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Transient dielectro-deformations of erythrocyte governed by time variation of cell ionic state

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Abstract

The possibility is shown to monitor the transient ionic state and volume of erythrocyte suspended in low ionic strength solution (LISS) by registration of the dielectro-deformation (DD) of a cell. The adequate theoretical basis is developed. Possible registration modes are considered analytically and numerically using the theory of dielectro-deformation of erythrocytes developed previously. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Erythrocyte ionic state; Electric field; Cell deformation

1. Introduction

Erythrocytes suspended in a low-conductivity isotonic solution can be deformed by high frequency (HF) electric field [1–4]. This phenomenon is termed the dielectro-deformation (DD) of erythrocytes. Depending on the basic field frequency and conductivity of external medium, both the elongation and shortening of cells in the field direction can be obtained [4]. In particular, the frequency range of $\sim 1 \div 5$ MHz and very small values of external conductivity are optimal for the observation of erythrocytes elongation [1–4]. The deformation is stationary if the field amplitude is kept constant [1,3,4], and varies in time if the amplitude is time-modulated [1,2]. The deformation value depends on mechanical, electrical, and geometrical parameters of erythrocyte [1–6]. Hence, DD phenomenon can be used in two ways: either to monitor erythrocyte mechanical properties [1], or to measure the value and frequency dependence of erythrocyte internal conductivity [6,8]. However, to obtain large deformation values and to avoid the preparation heating, the cells should be placed into a low ionic strength solution (LISS) [1,3]. Such environment leads to a transient ionic state of erythrocyte due to the efflux of cytoplasmic ions. As a consequence, cell volume and internal conductivity become time dependent [9]. This, in turn, should result in a time variation of the cell deformation even under constant HF amplitude, due to the strong dependence [6,8] of DD both on cell volume and on internal conductivity. The present work considers quantitatively the time evolution of the stationary DD and the effective relaxation time of dynamic DD governed by the transient ionic state of erythrocyte. The analysis and numerical results are referred to the frequency range of $\sim 1 \div 5$ MHz optimal for observation of these effects.

2. Results and discussion

Consider an erythrocyte placed in a spatially homogeneous HF electric field with the basic frequency ω and field amplitude E. According to the general DD theory [6,8], the shape of erythrocyte in HF electric field is approximated by a three-axis ellipsoid with the field-dependent semi-axes a, b, $\geq c$ and invariable volume V and surface area S. The a-axis is parallel to the field direction. In such approximation the field-dependent shape of erythrocyte is defined uniquely the cell shape parameter $\kappa = a/b$. The stationary deformation curve $\kappa_0(E)$ of erythrocyte is described by an implicit algebraic equation [6,8].

$$\kappa_S(\kappa_0) = \left[1 + 2\frac{K_c}{\mu S} f_b(\kappa_0) - 2F(\omega)\,|\,\chi(\omega,\kappa_0)\,|^2 \left|\frac{\partial N_a}{\partial \kappa}(\kappa_0)\right| \frac{VE^2}{\mu S}\right]^{-1/2} \quad (1)$$

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Here, $N_a(\kappa)$ is the depolarization factor of the ellipsoid associated with its a-axis [6], K_c is bending elasticity modulus and μ is the shear elasticity modulus of erythrocyte membrane. The function $f_b(\kappa)$, defined as the minus κ-derivative from the known Helfrich's expression for the membrane bending energy, describes the shape dependence of the membrane bending stiffness [8]. The general expressions for $F(\omega)$ and $\chi(\omega, \kappa)$ in the whole frequency range are given in Refs. [2,6]. In particular range $\omega = 10^6 \div 10^7$ rad s^{-1} is considered in this paper, the product $F(\omega)\,|\,\chi(\omega, \kappa)\,|^2$ is practically independent of ω and is described approximately by the expression [2,6].

$$F(\omega)\,|\,\chi(\omega,\kappa_0)\,|^2 \approx \frac{\varepsilon_0\varepsilon_e\left(\frac{\sigma_i^2}{\sigma_e^2} - 2\frac{\varepsilon_i}{\varepsilon_e} + 1\right)}{4\left[1 + \left(\frac{\sigma_i}{\sigma_e} - 1\right)N_a(\kappa_0)\right]^2} \tag{2}$$

