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Effect of the shape of human erythrocytes on the evaluation of the passive electrical properties of the cell membrane

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Abstract

The possible influence of the cell shape on the derivation of the passive electrical parameters of a biological cell membrane is discussed in light of two different models which describe the cell as a shelled ellipsoidal particle and as a biconcave disk obtained by the revolution of the Cassini oval, respectively. Whereas within the first model, the Laplace equation can be solved analytically, in the second one a numerical algorithm based on the boundary element method has been employed. We have compared the results obtained by these two different models in the case of normal human erythrocyte cell membrane, using radiowave dielectric spectroscopy measurements. Our findings show that, although in principle the cell shape might deeply affect the evaluation of the passive electrical parameters of the cell membrane, in the case of the erythrocyte shape modelled by the Cassini curve, only small deviations are evidenced in comparison to the values derived, as usually done in the dielectric spectroscopy of biological cell suspensions, from an ellipsoidal model analysis. This result gives further support to the reliability of the data reported in the literature based on an ellipsoidal shape erythrocyte model.

Keywords: Cell shape; Passive electrical properties; Cell membrane

1. Introduction

The passive behavior of a biological cell membrane under the influence of an external electric field is characterized by two parameters, i.e., the permittivity ε_s and the electrical conductivity σ_s, which take into account both the dynamical ionic transport processes and the structural ionic and polar group arrangements of the cell membrane phase. The study of the passive electrical properties of the cell membrane is an area of active interest, yielding a lot of information on the structure and physiology of cells and different cell compartments. Radiowave dielectric spectroscopy measurements have been proved to be a method suitable to furnish quantitative data, directed to the determination of significant properties of these biological systems [1–3]. With the recent appearance of commercially available automated electrical impedance analyzers, the frequency domain dielectric spectroscopy of biological cell suspensions has expanded its power and has become a field of intensive research that is increasingly leading to practical and technological applications [4]. Moreover, the frequency-dependent response of biological cell suspensions can be investigated in details. This technique has been applied to different types of biological systems, ranging from human normal and pathological erythrocytes [5–7], lymphocytes [8,9], yeast cells [10] to plant protoplasts [11]. The electrical characterization of the erythrocyte cell membrane has been generally developed on the basis of different models, at a different degree of complexity, involving spherical [12] or ellipsoidal shaped particle models [13,14].

However, erythrocyte cell membrane is highly non-spheroidal, having a characteristic axial symmetric biconcave disk shape, with an approximately average disk thickness of about 2.5 μm and an average diameter of about

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7.8 μm. The non-sphericity of these cells, associated to a very high flexibility, is essential to the tolerance to deformation in the circulation. Since the cell membrane is a site of a high field amplification and the effective membrane parameters might strongly depend on the cell geometry, it is in principle uncertain how the cell shape can affect the accuracy of the prediction in the electrical behavior and, therefore, it can be understood that the analysis of the experimental data of non-spheroidal cell to the spherical or spheroidal cell model could yield inconsistent values for the dielectric properties of the cell components (cytosol and plasmatic membrane).

Geometrical effects on the dielectric behavior of arbitrarily shaped biological cells have been recently investigated by Gheorghiu [15,16] and by Vrinceanu et al. [17], who emphasized, on the basis of a numerical calculation, that the shape influence might become larger and larger as the difference between the electrical parameters of the inner and outer medium increases.

To address this problem also from an experimental point of view and to evaluate how the cell geometry might affect the dielectric response of an erythrocyte cell suspension, we have described the cell morphology as generated by the revolution of the oval of Cassini. Although there is no direct connection between this equation and the physical behavior of the cell membrane, the selecting of the Cassini equation, which contains parameters providing the possibility to vary the shape from spherical to ellipsoidal, was not only on the basis of pure convenience but also because the resulting surface is consistent with the visual observation of the cell shape.

In this paper, we focus on the dielectric properties of normal human erythrocytes in physiological saline solution (300 mosM) analyzed taking into account the effective biconcave disk shape of the cell membrane by means of a numerical algorithm based on the boundary element method. We compare the values of the passive electrical properties of the cell membrane and of the cytosol to the corresponding values obtained following the usual analysis based on the oblate spheroidal dielectric model of the erythrocyte cell. We found that, provided the characteristic dimensions of the cell are appropriately considered, the influence of the cell shape on the evaluation of the passive electrical parameters of the cell membrane, at least as far as described by the Cassini oval revolution, is rather moderate, the difference from the values derived from the usual analysis based on the ellipsoidal model being within few percent. The main differences consist in a higher electrical conductivity of both the cytosol (of about 10%) and the membrane (of about 15%). The difference could become larger in the case of different elongated particle shape or particle covered by shell of non-uniform and/or non-negligible thickness.

