1999
Accepted 1 July 1999

Abstract. We have previously proposed the osmofiltration method based on a modified Hanss hemorheometer to analyze distributions of erythrocytes in their ability to pass through membrane filters with 3 μm pores. Upon decrease in medium osmolality (u) the erythrocyte volume increases. When cell volume becomes $V = V_{cr}$ at $u = u_{cr}$, such cell loses its ability to pass through a 3 μm pore. The flow rate of erythrocyte suspension containing cells with different u_{cr} through a filter gradually decreases with decreasing medium osmolality. This rate becomes zero at some $u = \Omega$, when the number of non-filterable cells in the applied sample approaches the number of pores in filter. Experimental determination of the dependencies of the filtration rate on medium osmolality for various hematocrit values allows to obtain Ω for each hematocrit and, thereby, to assess the distribution of erythrocytes in u_{cr}. Here, we propose a simplified version of this method, which allows screening of the erythrocytes in heterogeneous suspensions for the distribution in u_{cr} by measuring Ω for only two hematocrit values, 0.1% and 1%. Applications of the proposed method are exemplified by analysing the erythrocyte populations of healthy donors, of patients with microspherocytosis, hemochromatosis and normal erythrocyte populations in an acidic environment.

Keywords: Erythrocyte, filterability, subpopulations

1. Introduction

The deformability of erythrocytes is the characteristic that determines the rate at which they pass through microcirculation capillaries or pores of membrane filters. The deformability depends on a complex interplay of a number of factors, including cell volume, surface area, their ratio, intracellular viscosity, and mechanical properties of the membrane [10,11]. The erythrocyte populations in healthy donors are known to be non-uniform with respect to the above mentioned characteristics. In the blood, erythrocytes were shown to have approximately Gaussian distributions in the MCV, surface area, and intracellular hemoglobin concentration [7,16]. The deformability (filterability) of erythrocytes also varies within the normal population. The distribution of rates at which normal erythrocytes flow through filter pores has been determined using a Cell Transit Analyzer (CTA); however, this method ignores the cells that filter much slower than most of the other cells of a population or do not filter at all, clogging the pores [17].

The known way to change erythrocyte volume without changing its surface area is to change the medium osmolality value [8]. Earlier, we used osmotic scanning as described in [4] for constructing and

*Address reprint requests to: Irene L. Lisovskaya, National Research Center for Hematology, Novozykovskii pr. 4a, Moscow 125167, Russia. Tel.: +7 95 212 5531; Fax: +7 95 212 8870; E-mail: Fazli@blood.ru.

0006-355X/99/$8.00 © 1999 – IOS Press. All rights reserved

validating the mathematical model describing the filtration process for a uniform erythrocyte suspension [1]. According to this model, the critical volume V_{cr} exists for a cell with a fixed surface area S (or critical volume-to-surface area ratio V_{cr}/S). After reaching this volume, the cell loses the ability to pass through a pore of a given diameter and length because of only geometric limitations. As the medium osmolality (u) decreases, the volume (but not the surface area) of the erythrocyte increases reaching the value $V_{cr}(V_{cr}/S)$ at some $u = u_{cr}$. Thus, for a hypothetical suspension consisting of identical cells, the filtration rate is zero for $u < u_{cr}$. An increase in u_{cr} corresponds to a decrease in the deformability typical, for example, of cells with increased isotonic volumes, but normal surface areas (swollen cells) or cells with decreased surface areas, but normal isotonic volumes. In the range $u > u_{cr}$, the filtration rate of a homogeneous suspension rapidly increases with increasing u, passes through a maximum at a near-isotonic value of osmolality, and then gradually decreases as the intracellular viscosity increases [1].

A real suspension that contains subpopulations differing in u_{cr}, (i.e., in the rheological properties) stops flowing through a filter at some $u = \Omega$. Note that $u = \Omega$ denotes the osmolality value at which a particular real suspension stops flowing, whereas u_{cr} is a characteristic of an individual cell or a subpopulation of identical cells, i.e., the osmolality value at which that particular cell or subpopulation stops passing through a pore. As such, u_{cr} cannot be addressed directly in a real suspension. Recently, we have developed a method of consecutively washing the filter for determining the distribution of erythrocytes in u_{cr} [9]. Using membrane filters with mean pore diameters of 3.1 μm, we showed that the distribution of u_{cr}, in suspension of normal erythrocytes had a maximum at u of about 200 mOsm/kg and a standard deviation σ of about 20 mOsm/kg. The distributions changed in response to stress factors. Abnormal distributions were also found in some pathologies. The data on changes of erythrocyte distributions in u_{cr}, seem to be useful from a practical point of view, e.g., in diagnostics and for assessing the efficacy of therapy. However, this method is too labor consuming to be used routinely.

