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Application of the method of dielectric spectroscopy to study the properties of blood serum of mice with malignant ascites

© Zh.A. Salnikova[1], A.P. Smirnov[1], A.A. Bogdanov[2,3], N.A. Verlov[2,3], R.A. Kastro[1]

[1] Herzen State Pedagogical University of Russia,
St. Petersburg, Russia
[2] St. Petersburg Clinical Scientific and Practical Center for Specialized Types of Medical Services (Oncological),
St. Petersburg, Russia
[3] State Research Institute of Especially Purified Bioproducts of the Federal Medical and Biological Agency,
St. Petersburg, Russia
e-mail: recastro@mail.ru

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The article presents the results of a study by the method of dielectric spectroscopy of high-frequency relaxation processes in the blood serum of intact mice and mice vaccinated with an oncological disease — Ehrlich's ascites carcinoma. Using the formalism of the electrical module, the relaxation parameters were calculated for the serum samples of the two studied systems.

Keywords: electrical module, relaxation parameters, oncological disease.

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Introduction

Dielectric spectroscopy is one of the most informative physical methods of study of the relaxation properties of dielectric media in a wide range of frequencies $f = 10^{-3}-10^{10}$ Hz [1–5]. In recent years, this method has been used widely in the examination of blood and its components [6–9]. The values of $\varepsilon'(f)$ and $\varepsilon''(f)$ for the whole blood and plasma of several oncologic patients measured in the wavelength range were presented in [9]. It was demonstrated that $\varepsilon'(f) = 80-90$; $\varepsilon''(f) = 70-15$.

Since blood serum (BS) features an increased electrical conductivity that conceals relaxation phenomena, we used the concept of the complex electrical module (a reciprocal of the complex permittivity) to reveal them. Experience shows that this method provides an opportunity to identify BS relaxation peaks, while the corresponding mathematical techniques allow one to determine the values of the key relaxation parameters. This method is applicable to all BS samples. It is of interest to compare the relaxation parameters of BS samples from healthy and pathological biological objects. The BS parameters are expected to differ, since the BS composition changes quantitatively and qualitatively in the case of evident pathology, thus altering the nature of intermolecular interactions between the elements of BS, which is reflected in a change in the numerical values of BS relaxation parameters. The idea of identifying and examining pathologic behavior by analyzing the temperature and frequency dependences of BS relaxation parameters is an appealing one.

The aim of present study was to identify the specific features of high-frequency dielectric relaxation and determine the relaxation parameters of BS of intact mice and mice with Ehrlich's ascites carcinoma.

1. Theory

1.1. Relaxation equations

The concept of complex permittivity $\varepsilon^*(f)$ is used in the method of dielectric spectroscopy to analyze the relaxation properties of dielectrics. This quantity is defined as $\varepsilon^*(f) = \varepsilon'(f) - i\varepsilon''(f)$, where f is the frequency of the applied electric field, i is imaginary unit, $\varepsilon'(f)$ is the real permittivity of the medium characterizing the degree of screening of the external electric field, and $\varepsilon''(f)$ is the imaginary permittivity characterizing the absorption of energy with its conversion into thermal energy (i.e., dielectric losses). The values of $\varepsilon'(f)$ and $\varepsilon''(f)$ are determined experimentally.

In the general case, relaxation phenomena are characterized by the Havriliak–Negami (H–N) equation [10]:

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{\left(1 + (i\omega\tau_0)^{1-\alpha}\right)^\beta}, \qquad (1)$$

where $\varepsilon_s, \varepsilon_\infty$ are the static and the high-frequency permittivity $\varepsilon'(f)$ (ε_s at $f \to 0$, ε_∞ at $f \to \infty$), $\omega = 2\pi f$ is the cyclic frequency, τ_0 is the most probable time of relaxation of the electrical response of molecular aggregates or molecules of the sample, α is the width of the spectrum of relaxation times, and β is the asymmetry of this spectrum. These parameters vary within the following intervals: $0 \le \alpha < 1$, $0 < \beta \le 1$. The higher α is, the greater is the frequency dispersion of numerical values of

relaxation times of molecules of the sample τ (i.e., the wider is the relaxation spectrum); the lower β is, the higher is the degree of asymmetry of the spectrum.

