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Separation of Cells From Plasma by Means of Ultrasonics

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This paper presents an analysis of use of ultrasonic standing wave in cell separation from bodily fluids based on the example of erythrocyte separation from plasma. It describes movement of red blood cells in plasma under the influence of the acoustic field (whose forces result from interaction of red blood cells with plasma as the vibrating medium) and under the influence of resistance forces in Stokes' and Oseen's approximation. The general properties of solutions of the motion equation are given. The solutions for the parameters of the ultrasonic wave and blood cells which are interesting in terms of practical applications in medical diagnostics are discussed. Time constants of the cell transportation to the regions of stable equilibrium in the field of ultrasonic standing wave are estimated. The formulas which determine the time needed to obtain the assumed concentration increase in plasma in nodes and/or anti-nodes of the standing wave are derived.

Keywords: ultrasound standing wave; medical diagnostics; body fluids; blood; red blood cells; cells separation; plasma.

1. Introduction

Contemporary acoustics has developed in an interdisciplinary field encompassing various disciplines which are key for many branches of modern technology (Kozaczka, 1988) and medicine (Śliwiński, 2003).

One of the core issues in acoustics is evaluation of influence of the acoustic field on particles suspended in a liquid medium. One of the new monographs dealing with the above mentioned topic is (Czyż, 2003), which presents an analysis of influence of the acoustic waves on a heterogeneous medium. Ultrasonic acoustic waves (Barnett, 2000) are important diagnostic tools. Due to their complex interactions with human tissues, they are widely applied in medicine.

Diagnostic medicine is based on physical sciences due to which it is possible, inter alia, to precisely specify the composition of human blood. Blood structure allows for the separation of red cells from plasma. Since blood cells perform a variety of biological functions and take part in many disease processes, it is of great importance for the diagnostics to define blood composition (Nowicki, 2010). Methods for separating blood into components are therefore widely researched.

The problem of motion of small particles in the ultrasonic field is interesting as the fundamental physical effects of sound. This motion of particles in ultrasonic standing wave consists in monotonically approaching the stable equilibrium point or quasi-periodical vibration with amplitude damping. The aim of the present paper is to extend the theory of the particles' motion in the ultrasonic field to cover medical problems: as an innovative method of separation of erythrocytes from plasma.

Mechanisms of separation of cells from blood plasma are very complicated. The conventional methods for such separation include centrifugation and filtration. While these methods are subject to constant

improvements, new, more innovative methods are also sought. Based on the short analysis of physical processes underlying traditional methods of separating blood into components, an innovative method using ultrasonic waves with parameters selected appropriately to the physical parameters of blood components, is presented in (Clement, 2004).

The topic is a subject of extensive research, both theoretical and experimental. Some more recent publications include (Benes *et al.*, 2003) describing experimental research on separation of cells from bodily fluids. The authors put forward a proposal of constructing an ultrasonic resonator in which ultrasonic standing wave could be applied. When the particles were subjected to the ultrasonic wave, changes in cell concentration in plasma have been observed within a short period of time. Cells concentrated in the nodes of the standing wave. Similar experiments were conducted by (Pashovkin, Sadikova, 2009). Reviewing the results of the research done on this topic in the available scientific literature proves the complexity of the phenomena related to the application of ultrasonic waves in medical and biological processes (Benes *et al.*, 2005).

Experimental and theoretical work undertaken on the abovementioned topics aim at finding an appropriate selection of conditions allowing for manipulation of the cell separation process while ensuring cell safety. For the methods using ultrasonic standing wave to create cell concentration areas it is important to provide a comprehensive description of the phenomena as well as to determine the time needed to obtain the assumed concentration increase in a certain area.

2. Human blood

2.1. Composition, properties, characteristics

From a physico-chemical point of view, blood is a suspension, i.e., a mixture of liquids and soft matter objects (cell components) which flows through all human organs; it is a source of information about the body condition and a fundamental diagnostic specimen which can be easily taken from the patient without causing any harm. Human blood is composed of cells: erythrocytes, platelets, and leukocytes, which are suspended in plasma. Plasma contains water (approx. 85%), organic materials (mainly proteins), organic compounds (such as glucose), and inorganic materials (mainly chlorine and sodium ions). The ratio of the volume of erythrocytes to the total volume of blood is called hematocrit (HCT). The hematocrit value is expressed as a percentage. The normal hematocrit for adult women ranges from 37% to 47% whereas for adult men it does from 43% to 54%. Erythrocytes have round, biconcave shape and an average diameter of 7 μm to 7.5 μm. They are classified as morphotic elements and have high elasticity due to which they become deformed while flowing through narrow capillaries.