Here, ε_0 is the dielectric permittivity of vacuum, ε_k is the relative static dielectric constant and σ_k is the specific direct current conductivity of cell cytoplasm ($k=i$) and surrounding medium ($k=e$). Note, that for typical experimental conditions $\sigma_i \gg \sigma_e$ and $\varepsilon_i \sim \varepsilon_e$, hence, the product (2) is essentially positive. This corresponds to the cell elongation ($\kappa \geq 1$). The function $\kappa_s = \kappa_s(\kappa)$ in Eq. (1) relates the variation of local shear strain parameter κ_s of a small membrane element to the variation of the cell shape parameter κ. A particular form of $\kappa_s(\kappa)$ was suggested [7].

$$\kappa_s(\kappa) = 1 + \frac{(\kappa-1)^{n+1}}{(\kappa-1)^n + w^n} \tag{3}$$

Here, w and n are adjustable parameters to be found from the comparison with the measured data.

A remarkable feature of elongation curve $\kappa_0(E)$ of erythrocyte is its saturation behavior at high field strength and the existence of the terminal point [6,3,8]. It is a consequence of purely geometrical prohibition of infinite elongation of a body under constant volume and surface area. The following estimates for the geometrical limit of the axes ratio κ_{gl} or for the relative elongation $(a/a_0)_{gl}$ can be obtained [6,8].

$$\kappa_{gl} \approx \frac{S^3}{16\pi V^2}; \quad \left(\frac{a}{a_0}\right)_{gl} \approx \frac{S^{3/2}}{4\sqrt{\pi}V} \tag{4}$$

It is essential, that these limiting values are independent of electrical properties of erythrocyte. On the other hand, the elongation value in the pre-saturation region strongly depends on the cytoplasm conductivity, namely, on the (σ_i/σ_e) ratio, as Eqs. (1) and (2) show. Thus, the saturation part of the measured elongation curve $\kappa_0(E)$ allows one to evaluate erythrocyte volume, while the theoretical assessment of the whole curve enables one to obtain the cell conductivity value and w, n parameters of $\kappa_s(\kappa)$. Such evaluation was demonstrated successfully [8] for the measurements made in Ref. [3].

When normal erythrocytes are placed into LISS, the efflux of cytoplasmic ions starts immediately. That leads to a time-dependent decrease both of internal conductivity and, through the osmotic mechanism, the cell volume. Considering the osmotic equilibrium between the cytoplasm and LISS, the following expression for the time dependence of cell volume $V(t)$ was obtained [9].

$$t = \frac{V_0 - V(t)}{PS} + \frac{V_c}{PS}\ln\frac{V_0 - V_c}{V(t) - V_c} \tag{5}$$

Here, P is the specific membrane permeability of ions maintaining the osmotic equilibrium, $V_0 = V(t=0)$, $V_c = V(t \to \infty)$. The fit of measured data to Eq. (5) with $S = 133$ μm^2 gave $P = 2.1 \times 10^{-10}$ m/s, $V_c = 50$ μm^3 and $V_0 = 88.3\%$ of physiologically normal volume of erythrocyte $V = 98$ μm^3 [9]. Using the standard approach of known Drude theory of conductivity, time dependence of cytoplasm conductivity $\sigma_i(t)$ can be also estimated. Denote by $N_{ion}(t)$ the total number of penetrable ions inside the cell, which are also responsible for cytoplasm conductivity, by N_{mol} the total number of large molecules such as hemoglobin, which do not penetrate the cell membrane. According to the Drude formula for conductivity, $\sigma_i \propto n_{ion}\tau_{eff}$, where $n_{ion} = N_{ion}/V$ and τ_{eff} is an effective time between successive collisions of a moving ion. Assuming the major part of ion's collisions to be with nonpenetrable molecules, we may consider $\tau_{eff} \propto (N_{mol}/V)^{-1}$. That gives:

$$\sigma_i(t) \propto \frac{N_{ion}(t)}{V(t)}\,\frac{V(t)}{N_{mol}} \propto N_{ion}(t) \tag{6}$$

From the osmotic equilibrium condition it follows, that

$$\frac{N_{ion}(t)}{N_{ion}(0)} = \frac{V(t) - V_c}{V_0 - V_c} \tag{7}$$

Combining Eqs. (6) and (7), we finally get an estimate of $\sigma_i(t)$ dependence.

