THEORETICAL AND EXPERIMENTAL APPROACHES TO STUDY MECHANICAL PROPERTIES OF BLOOD

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ABSTRACT

A great variety of instruments, methods and models to study hemorheological parameters under conditions close to physiological are currently used. An overview of the factors, determining hemorheological properties and models, describing the relationship between shear stresses, shear rate, viscosity and hematocrit is presented. Time dependent properties – blood thixotropy and viscoelasticity and the influence of the inner structure on the blood flow has been described. A method, based on dielectric properties of dispersed systems in Couette viscometric blood flow, previously described[1,2] and applied to investigate the kinetics of RBC aggregation has been presented. The experimental relationships show that the human blood conductivity is time, shear rate and hematocrit dependent under steady and transient flow conditions. The results show that valuable information could be received about the mechanical properties of blood, in particular about the kinetics of "rouleaux formation" and that this technique may be used to clarify the mechanism of dynamics of RBC aggregates. Thus a method, based on dielectric properties of dispersed systems in Couette viscometric blood flow could be applied to investigate the kinetics of RBC aggregation.

Keywords: Blood models, new instrument, electrorheological method

1. FACTORS, DETERMINING HEMORHEOLOGICAL PROPERTIES

Plasma viscosity, blood viscosity, RBC deformability and RBC aggregation are the main factors, determining hemorheological properties. The RBC aggregation is a fundamental parameter determining rheological properties of blood. This is dynamic phenomena observed *in vitro* и *in vivo* and responsible for increasing blood viscosity at low shear rates. Changing of these factors influences the circulation in various blood vessels and microvessels through their participation in the pathophysiology of atherosclerosis, promotion trombogenesis and complexity of pathogenesis of various vascular diseases.

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Viscosity of fluids is a property, which appears from the kinetic theory of fluids. When monitoring, the mean effect involving a large number of hypothetical fluid particles, the adjacent fluid layers slide on one another and that slipping is opposed by friction. The magnitude of this friction is dependent on the fluid constitution which is from the macroscopic point of view described by a flow property called viscosity. The apparent viscosity of Non--Newtonian fluids such as blood, as determined under steady flow conditions, depends on the shear rate, hematocrit, temperature etc. In addition to this under non-steady and oscillating flow conditions, this parameter is further influenced by the frequency and amplitude of the measurement system. To analyse their influence, several mathematical models are developed.

1.1. Relationship between shear stresses and shear rate

The blood flow is well known for its complex rheological behaviour. The non-Newtonain features, such as shear thinning and viscoelasticity of blood, have been widely observed – Chien et al, 1970[3]; Thurston, 1979[4], which are closely relevant to the deformation and the aggregation of red blood cells in the blood.

The relationship between the shear stress and the shear rate must be determined experimentally. To be most useful, this relationship must be expressed as a mathematical equation. Some such equations are the result of curve-fitting experimental data, while others are based on a model of the fluid. Some useful empirical relationships are given here:

(1) $$\tau = \eta.\dot{\gamma}$$

where τ is the shear stress [mPa], η – dynamic viscosity, [mPa.s], $\dot{\gamma}$ is the shear rate [s^{-1}]. For Newtonian fluids η = const; for Non-Newtonian fluids such as blood $\eta = \eta(\dot{\gamma})$

Ostwald-de-Waele's equation:

(2) $$\tau = k.\gamma^n$$

where τ is the shear stress [mPa], $\dot{\gamma}$ is the shear rate [s^{-1}], k and n are parameters

Herschel-Bulkley's equation:

(3) $$\tau = \tau_0 + a.\gamma^m$$

where τ is the shear stress [mPa], τ_0 – is the yield stress, [mPa], $\dot{\gamma}$ is the shear rate [s^{-1}], a and m are parameters.

