Analytical Description of the Transmembrane Voltage Induced on Arbitrarily Oriented Ellipsoidal and Cylindrical Cells

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ABSTRACT We present an analytical equation for the transmembrane voltage ($\Delta\phi$) induced by a homogeneous AC field on arbitrarily oriented cells of the general ellipsoidal shape. The equation generalizes the Schwan equation for spherical cells and describes the dependence of $\Delta\phi$ on field frequency, cell size and shape, membrane capacitance, conductivities of cytoplasm, membrane and external medium, the location of the membrane site under consideration, and on the orientation of the cell with respect to the field. The derivation is based on the fact that the cytoplasm and the Maxwellian equivalent body of the whole cell are both of a general ellipsoidal shape and must thus exhibit constant local fields. The constant fields allow for a relatively simple description of the potentials on the internal and external membrane sides, leading to $\Delta\phi$. For this, the properties of cytoplasm, membrane, and external medium have been introduced into a special, finite element model. We found that $\Delta\phi$ can be unambiguously defined for non-spherical cells, provided that the membrane thickness is thin in comparison to the cell dimensions.

INTRODUCTION

As far as we are aware, Fricke (1953) was the first person to express the direct current (DC) steady-state transmembrane voltage ($\Delta\phi$) for a cell of the general ellipsoidal shape with negligible membrane conductance and a highly polarizable cytoplasm. In Fricke's brilliant paper, he presented the $\Delta\phi$ induced at the poles of an oriented cell of the general ellipsoidal shape in its most universal form,

$$\Delta\phi_a = \frac{1}{1 - n_a} aE. \quad (1)$$

$\Delta\phi_a$, E, a, and n_a stand for the induced transmembrane voltage at the pole, the field strength, the semiaxis oriented in field direction, and the depolarizing factor along semiaxis a, respectively. Expressions for the depolarizing factors are given in the appendix. Depending on the axial ratio of the ellipsoid, the depolarizing factor can take on values varying between 0 and 1. For the spherical shape ($a = b = c = R$, $n_a = n_b = n_c = ⅓$), Fricke's equation can be reduced to the well-known expression,

$$\Delta\phi = 1.5RE. \quad (2)$$

The detailed $\Delta\phi$ equation published by Bernhardt and Pauly (1973) is based on the knowledge of Stratton (1941) and Fricke (1953). Models for the description of the impedance properties of cells and cell suspensions were pioneered by Schwan (for a summary see: Schwan, 1957). Other authors have applied the knowledge developed by Schwan (Neumann et al., 1989; Zimmermann, 1982). Although the main focus of Schwan's work was the frequency and time dependence of biological material properties, he also dealt with the induced transmembrane voltage. Analytical $\Delta\phi$ equations are commonly named after Schwan to honor his role in the research into electrical properties of biological cells (Marszalek et al., 1990; Schwan, 1983; H.P. Schwan, personal information). Several attempts to improve and extend the equations to specific electric properties of the media and cells of the spherical (DeBruin and Krassowska, 1999) and nonspherical geometry (Gimsa and Wachner, 1999; Jerry et al., 1996; Kotnik and Miklavcic, 2000) and to properties, like the surface conductance (Grosse and Schwan, 1992) or the influence of deformational forces on the membrane permeabilization (Sukhorukov et al., 1998) exist.

We think that a complete $\Delta\phi$ equation must describe the dependence of $\Delta\phi$ on i) cell size and shape, ii) field frequency, iii) the membrane capacitance, iv) the conductivities of cytoplasm, membrane and external medium, v) the site at the membrane, e.g. given by the angle dependence, and vi) the orientation of the cell with respect to the external field. Analytical expressions for the $\Delta\phi$ for cells of the general ellipsoidal shape, meeting all of the above demands (i)–(vi) have, to our knowledge, not yet been published. Recently, we considered the polarization of spheroidal cells (Gimsa and Wachner, 1999). We also derived an analytical equation for $\Delta\phi$ for two orientations of the symmetry axis, parallel and perpendicular to the external field. Our expression meets points (i)–(iv) but is restricted to the poles of the cell. Nevertheless, it also applies to oriented cells of the general ellipsoidal shape when the respective depolarizing factor is assumed. In this paper, we extend our expression to points (v) and (vi) to meet all above criteria. We consider a single shell cell model of the general ellipsoidal shape with an arbitrary orientation of the inducing field.

Received for publication 12 February 2001 and in final form 11 July 2001.

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0006-3495/01/10/1888/09 $2.00

THEORY AND DISCUSSION

A finite element ansatz for $\Delta\phi$ of the oriented single shell model

Biological cells are usually negligibly magnetizable, and they are small with respect to wavelength at frequencies below a few GHz. Under these conditions, potential distribution can be directly obtained by solving Laplace's equation, which is the basis of the following derivations. Explicit solutions require surfaces of the second degree (Maxwell, 1873; Stratton, 1941). The general ellipsoid is the most complex but finite surface of the second degree. For the single shell ellipsoidal model, an additional geometrical restriction applies: an explicit solution requires that the two interfaces of the shell can be described within the same coordinate system, which is determined by the foci of the ellipsoidal surfaces. For a given thickness of the shell, e.g., along a certain principal axis, the foci determine the confocal shell of nonconstant thickness. A feature of such models is that a homogeneous body, i.e., the Maxwellian equivalent body, of the same external geometry can be found for all frequencies. For a given frequency, the body possesses certain electrical properties and exhibits the same external field distribution as the shelled model. The effective internal field E_{loc} of the body is constant. Its surface potential is identical to the potential at the external membrane side of the cell model. The potential at the internal membrane side can be calculated from the cytoplasmic field E_{i} that is constant, because the cytoplasmic surface is also ellipsoidal. For the oriented ellipsoidal cell $\Delta\phi$ at the poles with a perpendicular orientation to the field vanishes. $\Delta\phi$ is at maximum at the pole pointing in field direction. Here the external field, E_{loc}, E_{i}, and, consequently, the transmembrane field are in parallel (Fricke, 1953; please compare to Figs. 1, and 4). For a negligibly thin membrane $\Delta\phi$ at the pole of semiaxis a, pointing in field direction, is given by the difference of the potentials at the external and internal membrane side,

