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The scientific monograph presents traditional and innovative approaches to modern scientific research. General issues of technical and physical and mathematical sciences, economic sciences, agrarian sciences, law and public administration, social communications, historical sciences, philological and pedagogical sciences, etc. are considered. The publication is intended for scientists, teachers, graduate students and students, as well as a wide audience.

CHAPTER «PHYSICAL AND MATHEMATICAL SCIENCES»

BIOPHYSICAL BIOMARKERS OF HUMAN ERYTHROCYTES FOR EFFICIENT DIAGNOSTICS OF STROKE

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Abstract. Timely and reliable diagnostics of acute and severe diseases like cancer, stroke and myocardial infarction is of a great importance for human health. Biophysical parameters of the red blood cells (RBC) can be used as biomarkers for early diagnosis together with chemical biomarkers and clinical tests. Compared to the latter, the studies on the blood sample are easier accessible and cheaper. *The purpose* of the paper is to demonstrate the high sensitivity of the dielectric properties of single RBC to the general chemical composition of the blood plasma due to the disease development and the available medical technique for their measurements based on the clinical studies. *Methodology* of the study is based on experimental measurements on the blood samples of healthy donors (control group) and patients with ischemic and hemorrhage stroke (experimental group) in the electromagnetic field of microwave frequencies at different temperatures between $T=0C$ and $54C$. *Results* confirmed early noticeable influence of the disease on the real and imaginary part of complex dielectric permittivity of the REC and their membranes than can be easily detected by the microwave dielectric spectroscopy (MDS). The variations in the dielectric parameters tend to zero after a successful treatment that can be used as

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an additional biomarker of a proper individual treatment of the disease. *Practical implications.* The MDS method in a range of temperatures can be recommended for early diagnostics of the disease severity and estimation of the treatment efficiency. The method is fast, simple and cheap, and it needs a sample of the venous blood only.

Value/originality. The effectiveness of the proposed method is based on a series of experimental studies and theoretical modeling and gives a new insight to the importance of dielectric properties of RBC that are the most movable cells in our bodies which carries on their membranes the influences of all biochemical markers produced by the disease.

1. Introduction

Red blood cells (RBC) and other isometric cells with diameters $d \sim 5-10 \mu\text{m}$ can be modeled as microparticles in agglomerates and aquatic suspensions, and their movement can be considered based on the mathematical models of microfluids with complex electromechanical properties [1]. Besides mechanical parameters (density, rigidity, fluidity), biological tissues are characterized by electric properties which are highly frequency, temperature and age dependent that is important for clinical diagnostics of pathologies. The surface density of the electric charge $\sigma_e = q/S$, where q is the electric charge, S is surface area, is an important characteristic of the cells, tissues and organs. The surface charge of RBC is produced by the charged proteins embedded into the membrane, dissociation of the surface molecules, and adsorption of ions from blood plasma. The charged particles form the electrical double layer (EDL) around the cells in the water-based solvents. The monomolecular layer of adsorbed ions (Stern layer) is surrounded by a diffuse layer of solvated ions (Gouy-Chapman layer) [1; 2]. In the native RBCs the membrane is also covered by glycoproteins forming the complex 3D structure (glycocalyx). The positive and negative charged groups are nonuniformly distributed in the glycocalyx that changes the structure of EDL. The Debye length for biological cells is $\kappa^{-1} = 8 \text{ \AA}$ at normal values of ionic strength $I_i = 0.5 \sum C_i z_i^2$ of the solvent, where C_i and z_i are concentrations and valency of ions.

Dielectric permittivity is an important characteristic of biological cells and tissues. It is a complex value $\varepsilon = \varepsilon' + i\varepsilon''$ because the tissues are not ideal dielectrics and some part of the electromagnetic energy is transformed

into heat due to the electric conductivity of the tissues. The dielectric loss $\varphi_e = a \tan(\varepsilon'' / \varepsilon')$ characterizes the amount of the energy spent to the heat transfer due to conductivity and dielectric losses. Dielectric permittivity of blood increases with concentration of RBC and blood plasma proteins.

