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Dielectric Dissipation Resonances of Human Blood due to Forced Low-Frequency Cell Oscillations

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Resumen
La mecánica física de membranas celulares es una característica especial de deformación y oscilación geométrica de células al exponerlas a un campo eléctrico alterno. La electrodeformación tiene relación con la inducción de un momento dipolar debido a cargas eléctricas en las bandas opuestas de la célula. La respuesta dieléctrica de dipolos inducidos es un fenómeno resonante, que debe ocurrir en frecuencias intrínsecas de la célula consecuencia de oscilaciones forzadas. Se mostró que armónicos de las frecuencias de oscilación resultan en la aparición de picos dobles- y hasta triples en espectros de impedancia a baja frecuencia. Nuestros cálculos son reconfirmados por resultados presentes en la literatura acerca de tal espectroscopía.

Abstract
The physical mechanics of cell membranes is an essential aspect of cell deformation and shape oscillations, when exposed to an external electric a.c. field. Dielectrodeformation is related to the induction of a dipole moment due to electric charges on the opposite boundaries of the cell. Dielectric responses of induced dipoles are resonance phenomena, which are expected to occur at intrinsic cell frequencies due to the forced cell oscillations. It has been shown, that higher harmonics of oscillation frequencies go along with the possible appearance of double- or even triple-peak formation in low-frequency impedance spectra. Literature data of experimental low-frequency impedance spectroscopy confirm this finding.

INTRODUCTION

Dielectric relaxation spectroscopy in frequency or time domain has gained considerable attraction for studying dielectric properties of biological cell- and particle suspensions Cell suspension measurements by dielectric relaxation spectroscopy in frequency or time domain provide for statistically averaged data of the cell ensemble appearingly without allowing access to an individual cell study [1-9]. Indeed are the cell suspension spectra known to show broad asymmetric loss peaks which are frequently found in liquids and solid-like situations known as so-called β-dispersion [2, 10], and commonly measured in the frequency range of kilo- to gigahertz.

The classical theory of dielectric relaxation was laid out by Debye and is based on the well-known dielectric relaxation function

$$\varepsilon^*(\omega) - \varepsilon(\infty) = [\varepsilon(0) - \varepsilon(\infty)] / (1 + \omega\tau_D). \quad (1)$$

Here $\varepsilon^*(\omega) = \varepsilon'(\omega) - i\varepsilon''(\omega)$ is the complex dielectric permittivity at the radian frequency ω of the alternating electric field, and $\varepsilon(\infty)$, $\varepsilon(0)$ denote, respectively, the dielectric response under an electric field of 'infinite' and zero frequency oscillation, and $i=(-1)^{½}$ is the imaginary unit. The parameter τ_D is called the Debye relaxation time. Due to strong simplifications in the Debye model of relaxation, that amount to neglect the effects of memory, inertia and interaction between molecules, the correct description of experimental results is not the rule [10].

The dielectric susceptibility $\chi(\omega)$ is in general a complex function of frequency,

$$\chi(\omega) = \chi'(\omega) - i\chi''(\omega) = [\varepsilon_r'(\omega) - 1] - i\varepsilon_r'' \quad (2)$$

where the imaginary part $\chi''(\omega)$ is the dielectric loss per radian arising from the material medium. The energy loss per unit time, i.e. the power loss, is given by the a.c. conductivity

$$\sigma(\omega) = \sigma_0 + \varepsilon_0\omega\chi''(\omega). \quad (3)$$

where σ_0 is the d.c. conductivity of the substance, and ε_0 is the vacuum permittivity.

2. POLARIZATION AND RELAXATION IN BIOLOGICAL SYSTEMS

Blood is an aqueous solution of many substructures, different in composition, geometrical arrangement and size. The interaction with an external electric field involves multiple relaxation processes, including interfacial polarization around the cells. The dielectric behavior turns out to be complicate.