In this study, a simpler method is substantiated and described. This osmofiltration method gives information on the distribution of erythrocytes in u_{cr}, by comparing the values of Ω observed in suspensions that have different hematocrit values. The method was used to characterize changes in normal erythrocytes placed in an acidic environment and to analyze patient erythrocytes for their rheological heterogeneity and for filterability of the major (modal) subpopulation.

2. Methods

2.1. Preparation of suspensions

Human erythrocytes were obtained from the blood of healthy donors ($n = 15$), patients with hereditary hemochromatosis ($n = 15$), and hereditary microspherocytosis (HS; $n = 7$). Triple blood pack systems (Baxter, S.A. de C.V., Morelos, Mexico) containing CPDA-1 in the primary bag were used for donor blood collection. Patient blood was drawn into 3.8% sodium citrate (1 vol to 9 vol blood). Measurements were performed within 24 h after venipuncture, but in one case, packed cells that were stored in a bag at 4°C for 3 weeks were used. Erythrocytes were sedimented by centrifugation and washed three times with HEPES-buffered saline (HBS; 145 mM NaCl, 5 mM KCl, 20 mM HEPES, pH 7.4, 300 mOsm/kg), with careful removal of the buffy coat.

The osmolality was determined with an OMKA 1 Ts-01 cryoscopic osmometer (Russia). The washed erythrocytes were suspended in HBS to a hematocrit of 40–50% and used within 6 h after preparation. The stock suspensions contained no more than 0.2 leukocytes and 5 platelets per 1000 erythrocytes.

Before measurements, the suspensions were diluted to a hematocrit of 0.1, 1, or 4% with HBS containing NaCl at concentrations that provided the desired value of osmolality in the final suspensions.

Erythrocyte concentrations were determined with a model DN Coulter counter.

Glutaraldehyde (GA)-treated erythrocytes were prepared by incubation of cells in suspension (hematocrit of 6–8%) in the presence of 0.012, 0.019, or 0.025% GA for 20 min at 22 ± 0.2°C. The cells were then washed three times with a tenfold volume of 0.01 M glycine in HBS.

The HBSs used to study the effects of pH on the filterability of erythrocytes and their distribution in u_{cr} had pH values of 6.8 and 7.4.

All solutions were filtered through 0.22-μm Millipore filters before use.

2.2. *Determination of erythrocyte filterability*

The filterability of erythrocyte suspensions was measured as a t_b/t_s ratio, where t_s and t_b are the times it took 250 μl of HBS with and without cells, respectively, to pass through a membrane filter at 22 ± 1°C and pressure of 6 cm H_2O. We used our modification of Hanss' hemorheometer (RF Patent 2052194) that allowed measuring t_b and t_s to an accuracy of 0.1 s with a sensor mounted into a lid closing the filled column from above. The sensor in this instrument is a system of three needle electrodes spaced at different depths that is coupled to a time-measuring system. The latter is started at the moment when suspension is allowed to flow out and is stopped when 250 μl has passed through the filter. The instrument was not thermostated, but sample temperature variation was insignificant because both buffer and suspension were kept at 22 ± 0.2°C in a water bath throughout the experiment. Membrane filters were made of a poly(ethylene terephthalate) film (thickness, 7 μm; mean pore diameter, 3.2 μm; Joint Institute for Nuclear Research, Dubna, Moscow oblast, Russia).

All measurements were performed in triplicate at 22 ± 1°C within 6 h after the erythrocytes had been washed. The t_b and t_s times were determined for each filter by allowing aliquots of buffer and then a suspension of a known hematocrit, respectively, to pass through it. Below, the relative flow rate t_b/t_s plotted against the resuspending medium osmolality u will be referred to as an osmofiltration curve. Extrapolating the descending portion of the curve to lower osmolalities by using a linear regression procedure, we estimated the osmolality value at which t_b/t_s becomes zero (Ω). In this way, it was possible to determine Ω to an accuracy of about 3% (±5 mOsm/kg).

2.3. *Determination of the percentage of nonfilterable cells in erythrocyte suspensions by the method of consecutive washing of a filter*

The percentage of nonfilterable cells in erythrocyte suspensions was determined by the method described previously [9]. Briefly, the average number of pores (N_0) in a particular filter was determined from the Poiseuille law. In our case (mean pore diameter of 3.2 μm), $N_0 = 1.5 \times 10^6/t_b$. If multiple aliquots of buffer were applied to a filter through which a small volume of the suspension under study containing the known number of cells (n) had been passed, all cells that could pass through the pores were washed out, whereas the nonfilterable cells remained within pores and clogged them. Eventually, all nonfilterable cells that were in the suspension aliquot entered the pores and the time buffer aliquots took in passing through the filter reached a constant value $t_{bf} > t_b$. Obviously, the number of clogged pores (and, hence, the number of nonfilterable cells in the aliquot) is $N = N_0 \times (t_{bf} - t_b)/t_{bf}$. The total number of erythrocytes in this aliquot is n, hence, the percentage of nonfilterable cells is $Z\% = N \times 100/n$ %.