Biological cells and tissues are characterized by dispersion of their electric conductivity k and dielectric permittivity ε in the range of low frequencies (LF) $f=10-10^3$ Hz (α – dispersion), radio frequencies $f=10^4-10^8$ Hz (β – dispersion), and high frequencies (HF, microwaves) with $f > 10^{10}$ Hz (γ – dispersion). In human blood and RBC suspensions α – dispersion is almost absent [2]. Low blood conductivity at LF is explained by high conductance ($\sim 10^{-2}$ F/m²) of the RBC membranes and the dispersion is determined by ionic diffusion processes at the cellular membranes.

Ischemic stroke (IS) is a clinical syndrome manifesting itself as a sharp disruption of the brain's local functions, lasting more than a day, or resulting in death as a result of a reduction in cerebral blood flow, thrombosis, or embolism associated with heart, vascular, or blood diseases. More than 140,000 people die each year from stroke in the USA, 152,000 in the UK and almost 100,000 in Ukraine [3; 4]. According to the World Health Organization 15 million people suffer a stroke worldwide each year. The mortality rises up to 35%, and 75-85% are related to the IS which is caused by the carotid stenoses in 30-50% of patients.

The methods for predicting the risk of ischemic disorders of cerebral blood flow include blood lipid profile determinations, magnetic resonance imaging, computed tomography, transcranial dopplerography, and others. These methods do not allow revealing the functional state of the microcirculatory system [5] or red blood cell (RBC) malfunctioning [6]. Indicators of haemostasis, kinetics parameters of aggregation and disaggregation of erythrocytes and their deformability are associated with blood flow through microvessels under different pathological conditions including cerebral ischemia. In addition, the deterioration of rheological properties of blood and parameters of haemostasis is in some connection with the clinical features: the stage, severity and extent of IS, as well as with the prognosis of the course of the disease [6]. Nevertheless at the moment there are practically no experimental and clinical studies devoted to the complex analysis of haemostatic and haemorheological factors with an

assessment of their effect on microcirculation in patients with thrombolytic therapy of IS, and also on the functional outcome of the disease. Thus, it seems relevant to study these blood indices in the acute period of IS, including intravenous thrombolytic therapy.

The study is aimed at experimental and theoretical investigation of biophysical parameters (electric conductivity, dielectric permittivity, dielectric loss) of live cells (RBCs and others) as sensitive biomarkers that could be introduced in the everyday medical practice for differential diagnostics of severe diseases (like ischemia) and the treatment results for a given patient. The practical research task of the study is detailed measurements of the dielectric parameters of RBCs by two-channel microwave dielectric spectroscopy (MDS) at a frequency $f=9.2$ GHz. Complex dielectric permittivity of RBC in the RBS suspensions of healthy donors has been compared with those of the patients with IS before and after the treatment prescribed.

2. Microwave dielectrometry technique in complex study of microparticles

MDS in the region of relaxation of free water molecules (γ -dispersion) allows studying the state of water in biological systems [7; 8]. Water determines the mobility of macromolecules (MM), allows them to associate and dissociate, makes possible the proton transfer and facilitates a large number of biochemical processes [9]. The water interacting with MM can be described as belonging to one of the following three types: free water, associated water and strongly bound structured water. The physical properties of the three types of water are different. The molecules of bound water have less mobility than molecules of bulk water. Immobilization of certain part of water in the hydration shells of the MM or cells in aquatic suspensions leads to a decrease in the permittivity of the solution/suspension compared to that of the pure solvent. Amount of the bound water depends on the conformations of the MM [10].

MD at GHz frequencies can distinguish the bulk water from the bound water. The dipole relaxation of free water molecules contributes most to the dielectric constant of biological tissues at microwaves, so the content of free water or redistribution of water molecules between free and bound state could be traced and thus the content of free water in a sample could be used as a biomarker of conformational transitions of MM.

The degree of water incorporation into macromolecules in turn is related to the state of the patient's cells, which depends on natural factors and medications administered. This connection is very complex and has not yet been fully understood. It is known that in cardiovascular diseases, hypertensive disease, and IS there is a gradual desensitization and a decrease in the number of β -adrenergic receptors due to prolonged activating effect of increased concentrations of neurohormones and a decrease in the number of viable myocardial cells, formation of cardiofibrosis, apoptosis or necrosis processes. Clinically, this is manifested by aggravation of symptoms and progression of the disease, a decrease in the effectiveness of the drugs.