Blood and its components play many important roles in the human body and all life processes (Łętowska, 2011). The bone marrow produces 2–2.4 million red blood cells (erythrocytes) in one second, with a maximal lifespan of 120 days (Strippoli *et al.*, 2013). White blood cells (leukocytes) are divided by structure and shape into five classes including: neutrophils, eosinophils, basophils, monocytes, and lymphocytes. Platelets, also known as thrombocytes, are important blood components. They have no cell nucleus and they are disc-shaped with an average diameter significantly smaller than that of other blood components (2–4 μm) (Gray, 2008).

Viscosity, defined as a fluid's internal resistance to flow, is an important parameter of every liquid. Viscosity η is the ratio of shear stress and shear rate. Shear stress is defined as the ratio of the force causing displacement of a layer to the area over which the force is applied. Shear rate is the ratio of layer displacement velocity and distance between layers. According to Newton law, the friction force between two layers of a fluid is directly proportional to the difference in velocity of the displacing layers and inversely proportional to the distance between the layers. Viscosity of fluid is the proportionality factor.

Whole blood is a non-Newtonian fluid, i.e., its viscosity depends on the shear rate. Some of important factors affecting the viscosity of blood include: shear rate (the viscosity increases with the decrease of the shear rate), temperature (with the rise of temperature viscosity decreases), HCT (the lower hematocrit, the lower viscosity).

Blood is a heterogeneous mixture. To determine average blood density several measurements taken at different points are needed.

2.2. Falling of blood cells in the gravitational field

Spontaneous fall of the red blood cells (erythrocytes) is known as sedimentation. The process of sedimentation is a result of gravitational force and it begins just after taking blood sample from a patient. Since erythrocytes are heavier than other blood components, they fall to the bottom of the tube and create dark purple suspension which is approximately 40% of the total tube volume. Above the layer of red cells, a creamy buffy coat is created by leucocytes and platelets. The next layer is made by plasma which is a straw coloured liquid. The component constitutes over 50% of the tube volume. It is after more than ten hours that sedimentation ceases. In order to accelerate this process, laboratory centrifuges are used for spinning a sample and separating blood into components. A compound called anti-coagulant is added to the blood sample to prevent it from clotting and enable centrifugation of

serum. Measuring the erythrocyte sedimentation rate (ESR) is known as Biernacki's Reaction (OB) and it is one of the most common tests taken. The value of ESR depends on many factors ranging from 7 mm/h to 15 mm/h for adult women and from 5 mm/h to 10 mm/h for men. The test should be performed at a constant temperature (around 20°C) without exposure to any external stimuli. Sedimentation rate while spinning depends on many factors such as the protein content, quantity of red blood cells as well as their shape and size.

2.3. Conventional methods for separating blood into components

Centrifugation is one of the traditional methods for separation of blood into components and it is based on application of the centrifugal force. The process uses differences in density of cells subject to test. A centrifuge is equipped with a rotor that regulates the velocity of spinning. Since the temperature affects separation of blood components significantly, thermoregulation systems are applied to keep it constant. A vacuum chamber in which a rotor is installed is an important part of a centrifuge. The vacuum eliminates friction that heats the rotor when in contact with air. Centrifugation requires samples to be put in hermetically sealed containers. While spinning, the centrifugal force that is hundreds of thousands times bigger than gravity is created in the rotor. The following forces act on the particles with a certain mass that are suspended in liquid: the centrifugal force, the frictional force which depends on the velocity of particle motion in the sample, and the buoyancy force which, just as the frictional force, acts opposite to the direction of the centrifugal force. At known centrifugal acceleration, sedimentation velocity is directly proportional to the product of the particle volume and the difference in density between the particle and the medium. It also depends on the friction coefficient. Due to centrifugation it is possible to shorten the time of blood fractionation. While spinning the sample, specific parameters must be set to carry out the whole process properly. Modern centrifuges are equipped with additional functions of acceleration or deceleration to ensure the required rotation velocity.

Filtration is a method of separating blood cells from plasma through mechanical retention of the soft matter objects components by porous membranes called filters. In this process, the type of membrane (filter) plays an important role as it creates an obstacle for the flowing blood cells. Blood components flow at different velocities from the feed solution through the membrane (blood) to the receiving solution (permeate). The process takes place in the membrane module to ensure the flow of separated fluid in a parallel or perpendicular direction to the membrane surface.