Casson's equation:

(4) $\tau^{0.5} = \tau_0^{0.5} + \mu_c^{0.5}.\dot{\gamma}^{0.5}$, for $\tau \geq \tau_0$
$\dot{\gamma} = 0$, for $\tau \leq \tau_0$

where τ is the shear stress [mPa], τ_0- is the yield stress of the order of 5 mPa, μ_c is a constant, $\dot{\gamma}$ is the shear rate [s^{-1}]. There is a critical hematocrit value H, ranging from 1,3 to 6,5%, below which there is no yield stress.

1.2. Effect of hematocrit (relative cell volume fraction) on viscosity

Einstein established the equation for the viscosity of suspensions of solid particles in terms of viscosity of

continous phase and fraction of particles given as,

$$(5)\quad \eta_s = \eta_p + \eta_p KH = \eta_p(1+KH)$$

Where η_s is the viscosity of suspension, the shape factor K for spherical particles and dilute suspension is equal to 2.5, H is volume concentration of particles.

Whittington and Harkness's equation is derived from (5)

$$(6)\quad \frac{\eta}{\eta_0} = \left\{ \frac{A}{(1-\log\gamma / \log\gamma_c)\beta} \right\} \varphi$$

Where η_0 is the blood plasma viscosity, γ is the shear rate, γ_c is the critical shear rate, β is shear sensitive constant, $\dot{\gamma}$ is shear rate [s^{-1}], φ is the relative cell volume or hematocrit.

Cokelet's equation for RBC suspensions is:

$$(7)\quad \frac{\eta_s}{\eta_p} = \frac{1}{(1-H)^{2.5}}$$

where η_s is the RBC suspensions viscosity, η_p is the viscosity of the continuous phase or the plasma, H is the relative cell volume or hematocrit.

2. VISCOMETRY

The rheological properties of fluids are measured in viscometers specially designed so that only one velocity component exists and does not vary in the direction of flow. In addition, except for tube viscometers, the fluid space in the viscometer is small enough so that all of the fluid is subjected to approximately the same shear rate. The rheological properties of a fluid may be divided into two classes: time independent in almost all *in vivo* and *in vitro* flows and time-independent rheological properties.

2.1. Capillary viscometers – Flow of Poiseuille

The solutions of the equations of motion provide in the case of Poiseuille flow the fluid viscosity. For a Newtonian fluid, which obeys Poiseuille equation and has a linear pressure-flow rate curve passing through the origin, there exists a unique coefficient of viscosity (at a constant temperature).

$$(8)\quad \eta = \frac{\pi a^4}{8Q}\frac{\Delta P}{L}$$

For the corresponding flow rate Q, pressure drop ΔP, capillary radius a, capillary length L

2.2. Rotational viscometers – Couette Flow

In the case of Couette flow

$$(9)\quad \eta = \frac{R_2^2 - R_1^2}{R_1^2 R_2^2}\frac{T}{4\pi\Omega L}$$

Where L is the length of the cylinder, Ω the angular velocity, R_2 and R_1 the radii of outer and inner cylinder respectively.

2.3. Time dependent properties – blood thixotropy and viscoelasticity

The blood flow in the blood vessels, especially aorta and large arteries as well as in the microcirculation va-