The microwave dielectrometers of different generations are presented in Figure 1a, b. The measurements are based on the resonator methods that use the resonant frequency f_r and the constant parameter Q (Q-factor) of a resonator that must be known for given equipment. The microwave generators (Figure 1a, c, e) influences the object at a study (a liquid or cells in the liquid) located in a thin tube (Figure 1b, d, f). The difference between the signals from the resonator with an empty tube inside (control) and the resonator with the filled tube (experiment) is measured. The obtained signal is filtered to eliminate noise and then treated by mathematical methods to compute the dielectric parameters of the sample studied. An alternative method is the microwave waveguide measurements conducted on the electromagnetic wave propagated and reflected in a waveguide with and without a sample in it. The positive and negative features of both methods and their value has been compared in [11].

The MDS method is an addition to *in vitro* studies of the cells [13–15]. Unlike many traditional research methods used in biology and medicine, this integrated approach makes it possible to study biological objects in real time and without interfering their native state. Analysis of the cell state based on their dielectric parameters is performed by evaluating the rearrangement of the membrane-receptor complex of the cell under the action of specific bioregulators relative to control (intact) samples.

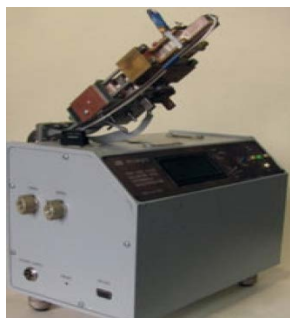
Human RBC was selected as a model for studying molecular mechanisms of control systems of cells due to the presence of adrenergic receptors in their membrane functionally and structurally similar to adrenoreceptors of vascular cells under conditions of acute disturbance of



a



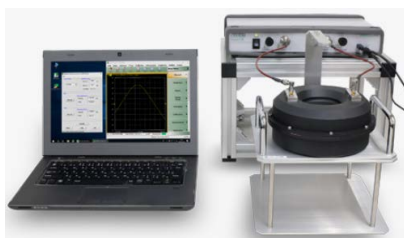
b



c



d



e



f

Figure 1. 2-channel microwave dielectrometer $f=9.2$ GHz (a) with the resonator in a thermostate (b) (adapted from [7; 13]); experimental dielectrometer (c) with two measurement cells (d) (adapted from [11]); modern computer-assisted microwave dielectrometer kit (e) and resonator with a sample inside (f) (adapted from [12]).

cerebral circulation, neurohumoral pathology of myocardium, and other organs [16]. The state of hypoxia of organs and tissues is an important mechanism in the pathogenesis of cerebral ischemia, which affects the structural and functional properties of erythrocytes that determine their oxygen transport function [17]. In addition, it is known that erythrocytes are involved in the pathogenesis of microcirculatory disorders in patients with cerebrovascular diseases.

The simplicity of organization of erythrocytes makes it possible to study the functional properties of cell membranes without interference imparted by intracellular membrane formations and organelles. Structural disturbances of erythrocyte membranes in various pathological processes can be extrapolated to other membrane systems. There are extensive literature data on methods for measuring the permittivity of biological substances [18], blood [19] and RBC [20; 21].

The state of cells and their reaction is expressed in a change in the ratio of free water and water bound by the macromolecules of the cell membranes. This ratio affects the complex value of the permittivity, since in bound water the mechanical moment is determined by the mass of the macromolecule. Dielectric parameters of blood parameters in clinical samples of patients make it possible to obtain information not only about the state of its vascular system, but also to determine the development of pathologies in the early stages, and the optimal path of treatment taking into account the pathology [20; 21]. The most informative measurements will be in γ -relaxation frequency range, including the frequency of free water relaxation ($f \sim 10$ GHz [22]), which determines the relationship between bound and free water and involves measurements in the frequency range of relaxation of water molecules. Another feature of these measurements is the need to minimize the volume of biological samples.

