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Analysis of dielectric spectra of eukaryotic cells by computer modeling

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Abstract An analysis of dielectric spectra, obtained by computer modeling, of spherical eukaryotic cells (lymphocytes in particular) is presented. The number of fitting parameters required to describe these spectra is determined. The influence of parameter variation on the spectral shape is illustrated.

Key words Eukaryotic cells · Dielectric spectra · Computer modeling

Introduction

The main two types of a variety of techniques for studying the dielectric properties of biological cells are impedance dielectric spectroscopy, in frequency or in time domain (Asami et al. 1989; Bordi et al. 1993; Irimajiri et al. 1978; Pauly and Schwan 1959), and electrokinetic techniques – dielectrophoresis and electrorotation (Gimsa and Wachner 1998; Huang et al. 1996; Jones 1995; Ziervogel et al. 1986). The first type is used for cell suspension measurements and gives the statistically averaged data of the cell ensemble, whereas the second group presents a single cell study. Practically all the methods use the unified physical presentation of a cell by shelled models (Asami et al. 1989; Irimajiri et al. 1979). In the present work we are considering the application of shelled models for biological cells in impedance dielectric spectroscopy (for simplicity we will call this method dielectric spectroscopy).

Dielectric spectroscopy provides a number of significant properties of cell suspensions, because of its capability for non-invasive and real-time measurements (Asami and Irimajiri 1984; Beving et al. 1994; Bordi et al. 1993). The dielectric parameters of the structural parts of biological cells can be determined by the measurement of dielectric spectra of cellular suspensions and subsequent calculation using a suitable dielectric model. The development of the dielectric cell model consists of two main parts. The first part is the description of the suspension spectrum by a so-called mixture model (Takashima 1989). The second is the modeling of the single-cell spectrum. In this study we deal only with spherical cells which can be described by a single- or double-shell model according to their biological make-up. The detailed description of these expressions can be found elsewhere (Asami et al. 1989).

These models all contain a large number of dielectric and geometric parameters, which leads to uncertainty in their determination during the fitting procedure. Moreover, one could argue that some of these parameters are not independent and should be obtained by additional methods. In this paper we present the analysis of model spectra to discuss the number of independent parameters in the aforementioned models. The sensitivity of the spectra to various parameters has been checked in order to decide which parameters have to be variable to achieve the best fitting of the experimental data.

Theoretical models

Number of fitting parameters for the single-shell model

The expression for the complex dielectric permittivity $[\varepsilon_c^*(\omega)]$ in the single-shell model contains five parameters: dielectric permittivity and conductivity of the cell membrane (ε_m and σ_m), dielectric permittivity and conductivity of the cell interior (cytoplasm) (ε_{cp} and σ_{cp}), and a geometrical parameter $v = (1 - d/R)^3$ where d is the thickness of the cell membrane and R is the radius of the cell. This expression is written as follows (Asami et al. 1989):

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$$\varepsilon_c^*(\omega) = \varepsilon_m^*(\omega)\frac{2(1-v)+(1+2v)E(\omega)}{2+v+(1-v)E(\omega)} \quad (1)$$

where $E(\omega) = \varepsilon_{cp}^*(\omega)/\varepsilon_m^*(\omega), \varepsilon_{cp}^*(\omega) = \varepsilon_{cp} - i(\sigma_{cp}/\varepsilon_0\omega)$, $\varepsilon_m^*(\omega) = \varepsilon_m - i(\sigma_m/\varepsilon_0\omega)$, and ε_0 is the dielectric permittivity of the vacuum.

It is possible to show that the single-shell model equation can be written as the sum of a single relaxation time process and a conductivity term[1]. This equation depends on only four parameters (dielectric strength, relaxation time, dielectric permittivity at high frequency, and low frequency conductivity), which are functions of the dielectric and geometric parameters of the single-shell model. This connection between the Debye-type spectrum and the single-shell model (Eq. 1) formulas allows one to conclude that only four independent parameters are required to describe the spectrum of a single cell. The fifth one can be expressed in terms of these four parameters. This expression can be derived by using the single-shell model relation (Eq. 1) in the range of low and high frequencies. Let us rewrite Eq. (1) as follows:

$$\varepsilon_c^*(\omega) = \varepsilon_m^*(\omega)\left(\frac{1+2v}{1-v}\right)\left(\frac{1+A}{1+B}\right) \quad (2)$$

where $A = 2(1-v)/(1+2v)E(\omega)$ and $B = (2+v)/(1-v)E(\omega)$. The frequency behaviors of parameters A and B in the range 10^4–10^{10} Hz for biological cells demonstrate the following intervals of their variations: $10^{-8} < |A| < 10^{-4}$ and $10^{-2} < |B| < 10^2$. Since parameter A is very small over a considerable frequency range it can be neglected, and Eq. (2) could be rewritten as follows:

$$\varepsilon_c^*(\omega) = \varepsilon_m^*(\omega)\left(\frac{1+2v}{1-v}\right)\left(\frac{1}{1+B}\right) \quad (3)$$

