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Dielectric study of blood serum of patients with oncohematological diseases

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Features of charge transfer and dielectric relaxation processes in blood serum samples of patients with oncohematological diseases and healthy donors were investigated by dielectric spectroscopy method. The observed features of the dielectric spectra for donors and patients, namely the decrease in conductivity in the high frequency region, the correlation between the degree indicator s and the total protein content, as well as changes in the spectrum of relaxation complexes indicate a change in the quantitative ratios of blood components in the presence of disease. This system rearrangement is the result of the fact that conformation of proteins (albumins and immunoglobulins) for patients is change.

Keywords: Dielectric spectroscopy, serum, chronic lymphocytic leukemia, multiple myeloma, charge transfer, dielectric relaxation.

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Introduction

Today there are many approaches to the measurement of electrophysical properties of biological systems defined by working conditions of the experiment. A great advantage among these methods has the dielectric spectroscopy (DS), that features quick determining of parameters for a large class of materials in a wide range of frequencies and temperatures [1–3].

The essence of the DS method consists in applying a low-amplitude disturbing sine signal to the system under test and studying the output response signal caused by this disturbance. Since the system response is conditioned by a combination of many factors, to achieve full understanding of the processes running under the impact of electric field, complex impedance data should be analyzed at the level of complex values of impedance ($Z^* = Z' + jZ''$), admittance ($Y^* = Y' + jY''$), dielectric permittivity ($\varepsilon^* = \varepsilon' + j\varepsilon''$) and electrical modulus ($M^* = 1/\varepsilon^* = M' + jM''$) [4].

The investigation of blood properties by the DS method makes it possible to obtain information on both the intramolecular motion of biological macromolecules in the blood and the character of their intermolecular interactions [5,6]. In recent years, the DS method has been used widely in the examination of blood and its components [7,8]. In [8] values of $\varepsilon'(f)$ and $\varepsilon''(f)$ are presented for the whole blood and plasma of several oncologic patients measured in the microwave region, values of dielectric permittivity $\varepsilon'(f) = 80-90$ and loss factor $\varepsilon''(f) = 70-15$ are obtained. However, in the specialized literature there are no works devoted to the investigation of blood serum electrical properties by the above-mentioned method. Investigations of whole blood and blood serum make it possible to study molecular processes taking place in the case of hematological neoplasms in conditions most closely resembling physiologic conditions.

Chronic lymphocytic leukemia (CLL) is one of the most widespread hemato-oncological diseases [9–11]. This is the most common type of leukemia among Caucasian people. The main change in the blood of CLL patients is caused by a significant (an order of magnitude greater than normal level) quantity of circulating tumoral lymphocyte

cells. Protein composition disturbances of the blood serum in CLL patients are extremely negligibly prominent as compared with the changes in multiple myeloma patient. Significant advances in the understanding of the role of different biological markers in CLL development and progress have resulted in that studying the nature of this disease becomes more and more relevant and important for the medical science [12–14]. Nevertheless some pathogenesis mechanisms of CLL are still not fully understood and require further investigation.

Multiple myeloma (MM) — a clonal B-cell lymphoproliferative disease with tumor plasma cells (TPC) as its substrate that secret a pathologic protein (so called M-protein or paraprotein), i.e. an immunoglobulin secreted by clonal transformed TPCs without functional characteristics of normal immunoglobulins. In case of myeloma, TPCs can also produce immunoglobulin light chains. It is known that MM accounted for 1% of all cancerous diseases and 10% of hematologic malignancies. In contrast to CLL, blood changes in MM are caused first of all by the disturbance of protein balance due to the prominent secretion of pathologic protein by the clonal plasma cells. Currently an active search is in progress for new approaches to disease diagnosis, as well as for additional factors that define clinical progress of the disease and require further investigation as well [15–17].