1.1. The erythrocyte shape

The shape of normal human erythrocytes is that of a biconcave structure (generally referred to as a biconcave disk) whose peculiar geometry is governed by different interacting processes. Measurements of the erythrocyte dimensions carried out by means of optical microscopic determination [18] (based on a significative statistics of cells at 300 mosM solution) gave the following values: average diameter (7.82±0.42 μm), average minimum thickness (0.81±0.35 μm), average maximum thickness (2.58±0.27 μm), cell volume (94±14 μm^3), cell surface (135±16 μm^2). The frequency histograms of these geometrical quantities are governed by normal distribution curves. In isotonic solutions, this peculiar shape can be described with good accuracy by the oval of Cassini that, in the (x, y) plane, reads

$$\left(y^2+x^2\right)^2-2a^2\left(x^2-y^2\right)=b^4-a^4 \tag{1}$$

where a and b are parameters which allow a whole range of various cell shapes, from spheres to prolate or oblate ellipsoids. In particular, the minimum thickness of the cell is given by $u=2\sqrt{b^2-a^2}$ the maximum thickness by $l=2\sqrt{a^2-(b^2/2a)^2}$ and the diameter by $v=2\sqrt{b^2+a^2}$.

It is worth noting that this equation, or its modified versions, has been previously employed to describe the erythrocyte shape by different authors [19,15]. More recently, Kralj-Iglič et al. [20] have extensively discussed the equilibrium shape of bilayer vesicles by using a modified Cassini function (with four parameters) and have determined the values of the model parameters from the geometrical constrains for the cell volume and area and by minimizing the membrane bending energy. This general approach yields many different cell shapes, ranging from discocytes, pear shaped cells, dumbbells cells to cells with spherical protusions and invaginations. In the present case, the Cassini equation has the advantage to depend on two parameters only, which provide the freedom needed to vary the cell shape (from a biconcave to a spheroidal particle). The same equation, with two free parameters, has been employed by Gheorghiu [15] in his analysis of the applicability of the ellipsoidal model.

2. Electrical properties of the cell membrane

Dielectric spectroscopy of a cell suspension allows the measurement of the electrical properties of the cell membrane, once the appropriate strategy for modelling the dielectric behavior of the whole cell is assumed [21]. Despite its highly heterogeneous structure, the most widely employed model replaces the cell with an effective particle of known geometry (size and shape) built up by different media of known dielectric and conductivity properties. The effective medium approximation (EMA) theory is then applied in order to obtain the macroscopic average properties of the cell suspension (mixture equation) as due to the sum of the contributions associated to the single cell. In the following, we briefly

review the ellipsoidal model, widely employed in the case of an erythrocyte cell.

2.1. Ellipsoidal particle suspension

The "single-shell" dielectric model considers a biological cell suspension as a collection of randomly oriented dielectric ellipsoidal particles of semiaxes a_0, b_0 and c_0, respectively, described by a complex dielectric constant $\varepsilon_p^*(\omega)$. Cells are covered with a thin shell of thickness d and complex dielectric constant $\varepsilon_s^*(\omega)$ (which represents the cell membrane) uniformly dispersed in a continuous medium of complex dielectric constant $\varepsilon_m^*(\omega)$ (which represents the extracellular solution). This model is based on the assumption that the three different media involved behave as isotropic non-dispersive dielectric materials, whose electrical parameters (the permittivity ε' and the electrical conductivity σ) are independent of the frequency of the applied electric field and, consequently, each medium is characterized by a complex dielectric constant given by

$$\varepsilon^*(\omega) = \varepsilon + \sigma_0 / i\varepsilon_0\omega \tag{2}$$

Despite its simplicity, this model has been successfully applied to different biological cell suspensions, in various experimental conditions and the values of the electrical parameters of the cell membrane, i.e., the permittivity ε_s and the electrical conductivity σ_s, have been well established.