3. Results

3.1. Substantiation and description of the method

Figures 1a and 1b show the osmofiltration curves for 0.1 and 1% suspensions of normal donor erythrocytes, respectively, before and after treatment with 0.012, 0.019 and 0.025% GA. The 0.1 and 1% suspensions of untreated control erythrocytes had the maximum t_b/t_s values of about 0.85 and 0.70, respectively. GA-treated cells are known to have an increased intracellular viscosity and membrane stiffness [12,15]. It is clearly seen that for each hematocrit, the curves and the Ω values (osmolality at which a suspension stops filtering) for control and GA-treated cells coincide in the hypotonic region. However, the observed Ω value was lower by approximately 15 mOsm/kg at a hematocrit of 0.1% than at a hematocrit of 1%: $\Omega(1\%) - \Omega(0.1\%) \approx 15$ mOsm/kg.

For each hematocrit, the curves for control and GA-treated cells diverged increasingly with increasing osmolality. The higher the concentration of GA, the lower the curve and the steeper its slope at high osmolalities. For 0.012% GA, a decrease in the maximum of the osmofiltration curve was observed only at a hematocrit of 1%. GA at higher concentrations changed the shape of the curves even at a hematocrit of 0.1%. These data suggest that within certain limits, Ω did not depend on the viscoelastic properties of the membrane and/or the entire cell (in contrast to other parameters of osmofiltration curves), but changed depending on the hematocrit value.

The dependence of Ω on the hematocrit value is also evident from comparison of the osmofiltration curves for suspensions of fresh (Fig. 2a) and 3-week stored (Fig. 2b) normal erythrocytes. The curves

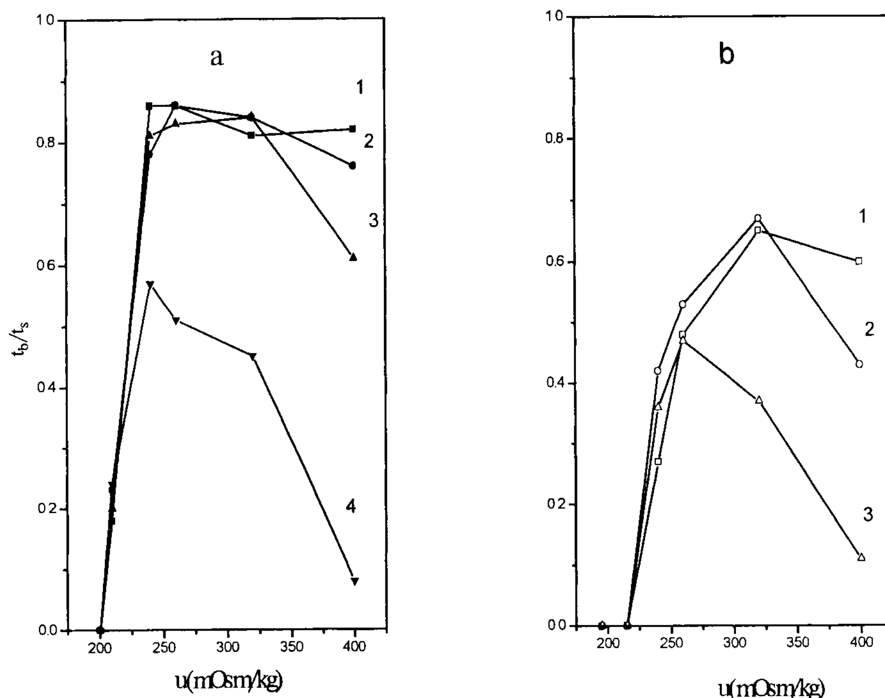

Fig. 1. Dependence of the relative filtration rate t_b/t_s on the medium osmolality u (osmofiltration curves) for normal erythrocytes (1) before and after treatment with (2) 0.012, (3) 0.019, or (4) 0.025% glutaraldehyde. Panel a: hematocrit of 0.1%, Panel b: hematocrit of 1%.

are shown for hematocrit values of 0.1, 1, and 4%. Note that Ω increased with increasing hematocrits. The flow rate of a fixed volume of a suspension (250 μl) becomes zero if the number of nonfilterable cells in this volume is approximately equal to the number of pores of the filter [9]. This holds at medium osmolality $u = \Omega$. As at a hematocrit of 1% the suspension contains about 2.5×10^7 erythrocytes and each filter contains $N_0 = 4-9 \times 10^5$ pores, the relative content of nonfilterable cells ($Z\% = 100 \times N_0/n$ %) at $u = \Omega(1\%)$ is 2–4%. If a hematocrit of the suspension of the same erythrocytes is 0.1%, a tenfold higher percentage of nonfilterable cells ($Z\%$ = 20–40%) is required. Therefore, $\Omega(0.1\%)$ is shifted to more hypotonic values. Conversely, an increase in the hematocrit to 4% leads to an increase in Ω: $\Omega(4\%) > \Omega\,(1\%)$.

