Cellular membrane potentials induced by alternating fields

Constantino Grosse*‡ and Herman P. Schwan§

*Instituto de Física, Universidad Nacional de Tucumán, 4000 San Miguel de Tucumán, Argentina; ‡Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina, Buenos Aires, Argentina; and §Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104 USA

ABSTRACT Membrane potentials induced by external alternating fields are usually derived assuming that the membrane is insulating, that the cell has no surface conductance, and that the potentials are everywhere solutions of the Laplace equation. This traditional approach is reexamined taking into account membrane conductance, surface admittance, and space charge effects. We find that whenever the conductivity of the medium outside the cell is low, large corrections are needed. Thus, in most of the cases where cells are manipulated by external fields (pore formation, cell fusion, cell rotation, dielectrophoresis) the field applied to the cell membrane is significantly reduced, sometimes practically abolished. This could have a strong bearing on present theories of pore formation, and of the influence of weak electric fields on membranes.

1. INTRODUCTION

Increasing interest in the effects of alternating electric fields on biological cells and cell membranes has been caused by several recent events:

First, effects of low frequency weak electric fields ($\sim 10^{-4}$ V/m in the medium) on tissues and cells have been reported (1–5). These effects are believed to be caused by primary field interactions with either membrane or glycocalic constituents (3). Recent concern has been expressed about the possible health effects of weak low frequency electric and magnetic fields (6).

Second, effects of comparably strong electric fields (~ 100 V/m in the medium) on cells have been abundantly demonstrated and used for cell manipulation. These effects include cell fusion (7, 8), rotation (9–12), alignment (13), dielectrophoresis, i.e., movement in an inhomogeneous field (14, 15), pore formation in the membrane (16), levitation (17), and cell deformation (18, 19).

The understanding of the interaction of "weak" fields is rather poor and remains controversial. Induced fields have been quoted to be too weak to cause membrane responses (20). More recent efforts have outlined under what circumstances membrane responses to weak fields may be possible in the presence of thermal noise limitations (21).

The theoretical understanding of the "strong" effects is fairly advanced. It is based on the understanding of the dielectric properties of biological cell suspensions (22–24) and cells (25). Mechanisms to account for observed dielectric properties and field interactions require a detailed knowledge of the potential distribution induced by the external imposed field.

Understanding of membrane responses requires knowledge of the induced membrane potential. Previous membrane potential theory was based on the applicability of the Laplace equation. The effects of space charges were only considered in the unperturbed case, i.e., the membrane potential as it exists on its own and caused by the charges of various cell components such as cytoplasmic proteins, spectrin, membrane molecular components, and glycocalyx (26–29), or centering the attention on the dielectric properties of the cell suspension (30).

In the following we calculate the membrane potentials induced by external alternating electric fields under a variety of circumstances. This includes the effects of membrane conductance, surface conductance, and space charges. Some of our results agree with previous work, but the effects of space charge and surface compartment are new. These effects reduce the membrane potential significantly if the cell is immersed in a weak electrolyte. This is usually the case when cells are manipulated by electric fields in order to avoid undue heating. Thus, previous estimates of induced membrane potentials and potentials causing membrane pore formation are too high.

2. INFLUENCE OF THE CONDUCTANCE OF THE CELL MEMBRANE AND OF THE SURFACE ADMITTANCE

The potential induced by an external field across the membrane of a spherical cell in suspension, is usually calculated using the following expression (31):

$$\Delta U = \frac{3/2 ER\cos\theta}{1 + i\omega RC_m(\rho_i + \rho_a/2)}, \qquad (1)$$

where:

E is the value of the external field in the electrolyte,
R is the radius of the cell,
θ is the polar angle measured with respect to the direction of the field,
ω is the angular frequency of the external field,
$C_m = \epsilon_m/h$ is the capacitance of the membrane per unit area,
ϵ_m is the absolute permittivity of the membrane,
h is the thickness of the membrane,
ρ_i is the resistivity of the interior of the cell,
ρ_a is the resistivity of the electrolyte.

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Thus, the membrane potential is independent of frequency until ω becomes comparable with the reciprocal of the time constant:

$$\tau = RC_m(\rho_i + \rho_a/2). \quad (2)$$

Under physiological conditions, typical values are of the order $C_m = 10^{-2}$ F/m^2 and $\rho_i, \rho_a = 1$ ohm m. Thus the frequency limit of the low frequency potential ranges for typical cell sizes ($R = 10^{-6}$–10^{-4} m) from the upper KHz to the lower MHz range.

The derivation of Eq. 1 is based on a series of assumptions: (*a*) The thickness of the membrane is much smaller than the radius of the cell: $h \ll R$. (*b*) The effect of the high frequency Maxwell-Wagner relaxation is neglected. (*c*) The membrane is perfectly insulating. (*d*) The conductivity of the electrolyte close to the cell membrane has the same value as far away from the cell. (*e*) The potential is everywhere a solution of the Laplace equation. All these assumptions are generally well justified for cells in aqueous electrolytes under physiological conditions. Nevertheless, assumptions *c*–*e* are no longer valid in the case of very weak electrolytes as those used in pore formation or electrorotation experiments.

In this section we shall still neglect any effects due to diffusion: for low conductivities, surface charges extend away from the interfaces forming volume charge distributions, so that the potential must be a solution of the Poisson equation.

We shall consider that the cell has a finite membrane conductance per unit area G_m, and that it is surrounded by a surface compartment characterized by a surface admittance per unit area Y_s. G_m is related to radial currents flowing through the membrane, while Y_s is related to tangential currents flowing around the cell.

The surface compartment is characterized by an enhancement of the ion density in the close neighborhood of the cell, which may be due to a number of different reasons. The most important is probably the layer of glycocalix, or wall, surrounding the cell which are rich in fixed charges which attract ions from the bulk electrolyte. Another contribution has its origin in the net charge which the interior of a living cell must have in order to establish its membrane potential (typically of the order of 0.1 V). The radial field of this charge attracts ions from the bulk electrolyte. A radial field is also created by hydrophilic head groups of the lipid molecules which make the membrane and dissociate on the outer cell boundary.