Useful and precise dielectric information may be obtained only when each relaxation process is extracted from the complicated overall behavior based on measurements at high resolutions, as well as over a large frequency range. The incidence of bound water molecules on dielectric properties was confirmed in biopolymers, such as DNA [11] and globular protein [12] at about microwave frequencies. It was found, that the relaxation strength for globular protein is in proportion to their surface area, which was related to the orientation of bound H_2O molecules on the surface.

Mixture formulas have been applied to obtain a description of the electric properties of complex liquids [13], which are based on the calculation of the electrical potential inside and outside the dispersed spherical particles [14] or in samples of cylindrical and elliptical shape [15]. A mixture formula for spherical particles was derived already by Maxwell and later extended [16,17]. This now coined Maxwell-Wagner-Sillars (MWS) theory can usually be applied only for dilute systems of spherical dispersed particles.

$$\varepsilon^*_{sus} = \varepsilon^*_{liq}[(2\varepsilon^*_{liq} + \varepsilon^*_{sph}) - 2p(\varepsilon^*_{liq} - \varepsilon^*_{sph})]\ [(2\varepsilon^*_{liq} + \varepsilon^*_{sph}) + p(\varepsilon^*_{liq} - \varepsilon^*_{sph})]^{-1}. \qquad (4)$$

ε^*_{sus} is the effective complex permittivity of the suspension, ε^*_{sph} the complex dielectric permittivity of the dispersed particles, ε^*_{liq} the complex dielectric permittivity of the suspending medium, and *p* is the volume fraction of the dispersed particles

The MWS-polarization theory may not deliver correct results, if the particle surface carries a high charge density. From a comparison of the erythrocyte size, which was calculated by use of both static and high frequency conductivity as well as visual data, it was concluded, that the surface of blood cells is covered by a non-conducting transparent layer, formed by organic material or by bound water molecules [9].

The interface polarization in the case of biological cells is related to the complex dielectric permittivity of the cell structural parts, which has led to the physical presentation of a cell by shelled models [3, 5, 7, 18]. Multilayer ellipsoidal cell models exposed to electric and magnetic fields up to 100 MHz have been studied [19, 20]. Given the spherical shape of lymphocytes with a thin cell membrane and a spherical nucleus, which by itself is covered by a thin nuclear envelope, a double-shell model has been assumed and characterized by both time domain dielectric spectroscopy [7] and computer modeling [8]. Every cell structural part (nucleoplasm,

nuclear envelope, cytoplasm, cell membrane) has been described by two electrical parameters, - electrical conductivity and permittivity, as well as the corresponding geometrical parameters (radii of nucleus and cell, thickness of inner envelope and membrane).

The approximate dielectric *(ε)* and conductivity *(σ)* data for lymphocytes given in [3, 5, 7, 9] are summarized in the following table 1.

	nucleoplasm	cytoplasm	nucleus envelope	outer cell membrane
ε	120	60	60	10
$\sigma\ (\Omega m)^{-1}$	1	0.5	$5\cdot10^{-3}$	10^{-5}
t [μm]	diameter 2.8	diameter 6.6	thickness 0.04	thickness 0.007

Table 1: Dielectric properties *(ε, σ)* and geometrical data *(t)* of lymphocyte cell structural parts

It is noticed, that the conductivity and the permittivity of nucleoplasm is about twice that of cytoplasm. This fact together with the volume differences of both can play an important role in the interpretation of bioimpedance spectra.

While lymphocytes possess a spherical double-shell structure, human erythrocytes are single-shelled and of discoidal shape. Nevertheless are dielectric responses in an external electrical field frequently treated within the framework of a spherical model. The highly oblate spheroid with semiaxes ***a=b>>c*** is transformed under the action of an electrical field in a three-axial ellipsoid with semiaxes ***a>b>>c*** (a is parallel to the external field). Table 2 contains some properties of human erythrocytes.

semi axes [μm] *a=b*	*c*	ε	$\sigma\ [\Omega m^{-1}]$	$\mu\ [Nm^{-1}]$	$\eta_s\ [Nsm^{-1}]$	$S\ [\mu m^2]$	$V\ [\mu m^3]$
3.8	0.7..1.4	60	0.4	$6.1\cdot10^{-6}$	$6\cdot10^{-7}$	134	94

Table 2: Dielectric, geometrical and mechanical properties of human erythrocytes. S, V, a are surface, volume, radius of the erythrocyte, respectively, μ - shear modulus of the membrane, η_s- friction coefficient.