In the absence of free charges within each dielectric medium, the solution of the Laplace equation $\nabla^2\psi(\vec{r}) = 0$ with the appropriate boundary conditions yields the expression for the complex dielectric constant of the single-shelled ellipsoidal particle suspension. The method is clearly detailed by Asami et al. [22] and we simply present the final expressions for the effective complex dielectric constant ε^* of the suspension that is given by

$$\frac{\varepsilon^* - \varepsilon_m^*}{\varepsilon^* + 2\varepsilon_m^*} = \frac{1}{9}\Phi \sum_{k=x,y,z} \frac{\varepsilon_{eq,k}^* - \varepsilon_m^*}{\varepsilon_m^* + \left(\varepsilon_{eq,k} - \varepsilon_m^*\right)A_{0k}} \tag{3}$$

where Φ is the fractional volume of the dispersed phase and $\varepsilon_{eq,k}^*$ (k=x, y, z) is the equivalent dielectric constant of the ellipsoidal particle surrounded by the shell and A_{0k} (k=x, y, z) are the depolarizing factors. Following Asami et al. [22], Eq. (3) completely describes the dielectric behavior of the suspension and gives the frequency dependence of both the permittivity $\varepsilon^*(\omega)$ and the electrical conductivity $\sigma(\omega)$. Details to describe the derivation procedure in multi-shelled ellipsoidal particle suspensions are reported by Asami in his recent review [23].

2.2. The boundary element method

The Laplace equation $\nabla^2\psi(\vec{r}) = 0$ furnishes a relatively simple analytical solutions only in the case of spherical or ellipsoidal geometry. For more realistic cell shapes, the electrostatic problem is more complex and must be solved numerically. Recently, Sekine [24] has taken into account the real shape (consistent with microscopic observation) of an erythrocyte cell applying the boundary element method (BEM) to the calculation of the electrical potential $\psi(\vec{r})$ outside a particle, under the influence of an uniform external electric field $E_0(\vec{r}) \equiv \left(E_{0x}, E_{0y}, E_{0z}\right)$. In this context, the effective medium theory approximation furnishes the final expression for the complex dielectric constant of the suspension. In particular, assuming a shell with a thickness d negligible small compared with the cell size and the electric field inside the membrane thickness to be uniform, Sekine [24] has shown that the electrical potential $\psi_m(r)$ in the external medium can be written in terms of the particle polarizabilities $P_{x,y,z}$ according to the relationship

$$\Psi_m(r) = \left(P_x x E_{0x} + P_y y E_{0y} + P_z z E_{0z}\right)\frac{1}{r^3} \tag{4}$$

satisfying the boundary conditions (continuity of the potential and the normal component of the displacement at the inner and outer membrane interfaces). Owing to the assumptions adopted, these conditions read

$$\Psi_s|_\Sigma = \Psi_m|_S + \varepsilon_m^*/\varepsilon_s^* d\,\partial\Psi_m/\partial r|_S \tag{5}$$

$$\varepsilon_p^*\,\partial\Psi_p/\partial r|_\Sigma + \varepsilon_m^*\,\partial\Psi_m/\partial r|_S = 0 \tag{6}$$

where $\Psi_p(\vec{r})$, $\Psi_s(\vec{r})$ and $\Psi_m(\vec{r})$ are the electrical potentials in the cytoplasm, membrane and extracellular medium, respectively, and Σ is the boundary cytoplasm–membrane surface and S the boundary membrane–external medium surface. Since the same functional form of Eq. (4) can be derived analytically from the Laplace equation in the case of ellipsoidal particles [22], the equating of these two equations results in the usual mixture equation, within the effective medium theory approximation.

Following the method developed by Sekine [24], we have considered the erythrocyte cell shape generated by rotating the Cassini oval along the y-axis (Eq. (1)). The numerical solution of the Laplace equation is calculated considering the particle surface divided into 8×8 isoparametric elements, resulting in a system of $2n$ complex coefficients linear equations, whose solution furnishes the value of the potential and of the electric field at the cell surface, in the center of each element. Once these values are known, the electric potential in the external medium at a distance large enough from the cell surface can be easily evaluated, which gives the final mixture equation for the heterogeneous system.

3. Experimental

3.1. Material

Red blood cells were obtained from healthy donors. The cells were washed in physiological saline solution (PBS,

290–300 mosM) and centrifuged for 5 min at 300×g three times and re-suspended in a PBS buffer at a controlled hematocrit Φ. Measurements have been carried out with Φ ranging from Φ=0.09 to Φ=0.15.