$$\Delta\phi_{\mathrm{a}} = (E_{\mathrm{loc}} - E_{\mathrm{i}})a. \tag{3}$$

In the DC case, the effective local field is amplified with respect to the external field for a cell with a nonconductive membrane. The maximum field amplification factor is related to the ellipsoid's axis ratio and can be expressed by the depolarizing factors (see Eq. 1 and the Appendix). These factors are obtained by solving Lapace's equation (Landau and Lifschitz, 1985; Osborn, 1945; Stille, 1944; Stoner, 1945; Stratton, 1941; see also Kotnik and Miklavcic, 2000). We recently introduced a related parameter, the influential radius, for modeling the polarization of spheroidal cells by a special, finite element ansatz (Gimsa and Wachner, 1999).

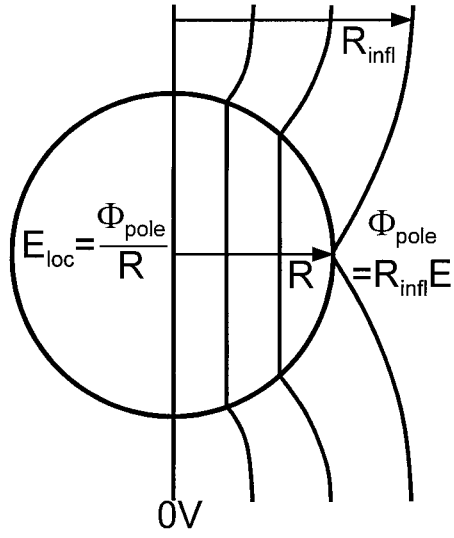

FIGURE 1 Sketch of the influential radius for a spherical, poorly polarizable object, e.g., an air bubble. The symmetry plane is assumed to be at 0 V. For a sphere, the depolarizing factor $n = ⅓$ and $R_{\mathrm{infl}} = 1.5$ R. The potential at the pole ϕ_{pole} corresponds to the undisturbed potential at distance R_{infl} from the symmetry plane. The constant local field can be calculated from ϕ_{pole}. Please note that (inside the object) the distances from each equipotential line to the 0 V plane are reduced by a factor of R/R_{infl} compared to a situation a great distance from the object or where the object is absent.

The influential radius a_{infl} is defined along the semiaxis a by the depolarizing factor n_{a} along that axis,

$$a_{\mathrm{infl}} = \frac{1}{1 - n_{\mathrm{a}}}\, a. \tag{4}$$

The maximum local field is given by $a_{\mathrm{infl}}\, E/a$. Figure 1 demonstrates the relations for a nonpolarizable sphere ($a = R$, $a_{\mathrm{infl}} = R_{\mathrm{infl}}$). For a cell, $\Delta\phi$ is at maximum for Fricke's conditions of a negligible membrane conductance and a highly polarizable cytoplasm (see Eq. 1; Fricke, 1953). The maximum at pole a is $a_{\mathrm{infl}}\, E$ (Please note that such conditions cannot as easily be met with alternating current (AC). At higher frequencies, when the membrane impedance decreases as a result of capacitive bridging, "metallic" cytoplasmic properties or an infinitely high permittivity are required).

Figure 2 demonstrates the relations of a finite element ansatz for modeling the membrane polarization (For details see: Gimsa and Wachner, 1999). Its geometry ensures the correct reflection of the potentials at the pole at the internal and external membrane side of the membrane. Only three elements of equal cross-sectional area are required. The impedance Z^* of each element is given by the geometry (cross-sectional area A and length l) and the electric properties (specific conductivity σ and permittivity $\epsilon\epsilon_0$),

$$Z^* = \frac{1}{\sigma^*}\frac{l}{A} \quad \text{with} \quad \sigma^* = \sigma + j\omega\epsilon\epsilon_0. \tag{5}$$