Despite their obvious importance, the available dielectric data even for aqueous solutions are rather limited, contradictionary and not always reliable. A major reason for this is the technical difficulty associated with the determination of the complex (dielectric) permittivity spectrum of electrolyte solutions. Their frequencies for dielectric relaxation at ambient temperature are close to or within the microwave region. Chelidze [9] reports for erythrocytes the most probable relaxation time at $\tau_0 = 2.65 \cdot 10^{-7}$ *s*, and for hemoglobin molecules $\tau_0 = 5.3 \cdot 10^{-8}$ *s*.

A shelled cell model with one or several membranes embracing electrolytes with mobile dipoles or charge carriers implies also the possibility for a mechanically vibrating oscillator. Given the elevated oscillator mass, this could happen only at far lower than microwave frequencies.

In the present study frequency-domain dielectric spectroscopy in the low frequency range of 1 Hz … 1 MHz was considered in order to test the viability of an oscillatory excitation of cell structures by a low intensity external electric field. Electrode polarization effects are known to be particularly strong at the lower end of the frequency spectrum and have to be appreciated carefully in order to avoid artifacts. On the other hand would we expect pronounced oscillations at more or less well defined frequencies, which should surmount the electrode polarization background. Mutual perturbation of the single oscillators can only be reduced by a sufficient dissolution of the samples.

3. FORCED CELL RESONANCE DIELECTRIC MODEL

The vibration of atoms in a molecule under the effect of an external radiation field, where atoms oscillate about their equilibrium positions, and at the same time interact with each other, is a standard textbook problem of electrodynamics. The double-shell lymphocyte bears similarities in the physical concept of motion, where the external force acts on both, the inner (nucleoplasma) sphere and the outer (cytoplasm) sphere. Given the different geometrical and dielectric characteristics, as outlined in table 1, the response of each might be different (Figs. 1 and 2). In the case of erythrocytes only one shell exists, and the oscillator model could be simpler, although the more complex shape makes it more difficult in the experimental approach (Fig. 3). Let us suppose, that a shelled spherical cell containing a certain dipole charge density is exposed to an harmonically variable electric field. For a spherical distribution of charge, the higher electric multipole moment is zero, but is positive for an elongated or prolate charge distribution and negative for an oblate or flattened charge distribution. It is easy to imagine, that the elastic properties of the nuclear envelope and the cell membrane provide for a restoring

force after a deformation due to the external field action on the mobile ions inside the sphere plasmas has occurred.

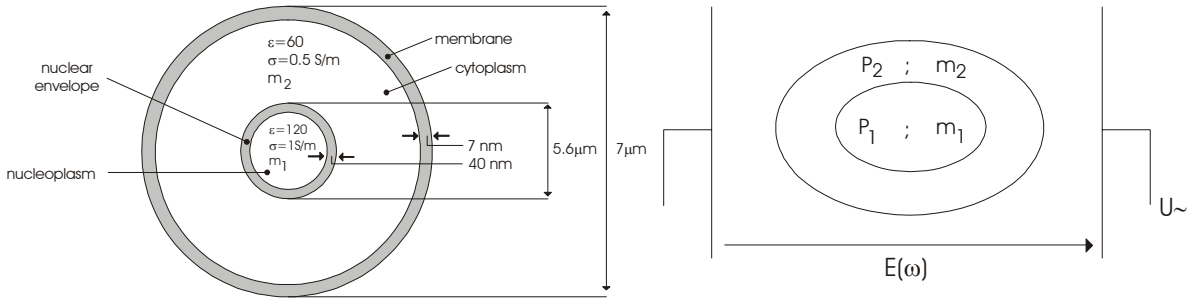

Fig. 1 (left): Double-shelled lymphocyte cell as proposed by Polevaya et al. (1999); ε is the dielectric constant, σ - specific electric conductivity, m_1 - mass of the inner nucleus ($m_1=9\cdot10^{11}$g), m_2- mass of the outer shell region ($m_2=6\cdot10^{11}$g), being $m_c= m_1+m_2$ the total cell mass.