The membrane potential can be calculated in a straightforward fashion by solving Laplace's equation in the four media of the system composed by the cell interior, the membrane, the conducting shell, and the unbounded external medium (Fig. 1).

The dielectric properties of each medium are characterized by a complex conductivity $K = \sigma + i\omega\epsilon$, where $\sigma = 1/\rho$ is the conductivity. For the membrane:

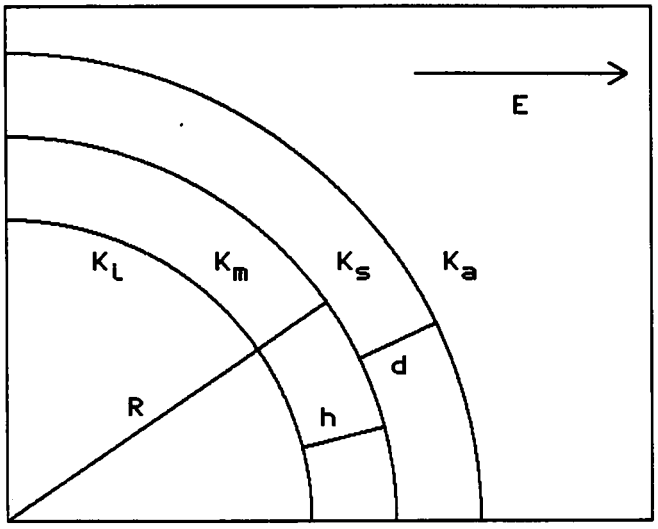

FIGURE 1 Schematic representation of the model used to represent a cell suspended in an electrolyte.

$$K_m = G_m h + i\omega C_m h, \quad (3)$$

while, for the conducting shell:

$$K_s = Y_s/d. \quad (4)$$

The potential in the four regions has the general form:

$$U_j = -A_j r \cos\theta + B_j R^3 \frac{\cos\theta}{r^2}, \quad (5)$$

with $B_i = 0$, and $A_a = E$.

The expressions for the coefficients A_j and B_j, determined from boundary conditions on the interfaces, are given in Appendix A. The membrane potential is calculated as:

$$\Delta U = U_m(R - h) - U_m(R)$$
$$= \left[A_m x \times B_m \frac{3\gamma - x}{1 - 3\gamma}\right] R\cos\theta, \quad (6)$$

where:

$$x = h/R \quad (7)$$
$$1 - 3\gamma = (1 - h/R)^3. \quad (8)$$

The general result for $h \ll R$ and $d \ll R$ is given in Appendix A, Eq. A17. The frequency dependence of this expression is represented in Figs. 2 and 3, where it is compared with Eq. 1.

A significant overall decrease of the membrane potential is observed, as well as the appearance of a small high frequency relaxation. This relaxation exists even in the simplest case corresponding to Eq. 1, but was neglected in the derivation of this equation. It has generally a very small amplitude and a relaxation time approximately equal to (32):

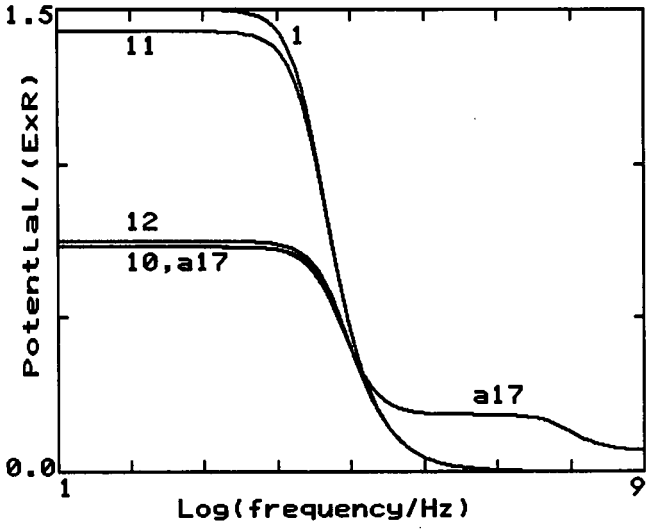

FIGURE 2 Influence of the membrane conductance and the surface conductance on the membrane potential, according to different theoretical expressions. The numbers above the curves correspond to equation numbers in the text. The values used in the calculations are: $\epsilon_i = \epsilon_a = 80 \times 8.85 \times 10^{-12}$ F/m, $\rho_i = 1$ ohm m, $\rho_a = 1{,}000$ ohm m, $C_m = 10^{-2}$ F/m^2, $G_m = 100$ S/m^2, $G_s = 10^{-9}$, S, $h = 10^{-8}$ m, $R = 10^{-6}$ m.

$$\tau_h = (\epsilon_i + 2\epsilon_a)/(\sigma_i + 2\sigma_a). \quad (9)$$

For cells under physiological conditions, and even for cells in low conductivity electrolytes (as long as they retain their internal ion concentration), the value of τ_h is many orders of magnitude smaller than the main relaxation time given in Eq. 2.

Neglecting the high frequency relaxation, and considering that $RG_m\rho_i \ll 1$, and that the surface admittance is real: $Y_s = G_s$, Eq. A17 reduces to:

$$\Delta U = \frac{3/2ER\cos\theta}{1 + \frac{\rho_a G_s}{R} + R(G_m + i\omega C_m)\left[\rho_i + \frac{\rho_a}{2} + \frac{\rho_i\rho_a G_s}{R}\right]}. \quad (10)$$

This result is a generalization of Eq. 1 to the case of cells with a nonvanishing membrane conductance, and with a surface conductance. It shows that the membrane potential decreases rapidly with both G_m and G_s when the resistivity of the electrolyte is high. The frequency behavior of this equation is also represented in Figs. 2 and 3.