Experimental study of the temperature dependences of the complex dielectric permittivity $\varepsilon = \varepsilon' + i\varepsilon''$ of polar liquids in the microwave range make it possible to obtain important information on the polarization processes, which can be used both for estimating theoretical ideas about the fundamental characteristics of substances and for practical purposes. A systematic study of the dielectric properties of RBC in the microwave region at different temperatures was practically not performed.

3. Materials and method

The dielectric characteristics of RBC in patients with IS were studied before and after the baseline therapy prescribed [23]. The study involved 20 patients aged between 38-45 years who underwent IS. The preliminary diagnosis was confirmed on the basis of the generally accepted European recommendations for the prevention and treatment of IS [24]. The diagnosis was based on the history, clinical symptoms of IS, neurological and somatic status, general and biochemical blood tests, CT scan of the brain, duplex scanning of brachiocephalic arteries, ECG, Echo-KG [25]. The classification of diagnosis of vascular lesions of the brain and spinal cord has been used [26]. The control group consisted of 20 healthy donors of the same age. The normal distribution test was carried out using the Shapiro-Wilk test [27]. To compare the changes in the dielectric parameters of the blood erythrocytes of patients before and after treatment, the Wilcoxon test was used [28].

Venous blood samples (2 ml) were stabilized with 0.1 ml of heparin solution with 5000 IU activity. RBC were separated from blood plasma and leukocytes by centrifugation (600g, 10 min) at 4°C and washed 3 times with phosphate-buffered saline (PBS: 150 mMNaCl, 8.1 mM Na₂HPO₄, and 1.9 mM NaH₂PO₄, pH 7.4). A standard erythrocyte suspension was prepared by diluting the re-suspended washed erythrocytes with medium and obtaining a suspension with cell concentration ~4.8×10⁶ cells/ml that was verified using Gorjaev's chamber.

The character of frequency dependence of the dielectric parameters of soft tissues is the same for all tissues of the human body, namely $\varepsilon'(f)$ is a decreasing function. The relaxation characteristics of free water molecules in blood do not differ from those of pure water and in the region of γ -dispersion can be described by the Debye equation [2; 7]

$$\varepsilon' = \operatorname{Re}(\varepsilon^*) = \varepsilon_{\infty} + \frac{\varepsilon_0 - \varepsilon_{\infty}}{1 + (f / f_d)^2}, \quad \varepsilon'' = \operatorname{Im}(\varepsilon^*) = \frac{\omega\tau(\varepsilon_0 - \varepsilon_{\infty})}{1 + (f / f_d)^2}, \quad (1)$$

where ε_0 and ε_{∞} are the high-frequency and low-frequency (static) permittivity (the asymptotic values of the dielectric constant at frequencies above and below the dispersion region respectively), f_d and f are the frequencies of the dielectric relaxation and the frequency of the external microwave field. In our calculations we assume $\varepsilon_{\infty} = 5.6$ (as the permittivity in the infrared range).

In the case of any polarization mechanism and single relaxation time τ Debye theory gives the following expressions

$$\varepsilon_0 = \varepsilon' + \frac{\varepsilon''^2}{\varepsilon' - \varepsilon_\infty}, \quad f_d = \frac{f(\varepsilon' - \varepsilon_\infty)}{\varepsilon''}. \quad (2)$$

The value ε_0 does not depend on the frequency and is smaller for concentrated solutions where the amount of bound water is bigger. The parameter f_d characterizes the mobility of molecules in the microwave field, and consequently the degree of its interaction with the environment. As long as we were not able to perform measurements at different frequencies we did scan our samples over the temperature. Based on [29] and using the dependence $f_d(T)$ [30] one can obtain

$$f_d = \frac{k_B T}{2\pi h} \exp\left(\frac{\Delta S(T)}{k_B} \exp\left[-\frac{\Delta H}{k_B T}\right]\right), \quad (3)$$

where k_B and h are Boltzmann and Planck constants, respectively, T is the absolute temperature, $\Delta S(T)$ is the entropy, ΔH is the enthalpy. The activation energy of the process of dielectric relaxation of water molecules was determined from the linear fit of the data in the coordinates $\ln(f)$ and $1/T$.

