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Theory of Broadband Dispersion of Permittivity of Biological Cell Suspensions

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Abstract—The classical Pauly–Schwan electrical model of the biological cell is generalized by considering the diffusion processes that occur due to the selective (with respect to ions of different signes of charges) conductivity of surface cell structures (cytoplasmic membrane and electrical double layer). The analytical theory of the dispersion of the permittivity of biological cell suspensions that cover a broad frequency band that includes three typical (for these systems) dispersion regions α, β, and γ is constructed using the generalized model, whereas the classical model describes only β and γ regions. Very large values of permittivity and their complete stipulation by the ionic selectivity of surface structures, which are characteristic for the region of α dispersion, predetermine the unique sensitivity of low-frequency dielectric spectrum to the effective conductivity of the membrane of a living cell, which can find important applications in biology and medicine.

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INTRODUCTION

Dielectric spectroscopy of the suspensions of biological cells is one of the most efficient nondestructive methods of the study of microorganisms and blood cells. The method consists of measuring the dependences of the real part of permittivity $\varepsilon'(F)$ and dielectric losses $\varepsilon''(F)$ on frequency F and determining (by means of theoretical interpretation of measurement results) the electrical characteristics of cell structures such as cell membrane, cytoplasm, and cell wall. This work is devoted to the generalization of current theory, which was only developed for high- and medium-frequency ranges of spectrum to the range of low frequencies. Very low electric fields are used in dielectric spectroscopy, which explains the applicability of this method to the study of living cells. The information content of this method is related to the presence of several dispersion ranges in spectra, the parameters of each range having different depences on the characteristics of cell structures.

Within the frequency range of 10^3 to 10^{10} Hz, three ranges of frequency dependence of the permittivity of the biological cell suspensions are observed, which are usually denoted by Greek letters in the increasing order as α, β, and γ ranges.

The theory of γ dispersion (the highest-frequency-range dispersion), developed by Maxwell [1] and modified by Wagner [2], is based on a model that represents the suspension of uniform spherical inclusions ("particles") with conductivity K_i and permittivity ε_i distributed in a medium with parameters K_e and ε_e, respectively. The physical nature of this dispersion range is related to the fact that the displacement current, which is negligibly low at low frequencies compared to conduction current, becomes more and more significant with an increase in frequency and finally exceeds the conduction current. Thus, at low frequencies, the polarizability of particles, together with their contribution to permittivity of suspension, $\delta\varepsilon'$, is determined by the ratio between conductivities of particles and medium, K_i/K_e, and, at high frequencies, it is determined by the ratio of their permittivities, $\varepsilon_i/\varepsilon_e$, thus determining frequency dependence $\delta\varepsilon'(F)$, i.e., the Maxwell–Wagner dielectric dispersion.

The influence of particle nonuniformity on this mechanism leads not only to the trivial broadening of Maxwell–Wagner band, but also to the emergence of a new dispersion range, which was revealed by Pauly and Schwan [3]. In their model of the biological cell, the Maxwell–Wagner uniform sphere is supplemented by an adjacent, nearly nonconducting thin spherical layer, which represents the cell membrane. This layer almost completely eliminates the penetration of current into the internal conducting medium until the frequency of external field increases so that the capacitive resistance of membrane becomes smaller than the effective ohmic resistance of medium through which the membrane is discharged. The equality of these resistances determines the critical frequency for the Pauly–Schwan β dispersion, which turned out to be much lower than the Maxwell–Wagner frequency due to the small (nanosized) thickness of membrane and, hence, due to its high capacity. Thus, the Pauly–Schwan model describes the β dispersion at frequencies of 10^5 to 10^7 Hz and, in the limiting case of high frequencies, also covers the Maxwell–Wagner dispersion.

However, at low frequencies (10– 10^4 Hz), a dielectric dispersion is almost always observed. This dispersion cannot be explained by the aforementioned classical models and is due to the mutual effect of electric and diffusion fluxes formed near the cell under the action of electric field. This mutual effect arises in rather widespread cases when the ionic selectivity of the conductivity of membrane structures takes place, i.e., when the current is transferred predominantly by ions of the same sign (e.g., by cations). Ionic channels of cell membrane, cell wall, and electrical double layer (EDL) can act as such structures. Due to ionic selectivity, the current between the cell and solution gives rise to the accumulation of neutral salt and its diffusion near the surface, while the concentration gradient of salt originates the electric current in membrane structures. The effect of this diffusion-controlled current gives rise to dielectric α dispersion at frequencies on the order of reciprocal time of the formation of diffusion fields around the polarized cell. The mechanism of the diffusion-controlled dielectric α dispersion, which was first proposed in [4–6] for the dilute suspensions of nonconducting spherical colloidal particles with a thin double layer, is now generally recognized (see, e.g. [7]) and is widely applied to describe low-frequency dielectric spectra of versatile systems. This work is devoted to the generalization of the Pauly–Schwan electrical model by accounting for the selective conductivity of membrane structures and, on this basis, to the development of the theory of the permittivity of cell suspension, which, along with medium- and high-frequency ranges of β- and γ dispersions, would describe the range of low-frequency α dispersion. Very large values of permittivity and their complete stipulation by the ion-selective conductivity of membrane structures, which are characteristic for the α dispersion, predetermine the unique sensitivity of low-frequency dielectric spectrum to the effective conductivity of the membrane of living cell that can be very important for the application in biology and medicine.