The analytical expression for the distribution function of relaxation times $G(\tau)$ with respect to τ_0 may be written in the following form [11]:

$$G(\tau) = \frac{\left(\frac{\tau}{\tau_0}\right)^{\beta(1-\alpha)} \sin(\beta\theta)}{\pi\tau\left[\left(\frac{\tau}{\tau_0}\right)^{2(1-\alpha)} + 2\left(\frac{\tau}{\tau_0}\right)^{(1-\alpha)} \cos\left(\pi(1-\alpha)\right) + 1\right]^{\beta/2}}, \tag{2}$$

where

$$\theta = \operatorname{arctg}\left[\frac{\sin\left(\pi(1-\alpha)\right)}{\left(\frac{\tau}{\tau_0}\right)^{(1-\alpha)} + \cos\left(\pi(1-\alpha)\right)}\right] \quad \text{and} \quad 0 \le \theta \le \pi.$$

At $\alpha = 0$ and $\beta = 1$, the H–N equation transforms into the Debye equation [12]. $G(\tau)$ then takes the form of delta function $\delta(\tau_0)$. This corresponds to the state when all molecules of the system have the same relaxation time τ_0.

At $\alpha \neq 0$ and $\beta = 1$, the H–N equation transforms into the Cole–Cole equation [13]:

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + (i\omega\tau_0)^{1-\alpha}}. \tag{3}$$

$G(\tau)$ is then a function symmetric with respect to τ_0.

At $\alpha = 0$ and $\beta \neq 1$, the H–N equation transforms into the Cole–Davidson equation [14]:

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{(1 + i\omega\tau_0)^{\beta}}. \tag{4}$$

$G(\tau)$ is then a function asymmetric with respect to τ_0.

Parameters α, β, τ, and $G(\tau)$ are the key relaxation parameters of the object under study. They are determined if relaxation peaks are found in the experimentally measured $\varepsilon''(f)$ dependence. If such peaks are not found, one cannot use this method to determine these parameters. In the present study, we determined them using the complex electrical module method. This method has already been applied to BS in our earlier study on chronic lymphocytic leukemia [15].

1.2. Complex electrical module method

Complex electrical module $M^*(\omega)$ is a reciprocal of the complex permittivity that is defined as follows [16]: $M^*(\omega) = M'(\omega) + iM''(\omega)$. Quantities $M'(\omega)$ and $M''(\omega)$ are the real and imaginary components of the complex electrical module. $M''(\omega)$ takes the form

$$M''(\omega) = \frac{\varepsilon''(\omega)}{\varepsilon'^2(\omega) + \varepsilon''^2(\omega)}. \tag{5}$$

The equation for $M''(\omega)$ may be derived from the H–N equation [17]:

$$M''(\omega) = \frac{M_\infty M_s A^\beta (M_\infty - M_s)\sin\beta\phi}{A^{2\beta}M_s^2 + 2A^\beta(M_\infty - M_s)M_s\cos\beta\phi + (M_\infty - M_s)^2}, \tag{6}$$

where

$$M_\infty = \frac{1}{\varepsilon_\infty}, \quad M_s = \frac{1}{\varepsilon_s},$$

$$A = \left[1 + 2(\omega\tau_0)^{1-\alpha}\sin\frac{\pi\alpha}{2} + (\omega\tau_0)^{2(1-\alpha)}\right]^{1/2},$$

$$\phi = \operatorname{arctg}\left[\frac{(\omega\tau_0)^{1-\alpha}\cos\frac{\pi\alpha}{2}}{1 + (\omega\tau_0)^{1-\alpha}\sin\frac{\pi\alpha}{2}}\right].$$

Parameters α, β, τ_0 have the same physical meaning as in Eq. (1).

2. Experimental procedure

CD-1 mice (female, weight: 22–24 g) from the „STEZAR“ breeding station were used in the experiment. Animals were housed in cages (5 mice in each cage) with free access to water and food (combined feed „Laboratorkorm“). Their zoosanitary parameters at the start of the experiment were within normal limits. The following groups were formed for the study: a group of intact mice (10 animals) and an experimental group (5 animals subjected to the abdominal injection of a suspension of Ehrlich's ascites carcinoma cells with a concentration of $3 \cdot 10^5$ cells per mouse). Blood sampling in the experimental group was performed on the fifth day after the injection. All animals were anesthetized with an intramuscular injection of Zoletil 100® (2 mg/kg) prior to blood sampling. The obtained blood samples were introduced into VACUETTE vacutainer tubes 2.0 mL in volume with a coagulation activator and centrifuged for 10 min at 2000 rpm. Blood serum was then sampled. The parameters of samples were measured on the day of sampling without refrigeration. All applicable international, national, and/or institutional guidelines for animal care and management were observed.