The dielectric permittivity of the RBC suspensions was measured according to the test sample-study design. The individual features of donors affect measured values of ε' and ε'' . These features include the haematocrit, the concentration of ions, the distribution of RBC in size and shape. To compensate for these changes we worked with suspensions of washed RBC. To increase the accuracy each measurement was repeated at least 5 times, after which the average value was found.

A cylindrical resonator with operating frequency $f=9.2$ GHz has been used for the measurements. The water–acetone mixtures with different molar fractions of water and known values of ε' and ε'' have been used for calibration of the capillary. The RBC suspension was collected in a capillary with $d=2$ mm and placed in the resonator, and a shift between the resonant frequencies of the empty and loaded resonator was recorded. The attenuation due to sample loading in the resonator was also recorded for further calculations of the ε'' values. The values ε' and ε'' have been calculated from the measured frequency shifts and attenuation factors by using regression equation for calibrated water-acetone mixtures with

known values of ε' and ε'' . The value ε'' was corrected for electrical conductivity due to the presence of inorganic ions in the medium. The electrical conductivity of the RBC suspensions was measured by a bridge method at a frequency of 1 kHz at room temperature. The absolute error in a value of ε' did not exceed ± 0.2 , for the ε'' the error did not exceed ± 0.5 . The temperature of the samples in the capillary varied in the range from 2 to 45°C and was measured with a copper-constantan thermocouple with an accuracy of $\pm 0.1^\circ\text{C}$.

The hydration level of the RBC has been computed as [23]

$$\Delta\varepsilon_0 = \varepsilon_0^{PBS} - \varepsilon_0^{RBS} ,$$

where $\Delta\varepsilon_0$ is the decrement of the static permittivity of the RBC suspension relatively the PBC.

4. Results and discussion

4.1. Temperature effects

The temperature dependence of ε' and ε'' for the RBC suspension in PBS of healthy donors and patients with IS before and after therapy are presented in Table 1. As it is known for PBS ε' value increases while ε'' decreases monotonously with temperature [1,10]. For all studied temperatures the magnitudes of the permittivity ε' and dielectric losses ε'' in RBC suspensions of ISB patients were significantly higher compared to healthy donors. The applied treatment reduces this tendency for ε' and ε'' , a less significant increase in these parameters is observed for ISA samples in comparison with the RBC suspensions of healthy donors.

The temperature dependences of the static permittivity of the erythrocyte suspension of healthy donors and patients with IS before and after the therapy prescribed are shown in Figure 2. The results show that stroke leads to the increase in the static dielectric permittivity of erythrocytes suspensions compared to that of healthy donors practically over the entire temperature range (Figure 2). For a given frequency $f = 9.2$ GHz the static dielectric permittivity decreases when increasing temperature. With rise in temperature both the strength and extent of the hydrogen bond decrease. This promotes the reorientation of dipoles and allows the water molecule to oscillate at higher frequencies; temperature as well reduces the drag to the rotation of the water molecules [10].

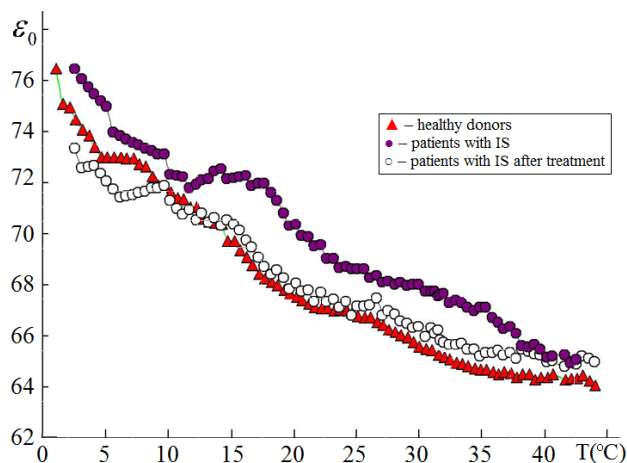


Figure 2. Temperature dependence of static permittivity ϵ_0 for the RBC suspension in 0.15 M sodium phosphate buffer