3.2. Dielectric and conductometric measurements

The dielectric and conductometric properties of the erythrocyte cell suspensions were measured by means two radiofrequency Impedance Analyzers, Hewlett-Packard model HP4192A in the frequency range from 1 kHz to 10 MHz and model HP4191A in the frequency range from 1 MHz to 1 GHz. The impedance data as a function of frequency were converted into the dielectric parameters by means of an appropriate equivalent circuit. Details of the conductivity cell and the calibration procedure are given elsewhere [25]. Owing to the high electrical conductivity of the cell suspension, in the low-frequency tail of the frequency range investigated, a marked electrode polarization effect appears, partially masking the contribution of the interfacial relaxation, associated to the heterogeneity of the system. The dielectric spectra have been corrected for this spurious effect, considering the electrode polarization mechanism as due to a constant phase angle element, resulting in an additional impedance $Z_p(\omega)=K(i\omega)^{-\alpha}$ in series with the sample impedance $Z_s(\omega)$. The details of this procedure and a critical evaluation of its influence on the dielectric results are reported elsewhere [26]. The overall accuracy of the experimental setup on both the permittivity $\varepsilon(\omega)$ and the electrical conductivity $\sigma(\omega)$ is within few percents over the whole frequency range investigated.

4. Results and discussion

In this section, we will present the analysis of the dielectric data of the erythrocyte suspensions we have investigated. Once the electrode polarization effect has been corrected (and removed) in the low-frequency tail of the spectrum, the remaining contributions refer to the interfacial polarization effect, at the intermediate frequency range, and to the orientational relaxation of the aqueous phase at the high-frequency tail of the range investigated. We will discuss the interfacial relaxation and determine its dielectric parameters. The next step is to compare and briefly discuss the results we obtained from the analysis of the data based on the two structural models we employed, i.e., the ellipsoidal cell model and the Cassini shape cell model.

4.1. Phenomenological analysis

In the case of a single-shelled spheroidal particle, with two different interfaces, i.e., the cytosol–membrane and the membrane–extracellular medium interfaces, the general expression of the Maxwell–Wagner polarization effect predicts two contiguous relaxation regions, each of them can be described by a Debye-type relaxation function, according to

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\Delta\varepsilon_1}{(1+(i\omega\tau_1))} + \frac{\Delta\varepsilon_2}{1+(i\omega\tau_2)} + \frac{\sigma_0}{i\omega\varepsilon 0} \qquad (7)$$

where $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ are the dielectric strengths, τ_1 and τ_2 are the relaxation times. Finally, ε_∞ is the high-frequency limit of the permittivity $\varepsilon'(\omega)$ and σ_0 the low-frequency limit of the electrical conductivity (d.c. conductivity).

Fig. 1 (panels A and B) shows a typical example of dielectric spectrum of an erythrocyte suspension in the frequency range investigated. The data have been analyzed by means of a complex non-linear least-squares minimization procedure using the Levenberg–Marquardt algorithm [27] which fits the real and imaginary parts of the complex dielectric constant simultaneously. The method, thanks to the constrains imposed by the dispersion relation between the real and imaginary part of the complex dielectric constant, allows a reliable estimate of the parameters of the dispersion, resulting in a fitted curve that describes in a very satisfactory way the experimental data over the whole frequency range where the interfacial polarization (Maxwell–Wagner effect) occurs.

Once the data have been corrected for the electrode polarization effect and the dispersion of the aqueous phase at the higher tail of the frequency range investigated is properly accounted for, we have fitted the data employing a relaxation model composed by two contiguous Cole–Cole relaxation functions (with β_1 and β_2 parameters taking into account the distribution of the relaxation times) and through the above-stated minimization algorithm, we have derived the dielectric parameters associated with the two dispersions. These data are reported in Table 1. As can be seen, both the two dielectric dispersions are rather accounted for by a Debye-type relaxation function, the β values being close to zero, indicating for the two dispersions a single exponential decay time. This finding agrees with predictions of Eq. (7), the small values of β being probably attributable to the size cell distribution. Within the theory of heterogeneous systems, Hanai et al. [28] discussed the number of the dielectric relaxations associated with the different interfaces present in the system and attributed the first relaxation at lower frequencies (P-relaxation) to the interfacial polarization of the outer surface and the second one at higher frequencies (Q-relaxation) to the inner surface. They stated that, as a general rule, spherical shelled structures (with two interfaces) give rise to two dielectric relaxations. However, for a shell thickness negligible in comparison with cell size, the strength of the Q-relaxation is generally small. For non-spherical shelled objects (oblate ellipsoids), Asami et al. [29] attributed the two relaxations to the interfacial polarization along the two different axes of the ellipsoid. Our analysis ensures that, whatever the physical origin of the dielectric dispersions