In the case when there is no surface conductance, Eq. 10 simplifies to:

$$\Delta U = \frac{3/2ER\cos\theta}{1 + R(G_m + i\omega C_m)\left[\rho_i + \frac{\rho_a}{2}\right]}. \quad (11)$$

Since values of G_m are typically in the range 10^{-1} to 10^3 S/m^2 (33), the effect of the membrane conductance is usually small under physiological conditions. However, if the resistivity of the medium is high and the cell size is large, then $RG_m(\rho_i + \rho_a/2)$ is no longer small compared with 1, and time constant as well as membrane potential decrease with increasing membrane conductance. Consider a cell with a radius of 10^{-5} m and a medium resistivity of 10^3 ohm m as often used in electrical cell manipulation experiments. Then, for $G_m = 100$ S/m^2, the membrane conductance reduces the membrane potential by a factor of 2.

In the case when the membrane conductance can be neglected, but there is a surface conductance, Eq. 10 reduces to:

$$\Delta U = \frac{3/2ER\cos\theta}{1 + \frac{\rho_a G_s}{R} + i\omega RC_m\left[\rho_i + \frac{\rho_a}{2} + \frac{\rho_i\rho_a G_s}{R}\right]}. \quad (12)$$

This expression shows that the membrane potential can be strongly diminished by the presence of a surface conductance. The determining factor of this change is the quotient $\rho_a G_s/R$. Therefore, the effect is more pronounced the smaller the size of the cell is, and the higher is the resistivity of the electrolyte.

While the coefficients G_s/R and RG_m do not enter Eq. 12 and 11, respectively, in the same fashion, their influence on the membrane potential is analogous. For $\rho_a \gg \rho_i$ and as long as $\rho_i G_s/R \ll 1$ (which is true in all practical cases), we find that if:

$$G_s/G_m = R^2/2, \quad (13)$$

then membrane and surface conductances have equal effects on the membrane potential. This equivalence is identical to the effect of membrane and surface conductances on the total cell conductance, or on the conductance of a suspension of cells (34). For example, if we assume surface conductance and membrane conductance values of the order of 10^{-9} S, and 10 S/m^2, respectively, Eq. 13 holds for $R = 14\ 10^{-6}$ m. This illustrates

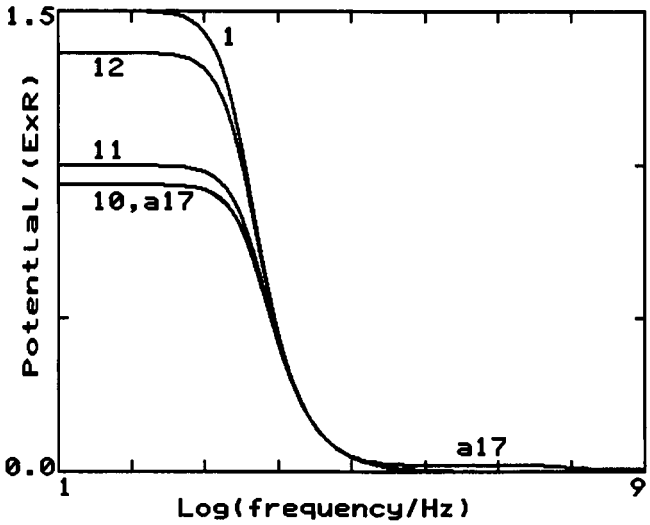

FIGURE 3 Same as in Fig. 2, but for a bigger cell: $R = 10^{-5}$ m.

that for typical cell sizes, both surface and membrane conductances may affect the membrane potential. Thus, at least from an external observer's point of view, it is impossible to separate membrane and surface contributions without additional independent information.

Figs. 2 and 3 illustrate how these two contributions depend on the cell size: the decrease of ΔU is mainly due to surface conductance for a small cell. On the contrary, for a bigger cell, this decrease corresponds almost entirely to the membrane conductance.

A value of 10^{-8} S for the surface conductance is not infrequently noted for cells (35). However, values tenfold larger have been reported for the conductivity of the walls surrounding some cells. Under such circumstances, the membrane potential is entirely abolished since the cell is effectively shielded by the highly conducting external wall or glycocalix.

3. INFLUENCE OF THE DIFFUSION OF IONS IN THE ELECTROLYTE

Symmetric limit

We now take into account the effect of the diffusion of ions in the electrolyte surrounding the cell. In order to be able to determine the potentials with the help of Laplace's equation and boundary conditions on the interfaces, the volume charge density must vanish everywhere except on the boundaries themselves. This condition is never rigorously true due to the diffusion of ions which spread out away from the boundaries a distance of the order of the Debye screening length. The reciprocal of this length in a symmetric medium with absolute permittivity ϵ and conductivity σ has the value:

$$\chi = \sqrt{\frac{\sigma}{\epsilon D}}, \tag{14}$$

where D is the diffusion coefficient (typically of the order of $2\ 10^{-9}$ m^2/s for small ions).

Laplace's equation can nevertheless be used as long as the thickness of the diffusion layer is much smaller than the smallest characteristic length in the system: the thickness of the cell membrane. This condition is usually fulfilled both in the interior of the cell and in the external medium since, under physiological conditions, the Debye length is smaller than 10^{-9} m.

However, in many experimental situations in which cells are subjected to strong fields, the conductivity of the electrolyte needs to be very low to avoid heating. The Debye length in the external medium increases correspondingly, and the product $\chi_a h$ approaches unity.

If the enhancement of the ion density in the surface compartment were only due to radial fields of charges located in the interior of the cell and on its surface, it would be necessary to take into account the diffusive nature of this layer and treat this compartment as part of the external medium. The real situation is quite different: the enhanced conductivity is mainly due to an excess of free ions moving among the charges fixed on the glycocalix layer, or wall, which have characteristic thicknesses nondependent on the value of the Debye screening length. Because of this, we shall continue treating the surface compartment separately from the bulk electrolyte, and consider that all field induced charge densities are located outside it.