The dielectric spectra of BS samples were measured using a „Concept-81“ (Novocontrol Technologies GmbH) spectrometer that is designed for the study of dielectric and conducting properties of a wide range of materials (liquids included) [18]. The volume of the measurement cell was 60 μL. Measurements were performed at frequencies $f = 10^6 - 1.5 \cdot 10^9$ Hz at room temperature ($T = 20^\circ$C).

3. Results and discussion

The frequency dependences of real $\varepsilon'(f)$ and imaginary $\varepsilon''(f)$ parts of permittivity for BS samples from intact mice and mice with Ehrlich's ascites carcinoma were measured. The frequency dependence of electrical module $M''(\omega)$ for each animal was plotted in accordance with formula (5). The results for BS samples from intact mice and animals injected with Ehrlich's ascites carcinoma cells are presented in Figs. 1 and 2, respectively. The experimental data were approximated with a curve in accordance with (6). Relaxation parameters α, β, τ_0 were determined empirically by finding the best fit between the experimental data and the theoretical curve of $M''(\omega)$. The results of approximation

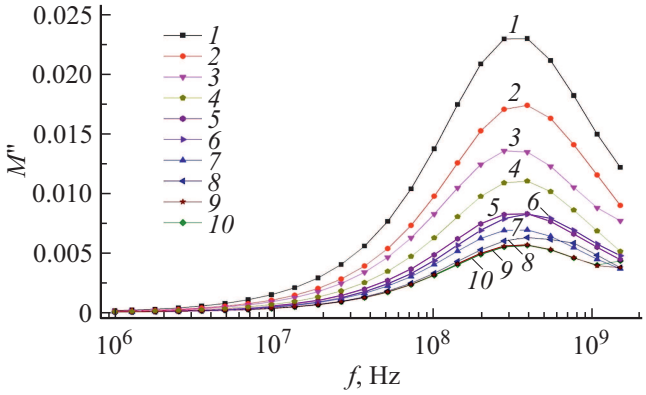

Figure 1. Frequency dependence of the imaginary part of electrical module $M''(f)$ of BS samples from intact mice. Numbers *1–10* correspond to different animals (see table).

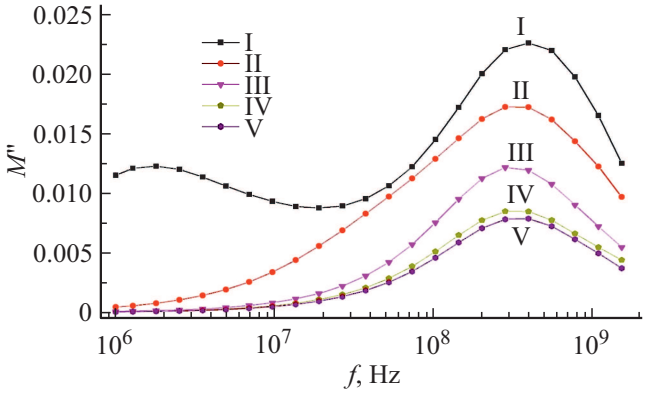

Figure 2. Frequency dependence of the imaginary part of electrical module $M''(f)$ of BS samples from mice injected with Ehrlich's ascites carcinoma cells. Numbers I–V correspond to different animals (see table).

are listed in the table. In the case of sample I (Fig. 2), the results of approximation only for the high-frequency (right) relaxation peak are presented. Parameter α turned out to be zero ($\alpha = 0$) for all BS samples from intact mice at $T = 20°\mathrm{C}$, while parameter $\beta \neq 1$. Therefore, their relaxation spectrum is characterized by Cole–Davidson equation (4). This demonstrates that BS molecules have differing relaxation times τ that are distributed over the horizontal scale asymmetrically with respect to τ_0 (i.e., function $G(\tau)$ is asymmetric).