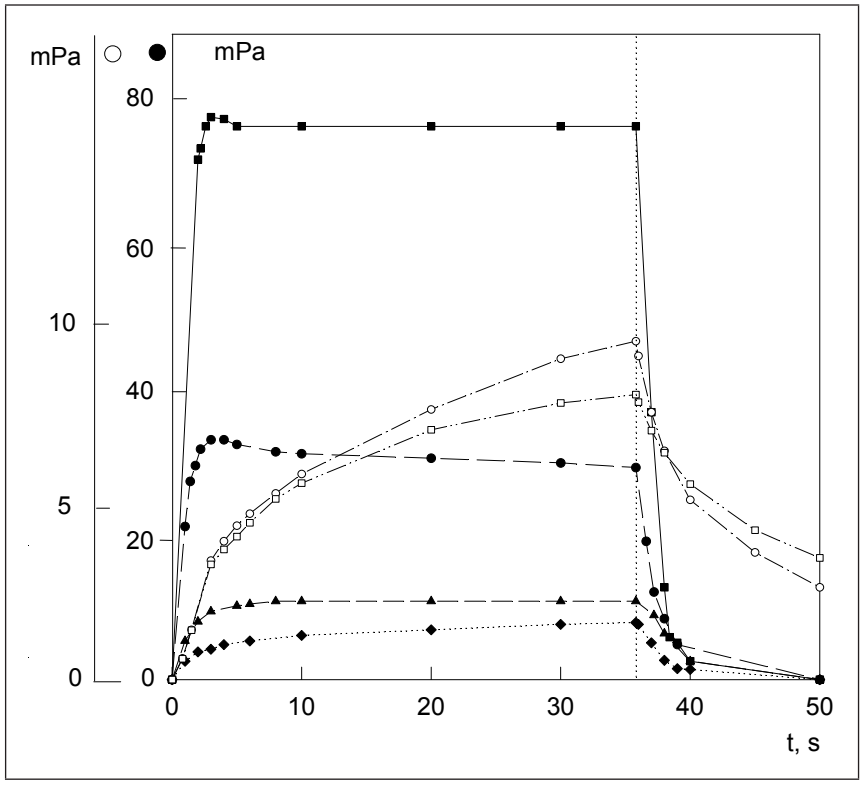


Figure 1. Rheograms at rectangular change of shear rates from 0 s^{-1} to 0.0596 s-1 to 0 s^{-1} for patients with CVD (°), from 0 to 0.0237 s^{-1} to 0 s^{-1} (□), from 0 to 5.96 s^{-1} to 0 s^{-1} (■), from 0 to 1.285 s^{-1} to 0 s^{-1} (●), from 0 to 0.277 s^{-1} to 0 s-1 (▲), from 0 to 0.1102 s^{-1} to 0 s^{-1} (♦); H = 43 %, T = 37°C, Low Shear 30 Contraves[5]

ries periodically. The geometrical complexity of the circulation system gives rise to areas where blood flow is significantly accelerated and there are varying transient flow conditions. The alteration of blood flow with time is typical for viscoelastic fluids and this phenomenon is related with the influence of the inner structure on the blood flow. In the case of thixotropy (Fig.1) when the flow is stopped, the particles relate each other and the process of destroying and creating of the relations is balanced. The forces of connections are weak. The higher shear rate leads to decreasing the size of the related structural units, corresponding to the less resistance to flow.

Similarly, the macromolecules of the viscoelastic fluid form set with temporal connections and destroying and creating of the relations is balanced. When the fluid is sustained to the larger shear rates, more macromolecules are destroying for a unit time. Consequently the thixotropy and viscoelasticity are related with similar time effects, reflecting in the shear rate effects. From this point of view the theories are developed, describing the mechanisms of thixotropy and viscoelasticity.

The properties of blood in the ground state of equilibrium can be measured by application of very small forces and deformations that do not disrupt the internal structure. By using a small sinosoidal perturbation at a fixed radian frequency, ω, the viscoelasticity in the ground state is obtained. These properties can be measured over a wide range of frequencies. The complex coefficient of viscosity, $\eta^*(\omega)$, describing the viscoelasticity, is the complex ratio of the shear stress, τ, to the shea rate $\dot{\gamma}$. The coefficient is divided into its real, energy dissipative part, η_V, and its imaginary, energy storage part, η_E.

The steady dynamic viscosity is determined using a rotational viscometer Low Shear 30 Contraves under steady viscometric flow within a shear rate range from 0,0237 s^{-1} to 128,5 s^{-1}. The Low Shear 30 Contraves can be switched from rotation to oscillation (Low Shear 30 sinus) using a rigidly mounted additional driving device (oscillation drive).