At the very low frequency branch the parameter B is very small and can be neglected. Hence, the low-frequency behavior of complex dielectric permittivity can be presented as follows:

$$\varepsilon_{c(low)}^*(\omega) \cong \varepsilon_m\left(\frac{1+2v}{1-v}\right) - j\frac{\sigma_m}{\omega\varepsilon_0}\left(\frac{1+2v}{1-v}\right) \quad (4)$$

At the high-frequency branch the parameter $B >> 1$ and after simple transformations the complex dielectric permittivity of the cell spectrum below the frequency $\omega_{cp} = \sigma_{cp}/(\varepsilon_{cp}\varepsilon_0)$ is:

$$\varepsilon_{c(high)}^*(\omega) \cong \varepsilon_{cp} - j\frac{\sigma_{cp}}{\omega\varepsilon_0} \quad (5)$$

Thus, expressions (3) and (4) show that in the fitting procedure of the spectrum using the single-shell model, the following set of four parameter combinations are obtained:

$$\varepsilon_m\left(\frac{1+2v}{1-v}\right), \quad \sigma_m\left(\frac{1+2v}{1-v}\right), \quad \varepsilon_{cp}, \quad \text{and} \quad \sigma_{cp} \quad (6)$$

Here the first two terms indicate that one of three parameters (ε_m, σ_m, and v) must be obtained by an independent method and fixed during the fitting procedure, whereas another two parameters can be fitted. Usually, the geometrical parameter v is fixed, because it can be obtained by using the values of the cell radius (R) and the cell membrane thickness (d), which are evaluated independently. The parameters ε_{cp} and σ_{cp} in the set (6) can be calculated directly from the fitting procedure.

It can be shown analytically that the expression for the model spectrum of a cell suspension, represented by the combination of the well-known Maxwell-Wagner mixture formula (Takashima 1989) and the relation (1) for the single-shell model, can be presented as the sum of two Debye-type processes and a conductivity term. It coincides the ideas behind the work of Irimajiri (Irimajiri et al. 1979) that every shelled particle interface gives a single Debye-type dispersion. It is true also for the simplest case of homogeneous particles without a shell, where a single interface generates a single Debye-type dispersion. In the case of a one-shell particle suspension there are two interfaces (cytoplasm-cell membrane and cell membrane-suspending medium interfaces). Hence, in the single-shell particles the two Debye-type dispersions should be observed. However, it was shown (Pauly and Schwan 1959) that for biological cells these two dispersions degenerate to a single one. To confirm this conclusion we performed the numerical evaluation of these dispersion parameters for the cell suspension described by a single-shell model. For this calculation we used realistic parameters for a plasma membrane and

Table 1 The variation interval of cell phase parameters in double-shell model

Parameters[a]	The experiential limits		Reference parameters
	Lower limit	Upper limit	
ε_m	1.4	16.8	5.8
σ_m (S/m)	8×10^{-8}	5.6×10^{-5}	8.7×10^{-6}
ε_{cp}	60	77	60
σ_{cp} (S/m)	0.033	1.1	0.48
ε_{ne}	6.8	100	41
σ_{ne} (S/m)	8.3×10^{-5}	7×10^{-3}	3×10^{-3}
ε_{np}	32	300	120
σ_{np} (S/m)	0.25	2.2	0.95
R (m)	3.5×10^{-6}	10.5×10^{-6}	7×10^{-6}
R_n (m)	2.95×10^{-6}	8.85×10^{-6}	5.9×10^{-6}
d (m)	3.5×10^{-9}	10.5×10^{-9}	7×10^{-9}
d_n (m)	2×10^{-8}	6×10^{-8}	4×10^{-8}
v_1	0.98805	0.999	0.99703
v_2	0.4	0.8	0.60398
v_3	0.95183	0.99043	0.99