COMPLEX PERMITTIVITY OF DILUTE SUSPENSION: GENERAL INFORMATION

Considering the response to the action of sinusoidal external electric field with frequency ω, we will present the dependence of the field on time using the complex multiplier

$$E(t) = E(\omega)e^{i\omega t}. \quad (1)$$

The known advantage of this presentation is that all values caused by the field and linear on it are characterized by the same time dependence

$$X(t) = X^*(\omega)e^{i\omega t}, \quad (2)$$

where the asterisk denotes the complex quantity. If, for a certain parameter X, the relaxation frequency is close to ω, variations of this value will be out of phase with the field that corresponds to the appearance of the imaginary part of multiplier $X^*(\omega)$, as well as to the dependence of the latter on frequency. Using the presentation set by Eqs. (1) and (2), the total electric response of a system can be characterized by its complex permittivity

$$\varepsilon^*(\omega) = \varepsilon(\omega) - \frac{iK(\omega)}{\omega} \quad (3)$$

or by complex conductivity

$$K^*(\omega) = K(\omega) + i\omega\varepsilon(\omega). \quad (4)$$

It should be emphasized that, in formulas (3) and (4), permittivity $\varepsilon(\omega)$, and conductivity $K(\omega)$, of suspension are real functions of frequency.

Contributions $\delta\varepsilon^*(\omega)$ and $\delta K^*(\omega)$ from inclusions (dispersed particles or cells) to the complex permittivity and conductivity of their dilute suspensions are determined by expressions

$$\delta\varepsilon^*(\omega) \equiv \delta\varepsilon(\omega) - \frac{i\delta K(\omega)}{\omega} = \varepsilon^*(\omega) - \varepsilon_e^*(\omega), \quad (5)$$

$$\delta K^*(\omega) \equiv \delta K(\omega) + i\omega\delta\varepsilon(\omega) = K^*(\omega) - K_e^*(\omega). \quad (6)$$

Quantities

$$\varepsilon_e^*(\omega) = \varepsilon_e - \frac{iK_e}{\omega} \quad (7)$$

and

$$K_e^*(\omega) = K_e + i\omega\varepsilon_e \quad (8)$$

present the complex permittivity and complex conductivity of dispersion medium, respectively (quantities without asterisks, namely, ε_m, K_m,, $\delta\varepsilon(\omega)$, $\delta K(\omega)$, are real quantities).

Functions $\delta\varepsilon^*(\omega)$ and $\delta K^*(\omega)$ can be determined via the superposition of long-range electric fields of polarized inclusions

$$\delta\varepsilon^*(\omega) = \frac{4\pi}{3}\phi\varepsilon^*(\omega)\gamma_p^*(\omega), \quad (9)$$

$$\delta K^*(\omega) = \frac{4\pi}{3}\phi K_m^*(\omega)\gamma_p^*(\omega). \quad (10)$$

Here, $\gamma_p^*(\omega)$ is the dipole coefficient of inclusions and ϕ is their volume fraction in suspension.

The dipole coefficient $\gamma_p^*(\omega)$ enters into expression for the distribution of electric potential around the polarized inclusion at large distances from its center

$$\varphi_m^* = -Er\cos\theta + \frac{\gamma_p^* a^3 E}{r^2}\cos\theta, \quad (11)$$

where a is the inclusion radius. Here, the first term in the right-hand side is the potential of uniform external field at point $\{r,\theta\}$, r is the distance from the particle center, and θ is the angle between the radius vector of

the point and the direction of external field. The second term of formula (11) characterizes the deviation of the potential outside the double layer of inclusion from the potential of uniform external field.

According to [1–6], the frequency dependences of the permittivity and conductivity of suspension, $\varepsilon(\omega)$ and $K(\omega)$, respectively, (in a frequency range in which characteristics of dispersion medium can be considered to be frequency-independent parameters) are related to the frequency dependence of dipole coefficient $\gamma_p^*(\omega)$. Note that, in the low-frequency range, in which strong inequality

$$\omega \ll K_e/\varepsilon_e, \tag{12}$$

is fulfilled, the absolute value of the imaginary part of complex permittivity of dispersion medium, K_e/ω, exceeds its real part by many times. As is known [4–6], this leads to very large values of low-frequency permittivity of suspensions, which are determined by the contribution of factor $\frac{K_e}{\omega}\mathrm{Im}\gamma_p^*(\omega)$ to the real part of Eq. (9).

Note that the symmetric phenomenon of the very large contribution of cells (particles) to the real part of suspension conductivity, $\delta K(\omega)$, can be expected (see [8]) in the high-frequency range of dispersion. This is connected with the contribution of term $\varepsilon_e\omega\mathrm{Im}\gamma_p^*(\omega)$, which can be very large if there is a mechanism that gives rise to the frequency dependence of dipole coefficient in the range of very high frequencies ($\omega \gg K_e/\varepsilon_e$).

REGIONS OF PAULY–SCHWAN AND MAXWELL–WAGNER RELAXATIONS: MEDIUM AND HIGH FREQUENCIES

There are different mechanisms that affect the polarization of inclusions, including both common uniform particles of the dispersed phase and biological cells in suspension. Each of these mechanisms is always connected with a certain property that is typical of either the internal structure of the inclusion, dispersion medium, or the dispersed phase- dispersion medium interface. The widely known mechanism of dielectric dispersion, i.e., the Maxwell–Wagner dispersion, is associated with the contact between phases (inclusion and medium with different volume conductivities and dielectric permittivities). Let us denote the conductivity and permittivity of inclusions by K_p and ε_p, respectively, while those of medium are denoted by K_e and ε_e, respectively. If deviations of these values are different, i.e., if

$$\frac{\varepsilon_e}{K_e} \neq \frac{\varepsilon_p}{K_p}, \tag{13}$$

the continuity condition for the normal component of current density and that for electrostatic induction vector on both sides of the surface become incompatible, thus leading to the formation of free ionic charge near the surface. The duration of this process, which, in dilute suspensions, takes time $\tau_{MW} = \frac{2\varepsilon_e + \varepsilon_p}{2K_e + K_p}$, is precisely responsible for the Maxwell–Wagner dielectric dispersion with critical frequency $\omega_{MW} = 1/\tau_{MW}$. For many systems, time τ_{MW} is close to the relaxation time of electrolyte, $\tau_{MW} = \frac{2\varepsilon_e + \varepsilon_p}{2K_e + K_p} \approx \frac{\varepsilon_e}{K_e}$.