Apparent steady shear viscosity was obtained started from a shear rate of 128,5 s^{-1} and decreasing the shear rate in different number of steps, thus realizing quasi non-steady flow conditions. Complex dynamic viscosity is determined by measuring

the angular displacement δ varying the frequency from 0,000222 Hz up to 1,63 Hz at a firmly set amplitude of ±30º.

Human blood exhibits non--Newtonian viscosity and non-linear viscoelasticity, depending on shear rate and frequency. The results of experiments under non-steady flow conditions show that the shear stresses are increasing and viscosity decreasing with increasing shear rate or frequency (Fig.2).

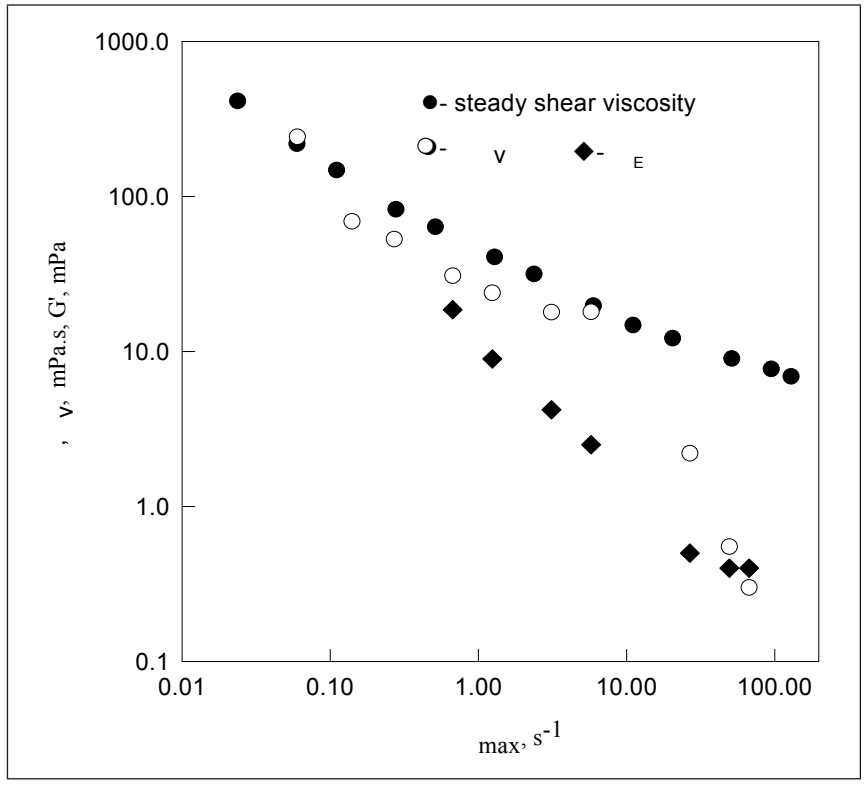


Figure 2. The experimental shear rate dependence of steady shear viscosity (filled circles) and oscillatory shear rate dependence of viscous part (η_v) of the complex viscosity (hollow circles) and elastic component (η_E - filled rombus) of human blood of patient with CVD; (H =45%,T=37±0.1°C), Low Shear 30 sinus Contraves[5]