The dependence of the % of nonfilterable cell ($Z\%$) on osmolality in suspensions of fresh (Fig. 2a, dotted line) and stored (Fig. 2b, dotted line) erythrocytes were directly measured by the method of consecutively washing the filter. These plots represent the cumulative distributions of erythrocytes in u_{cr}. As is evident, $Z = 50\%$ was observed at a medium osmolality $u \approx 180$ mOsm/kg for fresh cells and at $u \approx 200$ mOsm/kg for stored cells. Therefore, we conclude that the distribution of stored cells is shifted to the right relative to that of fresh cells. Note that despite the different Ω values observed at the same hematocrit for fresh and stored erythrocytes, in both cases $\Omega(0.1\%)$ corresponds to Z of about 30%, $\Omega(1\%)$ to Z of about 3% and $\Omega(4\%)$ to Z less than 1%. Thus, the direct measurements confirmed the estimates of the relative content of nonfilterable cells ($Z\%$) made above.

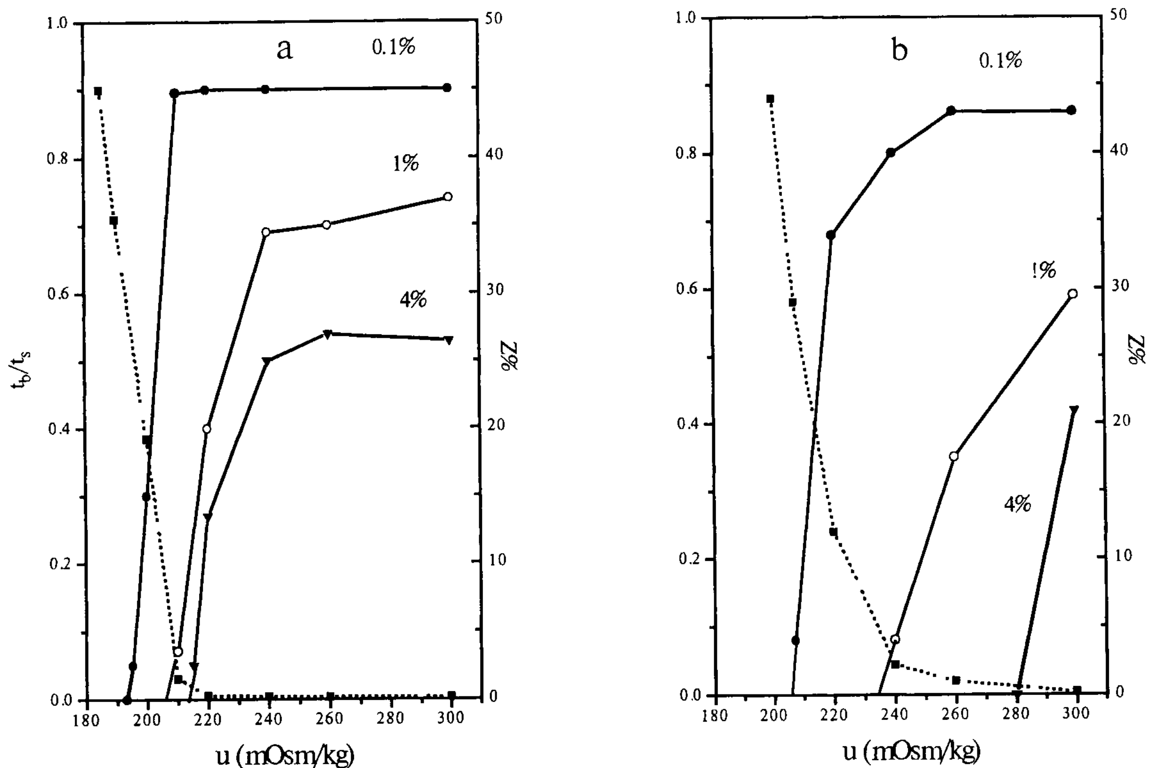

Fig. 2. Dependence of the relative filtration rate t_b/t_s (solid lines) and the percentage of nonfilterable cells $Z\%$ (dotted lines) on the medium osmolality (u) for normal erythrocytes. Panel a: fresh erythrocytes. Panel b: erythrocytes stored for three weeks. The hematocrit value of the suspension is indicated for each osmofiltration curve.

If we assume that (i) $Z = 30\%$ at $u = \Omega(0.1\%)$ and $Z = 3\%$ at $u = \Omega(1\%)$ and (ii) the distribution of erythrocytes in u_{cr} is normal, then $[\Omega(0.1\%) - M] = 0.5\sigma$, where M is the distribution center and σ is the standard deviation (Fig. 3). In other words, $M = u_{cr}$ for the major erythrocyte subpopulation. On the other hand, $[\Omega(1\%) - M] = 1.9\sigma$ for the normal distribution [5].