[a] Symbols: ε dielectric permittivity, σ DC conductivity; subscripts: m cell (plasma) membrane, cp cytoplasm, ne nuclear envelope, np nucleoplasm

[1] Here and below we use in that case the term "Debye-type process", i.e. the dielectric spectrum can be described by a Debye formula (Grant et al. 1978)

cytoplasm (see Table 1), a volume fraction $p = 0.06$, and parameters of supernatant ($\varepsilon_{sup} = 65$, $\sigma_{sup} = 0.16$ S/m). The following results for both dispersions were obtained: $\tau_1 = 3.84 \times 10^{-7}$ S; $\Delta\varepsilon_1 = 1.04 \times 10^3$; $\tau_2 = 2.1 \times 10^{-9}$ S; $\Delta\varepsilon_2 = 2.07$; $\varepsilon_{inf} = 64.7$. Thus, our numerical model experiment has shown that the dielectric strength of the second, high-frequency (HF) dispersion is about 2–3 orders of magnitude smaller than the dielectric strength of the low-frequency one and can therefore be neglected.

Number of fitting parameters for double-shell model

The degeneration of high-frequency dispersion in the spectrum of a single-shell particle suspension, as described in the previous section, can be extrapolated to the more complicated system of cells with a shelled nucleus, described by the well-known double-shell model (Asami et al. 1989). In this model, every cell phase (membrane, cytoplasm, nuclear envelope, and nucleoplasm) can be depicted by two dielectric parameters (conductivity and permittivity). The volume relations between these phases are expressed by geometric parameters $v_1 = (1 - d/R)^3$, $v_2 = (R_n/(R-d))^3$ and $v_3 = (1 - d_n/R_n)^3$, where R and R_n are the radii of the cell and the nucleus, and d and d_n are the thicknesses of the cell membrane and the nuclear envelope. Thus, the double-shell model contains 11 parameters.

The same assumptions for omitting the HF dispersion of the one-shell (i.e. prokaryotic) cell can also be applied to the nucleus with an envelope (for the eukaryotic cell). Thus, practically every shell adds a single Debye-type dispersion to the spectrum. Therefore, by analogy to the single-shell case, the expression for the spectrum of a suspension of double-shell particles can be described by the sum of two Debye-type dispersions and a conductivity term. The number of parameters has increased up to six phenomenological parameters (two values of dielectric strength, two relaxation times, dielectric permittivity at high frequency, and low-frequency conductivity). Thus, it follows that six independent parameters are enough to describe the double-shell cell suspension spectrum and, therefore, only six parameters can be found from applying the fitting procedure to the experimental spectrum. The other five parameters have to be obtained by some independent method and must to be fixed during the fitting procedure.

Computer analysis of double-shell model

The analysis of dielectric and geometrical parameters for white blood cells was done in order to build the spectrum for a double-shell cell model. The intervals of cell phase parameter variations obtained from different experimental studies (Asami et al. 1989; Bordi et al. 1993; Cametti et al. 1995; Irimajiri et al. 1978; Irimajiri et al. 1987; Polevaya et al. 1999; Ziervogel et al. 1986) are presented in Table 1. The reference values used in the model spectrum are listed in the last column. Following Asami (Asami et al. 1989) that the volume of the nucleus is equal to 60% of the cell volume ($R_n = 0.6^{1/3}R$) and using the thicknesses and radii of structural cell parts (see Table 1), the geometrical parameters (v_1, v_2, v_3) were calculated. The variation intervals of the radii and thicknesses were estimated under the assumption that experimental error does not exceed 50%.

The parameters were changed individually in order to evaluate their influence on the simulated spectrum. The variations of the values were implemented either in the framework of the limits in Table 1 or by noticeable spectrum alteration.

The results of these calculations are presented in Figs. 1–4. Note that the dielectric spectrum of one cell cannot be measured directly. It can only be calculated from the experimental cell suspension spectrum by using the mixture formula.