As for the internal medium, the equilibrium ion concentration depends on the particular cell considered: some retain their high internal conductivity, while others freely equilibrate internal and external ion concentrations. In what follows, and for sake of simplicity, we shall limit our discussion to the first case in which the inequality $\chi_i h \gg 1$ holds.

The system to be considered corresponds to the one represented in Fig. 1, except for the assumption that the conducting shell surrounding the cell is thin, and that its surface admittance is real. The general expressions for the potentials in the internal medium and in the membrane are the same as in the preceding case. On the contrary, the potential in the external medium must be now the solution of the Poisson rather than the Laplace equation:

$$\nabla^2 U_a = -\frac{\mu e}{\epsilon_a}, \tag{15}$$

where μe is the charge density induced by the external field. It's expression is (36, 37):

$$\mu e = Q \exp(-q\chi_a r)\left[\frac{1}{q\chi_a r} + \frac{1}{(q\chi_a r)^2}\right]\cos\theta, \tag{16}$$

where e is the absolute value of the charge of the ions, Q is a coefficient to be determined from boundary conditions, and

$$q^2 = 1 + i\omega\epsilon_a/\sigma_a. \tag{17}$$

The general form for the potential in the electrolyte becomes:

$$U_a = -Er\cos\theta + B_a R^3 \frac{\cos\theta}{r^2} - \frac{\mu e}{\epsilon_a \chi_a^2 q^2}. \tag{18}$$

The general expression of the membrane potential for $h \ll R$ is deduced in Appendix B, Eq. B14. The behavior of this expression is represented in Figs. 4 and 5. It can be seen that diffusion further decreases the membrane potential.

For $\omega = 0$, and considering that $RG_m\rho_i \ll 1$, Eq. B14 reduces to:

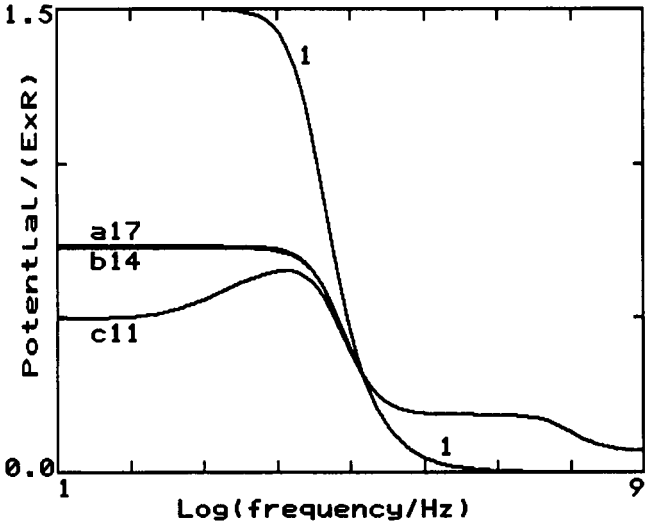

FIGURE 4 Influence of the diffusion of ions in the electrolyte on the membrane potential. Same values as in Fig. 2.

$$\Delta U = \frac{3/2ER\cos\theta}{1 + \frac{\rho_a G_s}{R} + RG_m\left[\rho_i + \frac{\rho_a}{2} + \frac{\rho_i\rho_a G_s}{R}\right] + \frac{RC_m - \epsilon_a\rho_a(RG_m + 2G_s/R)}{\epsilon_a[G(0) + Z^2\rho_a G_s/R]}}, \quad (19)$$

where

$$G(0) = \frac{Z^2 + 2Z + 2}{Z + 1} \quad (20)$$

$$Z = \chi_a R. \quad (21)$$

This expression shows that the decrement of the membrane potential due to diffusion is especially important when the surface conductance is low. This can be appreciated comparing Figs. 4 and 5.

The reason why diffusion decreases the value of the membrane potential becomes clear in the limiting form of Eq. 19 corresponding to $Z \gg 1$, and considering that there is no conducting layer surrounding the cell:

$$\Delta U(0) = 3/2ER\cos\theta\,\frac{\epsilon_a\chi_a}{\epsilon_a\chi_a + \epsilon_m/h} = U\frac{C_d}{C_d + C_m}. \quad (22)$$

In the last tem, U is the total static potential drop across the membrane together with the diffusion layer, Eq. 1, while C_d is the capacitance of the diffusion layer which has a permittivity ϵ_a and a thickness $1/\chi_a$. This expression shows that the capacitance of the membrane is in series with the capacitance of the diffusion layer. For high resistivity electrolytes C_d decreases and becomes comparable to C_m (for ρ_a = 1,000 ohm m and $D = 2\ 10^{-9}$ m^2/s, $C_d = 2\ 10^{-2}$ F/m^2).

4. INFLUENCE OF THE DIFFUSION OF IONS IN THE ELECTROLYTE

Asymmetric limit

In the preceding section we tacitly assumed that the conducting layer is symmetric with respect to the two types of ions. This is not necessarily the case, since the charges fixed in the glycocalix, or the cell wall, are mainly of a single sign. Furthermore, the radial field of the cell due to charges in its interior and its surface, attracts ions of a single sign: the counterions. On the contrary, coions, are repelled from the vicinity of the membrane.

This sort of asymmetry strongly influences the dielectric behavior of suspensions of charged particles (38–44). It leads to a very strong increase of the permittivity at audio frequencies: the α relaxation. We shall now consider its effect on the membrane potential.

Any real cell is neither totally symmetric nor asymmetric: its conducting layer is mainly composed of ions of a single sign, but with some fraction made of ions with the opposite sign. Instead of dealing with this general case which includes an unknown parameter: the degree of asymmetry, we shall now consider a totally asymmetric system. The results obtained, combined with those of the preceding section, will provide bounds for any real situation.