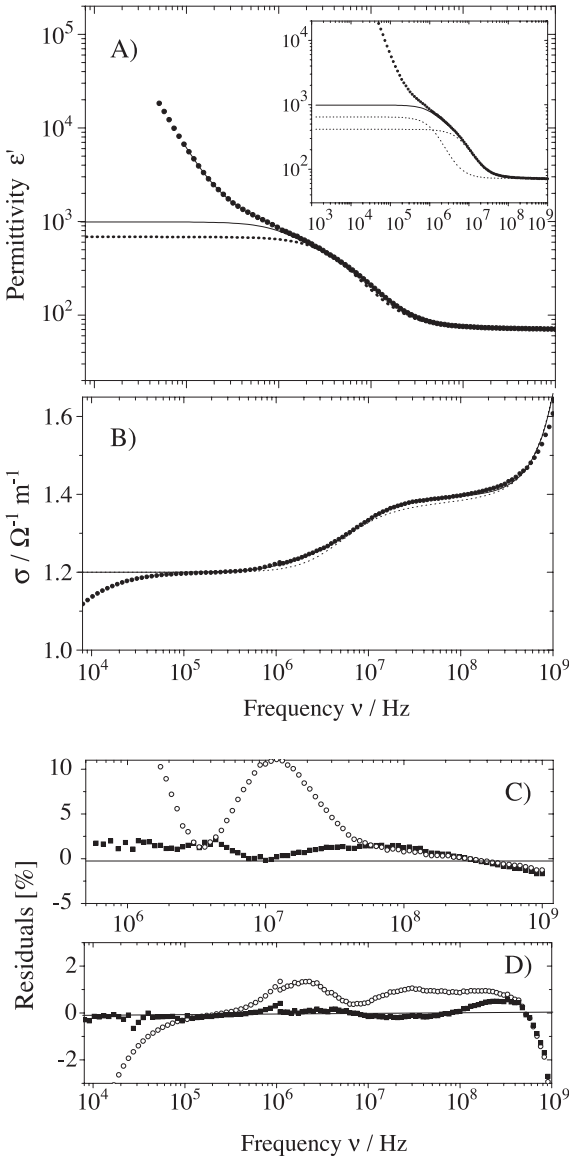

Fig. 1. The dielectric spectrum of an erythrocyte cell suspension at a hematocrit of Φ=0.165, at the temperature of T=25 °C. (A) The permittivity ε' as a function of frequency. In the low-frequency tail, the electrode polarization effect predominates. (B) The electrical conductivity σ as a function of frequency. In the low-frequency tail, the effect of electrode polarization produces a decrease of the conductivity values. Full lines: Values corrected for the electrode polarization effect and calculated on the basis of two contiguous Cole–Cole relaxation functions. The corresponding dielectric parameters are listed in Table 1. Dotted lines: Values corrected for the electrode polarization effect and calculated on the basis of a single Cole–Cole relaxation function (shown for comparison). In the inset of panel A, the two separate contributions to the permittivity, associated to the two Cole–Cole relaxation functions, are shown. (C) Residuals of the permittivity $\varepsilon'(\omega)$ as a function of frequency. (■) Values calculated on the basis of two contiguous Cole–Cole functions; (○) values calculated on the basis of a single Cole–Cole function. (D) Residuals of the electrical conductivity $\sigma(\omega)$ as a function of frequency. Symbols as in panel C.

may be, the system behaves correctly with respect to heterogeneous system theory and that the right number of interfacial polarizations contributes to the observed overall dielectric dispersion.

Table 1
Dielectric parameters of two contiguous Cole–Cole relaxation functions derived from a simultaneous fit of the permittivity and the electrical conductivity data

$\Delta\varepsilon_1$	$\Delta\varepsilon_2$	ε_∞	τ_1 [μs]	τ_2 [μs]	β_1	β_2	σ_0 [Ω^{-1} m^{-1}]
575.0	344.0	71.79	0.142	0.0212	0.005	0.011	1.200

In the next section, we will consider the two above-stated models in order to evaluate the membrane electrical parameters.