Fig. 2 (right): Oscillator model under the action of an external electric field. Elastic membranes provide for a restoring force of ellipsoidally deformed cells due to the field polarization. P_1 and P_2 are polarization vectors in the different cell regions.

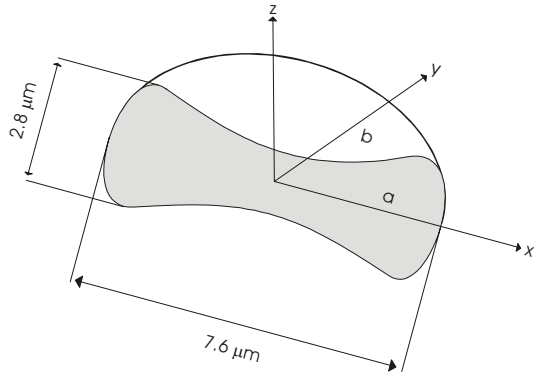

Fig. 3: Discoid shape of an erythrocyte of diameter 2R=7.6μm and thickness at the rim 2L=2.8 μm. ***a, b*** represent semi axes ***(a=b=R)*** in a spheroid approach with small semi axis c in direction of z.

In the cases, where isolated electrical charges oscillate between two preferred localized sites, represented by a potential double well, or where inertialess dipoles are constrained to assume one of two discrete orientations in space, the Debye relaxation time in the equation of motion for such charge carriers under the action of a time-dependent electric field is equal to the reciprocal natural frequency of oscillations,

$\omega_0=1/\tau_0$. The temperature dependence of $\tau_0(T)$ is in general related to a clearly defined activation energy W as

$$\tau_0(T) = 1/\omega_0 = \tau_{pe} \cdot exp(+W/kT), \quad (5)$$

where W is the barrier height of the double well, ω_0 denotes the loss peak frequency, and τ_{pe} is a suitable pre-exponential factor. Rotating dipoles and hopping charge carriers cause a temperature dependence of the susceptibility $\chi \sim 1/T$, known as the Curie law.

The periodic deformation of cells in an electric field $E_0(\omega)$ with frequency ω is determined by the distribution of the electrical forces applied and the mechanical forces generated in the deformed membranes and in the adjacent layers of the cell. The mechanical forces include elastic and viscous shear stresses in the membranes. The axis relation $q=R/L$ with L the longer half axis of the field-deformed ellipsoid of radius R (i.e. $q=1$ corresponds to a spheroid) characterizes both, the elongation (contraction) deformation and the forces, which determine the deformation [25].

The electric ponderomotive force is caused by the applied time-dependent field $E_0(t)$, and contains the dielectric properties of the cell:

$$F_{pond}(\omega, q) = V \cdot F(\omega) \left| E_{loc}(\omega, q) \right|^2 \cdot \frac{\partial f_L(q)}{\partial q}. \quad (6)$$

Here $f_L(q)$ is the depolarizing factor in direction of the main axis L parallel to the external field vector E_0. The local field $E_{loc}(\omega, q)$ is the value of the applied field inside the ellipsoid:

$$E_{loc}(\omega, q) = \Phi(\omega, q) \cdot E_o \quad (7)$$

$$\Phi(\omega, q) = \frac{\varepsilon_m^*(\omega)}{\varepsilon_m^*(\omega) + [\varepsilon_p^*(\omega) - \varepsilon_m^*(\omega)] \cdot f(q)} = \alpha(q) \cdot \varepsilon_m^*(\omega) \quad (8)$$