Except for the asymmetry, the system to be considered is the same as in the preceding section. The general expressions for the potential in the three regions are also the same (Eqs. 5 and 18). The only change is in the field induced variations of the ion densities of positive and negative ions, whose magnitude need not be equal anymore:

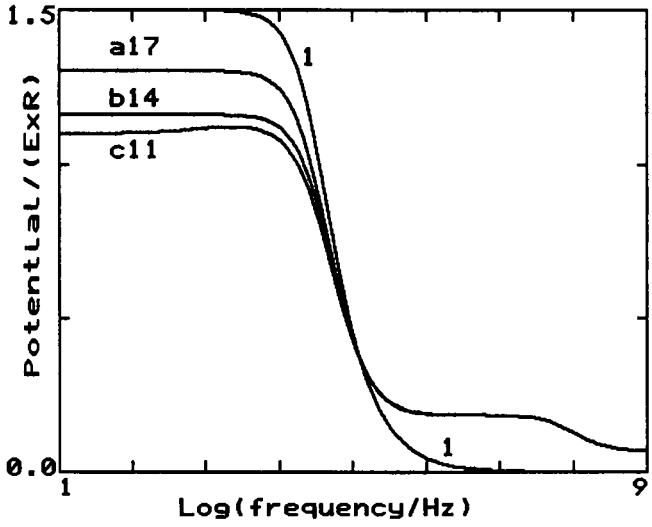

FIGURE 5 Same as in Fig. 4, but for a lower surface conductance: $G_s = 10^{-10}$ s.

$$\mu^{\pm} = \frac{\eta \pm \mu}{2}, \tag{23}$$

where η is the total change of the ion density (36, 37):

$$\eta e = P \exp(-p\chi_a r)\left[\frac{1}{p\chi_a r} + \frac{1}{(p\chi_a r)^2}\right]\cos\theta. \tag{24}$$

P is a coefficient to be determined from boundary conditions, and

$$p^2 = i\omega\epsilon_a/\sigma_a. \tag{25}$$

In the symmetric case η reduces to zero.

The general expression of the membrane potential for $h \ll R$ is deduced in Appendix C, Eq. C11. The main qualitative difference between this expression and all the preceding ones, is that it is no longer a monotonic function of the frequency. At low frequencies corresponding to the α relaxation, the membrane potential increases due to the frequency dependence of H. For $\omega = 0$, and considering that $RG_m\rho_i \ll 1$, it reduces to:

$$\Delta U = \frac{3/2ER\cos\theta}{1 + \frac{\rho_a G_s}{R} + RG_m\left[\rho_i + \frac{\rho_a}{2} + \frac{\rho_i\rho_a G_s}{R}\right] + \frac{RC_m - \epsilon_a\rho_a(RG_m + 2G_s/R - Z^2\rho_a G_s^2/R^2)}{\epsilon_a[G(0) + Z^2\rho_a G_s/R]}}. \tag{26}$$

This expression, compared with the corresponding result for the symmetric case Eq. 19, shows that asymmetry always reduces the static value of the membrane potential. Furthermore, the effect of diffusion increases now with the surface conductance as can be seen comparing Figs. 4 and 5. For $G_s = 0$, Eqs. 19 and 26 become equal, while for $G_s \to \infty$, the membrane potential in the asymmetric limit has half the value of ΔU corresponding to the symmetric case.

The reason why diffusion decreases the value of ΔU is different in the two limits. In the symmetric one, it happens because the total potential drop occurs over both the membrane and the Debye layer. In the asymmetric case, the charge cloud further modifies the total potential drop which occurs across the cell. This effect can be easily appreciated comparing the dipolar field coefficient given in Eqs. B9 and C7. For $\omega = 0$, and in the limit $G_s \to \infty$, these equations lead to $B_a = E$ in the symmetric case, and to $B_a = E/4$ in the asymmetric one.

5. DISCUSSION

Measurements of the dielectric properties of suspensions have shown the surface conductance to be frequency dependent. This necessitates the existence of a corresponding surface capacitance, as may be predicted from the linear Kramers-Kronig relationships. It is therefore more appropriate to introduce the concept of a surface admittance, which is composed of a parallel combination of frequency dependent surface conductance and capacitance (38).

The static (DC) contribution to the surface conductance, as well as the additional contribution at higher frequencies, have been explained by the additional ion concentration near the charged surface, and the movement of these counterions under the influence of an alternating field. This relaxational behavior has been the subject of many experimental and theoretical studies (39–43) motivated by the unusual dispersive dielectric properties of particle and cell suspensions observed at low frequencies (23, 38). Further support of the usefulness of the concept of a surface admittance is provided by the recognition that cells are surrounded by a surface environment rich in fixed charges and extending over distances comparable to, or larger than, the Debye length (glycocalix). Even thicker envelopes exist for cells surrounded by a cell wall.

The frequency dependence of the surface admittance has been investigated from measurements of the dielectric properties of cell suspensions (23, 45, 46). They typically occur at $\sim$100 Hz, and variations of 10^{-8} S, and more, in the surface conductance are estimated. Independent confirmation on the order of 10^{-8} S surface conductance values has been provided using electrorotation measurements (35).

These changes must be taken into account when the membrane potential is calculated. Our results show, however, that such calculations are usually not justified. The problem stems from the possibility that different physical mechanisms could determine a similar frequency dependence of the surface admittance. In particular, we shall now show that when this dependence is due to diffusion effects as suggested above, the very concept of a frequency dependent surface admittance should be avoided in the calculation of the membrane potential.

Let us assume that a cell can be well represented by a model which includes a frequency independent surface conductance and diffusion effects, as considered in sections 3 or 4. The dielectric behavior of the suspension can then be easily deduced (42) from the dipolar field coefficient B_a, Eq. B9 or C7. Fitting this theoretical behavior to the experimental data, the value of the frequency independent surface conductance G_s could be finally obtained.