The frequency dependence of the most important characteristic of inclusions, i.e., their dipole coefficient, which, according to equalities (9) and (10), determines the contributions of inclusions to the complex permittivity and complex conductivity of dilute suspension, is defined in the Maxwell–Wagner theory by the following equation:

$$\gamma_p^*(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \equiv \frac{K_p^* - K_m^*}{K_p^* + 2K_m^*}. \tag{14}$$

The theory of Maxwell–Wagner dielectric dispersion does not take into account any specific surface properties. In this theory, the surface is a mathematical notion, i.e., it is an infinitely thin interface between homogeneous media with different conductivities and dielectric permittivities. This model is often sufficient to describe the dielectric properties of suspensions of nonconducting particles or particles with ionic conductivity (particles of ionites or ionic crystals) in electrolyte solution at sufficiently high frequencies. However, the real surface is never infinitely thin, but rather presents a surface layer of finite thickness in which parameters K and ε vary from K_p and ε_p in the depth of inclusion and to K_e and ε_e in the depth of dispersion medium. If this change occurs monotonically and the size of the inclusion greatly exceeds the thickness of the thin surface layer, the nonuniformity of the latter can be ignored. Naturally, this cannot be done in the case of thick surface layer, which will not be considered here. However, if the ε and K parameters are changed in a highly nonmonotonic manner, even a very thin surface layer can quantitatively or even qualitatively affect the frequency dependence of the dipole coefficient of inclusion and (in accordance with relations (9) and (10)) can affect the frequency dependences of the permittivity and conductivity of suspension.

The strong nonmonotonic character of electrical parameters is characteristic of all biological cells, the internal content of which is cytoplasm with a rather high conductivity ($K_i \approx 1\ \Omega^{-1}\ \mathrm{m}^{-1}$), which is always surrounded by a thin (with thickness of several nanometers), nearly nonconducting layer (cytoplasmic membrane). If the cell is in an electrolyte solution, the thin,

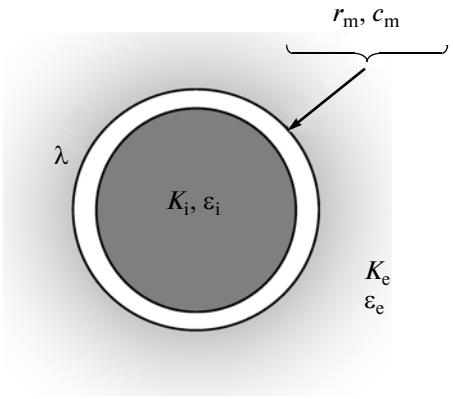

Fig. 1. Pauly–Schwan electrical model of biological cell. Dark gray shading denotes the cytoplasm with conductivity K_i and permittivity ε_i; white color denotes the external solution with conductivity K_e and permittivity ε_e; white band refers to cytoplasmic membrane with capacity c_m and resistance r_m per unit surface; gray diffuse layer outside the membrane refers to cell wall, which, along with diffuse part of EDL, is characterized by surface conductance λ.

nonconducting cytoplasmic membrane will separate the conducting cytoplasm from the conducting ($K_e \approx 10^{-2}$–$10^{-1}\ \Omega^{-1}\ m^{-}$) external medium (Fig. 1).

In this case, the current distributed on the exterior of the membrane under the action of an external electric field of rather low frequency will hardly reach the cytoplasm; hence, the cell will resemble a nonconducting inclusion (with zero effective conductivity ($K_{pef} = 0$). However, as the frequency increases to the critical value ($\omega = \omega_\beta$ for the β dispersion), the capacitive resistance of membrane decreases so that the density of the current entering the cell becomes equal to the density of the current in solution far from the cell and the cell behaves as an isoconducting inclusion ($K_{pef} = K_e$). Furthermore, when the frequency increases to values corresponding to $\omega \gg \omega_\beta$, the capacitive resistance of the membrane becomes negligibly low and the cell behaves as an inclusion with the same effective conductivity and permittivity as the interior (cytoplasm) of the cell as follows: $K_{pef} = K_i$ and $\varepsilon_{pef} = \varepsilon_i$. Finally, a further increase in frequency gives rise to conditions for a common Maxwell–Wagner dispersion. Formally, this frequency dependence of cell polarization is reflected (see [9]) via its effective complex conductivity (Fig. 2) as follows:

$$\gamma_p^*(\omega) = \frac{K_{pef}^* - K_m^*}{K_{pef}^* + 2K_m^*}, \tag{15}$$

$$K_{pef}^* = \frac{i\omega c_{mef}^* a}{1 + i\omega\left(c_{mef}^* a / K_i\right)} + \frac{2\lambda}{a}. \tag{16}$$

Here, λ denotes surface conductivity due to the excess of mobile ions in such structures outside the