In filtration porous membranes are used in which the separation is based on the so called sieve effect. The size of the pores is decisive for effective separation. Transportation through the membrane is due to the application of a proper driving force. Membrane performance is characterised by two parameters: permeate stream describing membrane efficiency and effectiveness reflecting the membrane's filtration capacity.

3. Ultrasonic method of separation of erythrocytes from plasma

3.1. The theory outline

Analysis of separation of erythrocytes from blood plasma using selected parameters of ultrasonic wave and particles (erythrocytes) suspended in plasma is based on a mathematical model, which assumes the following:

- no interaction between particles of approximately spherical shape;
- uniform, even initial cell concentration in liquid;
- motion of a single particle caused by influence of frictional forces that depend on the velocity, and forces of the acoustic field (drift forces).

In the field of ultrasonic standing wave, motion of a particle suspended in liquid is a sum of rapid vibrating movement and progressive movement towards the liquid medium. A progressive component of this movement is called drift. A few mechanisms are responsible for cell drift in the liquid medium subjected to the standing wave field (Sadikova *et al.*, 2006). Drift forces direct particles to the regions where the potential of drift forces is minimal. Different types of drifts: R-type, L-type, and A-type have been analysed and described by (Czyż, 2003).

The radiation drift (R-type drift) is connected with the radiation pressure acting on the particle as a result of momentum carried out by an ultrasonic wave diffracted on the particle. This type of drift is important for rather large particles. For small particles of radii of order of microns many authors introduced different mechanisms. The most important models concern:

- the effect of variations of the viscosity in the field caused by local changes of the temperature during periodical compressions and decompressions of the medium, L-type drift;
- the effect of dependence of the medium velocity amplitude on instantaneous position occupied by a suspended particle, in which case the oscillatory motion of the particle is asymmetrical and results in a steady drift towards regions of smaller amplitude of medium oscillations, A-type drift.

3.2. Radiation pressure as drift mechanism

The R-type drift force is expressed by the following equation (Czyż, 2003):

$$F_{DR} = 2\pi k r_p^3 G\left(\frac{\rho_g}{\rho_p}\right)\overline{E}\sin(2kx_0), \tag{1}$$

where x_0 is the instantaneous equilibrium position of the particle (erythrocyte), ρ_p, r_p is the density and radius of erythrocyte, ρ_g is the density of plasma, $\overline{E}$ is the average density of wave energy, k is the number wave.

The density factor $G\left(\frac{\rho_g}{\rho_p}\right)$ equals:

$$G\left(\frac{\rho_g}{\rho_p}\right) = \left[1 + \frac{2}{3}\left(1 - \frac{\rho_g}{\rho_p}\right)\right]\left(2 + \frac{\rho_g}{\rho_p}\right)^{-1}. \tag{2}$$

The factor depends on the relative density of the particle in the dispersed phase interacting with the medium. The density factor changes the sign when $G\left(\frac{\rho_g}{\rho_p}\right) = \frac{5}{2}$, see Fig. 1.

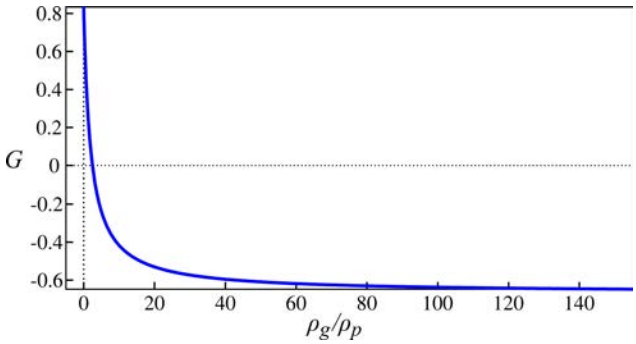

Fig. 1. Density coefficient G as the function $G\left(\frac{\rho_g}{\rho_p}\right)$.

3.3. Periodical viscosity changes of vibrating medium

L-type drift force is expressed as follows (Czyż, 2003):

$$F_{DL} = 3\pi(\kappa - 3)\frac{\eta_0}{\rho_g c} r_p \mu_g^2 \overline{E}\sin(2kx_0), \tag{3}$$

where η is the viscosity of plasma, c is the velocity of wave propagation, κ is the the ratio $\frac{c_p}{c_v}$ where c_p is a specific heat in the constant pressure, c_v is a specific heat in the constant volume, μ_g is the flow-around coefficient. Other indices as above.