The purpose of this study was to identify features of the processes of charge transport and dielectric relaxation in samples of blood serum of CLL and MM patients and healthy donors by the method of dielectric spectroscopy. The work presents results of the study of dielectric spectra of blood serum (BS) of healthy donors and patients with hemato-oncological diseases.

Experimental procedure

To obtain BS samples, we used S-Monovette tubes (Sarstedt, Germany) with clot activator. The taken blood samples were kept in tubes for 20−30 min at a room temperature of (18−24°C), then centrifuged for 15 min at a speed of 3000 rotations/min in a Heraeus Labofuge 200 centrifuge (Thermo Scientific, USA). The samples were frozen and stored at a temperature of −30°C until the physical and chemical investigation activities.

The investigation of BS was carried out using a Abbemat WR/MW refractometer (Anton Paar, Austria) in the „Centre for Diagnostics of Functional Materials for Medicine, Pharmacology and Nanoelectronics“, resource center of the Scientific Park of SPSU. The operational principle of the instrument is based on the measurement of the angle of total internal reflection. Light was sourced from a LED with a wavelength of 589.3 nm. Volume of the BS sample under study was 0.35 ml. To investigate the BS refraction, we used built-in measurement scales of refraction index and total protein concentration in serum [18]. The total protein concentration in the BS and refraction index were determined at a standard temperature of $T = 17.5°C$. Accuracy of wavelength setting was 0.2 nm, accuracy of thermostating temperature at the prisma/sample interface was 0.03°C. Accuracy of refraction index measurement was 4.10^{-5}.

The dielectric study of the BS was carried out by the method of „voltmeter-ammeter “ (low frequency region, $f = 10^{-2}-10^{6}$ Hz) and microwave method (high frequency region $f = 10^{6}-10^{9}$ Hz) in the Herzen State Pedagogical University of Russia.

In the high frequency region the components of complex conductivity were determined by a „Concept-81“ spectrometer (Novocontrol Technologies GmbH, Germany) in a frequency range of $f = 10^{6}-10^{9}$ Hz using the microwave method with a coaxial system at a temperature of $T = 20°C$.

Frequency dependencies of BS samples dielectric parameters: components of complex dielectric permittivity $\varepsilon^*(f)$ and complex specific conductivity $\sigma^*(f)$ were calculated on the basis of impedance spectrum

$$Z_s^*(\omega) = Z_0 \frac{1 + r^*(l)}{1 - r^*(l)}, \tag{1}$$

using the WinDETA software program (Novocontrol Technologies GmbH, Germany). Here Z_0 — impedance of a waveguide with a length of l, $r^*(l)$ — complex reflection coefficient.

In the low frequency region the components of complex dielectric permittivity were determined in a frequency range of $f = 10^{-1}-10^{6}$ Hz at a temperature of $t = 20°C$ by so called method of „voltmeter-ammeter“. The essence of this method consists in the following: an alternative voltage is applied to the sample from the generator and current flowing through the sample and phase-delayed in relation to the voltage is measured. By the phase difference between the current and voltage the complex impedance is determined.

As experimental data, the values of the imaginary and real parts of the impedance of the cell with the measured sample were measured. The complex dielectric permittivity spectra were calculated from the impedance spectra

$$Z^*(\omega) = R + \frac{1}{i\omega C} = Z' + iZ'' = \frac{U_0}{I^*(\omega)} \tag{2}$$

using the following formulae:

$$\varepsilon^* = \varepsilon' - i\varepsilon'' = \frac{-i}{\omega Z^*(\omega)} \frac{1}{C_0}, \tag{3}$$

where $C_0 = \frac{\varepsilon_0 S}{d}$ — capacitance of empty cell, $C = \frac{\varepsilon\varepsilon_0 S}{d}$ — capacitance of cell with the sample.