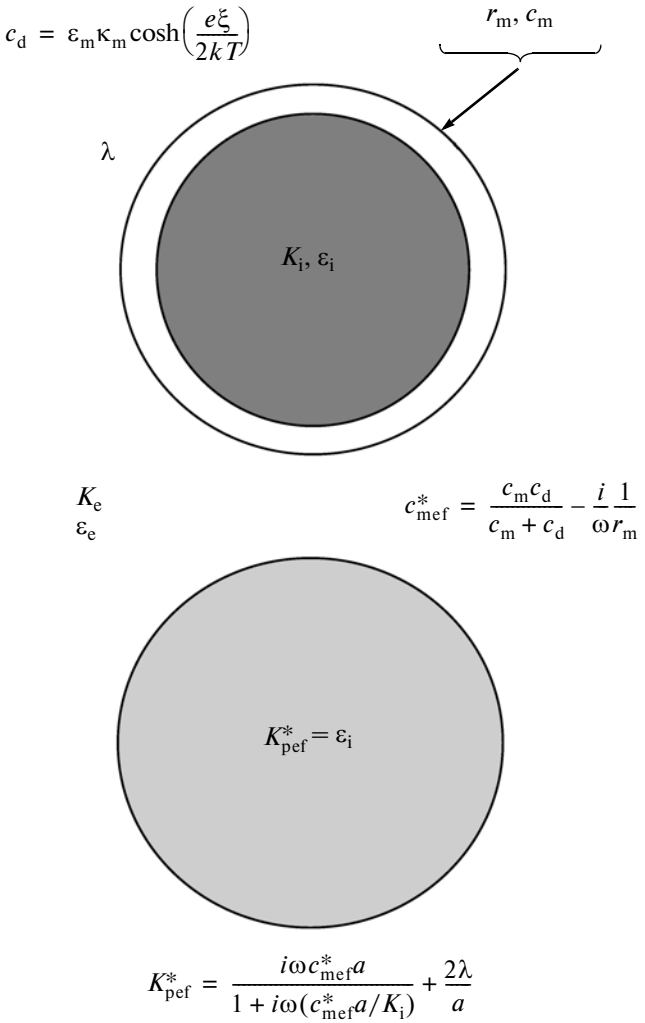

Fig. 2. Uniform particle with effective complex conductivity. K_{pef}^* is the analog of the electrical model of biological cell.

cytoplasmic membrane as the cell wall and the electrical double layer. The c_{mef}^* value is nothing other than the effective complex capacity per unit membrane surface

$$c_{mef}^* = \frac{c_m c_d}{c_m + c_d} - \frac{i}{\omega} \frac{1}{r_m}, \tag{17}$$

which depends on its capacity

$$c_m = \frac{\varepsilon_m}{h}, \tag{18}$$

its resistance

$$r_m = \frac{h}{K_m} \tag{19}$$

and (see [10] and [11]) the series capacitance c_d of the electrical double layer adjacent to the membrane

$$c_d = \varepsilon_e \kappa \mathrm{ch}\left(\frac{e\zeta}{2kT}\right). \tag{20}$$

Here, ε_m and K_m are the average values of the permittivity and conductivity of the membrane, respectively; h is its thickness; ε_e and κ – are the permittivity of external solution and the reciprocal Debye length in this solution, respectively; ζ is the equilibrium drop of potential in the diffuse part of double layer; e is the proton charge; k is Boltzmann's constant; and T is the absolute temperature.

Note that, under conditions when the dielectric dispersion of cell suspension can be measured, the conductivity of external solution becomes very low compared to that of cytoplasm. In addition, the time of the membrane charging is nearly independent of its low conductivity. Finally, rather rare cases are observed when the capacity of the diffuse layer is low enough to influence the effective capacity of the membrane. Taking into account all of these simplifying circumstances, the critical frequency of β dispersion can be written as follows:

$$\omega_\beta = \tau_\beta^{-1} \approx \frac{2K_e}{ac_m} = \frac{2K_e h}{\varepsilon_m a}. \quad (21)$$

Let us cite the expression of low-frequency limit of the Pauly–Schwan dispersion for the dipole coefficient of the cell

$$\gamma_p^1 = \lim_{\omega\to 0}\gamma_p^*(\omega) = \frac{K_{pef}^1 - K_e}{K_{pef}^1 + 2K_e} \quad (22)$$

which is important for further consideration.

The expression for the effective dc conductivity of the cell, K_{pef}^l, entering into Eq. (22), is derived from formulas (16) and (17) and is simplified due to strong inequality $K_i r_m \gg a$, which is always fulfilled for living cells with fairly large values of the conductivity of cytoplasm and the resistance of cell membrane as follows:

$$K_{pef}^1 \equiv \lim_{\omega\to 0} K_{pef} = \frac{aK_i}{a + K_i r_m} \underset{K_i r_m \gg a}{\approx} \frac{a}{r_m} + \frac{2\lambda}{a}. \quad (23)$$

LOW-FREQUENCY DIELECTRIC DISPERSION CAUSED BY CONCENTRATION POLARIZATION OF THE CELL: DIFFUSION-CONTROLLED α DISPERSION

Whereas the Maxwell–Wagner dielectric dispersion appears in connection with different spatial dependences of the local conductivity and permittivity, another typical factor for suspensions that gives rise to a low-frequency dielectric dispersion is the spatial dependence of transfer numbers of ions (cations $t^+ \equiv K^+/K$ and anions $t^- \equiv K^-/K = 1 - t^+$), when the relative contribution of specific ions, e.g., cations (t^+), to the local conductivity is different near the inclusion and in the depth of solution. The differences in transfer numbers near the inclusion surface and in the depth of solution result (during the flow of current from solution into surface structures) in the accumulation of ions of both signs and the emergence of electrolyte concentration gradient near the polarized inclusion. In turn, the effect of the electrolyte concentration gradient leads (upon the spatial dependence of transfer numbers) to the emergence of diffusion-controlled current that, in the final analysis, is responsible for the appearance of diffusion-controlled low-frequency dielectric dispersion. This mechanism is quite universal, above all because different inclusions in electrolyte solution almost always carry fixed charges on their structures that are screened by oppositely charged free ions (counterions), which contribute mostly to the conductivity within the Debye atmosphere (the diffuse part of EDL), whereas contributions of cations and anions in the depth of solution are comparable. The selectivity of ionic channels of cytoplasmic membrane is the specific mechanism for biological cells, which is also related to the strong spatial dependence of transfer numbers of ions. Thus, the inequality

$$\frac{K_{pef}^{\pm}}{K_{pef}} \neq \frac{K_e^{\pm}}{K_e} \quad (24)$$

is observed almost in any case without exception. If this inequality is fulfilled, the balance between the fluxes of ions of both signs is only possible upon the appearance of spatial-dependent additive $\delta c(\mathbf{r})$ to the electrolyte concentration near the polarized inclusions. In turn, the ratios of the diffusion fluxes of cations and anions controlled by the presence of $\delta c(\mathbf{r})$ additive will different inside and outside of the inclusion, provided that condition (24) is valid. This circumstance gives rise to the current through the surface of inclusion, thus affecting its dipole coefficient $\gamma_p^*(\omega)$. Note that this effect is related to the slow formation of the field of solution concentration $\delta c(\mathbf{r})$ and, hence, occurs precisely at low frequencies when inequality (12) is fulfilled. Correspondingly, this leads to gigantic values of the permittivity of these inclusions. This mechanism was described for the first time in [4–6] and was later [12] named " diffusion-controlled α dispersion."