3.4. Asymmetry of vibrating motion of medium in a standing wave

A-type drift force is expressed by the equation below (Czyż, 2003):

$$F_{DA} = -\frac{2}{3}\pi k r_p^3 \frac{\rho_p}{\rho_g}\mu_p^2 \overline{E}\sin(2kx_0), \tag{4}$$

where μ_p means the gust coefficient, the other indices are as above.

3.5. Comparison of effectiveness of different factors responsible for particle drift in ultrasonic standing wave

Apart from the above mentioned factors responsible for particle drift in the acoustic field, other factors may appear and lead to accidental displacements of particles; these, however, have been further omitted.

For comparison of different types of drift, A_D value was introduced as a relation of maximum drift force F_0 to the mass of the particle suspended in the liquid (this value is expressed as acceleration):

$$A_D = \frac{F_0}{m}, \tag{5}$$

where m is the mass of the particle (erythrocyte). Using the formulas (1)–(4) we can write A_D for the different types of drift. Calculating A_D value makes it is possible to compare the effectiveness of influence of different kinds of drift on the particle, i.e., erythrocyte in plasma. In calculations the following values for the parameters of blood cells and the ultrasonic wave have been adopted:

- density and radius of erythrocyte $\rho_p = 1080\ \mathrm{kg/m^3}$, $r_p = 4 \cdot 10^{-6}$ m respectively,
- density of plasma $\rho_g = 1070\ \mathrm{kg/m^3}$, viscosity of plasma $\eta = 4.62 \cdot 10^{-3}\ (\mathrm{N \cdot s})/\mathrm{m^2}$,
- velocity of wave propagation $c = 1550$ m/s, wave frequency 10^5–10^8 Hz,
- wave energy density $\overline{E_1} = 10\ \mathrm{J/m^3}$ and $\overline{E_2} = 100\ \mathrm{J/m^3}$.

Figure 2 presents the results of calculations illustrating the comparison of acceleration values for different types of drifts in the field of ultrasonic standing wave for frequencies ranging from $3 \cdot 10^5$–10^8 Hz.

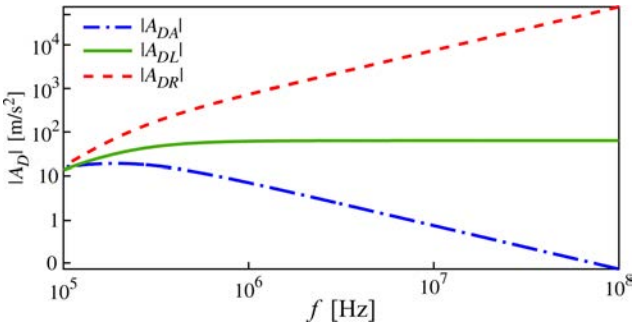

Fig. 2. Comparison of accelerations for $|A_{DR}|$ radiation drift, $|A_{DL}|$ viscous drift, and asymmetric drift $|A_{DA}|$ in the ultrasonic standing wave field, for frequencies ranging from 10^5 to 10^8 Hz.

Figure 2 shows that the relation of $|A_D(f)|$ for A-, L-, R-type drift forces acceleration values may be easily compared. It may be stated that R-type drift is predominant in the analysed case. The higher the

frequency of the wave, the bigger the difference in effectiveness of different types of drifts. The above mentioned mechanisms occur in the field of ultrasonic standing wave simultaneously and the motion of particles is caused by a resultant of these forces. The drift forces R-type, L-type, and A-type have different signs. In our discussion the problem of the sign of the drift force is of no importance. We do not take into account L-type and A-type drifts because they have very small values compared to drift R-type, see Fig. 2. In the ultrasonic standing wave field R-type drift force is responsible for the motion of blood cells. This type of drift should be therefore taken into account in further analyses as a mechanism of separating the erythrocytes from the plasma. In the ultrasonic standing wave field, $|A_{DR}|$ acceleration value for a particle radius depends strongly on other parameters of the particle and on the frequency and energy density of the wave.