3. ELECTRORHEOLOGICAL METHOD

3.1. Methods and Clinical Applications

The phenomenon of electrorheology refers to changes in the rheological behavior due to imposition of electric field. In general the electrorheological properties of blood are determined by a variety of factors including the electrical properties of blood cells and plasma, the fractional volume concentrations of the blood cells as well as the shape and orientation of the cells. When blood flows, all these complex factors may change and contribute in a different way to the overall impedance of the flowing blood. In physiological conditions human blood cells have a negative electrokinetic charge to maintain a physico-chemical stability of cellular suspensions. The interface of the suspended cells and blood plasma is the site of complex phenomena as the ionisation of solid phase molecules. Resulting spatial charge distribution induces the electric potential which plays a decisive role in determining cell rheological properties and cell interaction. In the flowing blood the red blood cells deform and rotate in plasma. The electrical resistivity of flowing blood depends on the direction of the applied electric field, but also on the orientation and deformation of the RBCs. On the other hand, the shape and arrangement of RBCs is influenced by the kinematics and dynamics of flow, respectively by the deformation rate and stress deformation. Therefore, by measuring the electric properties of blood, valuable information could be received about mechanical properties of blood during evaluation of some diseases, which affect modification of the local flow pattern and the rheological properties of blood.

Recently interest in using electrorheological methods for determination the mechanical properties of blood and blood cells increased. Blood electrical properties (impedance, permittivity, conductivity etc) have a wide range of applications in biomedical field. Therapies of malignant tumours ranging from actinotherapy, chemotherapy and angiogenesis inhibitors to surgical removal are routinely used. However, most of these therapies are toxic or invasive to the well being of the patient. Consequently, new therapies are examined including a therapy inhibiting blood supply to the tumour and thus inducing necrosis. To form a seal and block the flow, the electrorheological (ER) fluids and an electric field can be used. ER fluids have inner structure, which exhibit dipolar interaction when an electric field is applied turning the fluid temporarily into solid. Cancer cells exhibit altered local dielectric properties compared to normal cells, measurable as different electrical conductance and capacitance using electrical impedance scanning (EIS). EIS appears to be a promising new additional technology providing a rather high sensitivity for the verification of the suspicious breast lesions. Reduction of blood flow can lead to an increase of hypoxia and extra-cellular acidification. Additionally, if blood flow is chronically impaired, a cascade of tumour cell death will occur due to lack of nutrients and accumulation of catabolite products. Different mechanisms are involved in antitumor effectiveness of electrotherapy by low level electric current, which induces significant reduction of blood flow and oxygenation in tumours.

The investigation of the possibility of using the polymeric blood substitutes and biocompatible polymeric fluids as ER fluids consists of the determination of the basic parameters of the ER fluid sealing such as the sealing time, resistant pressure and the strength of the electric field that are important for designing the future studies on biological models.

A method based on dielectric properties of dispersed systems is developed to investigate RBC aggregation in blood and RBC suspensions. Measurements of capacitance and resistance are made in rectangular channel at low (0.2 MHz) and high (14 MHz) frequencies relative to the mid-point of the β-dispersion range. The conductance and capacitance of flowing and quiescent red blood cell (RBC) suspensions were made at a frequency of 0.2 MHz. The results demonstrate that the time-dependent changes in the conductance recorded during the aggregation process differ in nature for short linear rouleaux; branched aggregates and RBC network.