Compared to the PBS solutions, in the case of RBC suspensions the dependencies of $\epsilon_0(T)$ are of non-monotonous character with some deviations from monotonous (increase of ϵ_0 values at these temperatures in contrast to monotonous decrease of ϵ_0 values with rise in temperature) in temperature intervals 4–8, 12–15 and ~22–26°C for HD; 12–18°C for ISB; and 7–9°C, 25–27°C for ISA samples. Peculiarities in the same temperature regions were observed for RBC suspensions of different donors within each studied group. In the temperature ranges 12–20°C and 26–36°C the maximal statistically significant difference ($p < 0.05$) between values of ϵ_0 for ISB and HD/ISA groups are observed.

Non-monotonous changes in the static dielectric permittivity ϵ_0 of RBC suspensions with temperature are determined by the changes in the degree of water binding to erythrocytes and the redistribution of free and bound water in the vicinity of the cell surface due to the rearrangement or oxidation of membrane lipids or conformational changes of membrane proteins. Such changes of ϵ_0 can also arise from cell aggregation. Erythrocytes under conditions of impaired cerebral circulation adsorb fibrinogen and fibrin lysis products, impair their ability to deform and

lose part of the surface electric charge. All these factors increase the risk of cell disruption and spontaneous mutual aggregation. However in the temperature interval and cell concentration studied in the present work no formation of cell aggregates or associates was reported in the available literature. Moreover concentration dependencies of ε' and ε'' were linear in the cell concentration range studied (not illustrated) that evidences no cell aggregation in our experiments. Thus we attribute peculiarities observed on the ε' , ε'' and ε_0 temperature dependencies (Figure 2 and Table 1) to the cell membrane protein conformational change and membrane lipid rearrangements under the action of temperature. Profound increase of ε_0 in the range of $T=12-18^\circ\text{C}$ for ISB samples indicates dehydration of RBC membranes of ischemic patients at these temperatures.

It is known that the increment of static permittivity ε_0 of the cell suspension relative to the solvent is proportional to the hydration of erythrocyte cell membrane [29]. The degree of hydration of the erythrocyte membrane of ISB patients decreases in the temperature range from 5 to 10°C , in the ranges $T=12-18^\circ\text{C}$ and $26-36^\circ\text{C}$. In IS patients an increase in the static dielectric permittivity ε_0 may indicate a decrease in the thickness of the hydrated perimembrane unstirred layer of erythrocyte, decrease of the amount of bound water in it. Deviations from the monotonous decrease in the static dielectric permittivity ε_0 of erythrocytes of patients with stroke in the temperature ranges $5-10^\circ\text{C}$ and $37-44^\circ\text{C}$ indicate a decrease in the amount of free water and thus increase in the hydration of the cell membrane.

IS is associated with the activation of free radical oxidation of lipids in the erythrocyte membrane. The increase in the amount of free water molecules in the erythrocyte suspensions in the ranges of $T=12-20^\circ\text{C}$ and $26-36^\circ\text{C}$ is a consequence of the damage of cell membranes by reactive oxygen species. IS contributes to the fluidization of the lipid bilayer of the RBC membrane and the formation of channels of passive permeability due to the formation of hydroperoxides of fatty acids. Changes in the static dielectric permittivity ε_0 of RBC of patients after therapy approach the values of the static permittivity of healthy donors, which indicates a decrease in the amount of free water in the system, which is the “marker” of the disease.

Table 1

**Temperature dependence of the measured ϵ''' and ϵ'' values
for RBC suspensions of healthy donors and patients
with IS before and after treatment**