When considering the motion of a single particle suspended in liquid (erythrocyte in plasma) in the ultrasonic standing wave field, it is necessary to solve the Newton equation. In theoretical papers on the application of ultrasonic waves in medicine, approximate solutions are sometimes adopted instead of precise ones in order to present physical processes and show applicatory aspects of the topic. Assuming a general dependence of resistance forces on velocity and dependence of drift forces on the position, the following motion equation is obtained (Włoch *et al.*, 2015):

$$m\frac{\mathrm{d}^2x}{\mathrm{d}t^2} = -C_{St}\frac{\mathrm{d}x}{\mathrm{d}t} - C_{Os}\frac{\mathrm{d}x}{\mathrm{d}t}\left|\frac{\mathrm{d}x}{\mathrm{d}t}\right| + F_0\sin(2kx), \quad (6)$$

where C_{St} and C_{Os} are constants describing the resistance forces in Stokes' and Oseen's approximation, m is the mass of erythrocyte, F_0 is the drift force amplitude, k is the wave number, x is the position of erythrocyte measured along the wave propagation direction. We drop the index "0" in Eq. (6) which earlier denoted the mean position of the erythrocyte. It is a second order non-linear differential equation including five constants, which has been transformed to the form with only two constants by introducing the dimensionless θ instead of time and by appropriate transformation of variables:

$$y = \pi - 2kx, \quad (7)$$

$$\theta = \sqrt{2kA_D}\cdot t. \quad (8)$$

In the above equation the following indices have been introduced:

$$\frac{\mathrm{d}^2y}{\mathrm{d}\theta^2} + \alpha\frac{\mathrm{d}y}{\mathrm{d}\theta} + \beta\frac{\mathrm{d}y}{\mathrm{d}\theta}\left|\frac{\mathrm{d}y}{\mathrm{d}\theta}\right| + \sin y = 0. \quad (9)$$

In this way, an equation is obtained that contains only two constants:

$$\alpha = \left(\tau\sqrt{2kA_D}\right)^{-1}, \quad (10)$$

$$\beta = C_{Os}(2km)^{-1}, \quad (11)$$

where τ is the relaxation time,

$$\tau = \frac{2r_p^2\rho_p}{9\eta}, \quad (12)$$

k is the wave number

$$k = \frac{2\pi f}{c}. \quad (13)$$

Constant α depends on the amplitude of the drift force, the frequency of the wave. It depends on the parameters of the particle and the ultrasonic field. Constant β depends on the wave frequency and erythrocyte radius. It does not depend on the amplitude of the drift force, see Fig. 3.

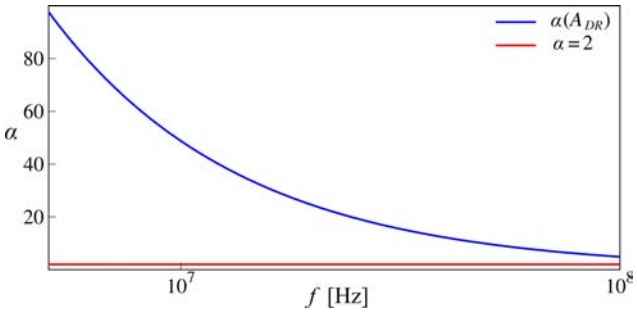

Fig. 3. Factor values α for A_{DR} depending on the wave frequency. The red line corresponds to $\alpha = 2$.

Stokes' drift represents resistance of the medium which depends on the instantaneous relative velocity of the red blood cell and describes the resistance force of the medium in sufficient detail, if Reynolds' number is less than one. In the analysed case, Oseen's correction may be omitted because of a very low value of $\beta \ll \alpha$, as shown in (Włoch *et al.*, 2015):

$$\frac{\mathrm{d}^2y}{\mathrm{d}\theta^2} + \alpha\frac{\mathrm{d}y}{\mathrm{d}\theta} + \sin y = 0. \quad (14)$$

The approximation of King-St. Clair assumes a balance between the force of Stokes' resistance and the drift force, omitting in the equation the component describing the inertia. Adopting this assumption can be justified when coefficient α in the equation satisfies inequality $\alpha > 2$, see Fig. 3. This relation is satisfied for the range of variation of the parameters related to erythrocyte, plasma, and ultrasonic wave field described in this paper. The Eq. (14) of motion of erythrocyte with consideration of adopted assumptions is as follows:

$$\alpha\frac{\mathrm{d}y}{\mathrm{d}\theta} + \sin y = 0. \quad (15)$$

It is a first order non-linear differential equation with separated variables. By adopting the initial conditions $y(0) = y_0$ the solution is obtained (Czyż, 2003):

$$y = \tan^{-1}\left[\tan\left(\frac{y_0}{2}\right)\exp\left(-\frac{\theta}{\alpha}\right)\right]. \tag{16}$$