This small cell could be also represented by a frequency dependent surface admittance, as considered in section 2. The dielectric behavior of the suspension, deduced now from the dipolar coefficient A12 and compared with the experimental data, would then lead to the function $Y_s(\omega)$.

Therefore, if the frequency dependence of the surface admittance were solely due to diffusion effects, this dependence could be easily obtained by equating the dipo-

lar coefficient, Eq. A12, with those given in Eqs. B9 or C7. In doing this, the value of K_s in Eq. A12 should be replaced using Eq. 4, and the limit $d \to 0$ should be taken.

The expression for $Y_s(\omega)$ so obtained would reproduce the dielectric behavior of the suspension. Nevertheless, this same expression should not be used to calculate the membrane potential with the help of Eq. A17. The reason for this is the following.

Since by hypothesis we are considering a cell which is well represented by a model which includes a frequency independent surface conductance and diffusion effects, its membrane potential should be given by Eq. B14 or C11. If we try to reproduce this membrane potential using a cell model with a frequency dependent surface admittance, the expression for this surface admittance should be obtained equating Eq. A17 to Eq. B14 or C11. But the function $Y_s(\omega)$ so obtained would be different from the one deduced equating the dipolar coefficients, as can easily be seen by simply comparing the way $Y_s(\omega)$ and G_s enter in the corresponding equations.

The expressions for $Y_s(\omega) = G_s(\omega) + i\omega C_s(\omega)$ deduced equating dipolar coefficients or membrane potentials, are only equal for very high frequencies, when $G_s(\omega)$ tends to G_s. In the static limit, the general results only have a simple form for big cells ($\chi_a R \gg 1$), and in the limit of high surface conductances ($G_s \gg R\sigma_a$):

(a) In the symmetric limit:

$G_s(0) = G_s \quad C_s(0) = -4\epsilon_a G_s/(\sigma_a Z^2)$ from dipolar coefficients

$G_s(0) = G_s \quad C_s(0) = \epsilon_a R/Z^2$ from membrane potentials.

(b) In the asymmetric limit:

$G_s(0) = R\sigma_a \quad C_s(0) = RZ^2\epsilon_a/2$ from dipolar coefficients

$G_s(0) = 2G_s \quad C_s(0) = -\epsilon_a Z^2 G_s/(4\sigma_a)$ from membrane potentials.

In the symmetric limit, the surface admittance changes very little with frequency. In the asymmetric one, the real part of the surface admittance becomes much lower than G_s when deduced by equating dipolar coefficients (38), while it increases to twice the value of G_s when the membrane potentials are equated.

This difference clearly shows that a frequency dependent surface admittance deduced from dielectric measurements should never be used in the calculation of the membrane potential. The knowledge of this potential requires a more detailed representation of the cell, including at least diffusion effects.

However, the sole inclusion of these effects, as done in sections 3 and 4, may still not be sufficient in some cases. The high permittivity values of cell suspensions observed at low frequencies are accounted for, according to our results, by large values of the surface capacitance, rather than the surface conductance. Therefore, our treatment might not be enough to explain some observed $G_s(0)$ values of more than 10^{-8} S. For these cases, even more elaborate models, possibly including extended charge distributions in the glycocalix, might be needed.

6. CONCLUSION

Membrane potentials induced by external alternating fields are usually derived from a Laplacian treatment, considering a cell surrounded by an insulating membrane. The primary intention of this article is to examine the assumptions underlying this traditional approach. We find that under circumstances of practical interest large corrections are needed, in particular, whenever the medium outside the cell has a low conductivity. They include corrections by inclusion of membrane conductance, surface admittance, and space charge effects.

Thus, in many cases where cells are manipulated by external fields in order to achieve pore formation, cell fusion, cell rotation, or levitation, the field applied to the cell membrane proper is significantly reduced, sometimes even entirely abolished. It appears to us, that this could significantly affect present theories about pore formation in cell membranes. Also, suggestions about the influence of "weak" electrical fields on membranes need to be reexamined.

APPENDIX A

The unknown constants A_i, A_m, B_m, A_s, B_s, and B_a, can be determined from boundary conditions on the three interfaces:

(a) *Continuity of the potential:*

$$U_i(R-h) = U_m(R-h) \tag{A1}$$

$$U_m(R) = U_s(R) \tag{A2}$$

$$U_s(R+d) = U_a(R+d) \tag{A3}$$

(b) *Continuity of the normal component of the complex current density:*

$$-K_i \frac{\partial U_i}{\partial r}\bigg|_{R-h} = -K_m \frac{\partial U_m}{\partial r}\bigg|_{R-h} \tag{A4}$$

$$-K_m \frac{\partial U_m}{\partial r}\bigg|_{R} = -K_s \frac{\partial U_s}{\partial r}\bigg|_{R} \tag{A5}$$

$$-K_s \frac{\partial U_s}{\partial r}\bigg|_{R+d} = -K_a \frac{\partial U_a}{\partial r}\bigg|_{R+d}. \tag{A6}$$

The solution for the unknown coefficients is:

$$A_i = \frac{3K_aK_sK_m(1+3\delta)E}{\text{Den2}} \tag{A7}$$

$$A_m = \frac{K_aK_s(K_i+2K_m)(1+3\delta)E}{\text{Den2}} \tag{A8}$$

$$B_m = \frac{K_aK_s(K_i-K_m)(1-3\gamma)(1+3\delta)E}{\text{Den2}} \tag{A9}$$

$$A_s = \frac{K_a[K_m(K_i + 2K_s) + 2\gamma(K_i - K_m)(K_s - K_m)](1 + 3\delta)E}{\text{Den2}} \tag{A10}$$

$$B_s = \frac{K_a[K_m(K_i - K_s) - \gamma(K_i - K_m)(K_s + 2K_m)](1 + 3\delta)E}{\text{Den2}} \tag{A11}$$

$$B_a = \frac{K_s[K_m(K_i - K_a) - \gamma(K_i - K_m)(K_a + 2K_m)] + \delta(K_s - K_a)[K_m(K_i + 2K_s) - 2\gamma(K_i - K_m)(K_m - K_s)]}{\text{Den 2}}(1 + 3\delta)E, \tag{A12}$$

where:

$$\text{Den2} = K_s[K_m(K_i + 2K_a) - 2\gamma(K_i - K_m)(K_m - K_a)] + \delta(K_s + 2K_a)[K_m(K_i + 2K_s) - 2\gamma(K_i - K_m)(K_m - K_s)] \tag{A13}$$

$$1 + 3\delta = (1 + d/R)^3. \tag{A14}$$

The expression for the membrane potential, Eq. 6, becomes:

$$\Delta U = \frac{3K_sK_a[xK_m + \gamma(K_i - K_m)]ER\cos\theta}{\text{Den2}} \tag{A15}$$