Therefore, it is possible to assess the distribution of erythrocytes in u_{cr} (the center M and the standard deviation σ) by determining the $\Omega(0.1\%)$ and $\Omega(1\%)$ values:

$$\sigma = [\Omega(1\%) - \Omega(0.1\%)]/1.4, \tag{1}$$

$$M = \Omega(0.1\%) - 0.5\sigma. \tag{2}$$

So, if the distribution in u_{cr} is close to normal, these parameters may be useful in assessing the distribution width and center position.

The value of $\Omega(0.1\%)$ and $\Omega(1\%)$ may be used to characterize the distribution of erythrocytes in u_{cr} even if this distribution is not exactly normal. The osmolality $u = \Omega(0.1\%)$ approximately corresponds to u_{cr} of the modal subpopulation, and the difference $\Omega(1\%) - \Omega(0.1\%)$ can be used as a rough estimate of the width of the erythrocyte distribution in u_{cr}. If necessary, the shape of the distribution can be refined by the results of additional measurements performed at intermediate hematocrit values.

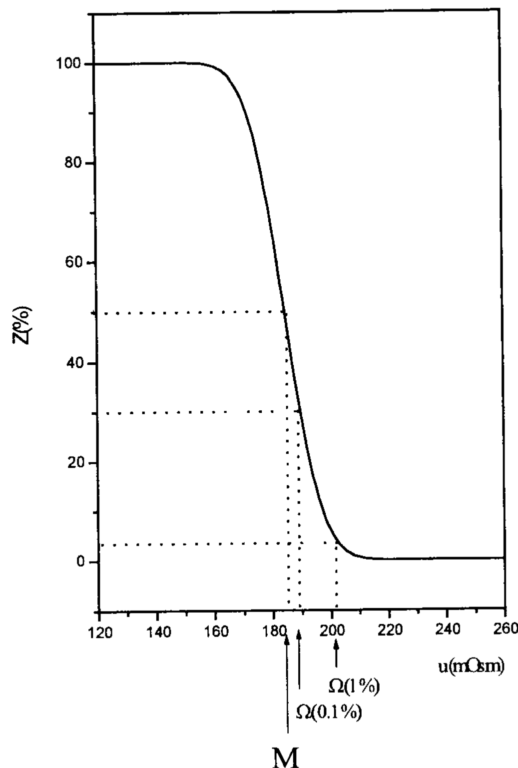

Fig. 3. Relationship between the parameters of normal cumulative distribution of erythrocytes in u_{cr} and the measurable parameters $\Omega(0.1\%)$ and $\Omega(1\%)$. The solid line indicates the simulated normal cumulative distribution of erythrocytes in u_{cr}: $M = 184$ mOsm/kg and $\sigma = 10$ mOsm/kg. The $u = \Omega(0.1\%)$ corresponds to $Z = 30\%$; $u = \Omega(1\%)$ corresponds to $Z = 3\%$.

Table 1

Critical values of osmolality for 1% and 0.1% erythrocyte suspensions from 9 healthy donors: $\Omega(1\%)$, $\Omega(0.1\%)$, their difference $\Omega(1\%) - \Omega(0.1\%)$, and the calculated erythrocyte distribution characteristics M and σ (the data are means ± SD)

$\Omega(1\%)$ mOsm/kg	$\Omega(0.1\%)$ mOsm/kg	$\Omega(1\%) - \Omega(0.1\%)$ mOsm/kg	σ mOsm/kg	M mOsm/kg
201 ± 7	189 ± 9	12 ± 5	9 ± 4	185 ± 8

The values of $\Omega(1\%)$, $\Omega(0.1\%)$, their difference $\Omega(1\%)-\Omega(0.1\%)$, and the σ and M values calculated according to Eqs (1) and (2) are shown in Table 1 for 9 healthy donors.

3.2. Applications of the method: Examples

3.2.1. Normal erythrocytes at pH 6.8

Earlier, using the method of consecutively washing a filter, we observed a parallel shift in the distribution of healthy donor erythrocytes in u_{cr} (15–20 mOsm/kg in the direction of larger osmolalities) at acidic pH values [9]. Figure 4 shows the osmofiltratrion curves obtained with erythrocytes of a healthy donor at pH values of 7.4 (curves 1 and 3) and 6.8 (curves 2 and 4) and hematocrit values of 0.1 (curves 1 and 2) and 1% (curves 3 and 4). It is known that in acidic media, erythrocytes increase in volume [6]; therefore, curves 2 and 4 are shifted to larger osmolalities compared to the control (curves 1 and 3). The value of this shift is the same for erythrocytes from the major and minor (about 3%) subpopulations: $\Omega(0.1\%$, pH 7.4) – $\Omega(0.1\%$, pH 6.8) is 20 mOsm/kg and $\Omega(1\%$, pH 7.4) – $\Omega(1\%$, pH 6.8) is also 20 mOsm/kg. Thus, the osmofiltration method gave results supporting those obtained earlier using another method [9].