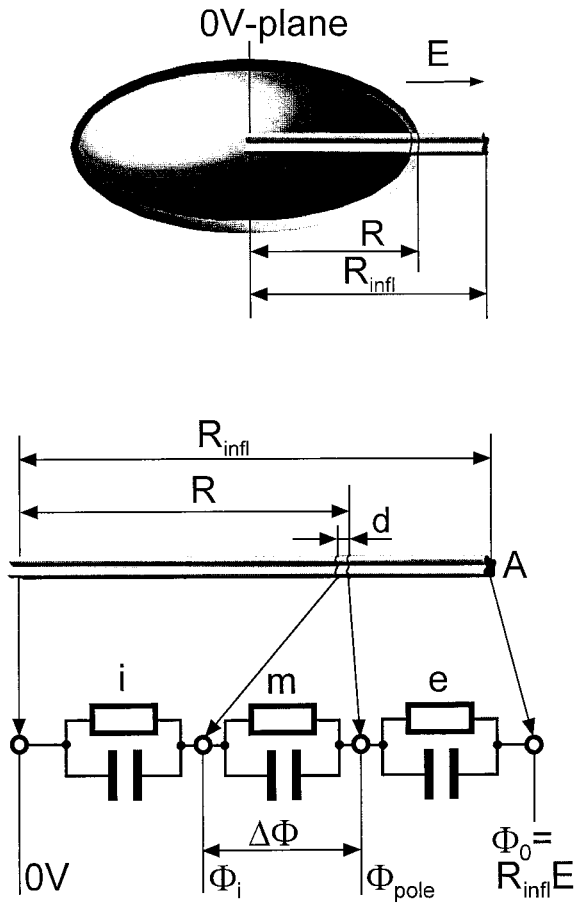

FIGURE 2 Oriented single-shell ellipsoid with sketches of the three finite elements and the RC-lump model for cytoplasm (*i*), membrane (*m*), and external medium (*e*), respectively. The symmetry plane of the model is assumed to be at 0 V. The elements possess the same cross-sectional area A. The length of the external medium element is given by the influential radius (R_{infl}). In the model, the potential ϕ_0 is fixed. ϕ_{pole} depends on the voltage divider properties of the elements. The maximum ϕ_{pole} is $\phi_0 = R_{\text{infl}}\,E$. The impedance of each element is given by Eq. 5 and can be modeled by an RC-pair.

σ^* and j are the complex, specific conductivity of the considered medium and $-1^{0.5}$, respectively. Eq. 5 applies to each resistor–capacitor (RC)-pair of the RC-lump model in Fig. 2. Starting from the finite element model, for a cell with semiaxis a oriented in field direction $\Delta\phi$ at the axis' pole can be expressed by the voltage divider properties of the lump model (compare to Figs. 1 and 2),

$$\begin{aligned}\Delta\phi_a &= aE_{\text{loc}} - aE_i \\ &= \frac{(Z_i^* + Z_m^*)\phi_{0,a}}{Z_{i,a}^* + Z_m^* + Z_{e,a}^*} - \frac{Z_i^*\phi_{0,a}}{Z_{i,a}^* + Z_m^* + Z_{e,a}^*} \\ &= \frac{Z_m^*}{Z_{i,a}^* + Z_m^* + Z_{e,a}^*} a_{\text{infl}}E. \end{aligned} \quad (6)$$

$\phi_{0,a}$ stands for the potential at the influential radius distance from the symmetry plane along axis a (compare to Figs. 2 and 3). The indices i, m, and e stand for the impedance of cytoplasm, membrane, and external medium, respectively, according to Eq. 5. Index a refers to the geometry along semiaxis a. Please note that no axis index must be assumed for the membrane impedance. The membrane elements can be assumed to possess the same geometry along each principal axis. The length l in Eq. 5 must be replaced by the respective expression according to Fig. 2. The cross-sectional area A is cancelled out.

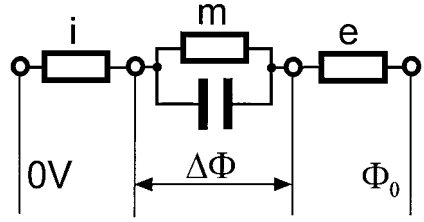

FIGURE 3 Reduced scheme for modeling $\Delta\phi$ in the range from DC up to frequencies above the capacitive membrane bridging. The permittive components of the internal and external media are cancelled out, allowing for a reduction in the number of parameters to be introduced into Eq. 6.

The parameter dependence of $\Delta\phi$ at the poles of the oriented single shell model

When $\Delta\phi$ is considered in the frequency range from DC up to frequencies of the capacitive membrane bridging (see below; Eq. 8) the permittive properties of the internal and external media can be neglected. Accordingly, the lump model of Fig. 2 reduces to that of Fig. 3.

Please note that the complete model, according to Eq. 6 and the scheme of Fig. 2, can be applied in cases where higher frequencies or nonregular membrane properties should also be considered. In this case, the permittive properties of the internal and external media and their possible structural (e.g., due to internal membrane systems) and nonstructural (e.g., due to Debye effects) dispersions can be included as well. The reduced scheme allows for a reduction in the number of parameters to be introduced into Eq. 6. A further simplification is possible by expressing the membrane properties by area-specific parameters. The properties of a membrane of thickness d are described by the area-specific capacitance C and conductance g. These values are given by $\epsilon_m\epsilon_0/d$, and σ_m/d, respectively. As long as C and g are kept constant, the model behavior will be largely insensitive to changes in membrane thickness. Physically, it is difficult to assume a membrane thickness lower than ϵ_0/C because this would lead to a relative membrane permittivity lower than unity. Nevertheless, for biological cells, C is approximately 10^{-2} F/m^2, leading to a lower membrane thickness limit of about 1 nm, negligibly low with respect to the cell size. Assuming a negligibly thin membrane, the cell and the cytoplasm would be of the same ellipsoidal shape, i.e., resemble surfaces of the second degree. Thus, the

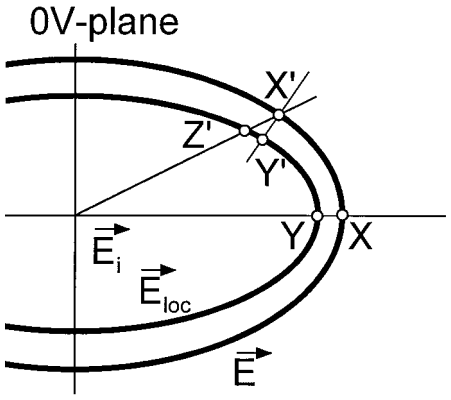

FIGURE 4 Schematic cut through an ellipsoidal cell with a confocal membrane thickness. The inducing external field E, the local field E_{loc}, and the cytoplasmic field E_i are oriented along a principal semiaxis with the pole X. $\Delta\phi$ is 0 V for all points of the symmetry plane oriented perpendicular to that semiaxis. The amplitude of E_{loc} is given by the potential at point X and its distance from the symmetry plane. The amplitude of E_i is given by the potential at point Y and its distance from the symmetry plane. $\Delta\phi$ at point X is defined as the potential difference $X - Y$. For $\Delta\phi$ at point X', see text.

equivalent body of the cell and its cytoplasm must exhibit a constant field (Maxell, 1873; Fricke, 1953; Landau and Lifschitz, 1985). When a principal axis, e.g., a, is oriented in parallel to the external field E, the local field E_{loc} and the cytoplasmic field E_i are also parallel to that axis. Fig. 4 demonstrates these relationships.