For $h \ll R$, $d \ll R$, and without making any assumptions regarding the values of the different K's, this expression reduces to:

$$\Delta U = \frac{3K_sK_a(K_i + xK_m)Eh\cos\theta}{K_s[K_m(K_i + 2K_a + 2yK_s) + 2x(K_m^2 + K_iK_a + yK_iK_s)] + 2yK_mK_a(K_i + 2xK_m),} \tag{A16}$$

where y is d/R.

We now consider that the conducting layer can be characterized by a surface admittance Y_s, Eq. 4. This leads to the following result for the membrane potential in the limit $d \to 0$:

$$\Delta U = \frac{3K_a(K_i + xK_m)Eh\cos\theta}{K_m(K_i + 2K_a + 2Y_s/R) + 2x(K_m^2 + K_iK_a + K_iY_s/R)}. \tag{A17}$$

APPENDIX B

The unknown constants A_i, A_m, B_m, B_a, and Q can be determined from the following boundary conditions: 1. Continuity of the potential on the two interfaces, Eq. A1 and:

$$U_m(R) = U_a(R). \tag{B1}$$

2. Continuity of the normal component of the complex current density on the inner interface, Eq. A4. 3. Discontinuity of the radial component of the displacement on the outer interface:

$$-\epsilon_a \left.\frac{\partial U_a}{\partial r}\right|_R + \epsilon_m \left.\frac{\partial U_m}{\partial r}\right|_R = \Gamma\cos\theta. \tag{B2}$$

In this expression Γ is the surface charge density induced by the external field in the conducting layer. 4. Continuity equation for the charge density in the conducting layer:

$$-\sigma_a \left.\frac{\partial U_a}{\partial r}\right|_R - eD \left.\frac{\partial \mu}{\partial r}\right|_R + \sigma_m \left.\frac{\partial U_m}{\partial r}\right|_R + 2\frac{G_s}{R}[-A_m + B_m]\cos\theta = -i\omega\Gamma\cos\theta. \tag{B3}$$

The left side of this equation represents the divergence of the current density. The first three terms correspond to the conduction and diffusion currents normal to the conducting layer. The fourth represents the tangential conduction current inside this layer. The right hand side is equal to minus the time derivative of the charge density in the conducting layer. 5. Value of the field induced ion density on the boundary of the conducting layer: This condition is not obvious since ions from the bulk electrolyte can freely exchange with the ions in the conducting layer. In the absence of an applied field, this layer is in equilibrium with its surroundings in a state which is characterized by a ratio of the ion densities in the neighboring regions. Any change in this ratio would lead immediately to a strong diffusive flow of ions tending to reestablish the equilibrium situation. Therefore, an applied field should also preserve this ratio (44, 47):

$$\frac{\mu(R, \theta)e}{N_a} = \frac{\Gamma\cos\theta}{dN_s}, \tag{B4}$$

where N_a and N_s are the equilibrium ion densities in the bulk electrolyte and in the layer, respectively. Assuming that the mobility does not change with the distance to the cell membrane, this condition reduces to:

$$\frac{\mu(R, \theta)e}{\sigma_a} = \frac{\Gamma\cos\theta}{G_s}. \tag{B5}$$

The solution for the unknown coefficients is:

$$A_i = \frac{3K_aK_mE}{\text{Den3}} \tag{B6}$$

$$A_m = \frac{K_a(K_i + 2K_m)E}{\text{Den3}} \tag{B7}$$

$$B_m = \frac{K_a(K_i - K_m)(1 - 3\gamma)E}{\text{Den3}} \tag{B8}$$

$$B_a = \frac{K_m(K_i - K_a + 2G_s/R) - \gamma(K_i - K_m)(K_a + 2K_m - 2G_s/R) - \dfrac{K_i\Delta - 2K_m\epsilon_aG_s/R - 2\gamma(K_i - K_m)(\Delta + \epsilon_aG_s/R)}{\epsilon_a\left[G + \dfrac{Z^2q^2G_s}{\sigma_aR}\right]}}{\text{Den3}}E \tag{B9}$$

$$\text{Den3} = K_m(K_i + 2K_a + 2G_s/R) - 2\gamma(K_i - K_m)(K_m - K_a - G_s/R) + 2\frac{(K_i\Delta - 2K_m\epsilon_a G_s/R) - 2\gamma(K_i - K_m)(\Delta + \epsilon_a G_s/R)}{\epsilon_a\left[G + \dfrac{Z^2q^2G_s}{\sigma_a R}\right]}, \quad \text{(B10)}$$

where:

$$G = -\frac{R}{\mu(R)}\left.\frac{\partial\mu}{\partial r}\right|_R = \frac{(qZ)^2 + 2qZ + 2}{qZ + 1} \quad \text{(B11)}$$

$$\Delta = \epsilon_m\sigma_a - \epsilon_a\sigma_m, \quad \text{(B12)}$$

and Z is given in Eq. 21.