Accuracy of system impedance determining in the course of measurement (which is the basis for calculation of all dielectric parameters) was 0.1% of the measured value. Values of relaxation parameters, as well as distribution functions of relaxation times $G(\tau)$ were determined by means of experimental curves approximation within the framework of the existing common theoretical models using the WinFit 3.3 software program (Novocontrol Technologies GmbH & Co), with an error of not more than 5%.

Results and discussion

Frequency dependence of the real part of specific conductivity σ' in the high frequency region ($f = 10^8 - 10^9$ Hz, Fig. 1) for both donors and patients is written as $\sigma' = A\omega^s$, where A — constant, $\omega = 2\pi f$ — rotational frequency. This dependence is typical for many disordered systems in the following cases: 1) transport by carriers excited to localized states near the edge of valence band or conduction band, 2) hop transport by carriers with an energy close to the Fermi level [19]. It is referred to the existence of hopping conductivity over localized states in the energy spectrum gap of the system under study. According to the model of correlated barrier hops (CBH-model) [20,21], charge carriers hop between energy states overcoming the potential barrier. In this case the power exponent s is related to the barrier height W_M as follows:

$$s = 1 - \frac{6kT}{W_M}. \qquad (4)$$

This conductivity is most likely connected with free ions of Na^+, K^+, Cl^- etc. As can be seen from Fig. 1, the BS conductivity of donors is higher than that of patients. It may be assumed that the BS conductivity of patients is changed due to disturbance of the interaction between free ions in the condition of changed protein composition. In this case noticeable disturbances of the blood proteome cause the changes in concentration of free ions in the serum in case of malignant processes development. The most noticeable is the change in quantities of two the most numerous proteins in the serum: albumin and certain types of immunoglobulins that provide the major portion of binding sites for inorganic ions. In particular, it is well known that one of main physiological functions of albumins is the transport of small molecules and metal ions in the blood flow, while pathological paraprotein can bind with calcium and copper ions, leading to the change in their concentrations in the blood serum [22].

Interesting is the fact that there is a correlation between values of the power exponent s and the total protein content N (Fig. 2) in donors and patients. However, an increase in s with growth of total protein content seems to be logical, because it means an increase in height of the potential barrier to be overcome by carriers in transition from one energy state to another (formula (4)) in case of disease. This circumstance also should result in a decrease in conductivity of the system. As a certain volume of statistical information is accumulated, the detected correlation and the dynamics of change in conductivity in the course of hemato-oncological diseases treatment can be used as a prognostic factor and a surrogate marker to estimate efficiency of the therapy.

In the low frequency region a dispersion of dielectric permittivity is detected, which is a reflection of intense polarization processes running in many systems in the region of low and infralow frequencies [3,4]. To analyze features of the observed relaxation processes, frequency

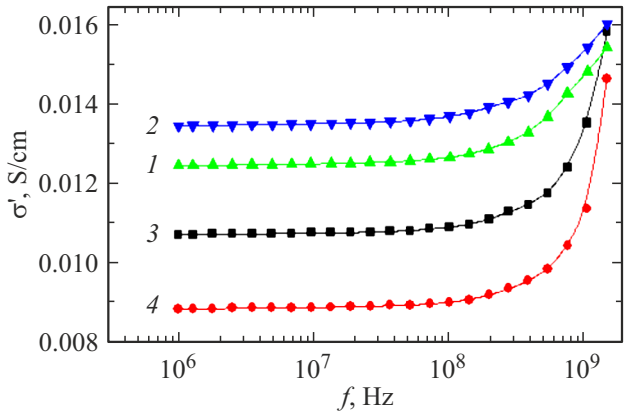

Figure 1. Specific conductivity σ' as a function of frequency for BS of two different donors and two different patients with chronic lymphocytic leukemia. In the figure: D_1 (curve *1*) and D_2 (curve *2*) — donors, P_I (curve *3*) and P_{II} (curve *4*) — patients.