T, °C	Erythrocytes (control)		Erythrocytes (ischemic stroke)		Erythrocytes (after therapy)	
	ϵ'	ϵ''	ϵ'	ϵ''	ϵ'	ϵ''
2	34,1±1,0	34,9±0,7	41,7±1,1*	33,2±0,8	35,0±1,0*	35,4±1,0*
5	42,0±1,2	33,3±0,8	44,2±0,7	31,8±0,9	42,6±1,4	34,0±0,9*
10	43,2±1,1	32,1±0,8	46,9±1,1*	30,4±1,0*	42,8±1,2	32,8±1,3
15	47,8±1,2	29,3±1,1	50,5±1,1	28,6±1,0	46,1±1,0	30,3±1,1
20	50,1±1,0	27,0±0,7	53,5±0,8	26,0±1,2	51,1±1,0	27,9±1,1*
25	52,8±1,3	25,3±1,1	56,9±1,1*	24,2±1,1	53,4±1,0*	25,5±1,1*
30	54,3±1,3	23,2±0,9	58,6±1,0*	22,1±1,0	53,6±1,3	23,4±1,1
35	55,4±1,1	21,2±1,0	59,4±0,9*	19,0±1,1	55,6±1,4	22,0±1,4
40	56,5±1,2	19,6±0,8	61,6±0,8*	18,4±0,7	58,9±1,1	19,2±1,2
44	57,0±1,0	18,3±0,4	62,9±1,2*	16,0±1,0*	60,8±1,0*	17,3±1,2

* – reliability of differences in comparison with control (healthy donors), $p \leq 0,05$

4.2. Time of Dielectric Relaxation Study

The dielectric properties of cell suspensions in the microwave range of frequencies are mainly determined by the presence of free water. Water interacts with molecules through hydrogen bond clusters. Cell suspensions exhibit a basic dispersion of the Debye type or close to the Debye type at microwaves. The mechanism of this dispersion is rotational diffusion of polar water molecules or their clusters [20].

The mean values of the frequency of the dielectric relaxation f_d of the water molecules in erythrocyte suspensions of the groups under study are shown in Figure 3. It can be seen that after the therapy, the temperature dependences for HD and ISA groups practically coincide in the temperature range from 2 to 26°C. The results [10] confirm the assumption that the dielectric properties of intracellular water are the same as in free water. According to our data, the mean value of the frequency of the dielectric relaxation f_d of water molecules in the erythrocyte suspension at a temperature of 20°C in the group of healthy donors is $f=15.1$ GHz, which is somewhat less than that of pure water (16.4 GHz). The obtained value is close to the value of f_d of bulk water molecules at 20°C (13.6 GHz) obtained in [29; 30] and close

to the data obtained by other authors ($f=16.7, 15.8$ and 12.6 GHz) given in the same papers. The value of the frequency of the dielectric relaxation f_d of erythrocyte water molecules in the group of patients in the acute period of IS is 17 GHz, which is somewhat higher than in pure water (16.4 GHz), and this can indicate the presence of some amount of free water and the disorganization of the erythrocyte membrane.

Frequency of dielectric relaxation of free water molecules f_d for water and thin electrolyte solution increases with temperature practically linearly [9]. Value of frequency of dielectric relaxation f_d characterises the mobility of free water molecules in external electromagnetic fields. The bound water is excluded from the total water relaxation in solution

because its mobility is less than mobility of free water. Thus in the case of RBC suspensions we have less amount of free water molecules and, consequently, lower value of f_d . Temperature dependencies of f_d for RBC suspensions have a number of kinks. In Fig. 3 four linear segments can be distinguished on the temperature dependences of f_d : below and above 12°C , 26°C and 36°C .

Arrhenius plots of the f_d allowed us to calculate activation energy of the dielectric relaxation for free water molecules in studied RBC suspensions. For the suspension of HD erythrocytes in the temperature ranges $2-12^\circ\text{C}$, $12-20^\circ\text{C}$ and $26-36^\circ\text{C}$, $36-45^\circ\text{C}$, observed kinks on $f_d(T)$ plots are accompanied by an increase in the activation energy by 11 kJ/mol at 12°C , decrease by 14 kJ/mol at 36°C and an increase in the activation energy in the temperature range $36-45^\circ\text{C}$ by 5.08 kJ/mol. For the suspension of ISB erythrocytes, activation energy in the range of 12°C increased by 10.47 kJ/