At point X, the pole of axis a of the ellipsoidal cell, $\Delta\phi$ is at maximum. This point has the largest distance from the symmetry plane, which is defined by the other two semiaxes. Because, at the pole, E, E_{loc}, and E_i, are in parallel, this also applies to the transmembrane field. $\Delta\phi$ at point X is given by Eq. 6. Using Eqs. 4 and 5, after some rearrangements, the following expression can be obtained (for details of the derivation see: Gimsa and Wachner, 1999):

$$\Delta\phi_a = \frac{a_{infl}E}{\left(1 + ag\left(\frac{1}{\sigma_i} + \frac{a_{infl} - a}{a\sigma_e}\right)\right)\sqrt{1 + \frac{f^2}{f_{c,a}^2}}}, \qquad (7)$$

with

$$f_{c,a} = \frac{1}{2\pi C}\left(\frac{\sigma_e\sigma_i}{a\sigma_e + (a_{infl} - a)\sigma_i} + g\right). \qquad (8)$$

f, $f_{c,a}$, σ_e, and σ_i stand for the external field frequency, the characteristic frequency of membrane polarization along semiaxis a, and the conductivities of the external and internal media. Please note that $f_{c,a}$ is the -3-dB frequency of the $\Delta\phi$ amplitude. Eqs. 7 and 8 were derived for area-specific membrane parameters. Assuming the respective influential radii, the equations are valid for the poles of cells of the general ellipsoidal, the spheroidal, and the spherical shape.

The site dependence of $\Delta\phi$ for the oriented ellipsoidal cell

As already pointed out in the introduction, a complete Schwan equation should also describe the angle dependence of $\Delta\phi$. In the following, we will derive an expression for the angle dependence. For surface points that are not poles of the ellipsoid, e.g., X' in Fig. 4, the definition of $\Delta\phi$ is not clear per se. Under geometric criteria $\Delta\phi$ may, e.g., be the potential difference $X' - Y'$ when Y' is, for example, defined by the shortest transmembrane distance. Similarly, $\Delta\phi$ may be the potential difference $X' - Z'$, when Z' is the crossing point of a line through the ellipsoid's center and X' with the internal membrane surface. For a given point X', other possibilities can also be conceived to define a reference point at the inner membrane surface. Nevertheless, at the ellipsoid's poles all $\Delta\phi$-definitions should be consistent and for any definition the following considerations should hold.

Let us assume $\Delta\phi$ for a given point X' at the external surface to be defined in two different ways, a correct and a slightly incorrect one, e.g., as the potential differences $X' - Y'$ and $X' - Z'$, respectively. In this case, for geometric reasons, a reduction of the membrane thickness reduces the distance of the points Y' and Z'. This in turn reduces the error in $\Delta\phi$ related to a possible potential difference $Y' - Z'$, more since the cytoplasmic conductivity is much higher than that of the membrane and the potential difference $Y' - Z'$ will thus be much smaller than $\Delta\phi$. As a result, any two different definitions of $\Delta\phi$ will approximate each other and match the correct value for a negligibly thin membrane. Furthermore, the voltage drop over a given distance within the cytoplasm is much lower than over the membrane for all points far from the cell equator. In practice, errors smaller than in the 1% range can be expected for cells with a semiaxis length and membrane thickness of the order of 10 μm and 10 nm, respectively.

These considerations show that Eq. 3 can be used to calculate $\Delta\phi$ when a is replaced by d, the distance of a given point to the symmetry (0 V) plane. Similarly, Eq. 7 can be normalized by the factor d/a, leading to

$$\Delta\phi_a = \frac{1}{\left(1 + ag\left(\frac{1}{\sigma_i} + \frac{a_{infl} - a}{a\sigma_e}\right)\right)\sqrt{1 + \frac{f^2}{f_{c,a}^2}}}\,\frac{a_{infl}}{a}\,Ed. \qquad (9)$$

Expressions for d are given below. For an ellipsoidal cell with the a-semiaxis oriented in field direction, the symmetry plane is determined by the semiaxes b and c. This plane is also the reference for the perpendicularly oriented local and cytoplasmic fields. These properties, up to now, have allowed us to avoid the vector notation that will be introduced for the general orientation.

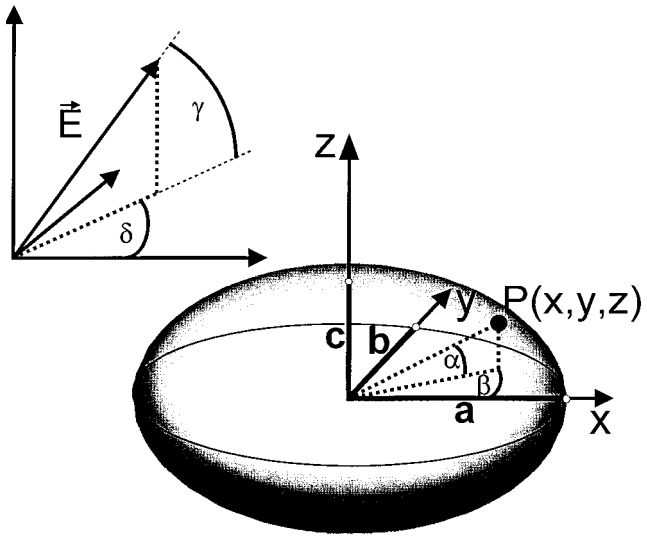

FIGURE 5 Sketch of a cell of the general ellipsoidal shape. The principal semiaxes a, b, and c, define the orthonormal coordinate system, x, y, z. $P(x, y, z)$ is a membrane surface point of the cell, defined in spherical coordinates (angles α and β). With respect to the cell, the homogeneous external field $\vec{E}$ has an arbitrary orientation, determined by the angles γ, and δ.