The factor $\alpha(q)$ has been calculated previously for the general case of a dielectric object with arbitrary shape [15]. The frequency dependence of the ponderomotive electric field force is given by $F(\omega)$

$$F(\omega)=\frac{\varepsilon_0\varepsilon_m}{4}\left[(\frac{\varepsilon_p}{\varepsilon_m}-1)^2(\omega\tau_e)^2+(\frac{\sigma_p}{\sigma_m})^2-\frac{2\varepsilon_p}{\varepsilon_m}+1\right]\cdot\left[1+(\omega\tau_e)^2\right]^{-1} \quad (9)$$

$$\tau_e=\varepsilon_0\varepsilon_m/\sigma_m. \quad (10)$$

Here ε_p and ε_m are the static relative permittivity of the cell and the suspending medium, σ_p and σ_m are the static conductivities, and ε_0 is the vacuum permittivity.

The elastic force of the cell membrane is given by

$$F_{ela}(q)=-½\cdot S\mu(1-q^2) \quad (11)$$

with S the surface area of the object and μ the shear elasticity modulus.

The friction force $F_{fr}(q, dq/dt)$ between the cell and the surrounding medium is

$$F_{fr}(q,dq/dt)=-S\cdot\eta_s q^2\frac{dq}{dt} \quad (12)$$

with η_s the surface viscosity coefficient of the membrane.

Due to the small mass of the object and low velocities of deformation dq/dt, it is safe to exclude the inertia term in the equation of cell motion. All forces acting on the cell will then fulfill the condition of instantaneous equilibrium; i.e., its sum must be equal to zero. Introducing $\tau_0=\eta_s/\mu$ as time of viscoelastic relaxation of the membrane, and summing up the forces given by ecs. (6, 11, 12), it follows

$$\left[1-F(\omega)\cdot\left|\Phi(\omega,q)\right|^2\cdot\left|\frac{\partial f(q)}{\partial q}\right|\cdot\frac{VE_0^{\,2}}{\mu S}\right]\cdot q^{-2}=1-2\tau_0\frac{dq}{dt}. \qquad (13)$$

This equation describes the time-dependent elongation (contraction) of the ellipsoid, depending on geometric *(V, S),* electrical *(ε_p, σ_p)* and mechanical *(μ, η_s)* cell parameters, as well as on experimental properties of the medium *(ε_m, σ_m)*. As can be appreciated in ec. (13), the cell deformation is caused not only by the electric field amplitude E_0, but by the dimensionless force parameters $(VE_o^2/\mu S)^{1/2}$. If we apply a time-dependent electric field $E_o(t)=E_0[1+\delta\cos\Omega t]$ of frequency Ω, modulation depth δ, and consider the periodic changes of the axis relation *q(t)* as forced oscillations of the ellipsoid, a solution of ec. (13) is found, which contains such oscillations not only at the frequency Ω but also at its harmonics 2Ω, 3Ω. Details of the calculations are given in [25]. For small-signal modulation $\delta<<1$ of the electric field amplitude, and using the abbreviations

$$\tau(E_o)=\tau_o/q_o\left(1+Bx_0^{\,2}/q_0^{\,2}\right) \quad ; \quad x_o^{\,2}=VE_o^{\,2}/\mu S$$

one gets

$$q(t)\sim\frac{\cos(\Omega t-\varphi_1)}{\left\{1+\left[\Omega\tau(\mathrm{E_o})\right]^2\right\}^{1/2}}+\frac{\delta}{2}\frac{\cos(2\Omega t-\varphi_2)}{\left\{1+\left[2\Omega\tau(\mathrm{E_o})\right]^2\right\}^{1/2}}+\cdots \qquad (14)$$

φ_1 and φ_2 are phase angles of the harmonics, and related to tan $\varphi_n=n\cdot\Omega\tau(E_o)$.

In the context of the present paper the frequencies Ω, 2Ω are of particular interest.