Figure 3 presents function $G(\tau)$ plotted based on the results of examination of BS samples *1* and *3*. The plots for BS samples from other intact mice are similar: the distribution of relaxators over relaxation times is asymmetric with respect to τ_0.

It can be seen from the table that parameter $\alpha \neq 0$ for BS samples from animals injected with Ehrlich's ascites carcinoma cells at $T = 20°\mathrm{C}$. Since $\beta = 1$ for samples I and II, their relaxation spectrum is characterized by Cole–Cole equation (3). This equation implies that molecules have

Relaxation parameters α, β, τ_0 for BS samples from intact mice and mice with Ehrlich's ascites carcinoma

Sample number*	α	β	τ_0 (*c*)
1	0.00	0.85	$1.03 \cdot 10^{-9}$
2	0.00	0.91	$1.38 \cdot 10^{-9}$
3	0.00	0.85	$8.42 \cdot 10^{-10}$
4	0.00	0.80	$8.30 \cdot 10^{-10}$
5	0.00	0.67	$4.52 \cdot 10^{-10}$
6	0.00	0.56	$4.44 \cdot 10^{-10}$
7	0.00	0.62	$6.12 \cdot 10^{-10}$
8	0.00	0.78	$5.34 \cdot 10^{-10}$
9	0.00	0.66	$5.32 \cdot 10^{-10}$
10	0.00	0.91	$1.05 \cdot 10^{-9}$
I	0.26	1.00	$1.82 \cdot 10^{-9}$
II	0.25	1.00	$5.95 \cdot 10^{-10}$
III	0.01	0.90	$5.72 \cdot 10^{-10}$
IV	0.01	0.82	$6.01 \cdot 10^{-10}$
V	0.01	0.98	$2.29 \cdot 10^{-8}$

Note. * *1–10* — intact mice, I–V — mice injected with Ehrlich's ascites carcinoma cells.

differing relaxation times τ distributed symmetrically with respect to τ_0 (i.e., function $G(\tau)$ is symmetric). Figure 4 presents function $G(\tau)$ for samples I and III. It can be seen that the distribution is symmetric. The visual difference between Figs. 4, *a* and 4, *b* is attributable to the fact that parameter α for sample I is higher. Function $G(\tau)$ for sample II looks the same as that for sample I. Function $G(\tau)$ for samples IV, V is the same as that for sample III. Samples III, IV, V have $\alpha \neq 0$ and $\beta \neq 1$. However, the average value of parameter β for them ($\langle\beta\rangle = 0.90$) is significantly closer to unity than the corresponding value for intact mice ($\langle\beta\rangle = 0.76$). Therefore, their relaxation spectrum, just as the spectrum for BS samples I and II, may be characterized approximately by Cole–Cole equation (3).

The presence of macromolecules with different relaxation times τ in serum samples follows from the theoretical description of dielectric relaxation for independent dipoles with several discrete orientation states [19]. If one takes the cooperative nature of reorientation of a macromolecule (dipole reorientation is executed in the form of isolated transitions, but the probability of reorientation or the reorientation activation energy depends on the orientation of neighbors [20]) into account, a spectrum of relaxation times τ emerges (see Figs. 3 and 4). The spectrum of relaxation times for more elongated molecules is asymmetric, since their jumps from one equilibrium position to the other depend on the orientation of neighbors and