4.2. Structural model analysis

The dielectric and conductometric spectra of the erythrocyte cell suspension have been analyzed on the basis of the oblate spheroidal model (analytical model, Eq. (3)) and on the basis of boundary element method (numerical model).

Both the two approaches contain a large number of dielectric and geometric parameters necessary to characterize the dielectric behavior. The two models require 10 parameters, i.e., the electrical parameters of the inner medium (the cytosol), ε_p and σ_p, the electrical parameters of the shell (the cell membrane), ε_s and σ_s, the electrical parameters of the external medium (the extracellular solution), ε_m and σ_m, besides the geometrical parameters, the semiaxes a_0 and b_0 in the ellipsoidal model, and the parameters u and v in the Cassini shape model, the thickness d of the cell membrane and, finally, the volume fraction Φ of the dispersed phase. These parameters are not completely independent [30] and some of them can be obtained by independent methods.

In the following analysis, ε_m and σ_m have been directly measured from the supernatant, once the corpuscular component of the suspension has been removed by centrifuging, the fractional volume Φ has been measured by means of a Cell Coulter method, the thickness d has been assumed to be equal to 75 Å [31]. As far as the cell dimensions are concerned, we have considered an oblate ellipsoid with semiaxis a_0=3.9 μm and b_0=1.7 μm within the analytical model (Eq. (3)) and a Cassini revolution oval with parameters

Table 2
Electrical parameters of the cell membrane and cytosol derived from the simultaneous fit of the dielectric data (permittivity and electrical conductivity) on the basis of the ellipsoidal and biconcave disk shape model

	ε_s	σ_s [Ω^{-1} m^{-1}]	ε_p	σ_p [Ω^{-1} m^{-1}]
Ellipsoidal model	9.5±0.5	(8.5±0.8)10^{-5}	51.5±0.5	0.640±0.050
Cassini oval model	8.8±0.5	(1.05±0.08)10^{-4}	50.9±0.5	0.730±0.050

The aqueous phase parameters are ε_m=80.0 and σ_m=1.52 Ω^{-1} m^{-1}. The membrane thickness is assumed to be d=75 Å.