The solution depending on the variables x and t is as follows:

$$x = \frac{1}{k}\tan^{-1}\left[\tan kx_0 \exp(2\tau A_{DR} kt)\right]. \tag{17}$$

The solution (17) depends only of the A_{DR} value, which is acceleration. It is therefore possible to determine the influence of gravity on the motion. After transformation of (17) in relation to time, the following is obtained:

$$t = (2k\tau A_D)^{-1}\ln\left(\frac{\tan kx}{\tan kx_0}\right). \tag{18}$$

When x_0 goes to zero, t goes to infinity, which means that the particles move asymptotically to the regions of stable equilibrium, it means the minimum of the drift force potential Φ_{drift}.

$$\Phi_{\text{drift}} = F_0 \sin(2kx) \tag{19}$$

and

$$F_{\text{drift}} = -\frac{\mathrm{d}\Phi_{\text{drift}}}{\mathrm{d}x}. \tag{20}$$

It means that

$$\Phi_{\text{drift}} = (2k)^{-1} F_0 \cos(2kx). \tag{21}$$

4. Cell concentration changes in plasma in the field of ultrasonic standing wave

Motion of cells (drift) in the regions between the anti-node and node of the standing wave at A_{DR} value acceleration causes changes of cell concentration in that region.

Denoting by $N = N(x,t)$ the number of particles per unit volume in a position described by coordinate x at instant t, the equation of conservation of the number of particles (the continuity equation) may be expressed as follows (Czyż, 2003):

$$\frac{\partial}{\partial x}(Nu_p) = -\frac{\partial N}{\partial t}, \tag{22}$$

where u_p is the velocity of the red blood cell. Within the scope of applicability of King-St. Clair approximation, the velocity of drift is obtained as follows:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = u_p = \tau A_{DR}\sin(2kx) = \nu_{DR}\sin(2kx), \tag{23}$$

where $\nu_{DR} = \tau A_{DR}$ is the amplitude of velocity of radiation drift and τ is the relaxation time. Assuming the initial concentration of cells in the zone between the node and anti-node N_0, after some transformations the distribution function is obtained:

$$N(x,t) = N_0\left[\sin^2(kx)\exp(-\delta t) + \cos^2(kx)\exp(\delta t)\right]^{-1}, \tag{24}$$

where $\delta = 2k\nu_{DR}$. The quantity δ^{-1} is the time constant of the cell concentration process in the regions of stable equilibrium. As shown by Eq. (24) concentration of cells in the region between the node and antinode changes exponentially. The equation allows to calculate the time needed to obtain the assumed concentration increase.

Based on the Eq. (24), it is possible to determine the quantity of δ^{-1} time constants of the exponential growth of the concentration in the regions of stable equilibrium (minimum of the drift force potential Φ_{drift}).

Figure 4 presents changes in cells concentration in the ultrasonic standing wave field as a function of position and time for wave frequencies $f = 10^7$ Hz. For calculations, it was denoted that the initial position of the red blood cell at instant $t_0 = 0$ is at half of the distance between the node and antinode. Drift acceleration A_{DR} has then the maximum value.

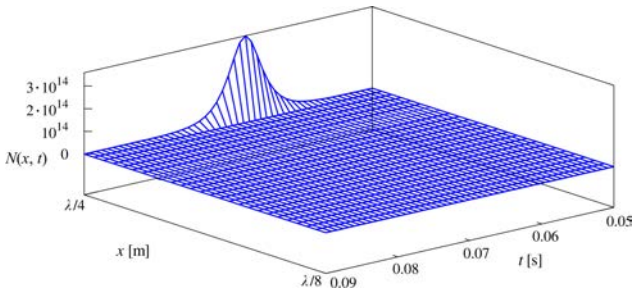

Fig. 4. Changes in plasma concentration of erythrocytes in the ultrasonic standing wave field as a function of position and time for $f = 10^7$ Hz.

As indicated by Fig. 4 exponential increase of cell concentration in the regions of stable equilibrium is obtained within fractions of a second.

5. Summary and conclusions

This paper presents an analysis of red blood cell motion in the field of ultrasonic standing wave. It states that R-type drift is a mechanism responsible for separation of erythrocytes from blood. Solutions of the equation of motion for the parameters of the acoustic field and blood components applied in medical diagnostics have been discussed. Solution of the continuity equation led to the estimation of time constants of the cell transportation to the regions of stable equilibrium where cell concentration increases exponentially. The outcomes of the analysis make a step forward for establishing the ultrasonic method for separation of blood

into components. The results still require experimental verification.

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