The influence of the dielectric permittivity variation for different cell components on the simulated cell spectrum is presented in Fig. 1. Note that the plasma membrane permittivity shows the maximum effect on the spectrum (Fig. 1a). The envelope permittivity (Fig. 1b) has a moderate influence, and the permittivities of the cytoplasm and nucleoplasm almost do not affect the

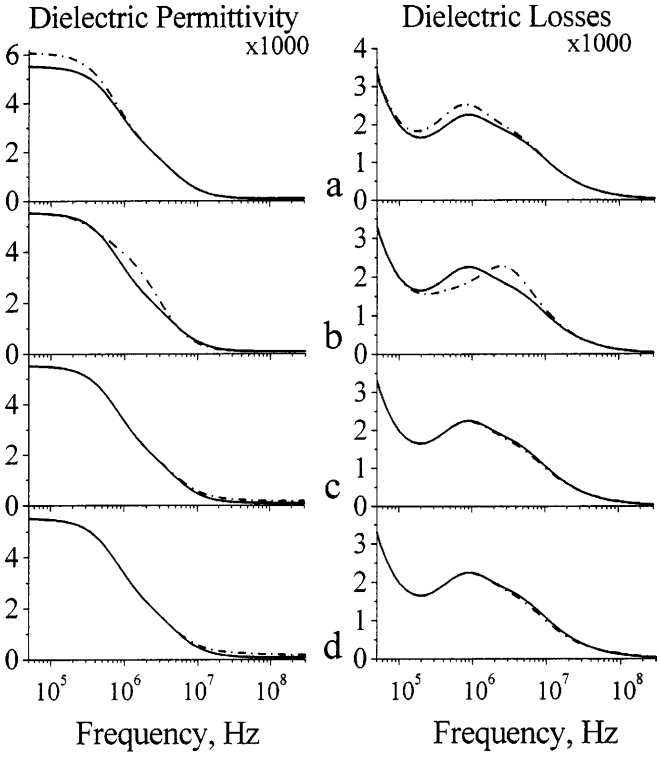

Fig. 1 The sensitivity of a cell spectrum to the permittivity of cell phases: **a** increase of plasma membrane permittivity by 1.1 times; **b** increase of nuclear envelope permittivity by 2 times; **c** increase of cytoplasm permittivity by 5 times; **d** increase of nucleoplasm permittivity by 3 times. *Solid line* indicates the spectrum with reference parameters. *Dashed line* indicates the spectrum with varied probe parameter

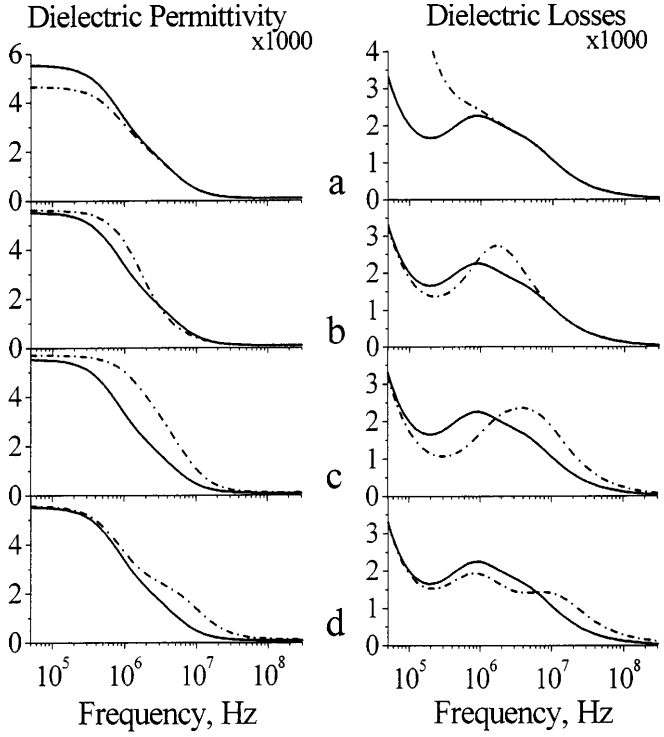

Fig. 2 The sensitivity of a cell spectrum to the conductivity of cell phases: **a** increase of plasma membrane conductivity by 5 times; **b** increase of nuclear envelope conductivity by 5 times; **c** increase of cytoplasm conductivity by 5 times; **d** increase of nucleoplasm conductivity by 5 times. *Solid line* indicates the spectrum with reference parameters. *Dashed line* indicates the spectrum with varied probe parameter