$$\frac{\sigma_i(t)}{\sigma_i(0)} = \frac{V(t) - V_c}{V_0 - V_c} \tag{8}$$

Eqs. (5) and (8) describe time variation of cell volume and internal conductivity due to the transient ionic state of erythrocyte. Substitution of these equations into Eqs. (2) and (1) gives the corresponding transient DD elongation of erythrocyte $\kappa_0(E, t)$. It can be used to monitor the ionic state of the cell. Two main registration modes are suggested here. In the first mode, a series of "instant" whole range elongation curves $\kappa_0(E, t_n)$ should be recorded by fast

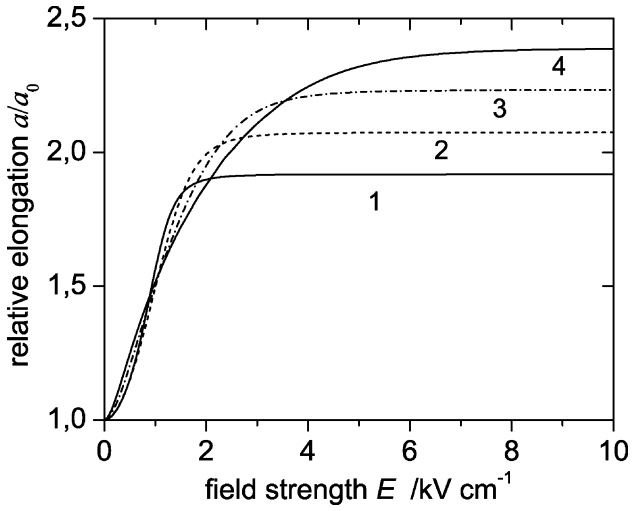

Fig. 1. Time evolution of stationary elongation curve resulted from transient ionic state of erythrocyte. Curves 1–4 are calculated for "instants" 0, 10, 20, and 30 min after cells' transfer into LISS.

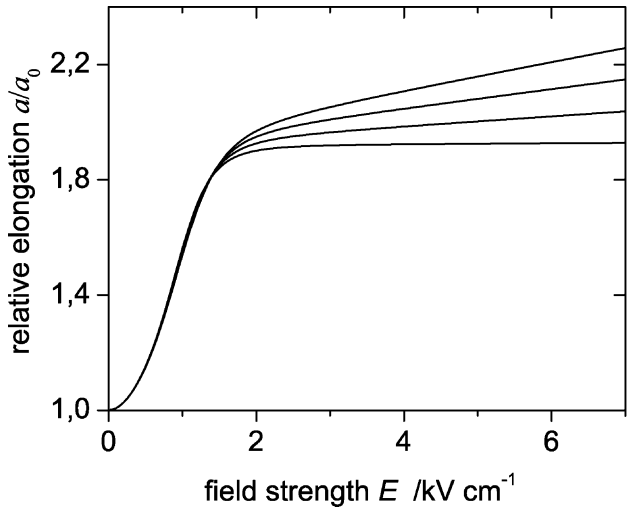

Fig. 3. Stationary elongation curves of erythrocyte calculated for slow linear time increase of field amplitude from zero up to maximum value during (bottom-up): 1, 10, 20, and 30 min counted from the instant of cells' transfer into LISS.

sweeps of HF amplitude E at consecutive moments t_n starting from the cells transfer into LISS. In the second mode, the time dependence $\kappa_0(t, E_0)$ should be registered for some pre-selected values of E_0. Fig. 1 shows a series of "instant" elongation curves calculated using Eqs. (1), (2), (5), (8) and geometrical relation between $\kappa_0(E, t_n)$ and $a(E, t_n)/a(0)$ for given S and V. The computation procedure was described elsewhere [8]. The cell and medium parameters were taken from Ref. [3] or estimated theoretically [8] for the measurements of erythrocytes elongation at 25 °C in Ref. [3]. The variation of w, n parameters of $\kappa_s(\kappa)$ dependence with the cell volume [8] was taken into account. In the saturation region, the calculated elongation $(a/a_0)_{gl}$ varies, practically, according to Eq. (4) with $V(t)$ taken from Eq. (5). In the pre-saturation region, cell elongation depends both on σ_i (increasingly) and V (decreasingly) according to Eqs. (1) and (2). Here, the dependence of $a(E)/a(0)$ curve on cell ionic state ("registration instant" t_n) is more complex and less pronounced. In particular, the counteractive influences of σ_i and V become mutually compensated near $E \sim 1$ kV cm^{-1}. Fig. 2 shows field dependence of effective relaxation time τ_{eff} of transient DD [7] related to the intrinsic relaxation time of the membrane material [8] $\tau_0 \approx 0.1$ s. These data also give the lower bound for the sweep time of HF amplitude in the first registration mode. Fig. 3 shows elongation curves for a slow registration mode, when cell ionic state changes appreciably in the course of $a(E)$ measurement. In this mode, the pre-saturation region of $a(E)$ dependence is even less sensitive to the time variation of σ_i and V. On the contrary, in the saturation region $a(E)$ increases quasi-linearly with E. According to Eq. (4), this results from the quasi-linear decrease of $V(t)$ observed in Ref. [9]. A minor influence of ionic state on the shape of $a(E)$ in the range $E < 1.5$ kV cm^{-1} explains the results of test measurements of elongation invariability with the time made in Ref. [3].