4. COMPARISON TO EXPERIMENTAL SPECTRA

If forced oscillations of cells give rise to a dissipative process at a peak frequency ω of the external field, one should expect peak repetitions at 2ω, and possibly at 3ω. Indeed

would such a peak sequence in a spectrum be indicative, that a forced oscillation of cells has occurred.

In contrast to the relaxation type response of dielectric objects with permanent dipoles, the dielectric response of induced dipoles are resonant phenomena, where energy resonance occurs at frequencies ω_0, which are easily calculated from the expressions for $\chi'(\omega)$ and $\chi''(\omega)$, and result in

$$\omega_0 \cong [2Nq^2 \Delta\omega/\varepsilon_0 m\chi']^{1/3}. \quad (15)$$

Here N is the number of induced dipoles per unit volume, m is the particle (oscillator) mass and q the elementary charge. $(\omega_0^2-\omega^2)=(\omega_0+\omega)(\omega_0-\omega)$ was substituted by $2\omega_0\Delta\omega$ as a first-order approximation for $\omega\approx\omega_0$.

In order to arrive at numerical values, we apply for $N \cong 10^{24}\ m^{-3}$, $m = 1.5 \cdot 10^{-10}\ g$, $\chi' = 120$, and $\Delta\omega = 200\ s^{-1}$, which correspond to realistic experimental parameters of a certain type of human blood cells. Here $\Delta\omega$ is the width of the single resonance line, and $\omega_0 \cong 1.7\ kHz$ is the experimentally determined [23, 24], and by ec. (15) reproduced resonance frequency.

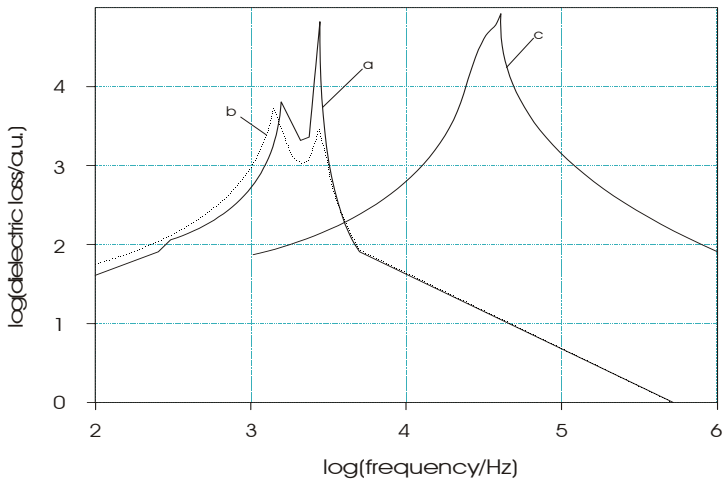

Fig. 4: Spectra of dielectric loss D vs. frequency ν [1/s] of human blood, as published by Vázquez et al. (1998) and Starostenko et al. (1999). The sample is described to be composed of (a) 0.1 ml blood + 20 ml H_2O; (b) 0.1 ml blood + 20 ml H_2O + 0.5 ml ethanol, introduced into a parallel-plate capacitor cell. (c) is a different donor.

A second peak should then be found at 3.4 kHz, which is actually seen in Fig. 4 of the mentioned spectra [23, 24]

5. DISCUSSION

The appearance of pronounced peaks in low-frequency impedance spectra of human blood suspensions with distilled water seems to support the forced oscillatory behavior of cells with the induction of dipole moments corresponding to the oscillation frequency of the external field. The dielectric responses of induced dipoles are resonant phenomena. The difference to other macromolecular structures arises from the fact, that the cell electrolytes are constrained in elastic membranes, which make them interact as a whole with the harmonically oscillating electric field. The elastic deformation of each single cell under the action of the slowly varying external field from spherical to oblate with harmonically forth and back changing the direction of the internal polarization vector makes the charges oscillate between two preferred localization sites, or the dipoles to assume one of two discrete orientations in space. Indeed would this picture represent an array of polarizable macrostructures with mutual interactions only by way of the counter-ions. The precise effect of the latter is not yet clear.

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