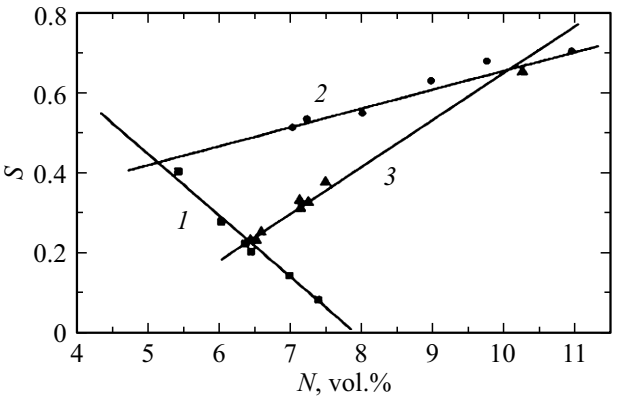

Figure 2. Correlation between the exponent of power law dependence of conductivity on frequency s and the total protein content in the blood serum N. D — donor (*1*), MM — multiple myeloma (*2*), CLL — chronic lymphocytic leukemia (*3*).

profiles of dielectric permittivity $\varepsilon^*(\omega)$ were approximated by the Havriliak-Negami function taking into account the conductivity contribution [4]:

$$\varepsilon(\omega) = \varepsilon' - i\varepsilon'' = -i\left(\frac{\sigma_0}{\varepsilon_0\omega}\right)^N + \sum_{k=1}^{3}\left[\frac{\Delta\varepsilon_k}{(1+(i\omega\tau_k)^{\alpha_k})^{\beta_k}} + \varepsilon_{\infty k}\right], \qquad (5)$$

where ε_∞ — high-frequency limit of the real part of dielectric permittivity, $\Delta\varepsilon$ — dielectric increment (the difference between low-frequency and high-frequency limits), $\omega = 2\pi f$ — cyclic frequency, α_{HN} and β_{HN} — shape parameters describing, respectively, symmetric ($\beta = 1.00$ — Cole–Cole distribution) and asymmetric ($\alpha = 1.00$ — Cole–Davidson distribution) extension of the relaxation function. On the basis of obtained values for α and β parameters, it is found that the DS behavior of BS samples

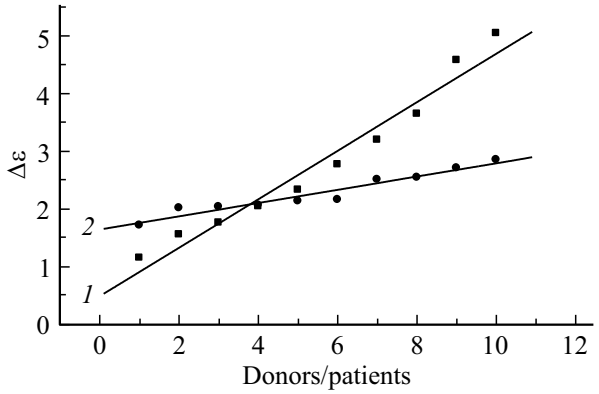

Figure 3. Dispersion of dielectric increment $\Delta\varepsilon$ for donors D (*1*) and MM patients (P) (*2*).

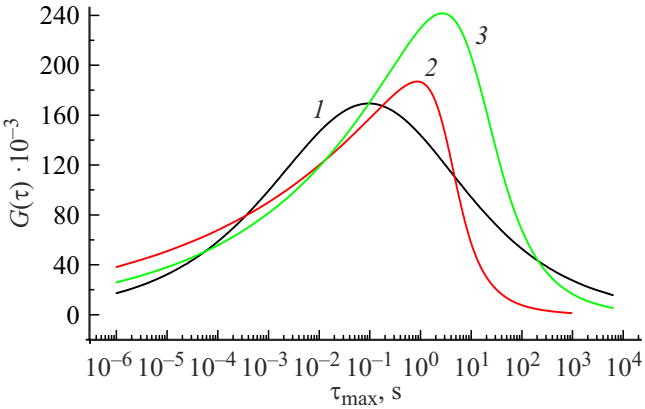

Figure 4. Function of relaxator distribution over relaxation time for donors D (curve *1*) and patients (MM — multiple myeloma (curve *2*), CLL — chronic lymphocytic leukemia (curve *3*)).