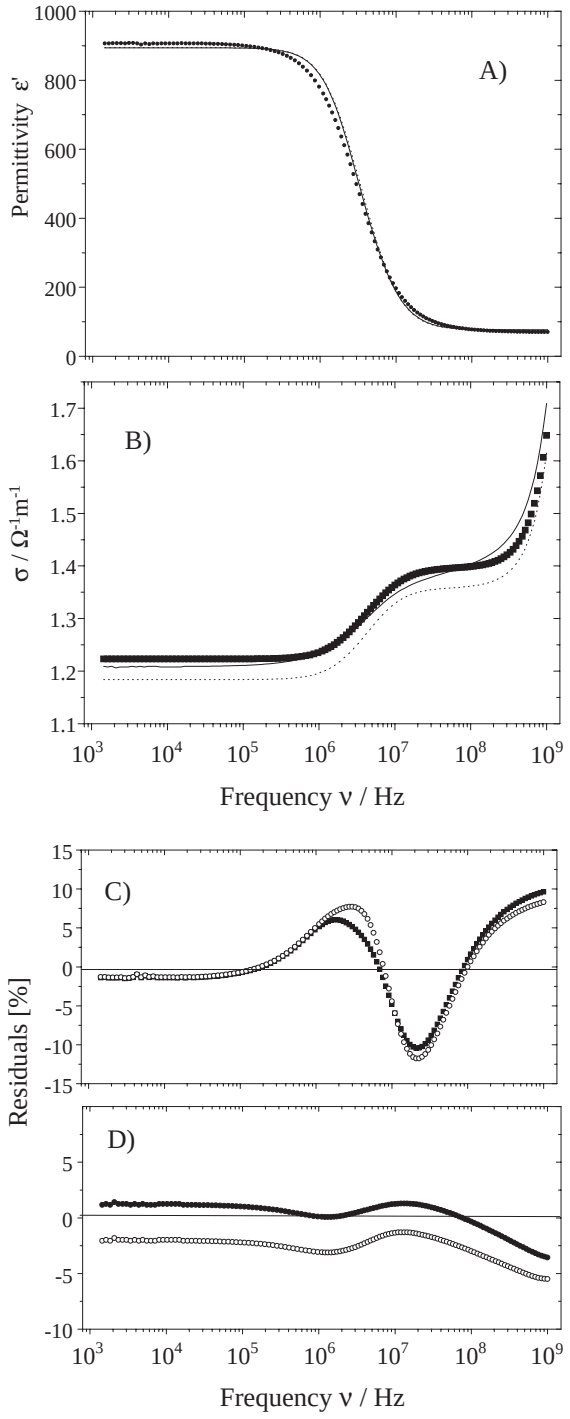

Fig. 2. The permittivity $\varepsilon'(\omega)$ (A) and the electrical conductivity $\sigma(\omega)$ (B) as a function of frequency for an erythrocyte suspension at a hematocrit Φ=0.136 and at the temperature of 25 °C. Full lines: Values calculated according to the boundary element method. Dotted lines: Values calculated according to the ellipsoidal cell dielectric model (oblate spheroid). The calculated values are obtained by fitting the two appropriate models to the permittivity and the electrical conductivity, simultaneously. The values of the phase parameters are shown in Table 2. Residuals of the permittivity $\varepsilon'(\omega)$ and the electrical conductivity $\sigma(\omega)$ as a function of frequency are shown in panels C and D, respectively. (■) Values calculated on the basis of the boundary element method (Cassini curve); (○) values calculated on the basis of the ellipsoidal cell dielectric model.

$u = 2\sqrt{b^2 - a^2} = 0.81$ μm and $v = 2\sqrt{a^2 + b^2} = 7.82$ μm. These values correspond to the average minimum thickness and the average diameter of the erythrocyte cell given by Evans [18], respectively. The remaining four parameters (ε_s, σ_s, ε_p, σ_p) have been derived by means of a non-linear least-squares fitting procedure. These values are shown in Table 2, for the two models investigated. Fig. 2 shows a comparison between the results of the analysis based on the ellipsoidal cell model (Eq. (3)) and the boundary element method using the erythrocyte shape given by the Cassini revolution oval. As can be seen, both the two models supply a satisfactory description of the relaxation spectra, especially if we keep into account the simultaneous fitting procedure in which the same set of parameters accounts for the permittivity and the electrical conductivity. However, should a decision be taken on the basis of the residues (Fig. 2, panels C and D), the boundary element method gives essentially the same description for the permittivity ε' in comparison to the ellipsoidal model, but a better approximation of the measured conductivity σ. In any case, note that differences are within some percent. As can be seen in Table 2, approximately the same set of parameters has been obtained from the two cell shape models, differences being confined within 10% for the conductivity of the cytosol σ_p and within 15% for the membrane conductivity σ_s.

5. Conclusion

Although the cell shape could in principle affect the evaluation of the passive electrical parameters of the cell membrane deduced from radiowave dielectric spectroscopic measurements, in the case of erythrocyte cells, this influence is moderate when the cell shape is modelled by a Cassini revolution oval with an appropriate choice of the shape parameters, in comparison with the usual ellipsoidal model.

We have compared the results usually obtained from the analytical solution of the Laplace equation, assuming a simplified oblate ellipsoidal geometry, and those obtained from a boundary element method, assuming the biconcave disk cell shape described by the Cassini revolution oval, which is consistent with the visual observation.

It can be expected that the use of a more realistic description of the membrane shape would lead to a significantly better description of the dielectric spectra and to a better evaluation of the passive electrical parameters of the cell membrane. On the contrary, as far as the erythrocyte shape is concerned, we find that the two approaches furnish essentially the same description of the experimental data and, moreover, the same set of electrical phase parameters for the cell membrane and cytosol within 10–15%. A similar conclusion has recently hypothesized by Gheorghiu [15], who, on the basis of a simple simulation of the dielectric profile in the radiowave frequency range, suggested that

ellipsoidal approximation is fairly good for random oriented cells as it happens when a cell suspension is investigated. Larger deviations could occur for single cells with particular orientation with respect to the external electric field. Our results, based on the contrary on the experimental analysis of the observed dielectric spectra over the whole frequency range where they fall and on the evaluation of the membrane passive electrical parameters, give a strong experimental supports to this view, enforcing the calculation carried out by Gheorghiu [15].

A final comment is in order. Although the effect of the cell shape on the electrical parameters are relatively small, the use of the Cassini geometry furnishes an overall improvement of the calculated values of both the electrical conductivity $\sigma(\omega)$ and the permittivity $\varepsilon(\omega)$, as seen from the residuals shown in Fig. 2. A more accurate analysis should require a more defined sample with a lower polydispersity. Extension of this approach to arbitrarily shaped cells will be the subject of future work.

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