The general case

In case of a general orientation, the constant local and cytoplasmic fields are not usually aligned to a semiaxis or to each other. Accordingly, their 0 V symmetry planes are tilted differently around the center point of the ellipsoid. Consequently, for a given point at the outer and inner membrane sides, different distances to the respective symmetry planes have to be taken into account to calculate the transmembrane voltage from the absolute values of the local and cytoplasmic field strengths, in analogy to Eq. 3. To overcome this problem, the vector notation will be used in the following. The principal semiaxes a, b, and c of the ellipsoidal cell are used to determine an orthonormal coordinate system, x, y, and z (Fig. 5).

The homogeneous external field $\vec{E}$ is orientated arbitrarily within this coordinate system with the orientation being determined by the angles γ and δ,

$$\vec{E} = \begin{pmatrix} E_x \\ E_y \\ E_z \end{pmatrix} = \begin{pmatrix} \cos\gamma\cos\delta \\ \cos\gamma\sin\delta \\ \sin\gamma \end{pmatrix} |\vec{E}|. \tag{10}$$

The local vector $\vec{P}$ of the membrane point under consideration shall be determined by the angles α and β,

$$\vec{P} = \begin{pmatrix} P_x \\ P_y \\ P_z \end{pmatrix} = \begin{pmatrix} \cos\alpha\cos\beta \\ \cos\alpha\ \sin\beta \\ \sin\alpha \end{pmatrix} |\vec{P}|. \tag{11}$$

In Eqs. 10 and 11, spherical coordinates are used, i.e., the coordinates of a surface point of a unit sphere are multiplied by the absolute length of the local vector. For a given surface point P_x, P_y, and P_z of the ellipsoid, the following equation holds:

$$\frac{P_x^2}{a^2} + \frac{P_y^2}{b^2} + \frac{P_z^2}{c^2} = 1. \tag{12}$$

The combination of Eqs. 11 and 12 leads to a general expression for the local vector of the membrane point:

$$\vec{P} = \begin{pmatrix} \cos\alpha\cos\beta \\ \cos\alpha\sin\beta \\ \sin\alpha \end{pmatrix} \times \frac{abc}{\sqrt{(a^2\sin^2\beta + b^2\cos^2\beta)c^2\cos^2\alpha + a^2b^2\sin^2\alpha}}. \tag{13}$$

Now, Eq. 3 can be written in a general form, to obtain the induced transmembrane potential of a cell with a negligibly thin membrane,

$$\Delta\phi = \vec{E}_{loc}\cdot\vec{P} - \vec{E}_i\cdot\vec{P} = (\vec{E}_{loc} - \vec{E}_i)\cdot\vec{P}. \tag{14}$$

The components of the local field $\vec{E}_{loc}$ and the cytoplasmic field $\vec{E}_i$, $E_{loc,a}$, $E_{loc,b}$, $E_{loc,c}$ and $E_{i,a}$, $E_{i,b}$, $E_{i,c}$, respectively, are induced along the three principal axes by the relevant components of the external field E_x, E_y, E_z,

$$\vec{E}_{loc} = \begin{pmatrix} E_{loc,a} \\ E_{loc,b} \\ E_{loc,c} \end{pmatrix} = \begin{pmatrix} \dfrac{Z^*_{i,a} + Z^*_m}{Z^*_{i,a} + Z^*_m + Z^*_{e,a}} \dfrac{a_{infl}}{a} E_x \\ \dfrac{Z^*_{i,b} + Z^*_m}{Z^*_{i,b} + Z^*_m + Z^*_{e,b}} \dfrac{b_{infl}}{b} E_y \\ \dfrac{Z^*_{i,c} + Z^*_m}{Z^*_{i,c} + Z^*_m + Z^*_{e,c}} \dfrac{c_{infl}}{c} E_z \end{pmatrix}, \tag{15}$$

$$\vec{E}_i = \begin{pmatrix} E_{i,a} \\ E_{i,b} \\ E_{i,c} \end{pmatrix} = \begin{pmatrix} \dfrac{Z^*_{i,a}}{Z^*_{i,a} + Z^*_m + Z^*_{e,a}} \dfrac{a_{infl}}{a} E_x \\ \dfrac{Z^*_{i,b}}{Z^*_{i,b} + Z^*_m + Z^*_{e,b}} \dfrac{b_{infl}}{b} E_y \\ \dfrac{Z^*_{i,c}}{Z^*_{i,c} + Z^*_m + Z^*_{e,c}} \dfrac{c_{infl}}{c} E_z \end{pmatrix}. \tag{16}$$