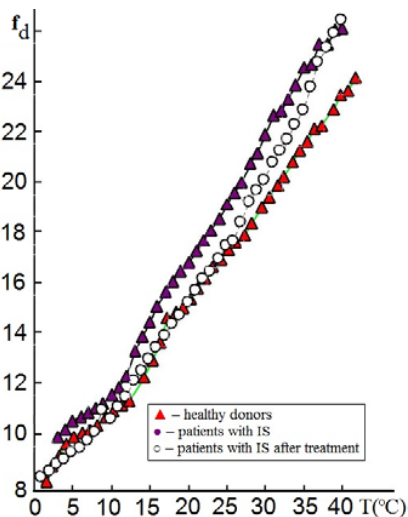


Figure 3. Temperature dependence of dielectric relaxation frequency f_d of the water molecules in RBC suspensions

mol and by 3.44 kJ/mol in the range of 36-45°C. The significant changes in the values of activation energy of the dielectric relaxation of water molecules, which approach the values of activation energy of the dielectric relaxation of water molecules of healthy donors has observed after therapy.

If the energy of the hydrogen bond is estimated at 12.5 kJ/mol [1], then in the RBC suspensions of healthy donors each water molecule forms 1.5 to 2.5 H-bonds with neighbouring molecules (Table 2). In the RBC suspensions of IS patients each water molecule forms 1-2 H-bonds with neighbouring molecules. Along with our data on the change in the activation energy of the process of dielectric relaxation of free water molecules in the erythrocyte suspension, the results presented here suggest that the reason for the change in the dielectric relaxation frequency of water molecules in the suspensions in patients with IS in comparison with healthy donors is an increase in the thickness of hydration layer of their RBC membrane.

Table 2

The activation energy of the dielectric relaxation of water molecules in RBC suspensions

	Control	IS	IS after treatment
T, °C	ΔE, kJ/mol	ΔE, kJ/mol	ΔE, kJ/mol
2-12	19,03±0,6	13,54±0,6*	19,05±0,3*
12-20	30,31±0,4	24,01±0,5*	29,82±0,6*
26-36	16,21±0,6	11,12±0,6*	16,39±0,4*
36-45	21,29±0,6	14,56±0,3*	23,16 ±0,6

Since the realization of the stress reaction of the organism is directly related to the functional state of the membrane-receptor complex of cells, investigation of the relationship of cerebrovascular disorders with the features of the membrane complex will allow personifying approaches to the prevention of development and progression of cerebral circulation disorders. The detected temperature-dependent changes in the dielectric parameters, namely ϵ_0 and f_d , of the PBS suspension of RBC of IS patients could be used as complementary diagnostic facility for clinical application. Further considerations and more sophisticated models of water relaxation with measurements at several frequencies in GHz range could bring new insights on the molecular mechanisms of ischemic stroke cellular effects.

5. Conclusions

Temperature-dependent changes in the dielectric parameters of the erythrocyte suspensions in patients with IS were studied. It was shown that the activation energy of dipole relaxation of water in RBC suspension of healthy donors and IS patients after therapy are close to each other, which indicates a similar molecular mechanism of disease development and recovery after the treatment. Both processes involve breakup of a certain number of hydrogen bonds between water molecules.

Temperature-induced structural rearrangements of erythrocyte membranes cause changes in the structure of the solvent. Structural transition of membranes of erythrocytes in patients in the temperature ranges $T=2-12^{\circ}\text{C}$ and $T=12-20^{\circ}\text{C}$ is accompanied by a change in the ratio of bound and free water, an increase in the frequency of dielectric relaxation of water in the suspensions of patients compared with healthy donors, which leads to an increase in the amount of free water. In the temperature ranges $2-12^{\circ}\text{C}$, $12-20^{\circ}\text{C}$, $26-36^{\circ}\text{C}$, $36-45^{\circ}\text{C}$ increase of the activation energy of dipole relaxation occur.

The structural transition in RBC membranes is preceded by the dehydration of the membrane components, which results in a decrease in the mobility of the water molecules in the erythrocytes as compared with the mobility of water in the surrounding physiological solution. The unidirectional character of the violations of the temperature dependence of the frequency of the dielectric relaxation of the water molecules of the erythrocyte suspension in patients with IS found during the study shows that these disorders can be considered as non-specific signs of involvement of the RBC into the complex set of changes in the body that accompany the development of cerebral circulation disorders.

The MDS is a promising method for the integral assessment of pathology, associated with ischemic stroke.

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