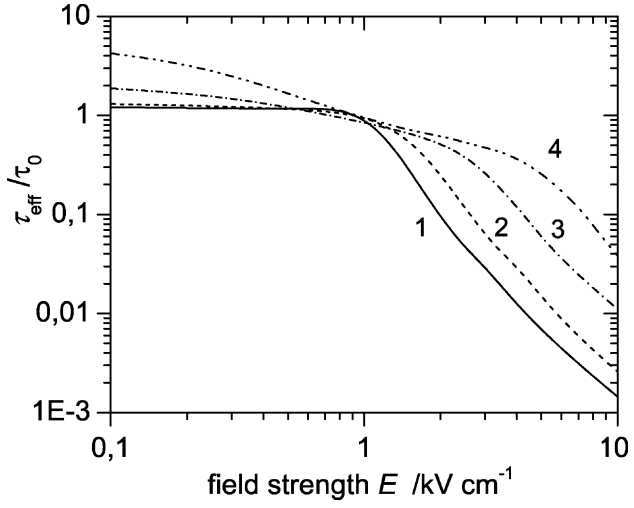

Fig. 2. Field dependence of effective relaxation time of erythrocyte dielectro-deformations, calculated for "instants" 0, 10, 20, and 30 min (curves 1–4) after cells' transfer into LISS.

3. Conclusion

The registration of time dependence of the saturation value of stationary DD curve of erythrocyte, under constant HF field amplitude, enables the direct measurement of time variation of erythrocyte volume, resulted from the transient ionic state of cell suspended in LISS. The registration of the whole-range DD curve allows one to also evaluate the time variation of cell internal conductivity.

References

[1] H. Engelhardt, E. Sackmann, On the measurement of shear elastic modulii and viscosities of erythrocyte plasma membranes by transient deformation in high frequency electric fields, Biophys. J. 54 (1988) 495–508.

[2] V.L. Kononenko, M.R. Kasymova, Forced low-frequency dielectro-deformational oscillations of erythrocytes, Biological Membranes, Archives of Soviet Science, Life Sciences Section (A cover-to-cover translation of Biologicheskie Membrany by Harwood Academic Publishers) 5 (1991) 429–457.

[3] M. Krueger, F. Thom, Deformability and stability of erythrocytes in high-frequency electric fields down to subzero temperatures, Biophys. J. 73 (1997) 2653–2666.

[4] V.L. Sukhorukov, H. Mussauer, U. Zimmermann, The effect of electrical deformation forces on the electro-permeabilization of erythrocyte membranes in low- and high-conductivity media, J. Membr. Biol. 163 (1998) 235–245.

[5] P. Pawlowski, M. Fikus, Bioelectrorheological model of the cell. 4. Analysis of the extensil deformation of cellular membrane in alternating electric field, Biophys. J. 65 (1993) 535–540.

[6] V.L. Kononenko, Dielectro-deformational spectroscopy of erythrocytes, in: R.F. Bonner, G.E. Cohn, T.M. Laue, A.V. Priezzhev (Eds.), Proceedings of SPIE, Biochem. Diagn. Instrum., vol. 2136, SPIE, Bellingham, 1994, pp. 163–174.

[7] V.L. Kononenko, T.A. Ilyina, Optical diagnostics of mechanical properties of red blood cells based on the deformation by high frequency electric field, J. Biomed. Opt. 4 (1999) 85–93.

[8] V.L. Kononenko, J.K. Shimkus, Stationary deformations of erythrocytes by high frequency electric field, Bioelectrochemistry 52 (2000) 187–196.

[9] J. Gimsa, T. Schnelle, G. Zechel, R. Glaser, Dielectric spectroscopy of human erythrocytes: investigations under the influence of nystatin, Biophys. J. 66 (1994) 1244–1253.