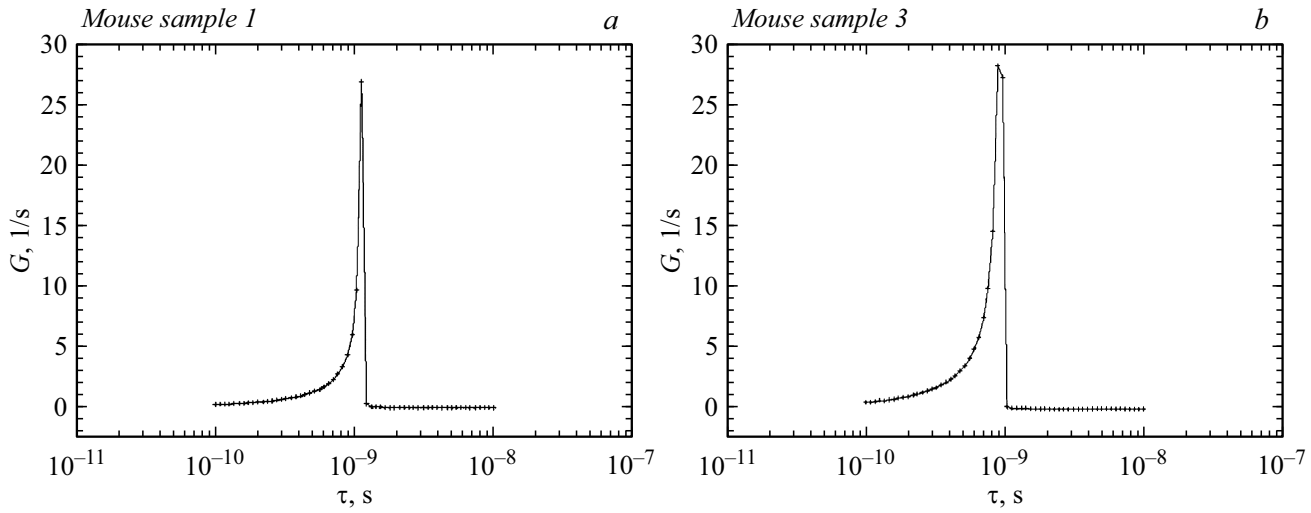

Figure 3. Distribution function of relaxation times $G(\tau)$ for intact mice: *a* — sample *1*, *b* — sample *3* (see table).

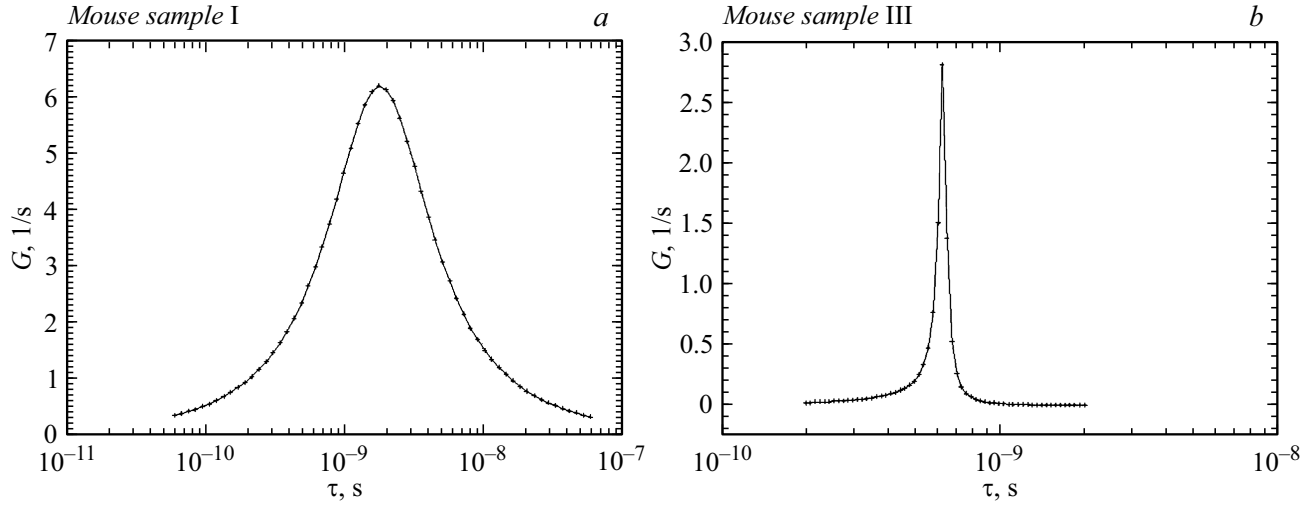

Figure 4. Distribution function of relaxation times $G(\tau)$ for mice injected with Ehrlich's ascites carcinoma cells: *a* — sample I, *b* — sample III (see table).

the energy of interaction with them. The spectrum for macromolecules with a near-spherical shape is symmetric, since the orientation of neighbors is insignificant in this case. Thus, the difference between the relaxation spectra of BS samples from intact mice and animals injected with Ehrlich's ascites carcinoma cells is probably attributable to a change in the conformation of protein macromolecules in the blood serum occurring during malignant processes.