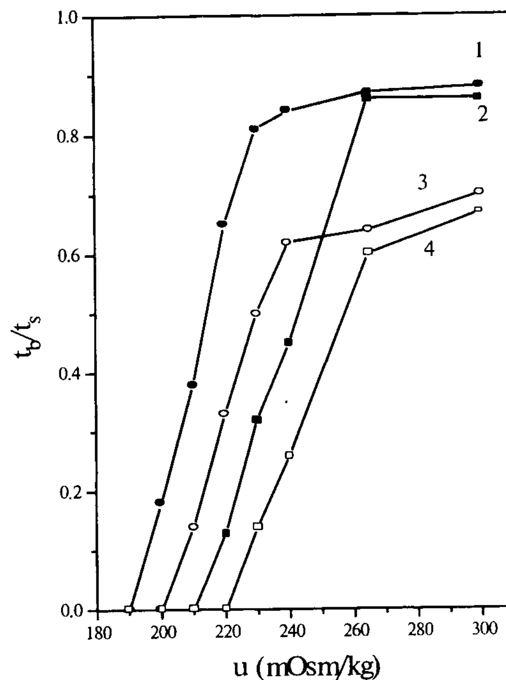

Fig. 4. Osmofiltration curves for normal erythrocytes resuspended at pH 7.4 (curves 1 and 3) or 6.8 (curves 2 and 4) and a hematocrit of 0.1 (curves 1 and 2) or 1% (curves 3 and 4).

Table 2

Critical values of osmolality of 1% and 0.1% erythrocyte suspensions for seven patients with hereditary microspherocytosis: Ω (1%), Ω (0.1%), and their difference Ω (1%) − Ω(0.1%)

Patient	Ω (1%) mOsm/kg	Ω (0.1%) mOsm/kg	Ω (1%) − Ω (0.1%) mOsm/kg
K1	280	234	46
V	286	232	54
B	264	226	38
S	310	220	90
K2	280	220	60
Ch	240	218	32
P	292	254	38
Mean ± SD	278.8 ± 22.1	229 ± 12.6	50 ± 21.6

3.2.2. Erythrocytes of patients with hematological disorders

The values of Ω(1%), Ω(0.1%), and their difference Ω(1%) − Ω(0.1%) determined for seven patients with hereditary microspherocytosis (HS) are shown in Table 2. All Ω(0.1%) and Ω(1%) values are appreciably greater compared to the corresponding control (Table 1).

The large difference Ω(1%) − Ω(0.1%) in HS patients indicates that their erythrocytes are more heterogeneous with respect to u_{cr} than erythrocytes of healthy donors: the distribution width, a quantitative measure of heterogeneity of erythrocytes in u_{cr}, is significantly greater in HS than in healthy individuals. This result is consistent with the data that in HS, the volume-to-area ratio of erythrocytes is decreased and their rheological properties correspondingly deteriorate [3].

In certain pathologies, we observed erythrocyte distributions in u_{cr} that were narrower than the distributions of normal erythrocytes, with Ω(0.1%) being sometimes equal to Ω(1%). This was the case in several patients with hereditary hemochromatosis (3 out of 15). Figure 5 shows the osmofiltratrion

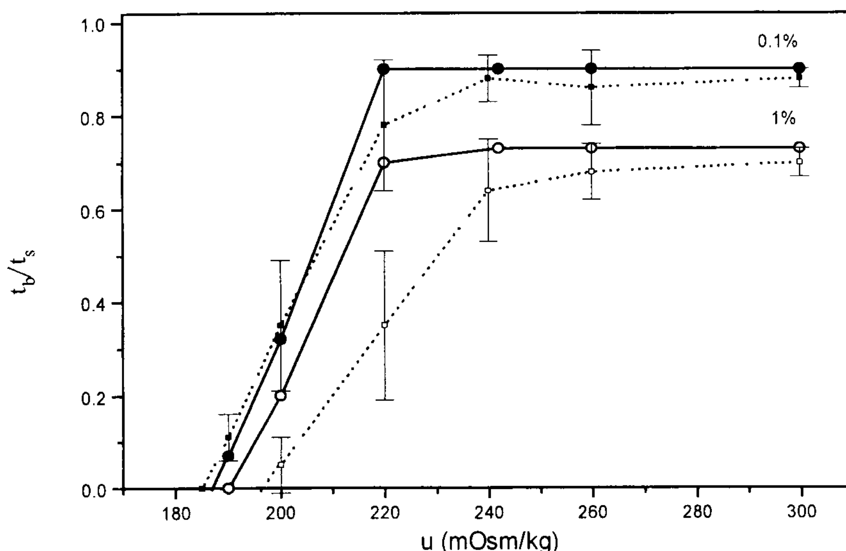

Fig. 5. Osmofiltration curves for erythrocytes of a patient with hereditary hemochromatosis. Dashed lines represent the mean data for 9 normal donors.

curves obtained with erythrocytes of a patient with hereditary hemochromatosis arbitrarily chosen of these 3 patients at hematocrit values of 0.1 and 1%.