is in line with the Cole–Davidson model for the case of asymmetrical distribution of relaxators over relaxation time ($\alpha = 1.0$, $\beta \neq 1.0$) [23]. In our case the detection of one maximum in the frequency dependence of the loss factor is suggestive for the existence of one relaxation process. In this case relationship (5), without taking into account the conductivity contribution, can be written as follows:

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\Delta\varepsilon}{[1 + (i\omega\tau)^\alpha]^\beta}. \qquad (6)$$

It must be noted that when switching from donor spectra to patient spectra, a change is observed in the distribution of relaxation complexes over relaxation time. It is possible to note a lower dispersion of dielectric increment values $\Delta\varepsilon$ in patients ($\Delta\varepsilon \approx 1.9-2.5$) as compared with donors ($\Delta\varepsilon \approx 1.0-5.0$) (Fig. 3).

We have investigated the behavior of the function of relaxator distribution over relaxation time $G(\tau)$. The value of $G(\tau)$ can be obtained as a result of inverse solution. For example, for Debye relaxation $G(\tau)$ is defined as

$$\varepsilon^*(\omega) = \varepsilon_\infty + (\varepsilon_s - \varepsilon_\infty) \int_0^\infty \frac{G(\tau)}{1 + i\omega\tau}\, d\tau, \qquad (6)$$

where ε_s — permittivity at extremely low frequencies, τ — relaxation time.

Figure 4 shows distribution function of relaxators by relaxation time $G(\tau)$ for samples of donors and patients. It can be seen that the behavior of $G(\tau)$ is corrupted, function symmetry is distorted, the peak is shifted towards higher times, i.e. changes are observed in the blood relaxator spectrum when switching from donors to patients.

The detected features of donor and patient DS, that is the conductivity decrease in the region of high frequencies, as well as the correlation between the power exponent s and the total protein content, and changes in the spectrum of relaxation complexes responsible for relaxation in the region of low frequencies indicate the change in quantitative relationship between blood components in the case of disease. This change of the system is caused by the fact that a change in conformation (secondary structure) of BS albumins and immunoglobulins takes place in patients [24,25]. The later circumstance, as shown above, can result in partial dimerization of serum proteins [26,27]. As a consequence, dipole moments of proteins and their dimers change towards their increase. At increase in dipole moment of BS structural units, a sharp increase in their intermolecular dipole–dipole interaction occurs, that may explain the clustering effects, i.e. the formation of protein oligomers in the patients? BS. Exactly around these clusters a part of free ions can be solvated resulting in decrease in BS conductivity in patients. The change in relationship between BS components can also explain the observed change in relaxator spectra.

Conclusion

In this study features of the processes of charge transport and dielectric relaxation in samples of blood serum of two groups of test persons (10 patients with hemato-oncological diseases and 10 healthy donors) are investigated by the method of dielectric spectroscopy. The capabilities of dielectric spectroscopy method are shown for studying of physical properties of biological objects in a wide range of alternating electric field frequencies. The detected relationships can be used as predictors of response to therapeutic treatment in case of CLL and MM. The following relationships are found.

1. A decrease in conductivity of blood serum in patients may be a consequence of change in concentration of free ions in case of malignant processes development.

2. There is a correlation between values of the power exponent s and the total protein content N in donors and patients. An increase in s with growth of total protein content means an increase in height of potential barrier to be overcome by carriers in the patient?s blood serum.

3. The observed change in relaxator spectrum of patient?s blood serum is related to the change in conformation of

albumin and globulin proteins (secondary structure) in case of hemato-oncological diseases.

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Conflict of interest

The authors declare that they have no conflict of interest.

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