For 1 : 1 symmetric electrolyte, the $K_e^{\pm}$ values are determined by the formulas

$$K_e^{\pm} = \frac{e^2 n_0}{kT} D^{\pm}, \quad (25)$$

where n_0 is the numerical concentration of ions of each sign in the bulk of electrolyte and D^+ and D^- are the diffusion coefficients of cations and anions, respectively. The known expression for the conductivity of electrolyte solution

$$K_e = K_e^+ + K_e^- = \frac{e^2 n_0}{kT}\left(D^+ + D^-\right) \quad (26)$$

follows from equality (25).

The analytical theory of the diffusion-controlled low-frequency dispersion of the permittivity of the suspensions of nonconducting charged spheres was developed in [4–6]. This theory is based on the approximation of local equilibrium between the $\delta c(\mathbf{r})$ values near the particle surface and changes in the parameters of double layer. For nonconducting particles, this approximation is quite reasonable if the EDL thickness is small compared to the particle radius

$$\kappa a \gg 1, \tag{27}$$

and the frequency is much lower than the critical frequency of the Maxwell–Wagner dispersion

$$\omega \ll \omega_{MW}. \tag{28}$$

For a biological cell, the condition of the local equilibrium of its membrane structures with adjacent solution leads to a more rigid constraint on the frequency of the external field. The latter should be low enough for the field period to considerably exceed the time needed to charge the cell membrane. This requirement is ensured by the following inequality:

$$\omega \ll \omega_{\beta}. \tag{29}$$

When generalizing the Pauly–Schwan model to describe, together with β and γ dispersions, the diffusion-controlled, low-frequency α dispersion, we should first take into account that the conductivities of both cytoplasm and membrane structures are ionic. Based on this fact and comparing the cell and uniform particle with conductivity K^1_{pef},, we should assume that the latter is also characterized by ionic character. Thus, considering the distributions of electric potential φ and electrolyte concentrations c around the biological cell under condition (29), we take advantage of the same system of equations and boundary conditions, which was used to describe the polarization of the uniform particle with ionic conductivity (ionite particles) [13, 14]. Using Eq. (23), we related the cell to the equivalent uniform particle with volume conductivity K^1_{pef}. With regard to the transfer numbers of ions in the bulk of equivalent particle, considering so far the most encountered case when the cell carries fixed negative charge and, correspondingly, when free carriers of charge in its membrane structures are predominantly cations, we assume that the effective conductivity of cell, K^1_{pef}, is caused by cations and the transfer number of anions is equal to zero. According to the last assumption, our system of equations is closed in the region outside the cell ($r > a$) and includes the following conservation equations for cations and anions:

$$\mathbf{j}^{\pm} = -\frac{1}{e}K_e^{\pm}\nabla\mu^{\pm}, \tag{30}$$

where the electrochemical potentials of cations and anions, $\mu^{\pm}$, are determined by electrolyte concentration c and electric potential φ

$$\mu^{\pm} = kT\ln c \pm ez^{\pm}\varphi, \tag{31}$$

with boundary conditions, the first of which reflects the continuity of cation fluxes on the cell surface, while the second condition takes into account the absence of the transfer of anions through this surface

$$K_e^{+}\nabla_n\mu^{+}\Big|_{r=a} = \frac{K^1_{\text{pef}}}{a}\mu^{+}\Big|_{r=a}, \tag{32}$$

$$j_n^{-}\Big|_{r=a} = 0. \tag{33}$$

Let us also use infinity conditions, the first of which accounts for the uniformity of external filed with strength E, whereas the second condition is responsible for the uniform concentration at a large distance from the cell

$$-\nabla\varphi\big|_{\infty} = \mathbf{E}_{\infty},\ \ \nabla c\big|_{\infty} = 0. \tag{34}$$

Solving the system of equations (30)–(34) with respect to φ and c and deriving the asymptotics of potential φ from its distribution at large distances from the cell, according to Eq. (11), we arrive at the frequency dependence of the dipole coefficient in the region of diffusion-controlled low-frequency α dispersion, which has the following compact form [7, 8]:

$$\gamma_p^{*1} = \frac{K^1_{\text{pef}} - K_e}{K^1_{\text{pef}} + 2K_e} - \frac{3R_h}{2B} + \frac{3R_h}{2B}\frac{i\omega\tau_\alpha}{1+\sqrt{2/S}\sqrt{i\omega\tau_\alpha}+i\omega\tau_\alpha}. \tag{35}$$

where

$$R_h = 2\frac{D^-}{D^+}\frac{K^1_{\text{pef}}}{K_e}\frac{K^1_{\text{pef}}}{K_{\text{pef}}+2K_e},$$

$$B = 4 + 2\frac{K^1_{\text{pef}}}{K_e}\frac{D^+ + D^-}{D^+},$$

$$S = 1 + \frac{D^-}{D^+}\frac{K^1_{\text{pef}}}{(K_{\text{pef}}+2K_e)},$$

$$\tau_\alpha = \frac{a^2\left(D^+ + D^-\right)S}{4D^+D^-}. \tag{36}$$

The first term in the right-hand side of Eq. (35) coincides with the right-hand side of Eq. (15) and, at the same time, is the high-frequency limit of diffusion-controlled α dispersion and low-frequency limit of the Pauly–Schwan dispersion of dipole coefficient. The second term presents the variation amplitude of the dipole coefficient for the low-frequency α dispersion. Thus, the first two terms taken together present the absolute low-frequency limit for all of three ranges α,

β, and γ dispersions of dipole coefficient in the generalized Pauly–Schwan model. The third, frequency-dependent term ensures the transition from the absolute low-frequency limit to the low-frequency limit of classical Pauly–Schwan dispersion.