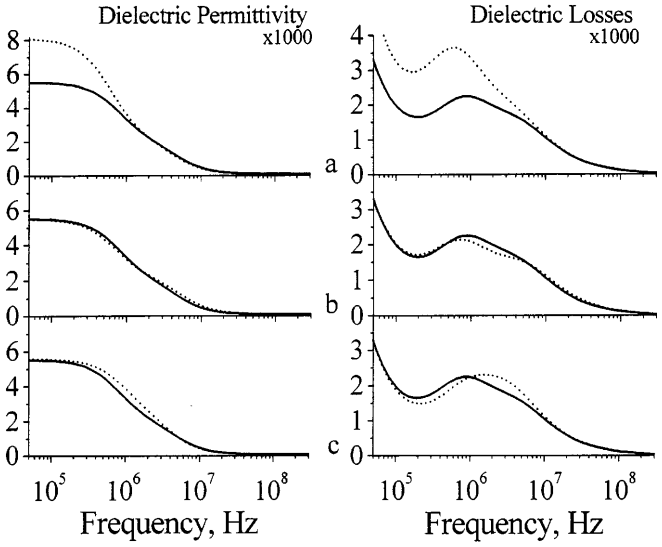

Fig. 3 The sensitivity of a cell spectrum to geometrical parameters: **a** increase of v_1 by 0.1%; **b** increase of v_2 by 33%; **c** increase of v_3 by 1%. *Solid line* indicates the spectrum with reference parameters. *Dashed line* indicates the spectrum with varied probe parameter

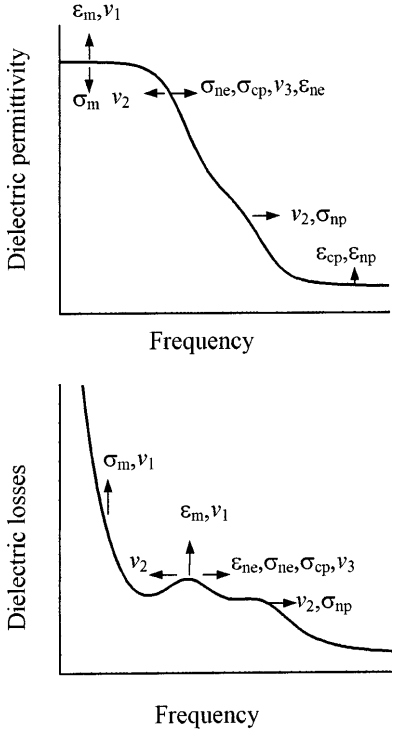

Fig. 4 The influence of double-shell model parameters on the cell spectrum. The *arrows* show the direction of spectrum changes. Symbols: ε dielectric permittivity, σ DC conductivity; subscripts: m cell (plasma) membrane, cp cytoplasm, ne nuclear envelope, np nucleoplasm

shape of the spectrum (Fig. 1c, d). Concerning the conductivities of the cell phases (plasma membrane, cytoplasm, nuclear envelope, and nucleoplasm), all have a strong influence on the different parts of the cell spectrum (Fig. 2). The effect of the geometrical parameters (v_1, v_2, and v_3) was tested over a small range, which results from the range of variation for R, R_n, d, and d_n for biological cells. Even the small variations of these parameters produce a noticeable change in the spectral shape (Fig. 3).

The schematic presentation of the position and extent of the influence of each parameter on the double-shell model spectrum is summarized in Fig. 4. This scheme shows that ε_m, σ_m, and v_1 affect the low-frequency part of the spectrum; ε_{ne}, σ_{ne}, σ_{cp}, and v_3 affect the low-frequency dispersion; and σ_{np} affect the high-frequency dispersion; v_2 has an influence on both dispersions; and ε_{cp} and ε_{np} affect the high-frequency part of the spectrum. Hence, the dielectric parameters of the exterior cell phases influence the low-frequency part of the spectrum, whereas the dielectric parameters of the interior cell phases affect the high-frequency region.

The analysis presented above allows formulating the criteria for the optimal choice of fixed parameters in the fitting procedure of the cell spectrum. When several parameters influence the spectrum within the same frequency interval, some of them must be fixed. From this point of view, all geometrical parameters (v_1, v_2, v_3) can be fixed; moreover, they can be measured by independent methods. Owing to the high sensitivity of the

spectrum to v_1, v_2, and v_3, it is important to evaluate them accurately.

The minimal distortion of the simulated spectrum due to significant variations of the model parameters can be another useful criterion for the selection of fixed parameters. The two parameters ε_{cp} and ε_{np} satisfy this requirement in the best way.

The approach presented in this paper was applied to the data analysis of lymphocyte spectra in a study of normal, transformed, and malignant cell suspensions (Polevaya et al. 1999).

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