Conclusion

The complex electrical module method allows one to determine relaxation parameters α, β, τ_0 and function $G(\tau)$ for BS samples from intact mice and mice with Ehrlich's ascites carcinoma. These parameters for intact and affected animals differ somewhat at a temperature of 20°C. These differences are likely to be more pronounced at physiological temperatures (35–41°C). The BS composition may change during disease development, thus affecting intermolecular interactions and, consequently, the BS relaxation properties. The interrelation between changes in relaxation parameters and the presence and stage of a disease (e.g., cancer) is a potential subject for future studies. Additional physical methods of research are needed to examine the issue of a probable change in the conformation of protein molecules in the blood serum during pathological processes. It is essential in this respect to perform correlation analysis of the data obtained by dielectric spectroscopy and the data from biochemical blood assays for various diseases.

The obtained data suggest that the dielectric spectroscopy technique may be applied in the future in diagnostics of diseases (e.g., cancers) based on the identification of specific features of molecular physical processes in body fluids.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] B.I. Sazhin, *Elektricheskie svoistva polimerov* (Khimiya, L., 1986) (in Russian).
[2] T. Blythe, D. Bloor, *Electrical Properties of Polymers* (Cambridge Univ. Press, 2005).
[3] Yu.A. Gorokhovatskii, E.A. Karulina, D.E. Temnov, *Fizika polimernykh dielektrikov* (Izd. Ross. Gos. Pedagog. Univ., St. Petersburg, 2013) (in Russian).
[4] R.A. Castro, A.I. Ignatiev, N.V. Nikonorov, A.I. Sidorov, M.V. Stolyarchuk. J. Non-Crystall. Solid., **461**, 72 (2017).
[5] N.A. Nikonorova, A.A. Kononov, H.T. Dao, R.A. Castro. J. Non-Crystall. Solid., **511**, 109 (2019).
[6] M. Wolf, R. Gulich, P. Lunkenheimer, A. Loidl. Biochim. Biophys. Acta, **1810** (8), 727 (2011).
[7] K. Asami. J. Non-Crystall. Solid., **305**, 268 (2002).
[8] T. Chelidze. J. Non-Crystall. Solid., **305**, 285 (2002).
[9] A.N. Romanov, E.Yu. Vinokurova, A.O. Kovrigin, A.F. Lazarev, V.A. Lubennikov, N.A. Romanova, S.A. Komarov, *Dielektricheskie kharakteristiki biologicheskikh zhidkostei cheloveka pri razvitii onkologicheskikh zabolevanii (mikrovolnovyi diapazon)* (Azbuka, Barnaul, 2008) (in Russian).
[10] S. Havriliak, S. Negami. J. Polymer Science Part C, **14** (1), 99 (1966).
[11] S. Havriliak, S. Negami. Polymer, **8**, 161 (1967).
[12] P.J.W. Debye, *Polar Molecules* (Chemical Catalog Company, Inc., 1929).
[13] K.S. Cole, R.H. Cole. J. Chem. Phys., **9**, 341 (1941)
[14] D.W. Davidson, R.H. Cole. J. Chem. Phys., **19**, 1484 (1951); **18**, 1417 (1950)
[15] Zh.A. Salnikova, L.V. Plotnikova, A.P. Smirnov, A.D. Garifullin, A.Yu. Kuvshinov, S.V. Voloshin. A.M. Polyanichko. AIP Conf. Proceed. **2308**, 030018 (2020); DOI: 10.1063/5.0035270
[16] N.G. McCrum, B.E. Read, G. Williams In: *Anealastic and Dielectric Effecrts in Polymeric Solids* (Willey, London, 1967), p. 108–111.
[17] Zh.A. Salnikova, A.A. Kononov. AIP Conf. Proceed. **2308**, 030017 (2020); DOI: 10.1063/5.0034028
[18] Electronic source. Description of a „Novocontrol Concept−81“ instrument. https://herzen.spb.ru/main/nauka/1297769731/1318945373/Dielectric
[19] H. Fröhlich, *Theory of Dielectrics: Dielectric Constant and Dielectric Loss* (Second Edition Oxford Univ. Press, 1958).
[20] Yu.Ya. Gotlib, A.A. Darinskii, Yu.E. Svetlov, *Fizicheskaya kinetika makromolekul* (Khimiya, L., 1986) (in Russian).