The expression for the membrane potential, Eq. 6, becomes:

$$\Delta U = \frac{3K_a[xK_m + \gamma(K_i - K_m)]ER\cos\theta}{\text{Den3}}. \quad \text{(B13)}$$

For $h \ll R$, and without making any assumptions regarding the values of the different K's, this expression reduces to:

$$\Delta U = \frac{3K_a(K_i + xK_m)Eh\cos\theta}{K_m(K_i + 2K_a + 2G_s/R) + 2x(K_m^2 + K_iK_a + K_iG_s/R)} + 2\frac{(K_i + 2xK_m)\Delta - 2\epsilon_a(K_m + xK_i)G_s/R}{\epsilon_a\left[G + \dfrac{Z^2q^2G_s}{\sigma_a R}\right]}. \quad \text{(B14)}$$

Because of the factor G, which is a function of the frequency, this result cannot be separated into a sum of single time constant relaxations.

APPENDIX C

The unknown constants A_i, A_m, B_m, B_a, Q, and P can be determined from the following boundary conditions. The first four are the same as in the preceding case: 1. Continuity of the potential on the two interfaces, Eqs. A1 and B1. 2. Continuity of the normal component of the complex current density on the inner interface, Eq. A4. 3. Discontinuity of the radial component of the displacement on the outer interface, Eq. B2.

The next two conditions are also the same, but they only apply now to the counterions. For definiteness we shall assume that the counterions are positive. 4. Continuity equation for the radial flow of counterions at the outer interface:

$$-\frac{\sigma_a}{2}\left.\frac{\partial U_a}{\partial r}\right|_R - eD\left.\frac{\partial\mu^+}{\partial r}\right|_R + \frac{\sigma_m}{2}\left.\frac{\partial U_m}{\partial r}\right|_R + 2\,G_s/R[-A_m + B_m]\cos\theta = -i\omega\Gamma\cos\theta \quad \text{(C1)}$$

The conductivities in this expression are divided by 2, because they only represent the flow of ions of a single sign. 5. Value of the field induced counterion density on the boundary of the conducting layer:

$$\frac{\mu^+(R,\theta)e}{\sigma_a/2} = \frac{\Gamma\cos\theta}{G_s}. \quad \text{(C2)}$$

Finally a new condition is added, which is analogous to condition 4 but written for the coions. 6. Continuity equation for the radial flow of coions at the outer interface:

$$-\frac{\sigma_a}{2}\left.\frac{\partial U_a}{\partial r}\right|_R + eD\left.\frac{\partial\mu^-}{\partial r}\right|_R + \frac{\sigma_m}{2}\left.\frac{\partial U_m}{\partial r}\right|_R = 0. \quad \text{(C3)}$$

The radial flow of coions must be continuous since they can neither move along the conducting layer nor modify its ion density.

The solution for the unknown coefficients is:

$$A_i = \frac{3K_aK_mE}{\text{Den4}} \quad \text{(C4)}$$

$$A_m = \frac{K_a(K_i + 2K_m)E}{\text{Den4}} \quad \text{(C5)}$$

$$B_m = \frac{K_a(K_i - K_m)(1 - 3\gamma)E}{\text{Den4}} \quad \text{(C6)}$$

$$B_a = \frac{K_m(K_i - K_a + 2G_s/R) - \gamma(K_i - K_m)(K_a + 2K_m - 2G_s/R) - \dfrac{K_i\Delta - 2K_m\epsilon_aG_s/R - 2\gamma(K_i - K_m)(\Delta + \epsilon_aG_s/R) + \dfrac{\epsilon_aZ^2G_s}{H\sigma_a^2R}\,\text{Num1}}{\epsilon_a\left[G + \dfrac{Z^2q^2G_s}{\sigma_aR} + i\omega\dfrac{\epsilon_aGZ^2G_s}{H\sigma_a^2R}\right]}}{\text{Den4}}E \quad \text{(C7)}$$

$$\text{Den4} = K_m(K_i + 2K_a + 2G_a/R) - 2\gamma(K_i - K_m)(K_m - K_a - G_s/R) + 2\frac{K_i\Delta - 2K_m\epsilon_aG_s/R - 2\gamma(K_i - K_m)(\Delta + \epsilon_aG_s/R) + \dfrac{\epsilon_aZ^2G_s}{H\sigma_a^2R}\,\text{Num1}}{\epsilon_a\left[G + \dfrac{Z^2q^2G_s}{\sigma_aR} + i\omega\dfrac{\epsilon_aGZ^2G_s}{H\sigma_a^2R}\right]}, \quad \text{(C8)}$$

where:

$$H = -\frac{R}{\eta(R)}\left.\frac{\partial\eta}{\partial r}\right|_R = \frac{(pZ)^2 + 2pZ + 2}{pZ + 1} \quad \text{(C9)}$$

$$\text{Num1} = 2\sigma_a G_s/R[K_m + \gamma(K_i - K_m)] + i\omega[K_i - 2\gamma(K_i - K_m)]\Delta. \quad \text{(C10)}$$

The expression for the membrane potential has the same form as in the preceding case, Eq. B13. For $h \ll R$, and without making any assumptions regarding the values of the different K's, this expression reduces to:

$$\Delta U = \frac{3K_a(K_i + xK_m)Eh\cos\theta}{K_m(K_i + 2K_a + 2G_s/R) + 2x(K_m^2 + K_iK_a + K_iG_s/R) + 2\dfrac{(K_i + 2xK_m)\Delta - 2\epsilon_a(K_m + xK_i)G_s/R + \dfrac{\epsilon_a Z^2 G_s}{H\sigma_a^2 R}\text{Num2}}{\epsilon_a\left[G + \dfrac{Z^2q^2G_s}{\sigma_a R} + i\omega\dfrac{\epsilon_a GZ^2G_s}{H\sigma_a^2 R}\right]}}, \quad \text{(C11)}$$

where

$$\text{Num2} = 2\sigma_a G_s/R(K_m + xK_i) + i\omega(K_i + 2xK_m)\Delta. \quad \text{(C12)}$$

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