3.2. Measuring and data acquisition system

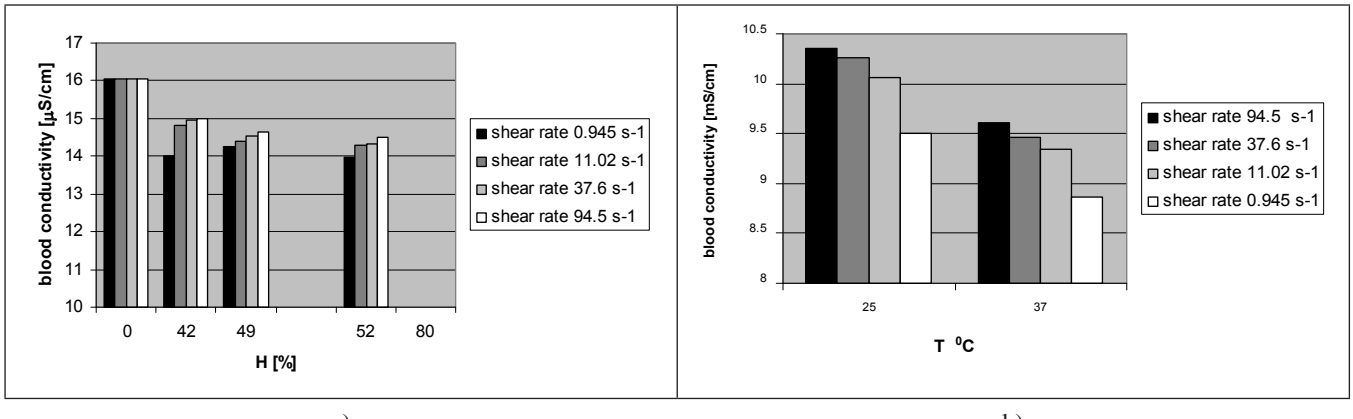
The conductometric method has been applied to develop measuring and data acquisition system for determination electrorheological properties of blood. The concurrent measurement system (MS), using a Contraves Low Shear 30 rheometer as a base unit was developed[1,2]. A resin made replica of the Couette type flow chamber of the LS 30 rheometer was made. It includes a pair of platinum electrodes in cylindrical ring shape, embedded into the wall.

It can be assumed that the electric field is homogeneous since the width of the flow slit is very small compared with the cylinder diameters[1,2]. The principal electronical scheme of the device for acquisition and processing of data (Data acquisition system) from the rotational viscometer Contraves Low Shear 30 is shown and described in details in[1,2].The conductometric method is based on the measurement of the AC current response resulting from a small amplitude (200 mV p-p) sinusoidal (2 kHz) AC voltage applied between a pair of platinum electrodes. The AC current is converted in voltage with the aim of a controlled gain I/E converter. Then the 2 KHz AC voltage signal is processed by a precise linear rectifier and then by a low pass filter in order to obtain a DC signal proportional to the measured electrical conductivity of the blood or other biological sample. This DC signal could be recorded by means of X-Y recorder or by a PC using a specially developed device and software for collection and processing of data from the rotational viscometer Contraves Low Shear 30 (Data acquisition system). The microprocessor module has been managed by the program, which has been installed in the PC, through interface RS 232. By means of appropriate graphic interface in the program the frequency of data acquisition has been given. The rate of data acquisition could be given from 256 μs to 15 min period between two measurements. These data graphically are visualized on the desktop of the PC as a function of time. The developed concurrent measurement system (MS), the device and software were used as a basis for series of experiments.

3.3. Results

We found the apparent viscosity dependence on hematocrit under electric field of 2 kHz. It has been observed that the blood conductivity depends on the applied shear rates (Fig.3a) and temperature (Fig.3b); due to flow the blood conductivity increases, when shear rate increases from 0,945 s-1 to 94,5 s-1 and temperature increases from 25ºC to 37ºC.

The time dependences of the blood conductivity on the regime of the applied viscometric flow have been stu-



a) b)

Figure 3. Experimental relationship between conductivity and a) shear rates from 94,5 s^{-1} to 0,945 s^{-1} for whole human blood samples with different native hematocrit and b) temperature for whole human blood (H=38%), measured by LS 30 Contraves at shear rate from 94,5 s^{-1} to 0,945 s^{-1}, T=37°C

died. To investigate cell transformation during aggregation processes under transient flow conditions, whole human blood was subjected to shearing to disperse all aggregates and the flow was stopped to allow RBC aggregation. The kinetics of the whole blood conductivity was measured. It was found that the whole blood conductivity increases with increasing shear rate (Fig. 4). After cessation of flow, the conductivity tends to fall to the same initial value. These results may be explained by the formation of branched aggregates at different flow conditions and this process could be qualified by the kinetic conductivity measurements. A time course of blood conductivity recorded under different flow conditions provides experimental description of RBC aggregation--desaggregation processes. The initially time-dependent changes must reflect the rupture of intercellular links. The break-up of intercellular links increases both the concentration of aggregates and the degree of branching. To investigate aggregation process in stasis and under flow conditions after subjected to shearing to disperse all aggregates RBC suspension was stopped or decreased to allow RBCs aggregation[6]. The relaxation part of the kinetic curve of blood conductivity reflects the RBC aggregation process (Fig. 4). This finding is consistent with the literature reports[7].