The dashed lines represent the mean data for nine normal donors. The rate of filtration of patient erythrocytes did not differ from that of normal erythrocytes at a hematocrit of 0.1%, but was significantly higher at a hematocrit of 1%. In this case, $\Omega(1\%) - \Omega(0.1\%)$ was less than 5 mOsm/kg, indicating that the erythrocytes were highly uniform with respect to their rheological properties.

4. Discussion

The difference between the isotonic and critical osmolality values ($300 - u_{cr}$) indicates to which extent the cell volume increases before the volume-to-surface area ratio reaches the value critical for a given filter (V_{cr}/S). In these terms, u_{cr} and, say, the MCD (minimum cylindrical diameter [2]) provide similar information on the erythrocyte geometric properties. The values of u_{cr} as a function of the MCD were calculated for two different erythrocyte surface areas using the mathematical model describing the filtration of an individual erythrocyte [1]. Figure 6 shows that for a given erythrocyte, a one-to-one correspondence exists between these parameters. Nevertheless, the geometry of an erythrocyte is not the sole parameter that determines u_{cr}. It also depends on erythrocyte behavior in response to varying medium osmolality. Evidently, this may be important for interpreting the data on real suspensions.

It is well known that the flow rate of an erythrocyte suspension through membrane filters depends to a large extent on the minor subpopulations of cells with decreased filterability [12,13]. However, this fact is often ignored in interpreting the data obtained using Hanss-type hemorheometers. Such data are commonly believed to characterize some "averaged" filterability typical of most cells in the suspension under study. In fact, at relatively high hematocrit values, the time it takes a fixed volume of a heterogeneous suspension to pass through a filter primarily depends on the minor fractions of poorly filterable

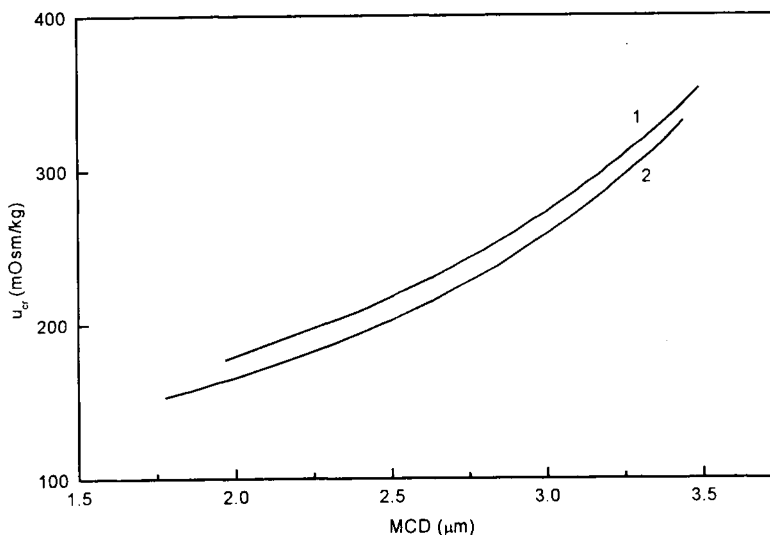

Fig. 6. Dependence of u_{cr} on the MCD calculated for the erythrocyte surface area of (1) 135 and (2) 150 μm^2 [1, 2]. The value of u_{cr} was calculated from eq.: $V_{cr} = H/0.6844 + f/u_{cr}$, where V_{cr} is erythrocyte critical volume, H is hemoglobin content per cell and f – a coefficient [1]. The parameters used in the calculations were as follows: $f = 15\,000$; 10 pg/cell $> H >$ 40 pg/cell. Filter parameters: mean pore diameter, 3.1 μm, thickness, 7 μm.

erythrocytes. For example, if the hematocrit value of a suspension is 8%, its 40-μl aliquot will not flow through a 3-μm Nucleopore filter (area, 80 mm^2; pore density, 1.8×10^4 mm^{-2}) at a nonfilterable cell content as low as 5%. Therefore, if the filterability of only 5% of all cells becomes abnormal, the experimentally measured t_s value will increase, despite the fact that for the other 95% of cells it does not change. The estimates obtained in this way may correlate with some erythrocyte parameters that represent the actual means for the suspensions (e.g., MCV or MCHC) [14]. However, such correlations (or their absence) should be interpreted with caution. Therefore, it is important to have an opportunity to evaluate the distribution of a heterogeneous erythrocyte population in rheological parameters.

In our previous paper [9], the method of consecutive washing of a filter was proposed to evaluate the erythrocyte distribution in u_{cr}. Here, a simplified, less time-consuming method is described to screen heterogeneous suspensions of normal, preserved, or pathological erythrocytes for their distributions in u_{cr} by measuring Ω at two hematocrit values, 0.1 and 1%. The results obtained demonstrate that both osmofiltration methods provide consistent and useful information on the properties of erythrocytes in the blood under study, but the second method is preferable for routine use.