DISPERSION OF DIPOLE COEFFICIENT AND DIELECTRIC DISPERSION WITHIN WIDE FREQUENCY RANGE

Now, we will derive the general formula for induced dipole moment, which should describe its frequency dependence in all three dispersion ranges, i.e., for diffusion-controlled α dispersion, Pauly–Schwan β dispersion, and Maxwell–Wagner γ dispersion. The path to this generalization is opened by the coincidence (see formula (35)) of the high-frequency limit of dipole coefficient derived analytically in the theory of low-frequency dispersion and the low-frequency limit of dipole coefficient derived in the Pauly–Schwan theory (22).

This coincidence permits us to take advantage of the superposition approximation proposed in [18]. The approximation consists of the replacement of the frequency-independent term $\frac{K_{ief} - K_e}{K_{ief} + 2K_e}$, which presents the high-frequency limit of Eq. (35) by the frequency-dependent dipole coefficient (15)

$$\gamma_p^* = \frac{K_{pef}^* - K_e^*}{K_{pef}^1 + 2K_e^*} - \frac{3R_h}{2B} + \frac{3R_h}{2B}\frac{i\omega\tau_\alpha}{1 + \sqrt{2/S}\sqrt{i\omega\tau_\alpha} + i\omega\tau_\alpha}. \quad (37)$$

The possible use of the superposition approximation is determined by the strong inequality

$$\tau_\beta \ll \tau_\alpha. \quad (38)$$

Under this inequality, it is unnecessary to take into account the mutual effect of the mechanism of bulk diffusion (inherent to low-frequency dispersion) and the capacitive current that passes through the cytoplasmic membrane (inherent to the Pauly–Schwan dispersion). It is possibledue to the fact that the frequencies at which the mechanism of bulk diffusion is significant for the polarization of particle $(\omega \approx 1/\tau_\alpha)$, turned out to be so low that the capacitive current is negligible compared to the conduction current. On the other hand, capacitive currents are significant at frequency $\omega \approx 1/\tau_\beta$, which is too high for the concentration fields and relevant diffusion fluxes to have time to form. In other words, capacitive currents and diffusion fluxes are comparable at frequencies for which two strong inequalities are fulfilled simultaneously, i.e., when $1/\tau_\beta \gg \omega \gg 1/\tau_\alpha$. However, at these frequencies, both capacitive currents and diffusion fluxes are insignificant; displacement currents are still too low, whereas concentration gradients no longer have time to be formed. This fact determines the smallness of the mutual effect of both relaxation mechanisms and, hence, the applicability of superposition approximation in which expression (37) was derived is also low. For the majority of typical systems, Pauly–Schwan relaxation time is given by formula (21). From relations (36) and (21) with allowance for the known expression that relates the reciprocal Debye length κ with the conductivity of the intercellular solution, K_e, its permittivity ε_e, and the diffusion coefficient of ions, D, we obtain the following estimate of the ratio of characteristic times of α and β ranges of dispersion:

$$\frac{\tau_\alpha}{\tau_\beta} = \frac{K_e}{D\varepsilon_m}ah = \frac{K_e\varepsilon_e}{D\varepsilon_e\varepsilon_m}ah = \frac{\varepsilon_e}{\varepsilon_m}\kappa^2 ah. \quad (39)$$

For the most of typical systems, the value of this ratio is on the order of 10^2–10^3. According to ratio (39), the strong inequality (38), which makes it possible to use superposition approximation, is closely connected with inequality (27), which is fulfilled when the thickness of Debye atmosphere is very small compared to the cell radius. It is important that both theories (diffusion-controlled low-frequency α dispersion and the Pauly–Schwan β dispersion) are applicable for this limiting case.

Substituting expressions (37) into Eq. (9) and accounting for presentation (7), we arrive at the frequency dependences of the contribution of cells to the complex dielectric permittivity of their dilute suspension, $\delta\varepsilon^*(\omega)$. The real part $\delta\varepsilon^*(\omega)$ corresponds to the real permittivity $\delta\varepsilon'(\omega)$:

$$\delta\varepsilon'(\omega) = \mathrm{Re}\,\delta\varepsilon^*(\omega). \quad (41)$$

With regard to imaginary part $\delta\varepsilon^*(\omega)$, after the elimination of the through conductance from this part, we obtain expression for dielectric losses $\varepsilon''(\omega)$, in the cell suspension

$$\varepsilon''(\omega) = -\left[\mathrm{Im}\,\delta\varepsilon^* - \frac{1}{\omega}\lim_{\omega\to 0}(\omega\,\mathrm{Im}\,\delta\varepsilon^*)\right]. \quad (42)$$

Figures 3–5 show the frequency dependences of the real part of the permittivity (monotonic curves with shelves) and dielectric losses (curves with maxima) in the dilute suspension of cells for broad frequency bands covering all three regions of dispersion, i.e., low-frequency α dispersion, the Pauly–Schwan β dispersion at medium frequencies, and the Maxwell–Wagner γ dispersion.

The values of the conductivity and permittivity of the intercellular solution (K_e and ε_e) and cytoplasm of cells (K_i and ε_i), cell radius a, thickness h, and permittivity ε_m of cytoplasmic membrane, as well as diffusion coefficients of ions, are presented in the table.