Using Eqs. 15 and 16, Eq. 14 becomes (compare to Eq. 6)

$$\Delta\phi = \begin{pmatrix} \dfrac{Z^*_m}{Z^*_{i,a} + Z^*_m + Z^*_{e,a}} \dfrac{a_{infl}}{a} E_x \\ \dfrac{Z^*_m}{Z^*_{i,b} + Z^*_m + Z^*_{e,b}} \dfrac{b_{infl}}{b} E_y \\ \dfrac{Z^*_m}{Z^*_{i,c} + Z^*_m + Z^*_{e,c}} \dfrac{c_{infl}}{c} E_z \end{pmatrix} \cdot \vec{P}, \tag{17}$$

where $\vec{P}$ is given by Eq. 13. This is the general expression of the induced transmembrane potential. Neglecting the permittivities of the cytoplasm and the external medium, the

impedance terms in Eq. 17 can be replaced by the respective term of Eq. 9. We thus obtain the final expression,

$$\Delta\phi = \begin{pmatrix} \dfrac{1}{\left(1 + ag\left(\dfrac{1}{\sigma_i} + \dfrac{a_{infl} - a}{a\sigma_e}\right)\right)\sqrt{1 + \dfrac{f^2}{f_{c,a}^2}}} \dfrac{a_{infl}}{a} E_x \\ \dfrac{1}{\left(1 + bg\left(\dfrac{1}{\sigma_i} + \dfrac{b_{infl} - b}{b\sigma_e}\right)\right)\sqrt{1 + \dfrac{f^2}{f_{c,b}^2}}} \dfrac{b_{infl}}{b} E_y \\ \dfrac{1}{\left(1 + cg\left(\dfrac{1}{\sigma_i} + \dfrac{c_{infl} - c}{c\sigma_e}\right)\right)\sqrt{1 + \dfrac{f^2}{f_{c,c}^2}}} \dfrac{c_{infl}}{c} E_z \end{pmatrix} \cdot \vec{P}, \tag{18}$$

where the characteristic frequencies of membrane polarization, $f_{c,a}$, $f_{c,b}$, and $f_{c,c}$, are given by Eq. 8. Please note that these characteristic frequencies vary along the three principal axes. As a result, for an arbitrarily oriented cell, the point of the highest induced transmembrane potential changes with increasing frequency. For frequencies approaching that of the membrane dispersion, the point is shifted toward the longest axis with the highest characteristic frequency (compare to Fig. 6).

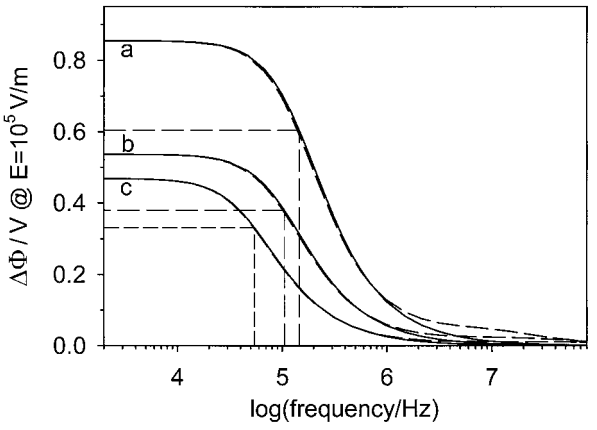

FIGURE 6 Frequency dependence of $\Delta\phi$ according to Eq. 9 at the three poles of a hypothetical, ellipsoidal cell for an external field strength of 100 kV/m (*solid line*). The three curves correspond to the three semiaxis *a*, *b*, and *c*, oriented in field direction. The curves are designated by the semiaxis oriented in field direction. For calculations, the geometry of chicken erythrocytes, obtained from microscopic measurements, was assumed (semiaxes length $a{:}b{:}c$ = 7.7:4:1.85 μm). A specific membrane capacitance and conductance of $C = 10^{-2}$ F/m^2 and g = 125 S/m^2, as well as internal and external conductivities of σ_i = 0.53 S/m, and σ_e = 0.01 S/m, respectively, were used. For comparison, also the curves of the complete model (Eq. 6) are plotted (*dashed lines*). The frequencies of the 3-dB decrease in the $\Delta\phi$ amplitude, with respect to the low-frequency plateau, are marked. Please note that these characteristic frequencies increase with the length of the axis oriented in field direction. The frequencies are given by Eq. 8.

Geometrical simplifications

The spheroidal and cylindrical shape

The reduction of the general ellipsoidal cell shape to a spheroidal shape ($b = c$) is a significant simplification, because it allows for the introduction of closed expressions for the depolarizing factors into Eq. 4 to obtain the influential radii for Eqs. 8, 17, and 18, respectively (see Appendix).

The cylindrical shape is the limiting case of an infinitely long spheroid ($b = c$; $a \gg b, c$) and the depolarizing factors for this shape are well defined ($n_a = 0$, $n_b = 0.5$, $n_c = 0.5$). These factors result in influential radii of $a_{infl} = a$, $b_{infl} = 2b$, and $c_{infl} = 2c$ (Eq. 4) which allow for a further simplification of Eqs. 8, 17, and 18, respectively. For the cylindrical shape, also the local vector expression (Eq. 13) can be simplified.

The oriented ellipsoidal cell

For oriented cells with the shape of a general ellipsoid, spheroid, cylinder, or sphere, a number of simplifications can be introduced. For the general ellipsoidal shape, we can start from Eq. 9. For example, when semiaxis *a* is oriented in the direction of the external field, the symmetry (0 *V*) planes of the local and the cytoplasmic fields are defined by the semiaxes *b* and *c*. Consequently, the distance *d* of the considered point to the symmetry plane is solely given by the *x*-component of Eq. 13 (Figs. 1 and 4),

$$d = \frac{abc \cos\alpha \cos\beta}{\sqrt{(a^2 \sin^2\beta + b^2 \cos^2\beta)c^2 \cos^2\alpha + a^2 b^2 \sin^2\alpha}}. \tag{19}$$

Like in Fig. 5, α describes the angle relative to the a–b plane, β, the angle within this plane. The external field is oriented at 0°, 0°. Introduction of Eqs. 8 and 19 into Eq. 9 results in a Schwan equation for oriented cells of the general ellipsoidal shape.