For each suspension, the erythrocyte distribution in u_{cr} characterizes the rheological properties of the major erythrocyte fraction (by the position of the distribution maximum) and the heterogeneity of the suspension (by the distribution width). However, like the MCD distribution, the distribution in u_{cr} does not carry information on all parameters determining cell filterability/deformability. As evident from Fig. 1, changes in the mechanical properties of the membrane (and the cell as a whole) caused by GA treatment significantly decreased the filtration rate but did not change the $\Omega(0.1\%)$, and $\Omega(1\%)$ values, i.e., the distribution characteristics.

The method described provides the possibility to quantitatively assess changes in the rheological properties of both major and minor subpopulations of normal and abnormal erythrocytes. This screening method is simple and sensitive to changes in this distribution in response to experimental stressful factors or in pathologies. Therefore, we believe that it can be successfully used in quality control of erythrocytes intended for transfusion, in diagnostics of a number of disorders, and control of the efficacy of therapy.

Acknowledgment

The authors thank Dr Rimma I. Volkova for helpful discussions during the preparation of the manuscript.

References

[1] F.I. Ataullakhanov, V.M. Vitvitskii, I.L. Lisovskaya and Ye.G. Tuzhilova, Analysis of the geometric parameters and mechanical properties of erythrocytes by the method of membrane nuclear filtration. 1. Mathematical model, *Biophizika* **39** (1994), 672–680.

[2] P.B. Canham and A.C. Burton, Distribution of size and shape in populations of normal human red cells, *Circ. Res.* **22** (1968), 405–422.

[3] J.A. Chasis, P. Agre and N. Mohandas, Decreased membrane mechanical stability and in vivo loss of surface area reflect spectrin deficiencies in hereditary spherocytosis, *J. Clin. Invest.* **82** (1988), 617–623.

[4] M.R. Clark, N. Mohandas and S.B. Shohet, Osmotic gradient ektacytometry: comprehensive characterization of red cell volume and surface maintenance, *Blood* **61** (1983), 899–910.

[5] G.A. Korn and T.M. Korn, *Mathematical Handbook for Scientists and Engineers. Definitions, Theorems and Formulas for Reference and Review*, McGraw-Hill Book Company, Inc., New York, Toronto, London, 1961.

[6] D.G. Legge and K. Shortman, The effect of pH on the volume, density and shape of erythrocytes and thymus lymphocytes, *Brit. J. Haematol.* **14** (1968), 323–335.

[7] V.L. Lew, J.E. Raftos, M. Sorette, R.M. Bookchin and N. Mohandas, Generation of normal human red cell volume, hemoglobin content, and membrane area distributions by "birth" or regulation, *Blood* **86** (1995), 334–341.

[8] J. Linderkamp and H.J. Meiselman, Geometric, osmotic and membrane mechanical properties of density-separated human red cells, *Blood* **59** (1982), 1121–1127.

[9] I.L. Lisovskaya, E.S. Shurkhina, V.M. Nesterenko, Yu.M. Rozenberg and F.I. Ataullakhanov, Determination of the content of nonfilterable cells in erythrocyte suspensions as a function of the medium osmolality, *Biorheology* **35** (1998), 141–153.

[10] N. Mohandas and J.A. Chasis, Red blood cell deformability, membrane material properties and shape: regulation by transmembrane, skeletal and cytosolic proteins and lipids, *Sem. Hematol.* **30** (1993), 171–192.

[11] W.H. Reinhart and S. Chien, Roles of cell geometry and cellular viscosity in red cell passage through narrow pores, *Am. J. Physiol.* **248** (1985), C473–479.

[12] W.H. Reinhart and P.W. Straub, Detection of less deformable erythrocytes in suspensions: evaluation of different rheological tests, *Clin. Hemorheol.* **8** (1988), 861–876.

[13] J. Stuart and G.B. Nash, Technological advances in blood rheology, *Crit. Rev. Oncol. Hematol.* **28** (1990), 61–93.

[14] J. Stuart, P.C.W. Stone, D. Bareford and Y.Y. Bilto, Effect of pore diameter and cell volume on erythrocyte filterability, *Clin. Hemorheol.* **5** (1985), 449–461.

[15] P.S. Vassar, J.M. Hards, D.E. Brooks, B. Hagenberger and G.V.F. Seaman, Physicochemical effects of aldehydes on the human erythrocyte, *J. Cell Biol.* **53** (1972), 809–818.

[16] R. Waugh, N. Mohandas, C. Jackson, T. Mueller, T. Suzuki and G.L. Dale, Rheologic properties of senescent erythrocytes: loss of surface area and volume with red blood cell age, *Blood* **79** (1992), 1351–1358.

[17] J.C. Zhu, P.C.V. Stone and J. Stuart, Measurement of erythrocyte deformability by cell transit analyser, *Clin. Hemorheol.* **9** (1989), 897–908.