The reported values are close to those determined in [15], which was devoted to the application of dielectric spectroscopy to the study of blood cells, both

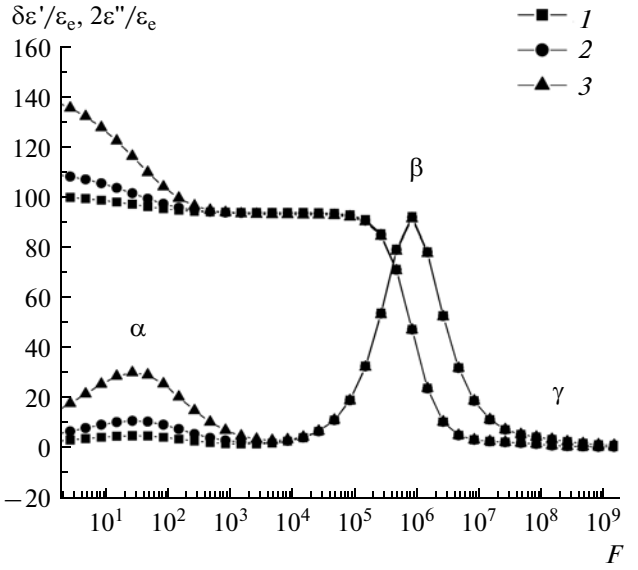

Fig. 3. Frequency dependences of permittivity of cell suspensions at different values of parameter K^1_{pef}/K_e: (*1*) 0.0025, (*2*) 0.005, and (*3*) 0.01.

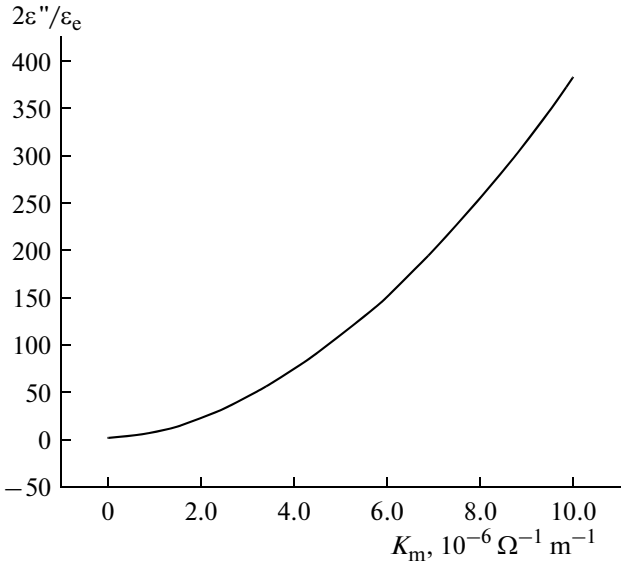

Fig. 4. Dependence of total increment of α dispersion of permittivity of cell suspension on conductivity of cell membrane K_m.

malignant and normal lymphocytes. Additional values of parameters are shown in figure captions.

Curves in Fig. 3 are plotted for three different values of the effective conductivity K^1_{pef}. of cells. Bearing in mind the rather large size of lymphocyte cells and the absence of the expressed cell wall and, correspondingly, the low surface conductivity λ, following the authors of [15], we ignore the influence of the latter parameter. Then, in accordance with formula (23), the effective conductivity is expressed via the cell radius and the electrical resistance of the unit surface of cytoplasmic membrane, r_m, as $K^1_{pef} \approx a/r_m$. Using Eq. (19) and tabular data, we obtain that the membrane conductivity $K_m \approx 2.3 \times 10^{-4} K^1_{pef}/K_e$ Ω^{-1} m^{-1} corresponds to each of K^1_{pef}/K_e ratios shown in Fig. 3. Thus, if the K^1_{pef}/K_e ratio and membrane conductivity K_m vary and remain smaller than 0.01 and 2.3×10^{-6} Ω^{-1} m^{-1}, respectively; then, as can be seen from Fig. 3, these variations are hardly exhibited in the β and γ ranges of dielectric spectrum. At the same time, so small values of the conductivity of cytoplasmic membrane significantly increase the increment of permittivity in the α range of the spectrum.

As the membrane conductivity increases, this increment also rapidly rises (Fig. 4). Comparing Figs. 3 and 4, we can see that, at $K_m > 4.5 \times 10^{-6}$ Ω^{-1} m^{-1}, the amplitude of α dispersion exceeds that of Pauly–Schwan β dispersion. Furthermore, the effect of the conductivity of membrane on its parameters is still quite low. This follows from both our calculations and data from [15], in which the membrane conductivity (varying from 1×10^{-5} to 5×10^{-5} Ω^{-1} m^{-1}) was determined by changes in the amplitude of β dispersion with 10% error.

Figure 5 demonstrates the broadband dielectric spectra of the cell suspension for the capacity of cytoplasmic membrane set by three values of its permittivity ε_m. As can be seen from this figure, the spectrum of dielectric losses is only sensitive to ε_m for the β disper-

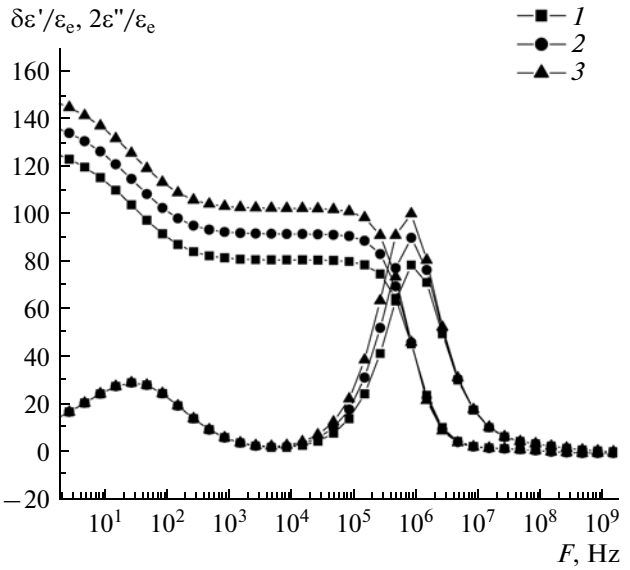

Fig. 5. Frequency dependences of permittivity of cell suspension at different values of parameter ε_m: (*1*) 7, (*2*) 8, and (*3*) 9. $K^1_{pef}/K_e = 0.01$.