Figure 6 presents the frequency dependence of $\Delta\phi$ at the three poles of a cell model of the general ellipsoidal shape according to Eq. 9. $\Delta\phi$ was calculated for a hypothetic cell with the geometry of chicken erythrocytes and the electric parameters of human erythrocytes at an external conductivity of 0.01 S/m. For these parameters, deviations of the curves obtained from Eq. 9 and from the complete model (Eq. 6) were found in the higher frequency range. Whereas the curves obtained from Eq. 9 smoothly approach the zero $\Delta\phi$, the complete model exhibits small, additional plateaus in the high frequency range. After a strong decrease, two more plateaus are reached. For our conditions, the first plateau is due to the capacitive bridging of the external medium element already at relatively low frequencies (at a

few MHz). The time constant of the external medium element is larger than that of the cytoplasmic element. The constant plateau level at very high frequencies is reached when the voltage divider properties are completely determined by the permittive properties of the media. As can be seen from Fig. 6, the $\Delta\phi$ amplitude and the -3-dB frequency differ for the axis oriented in field direction. The longer the axis in field direction, the higher the amplitude. This is the case, although the maximum field amplification factor along the oriented axis, given by $a_{\mathrm{infl}}/a = 1/(1 - n_{\mathrm{a}})$ decreases with the axis length (see Fig. 1 and Eq. 4). Obviously, the decrease of the amplification factor is overcompensated by the increase in the axis length. Experimentally, these relations can be conveniently tested in a rotating poration field that scans the cell in a plane around two principal axes (Gimsa et al., 1992). In parallel with the axis length, the -3-dB frequency is shifted toward higher frequencies. We suppose that the -3-dB frequency must be roughly related to the mean membrane curvature of the considered cell pole. Nonetheless, this relation is not fully understood and currently subject of more detailed investigation.

The oriented spheroidal cell

Reducing the oriented ellipsoidal shape to an oriented spheroidal shape allows for a further simplification. As discussed above, for spheroidal cells ($b = c$), closed expressions for the depolarizing factors can be given. Further, in the oriented case, Eq. 18 can be reduced to Eq. 9. When the symmetry axis a of the spheroidal cell is oriented perpendicular to the field, the geometrical expression for d, given by Eq. 19 is reduced to

$$d = \frac{ab \cos\alpha \ \cos\beta}{\sqrt{a^2 \sin^2\alpha + b^2 \cos^2\alpha}}. \tag{20}$$

For the parallel orientation of the symmetry axis to the field, Eq. 20 can further be reduced. In this case, points of equal $\Delta\phi$ form rings at the membrane surface around the symmetry axis. This feature reduces the calculation of the distance in between a surface point and the symmetry plane to the two-dimensional case of an ellipse with semiaxes a and b. To describe the equipotential rings, it is sufficient to define a single angle φ with respect to the symmetry axis. Eq. 20 can be transformed into

$$d = \frac{ab \cos\varphi}{\sqrt{a^2 \sin^2\varphi + b^2 \cos^2\varphi}}. \tag{21}$$

The spherical cell

For a sphere ($a = b = c = R$) Eq. 21 becomes the well-known expression,

$$d = R \cos\varphi. \tag{22}$$

Eqs. 8 and 9 can further be simplified using the relation $R_{\mathrm{infl}} = 3R/2$.

Electric simplifications

Simplifications of the model can also be introduced for certain electrical properties. For example, a zero membrane conductance can be assumed (see Eqs. 8 and 9 or 18). The strongest electrical simplification that can be thought of was introduced by Fricke, leading to Eq. 1 (Fricke, 1953). Fricke assumed an oriented ellipsoidal cell with negligible membrane conductance and a highly polarizable cytoplasm. In this case, the whole cytoplasmic surface will be at the same potential (e.g., 0 V). Thus, $\Delta\phi$ at any surface point is directly given by the potential at the surface that can be calculated from the constant local field and the distance to the symmetry plane (see Fig. 1). According to these considerations, the angle dependence of $\Delta\phi$ can easily be introduced into Eq. 1 by exchanging a for the respective expression for d. d is given by Eqs. 19, 20, 21, and 22, for cells of the shape of a general ellipsoid, a spheroid oriented with the symmetry axis perpendicular, or in parallel to the field, and a sphere, respectively. It should be mentioned that Kotnik and Miklavcic (2000) applied Fricke's condition to a spheroidal cell with the symmetry axis in parallel to the field. Accordingly, their result is identical to the combination of Eqs. 1 and 21 for the respective depolarizing factors (Eqs. A4 and A5). Using Eq. 20 instead of Eq. 21 leads to the solution for the perpendicular orientation of the symmetry axis to the field, missing in their paper (The respective depolarizing factor for the other axis orientation can be obtained from the relation of the three factors. See Appendix). For Fricke's condition also a solution for the arbitrary orientation of the general ellipsoidal shape can easily be derived. For an infinitely high membrane impedance, Eq. 17 can be significantly simplified. Introducing Eq. 13 into Eq. 17 leads to

$$\Delta\phi = \begin{pmatrix} \frac{a_{\mathrm{infl}}}{a}\cos\alpha\cos\beta \\ \frac{b_{\mathrm{infl}}}{b}\cos\alpha\sin\beta \\ \frac{c_{\mathrm{infl}}}{c}\sin\alpha \end{pmatrix} \begin{pmatrix} \cos\gamma\cos\delta \\ \cos\gamma\sin\delta \\ \sin\gamma \end{pmatrix} \times \frac{|\vec{E}|\, abc}{\sqrt{(a^2\sin^2\beta + b^2\cos^2\beta)c^2\cos^2\alpha}}. \tag{23}$$

It should also be mentioned, that from a physical point of view, Fricke's condition reduces the $\Delta\phi$-problem to the simple problem of the potential distribution at the surface of

an ellipsoidal cavity in a dielectric, leaving a cell model that misses almost any physiological property.