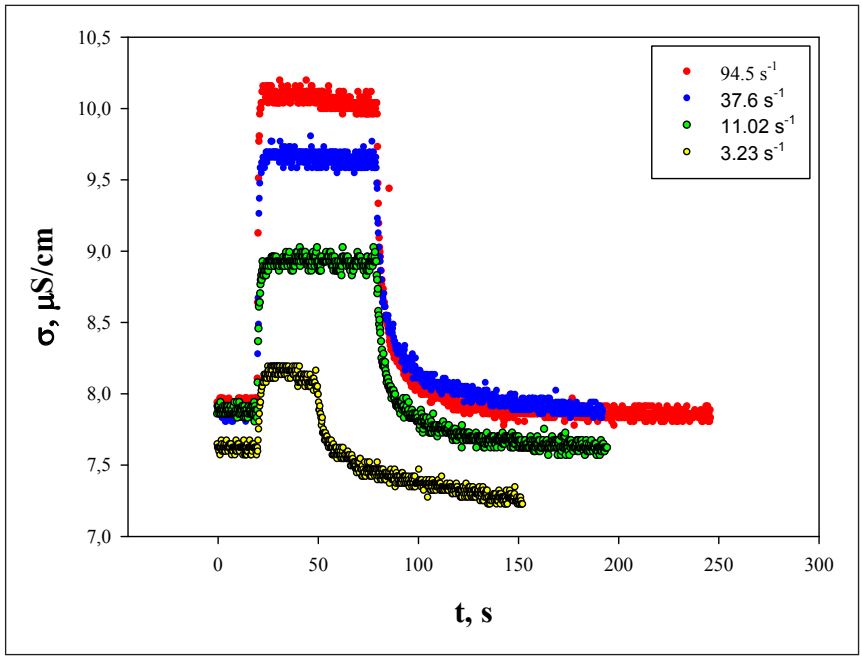


Figure 4. Experimental relationship of changes of conductivity with time under trapezium-shaped changes of shear rates from 3,23 s-1 to 94, 5 s-1 for whole human blood (H=39,6%), measured by LS 30 Contraves, T=370C

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REFERENCES

1. Antonova N., Riha P., Zlatev R., Ivanov I., 2005, Experimental relationships between the blood conductivity and blood rheological properties, 3rd European Medical & Biological Engineering Conference EMBEC 2005 Conference, November 20-25, 2005, Prague, Czech republic – IFMBE Proceedings Series, 11 (1), 4247-4252
2. Antonova N., Riha P., 2006, Studies of electrorheological properties of blood, J. Clin. Hemorheology and Microcirculation, 35, 19-29.
3. Chien et al, 1970, Shear dependent deformation of erythrocytes in rheology of human blood, American Journal of Physiology 219, 136–142.
4. Thurston, 1979, Rheological parameters for the viscosity, viscoelasticity and thixotropy of blood. Biorheology 16, 149–162.
5. Antonova N., Blood viscoelastic and thixotropic properties, 1998, J. Theoretical and applied mechanics, 28,3, 42-54.
6. Antonova, N., Riha, P., Ivanov I., 2008, Time dependent variation of human blood conductivity as a method for an estimation of RBC aggregation, J. Clinical Hemorheology and Microcirculation, 39, 1-4, 69-78.
7. Pribush A., Meyerstein D., Meyerstein N., 2004, Conductometric study of shear-dependent processes in red cell suspensions. II. Transient cross-stream hematocrit distribution, Biorheology 41, 29-43.