Values of parameters of systems used in calculations

K_e	ε_e	K_i	ε_i	a	h	ε_m	D_{eff}
0.1 Ω^{-1} m^{-1}	$78.5\varepsilon_0$	1 Ω^{-1} m^{-1}	$60\varepsilon_0$	3×10^{-6} m	7 nm	$7\varepsilon_0$	2×10^{-9} m^2/s

sion within the 2×10^4–2×10^6 Hz range. With regard to the spectrum of the real part of permittivity, outside the range of β dispersion, at lower frequencies (the α dispersion), the $\varepsilon'(F)$ curves do not change their patterns upon variations in ε_m, but, as a whole, exhibits a greater shift with increasing ε_m. In the range of frequencies higher than 2×10^6 Hz, $\varepsilon'(F)$curves are not sensitive to changes in ε_m.

The broadband dielectric spectra in Fig. 6 are plotted for three different values of the ratio of conductivities of cytoplasm, K_i, and intercellular solution, K_e. As can be seen, the effect of cytoplasm conductivity is only significant within 2×10^7–2×10^9 Hz frequency range, i.e., in the range of Maxwell–Wagner γ dispersion. The absence of this effect at lower frequencies is associated with the fact that, due to the low conductivity of cell membrane, only a small portion of current that streamlines the cell flows into its cytoplasm. The situation only changes at frequencies above the range of the β dispersion when the capacitive resistance of membrane becomes small enough to bypass its high ohmic resistance.

CONCLUSIONS

As can be seen from a comparison of Figs. 3–6, the dielectric spectrum in the range of diffusion-controlled α dispersion is much more sensitive to the effective resistance of cell membrane structures than in the ranges of β- and γ dispersions, the first of which is particularly sensitive to the variations in membrane capacity, while the second, to the electric characteristics of cytoplasm.

At present, the application of dielectric spectroscopy to determining the electrical characteristics of cell structures is primarily reduced to the measurement and interpretation of the characteristics of Pauly–Schwan β dispersion. However, as can be seen from a comparison of Figs. 3–6, the low-frequency α dispersion is, as a rule, much more sensitive to the fairly low membrane conductivity. Furthermore, changes in the parameters of the β dispersion are also caused (and even to a greater extent) by the effect of the membrane capacity, thus leading to a difficult problem of the separation of contributions of these parameters. Thus, the expansion of the interval of dielectric measurements of cell suspensions by incorporating the range of α dispersion enables us to considerably enhance the sensitivity of the method to the conductivity of the membrane and improve the separate measurement of main electrical characteristics of cell structures. The major obstacle on this path is the concealment of a useful signal by an electrode polarization, the effect of which is the stronger the lower is the signal frequency and the higher is the conductivity of the intercellular solution,. The latter circumstance, which particularly hampers the use of this method in

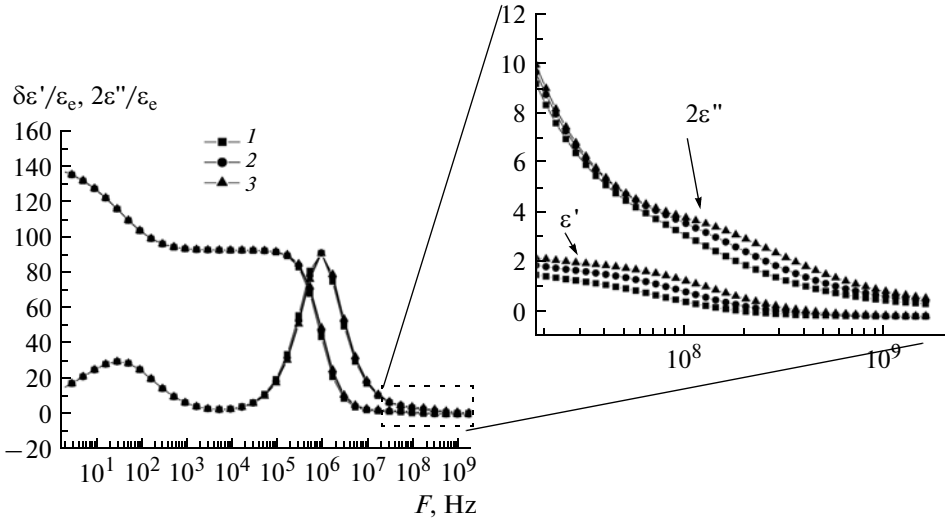

Fig. 6. Frequency dependences of permittivity of cell suspension at different values of parameter K_i/K_e: (*1*) 8, (*2*) 10, and (*3*) 14. $\varepsilon_m = 7\varepsilon_0$,.Values of other parameters are the same as in Fig. 4.

studying blood cells, has been recently overcome in [15] and [16]. The authors of these works replaced the intercellular medium with saccharose solution, which is similar to an isotonic solution and is characterized by lower (by order of magnitude) conductivity. In combination with some other additives, the saccharose solution ensures the conservation of the cell morphology over the course of at least 1 h. The combination of this method with the results of classical Schwan work [17] and current studies [18] on the modification of the design of measurement cells show the promise of the significant extension of the frequency range of dielectric spectroscopy of cell suspensions. The quantitative correlation between the specific features of broadband dielectric spectrum and the electrical characteristics of cell structures established in this work will promote the improvement of the information content of dielectric spectroscopic studies in biophysics and medicine.

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