SUMMARY

The presented $\Delta\phi$ expression (Eqs. 17 or 18) is the complete Schwan equation fulfilling demands (i)–(vi), raised in the Introduction. The derivation is based on the influential radii (Fig. 1, Eq. 4) that allowed us to put up a lump model that almost precisely reflects the Laplace solution (for details see Gimsa and Wachner, 1999). The advantage of this approach is the easy simplification of the mathematical problem to be solved. Canceling the permittive elements of the cytoplasm and the external solution and introducing area-specific membrane properties lead to Eq. 18. Thus, Eq. 18 contains the area-specific conductance and capacitance of the membrane and the conductive properties of the internal and external media. These parameters can be replaced by other physiological properties, like permeabilities, ion concentrations, etc. (DeBruin and Krassowska, 1999; Gimsa et al., 1989). Long cylindrical cells or axons can be modeled by the limiting case of the spheroidal shape, an infinitely long cylinder. Principally, Eq. 18 can also be extended by a surface-conductance term according to Grosse and Schwan (1992). Nevertheless, the surface conductance introduces lateral membrane currents that are neglected in the finite element ansatz.

Under certain experimental conditions, the cell properties may be subject to a time-dependent change. The cytoplasmic conductivity may, for example, change due to ion leakage through field-induced membrane pores (Gimsa et al., 1989). Modeling these relations may, e.g., be important in investigations on the dielectric membrane breakdown (DeBruin and Krassowska, 1999; Marszalek et al., 1990). An AC field below the frequency of membrane dispersion may, in parallel to the $\Delta\phi$ induction, induce a compression of the cell (Sukhorukov et al., 1998). Such time-dependent geometry changes, in turn, influence the induced $\Delta\phi$ and can be modeled by Eq. 9. The time-dependent charging and discharging of the membrane can be modeled when the frequency dependence is transformed into a time dependence.

APPENDIX:

THE DEPOLARIZING FACTORS

For spheroids, analytic equations for the depolarizing factors were first derived by Stratton (1941) and more detailed by Stille (1944). The depolarizing factors were extended to the general ellipsoidal shape in 1945, independently by Stoner (1945) and Osborn (1945). Depending on the axial ratio of the ellipsoid the depolarizing factor along a given principal axis can take on values between 0 and 1. For the general ellipsoid with the three principal axes a, b, and c and $a > b > c$, the depolarizing factors n_a, n_b, and n_c depend on the axis ratios $\beta = b/a$ and $\delta = c/a$. They are given by

$$n_a = \frac{\beta\delta}{\sqrt{1-\delta^2\,(1-\beta^2)}}\,(\mathrm{LF}(k,\psi) - \mathrm{LE}(k,\psi)),$$

$$n_b = -n_a + \frac{\beta\delta}{\sqrt{1-\delta^2\,(\beta^2-\delta^2)}}\,\mathrm{LE}(k,\psi) - \frac{\delta^2}{\beta^2-\delta^2},$$

$$n_c = -\frac{\beta\delta}{\sqrt{1-\delta^2\,(\beta^2-\delta^2)}}\,\mathrm{LE}(k,\psi) + \frac{\beta^2}{\beta^2-\delta^2}. \qquad \text{(A1)}$$

LF and LE are the elliptical integrals that are functions of k and Ψ. k and Ψ also depend on the axis ratios according to

$$k = \sqrt{\frac{1-\beta^2}{1-\delta^2}}, \quad \text{and} \quad \psi = \arccos(\delta). \qquad \text{(A2)}$$

The elliptical integrals are then

$$\mathrm{LF}(k,\psi) = \int_0^{\psi} \frac{1}{\sqrt{1-k^2\sin^2\phi}}\,\mathrm{d}\phi,$$

$$\mathrm{LE}(k,\psi) = \int_0^{\psi} \sqrt{1-k^2\sin^2\phi}\,\mathrm{d}\phi. \qquad \text{(A3)}$$

The sum of the depolarizing factors along the three principal axes is always unity ($n_a + n_b + n_c = 1$). For the sum of the relative influential radii from Eq. 4, it follows that $a/a_{infl} + b/b_{infl} + c/c_{infl} = 2$. For numerical values of the depolarizing factors refer to Bernhardt and Pauly (1973) or Fricke (1953).

For oblate and prolate spheroids closed, explicit expressions can be obtained (see, e.g., Stille 1944). For the oblate case ($a < b$) the factor n_a along the symmetry axis a is

$$n_a = \frac{1+e^2}{e^3}\,(e - \arctan e) \quad \text{with} \quad e = \sqrt{(b/a)^2 - 1} \qquad \text{(A4)}$$

and for the prolate case ($a > b$):

$$n_a = \frac{1-e^2}{2e^3}\left(\log\frac{1+e}{1-e} - 2e\right) \quad \text{with} \quad e = \sqrt{1-(b/a)^2} \qquad \text{(A5)}$$

In Eqs. A4 and A5, e stands for the eccentricity of the spheroid. From the sum of the depolarizing factors along the three principal axes being unity, it follows that, for the spheroid, $n_a = 1 - 2n_b$ with a being the symmetry axis. For spheres ($a = b = c$), all factors are 1/3.

We are grateful to Ms. Ch. Mrosek, Ms. S. Gauter, and Mr. S. Lippert for their assistance and to Dr. U. Gimsa and Mr. R. Sleigh for help with the manuscript. Dr. H. P. Schwan, Dr. J. H. Bernhardt, and Dr. P. Marszalek are acknowledged for fruitful discussions on the history of the transmembrane voltage equations.

D.W. is grateful for a grant by Nachwuchsförderungsgesetz des Landes Berlin (NaFöG).

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