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Sensitivity of transmembrane voltage induced by applied electric fields—a theoretical analysis

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Abstract

The sensitivity of induced transmembrane voltage to extracellular conductivity, membrane conductivity, cytoplasmic conductivity, cell radius and electric pulse duration has been studied. The study showed that variations of membrane conductivity, cytoplasmic conductivity and cell radius within the ranges of their physiological values do not influence induced transmembrane voltage substantially, provided that extracellular conductivity also corresponds to the physiological conditions, and duration of the electric pulse is in range of 10 μs or longer. However, when extracellular conductivity is reduced to the values typical for a ''low conductivity'' medium, the induced transmembrane voltage decreases considerably, while the charging time of the membrane increases up to the range of 1 ms. This increases the necessary amplitude and duration of electric pulses used for electroporation. In a ''low conductivity'' medium, the induced transmembrane voltage is also much more sensitive to variations in cell radius, membrane conductivity, and cytoplasmic conductivity. Such a medium is used in many in vitro studies of the effects of an electric field upon single cells. Our study shows that in these cases, in order to evaluate the induced transmembrane voltage, it is important to consider the values of conductivities and radii of cells used in the experiment, as well as the duration of the electric pulses used. © 1997 Elsevier Science S.A.

Keywords: Electric field stimulation; Electroporation; Transmembrane voltage; Low conductivity medium; Pulse duration

1. Introduction

Most known biological effects of externally applied electric fields are based on a field-induced change of the transmembrane voltage (also called transmembrane potential, or transmembrane potential difference) [1–4]. This can produce a variety of profound biochemical and physiological responses in cells, tissues, and whole body. When the cell is exposed to high-intensity pulses of an electric field, a supraphysiological transmembrane voltage is induced, causing formation of pores in the membrane and leading to increased membrane permeability. This phenomenon, called electroporation, is used for gene transfection [5], preparation of monoclonal antibodies in immunochemistry [6], electrochemotherapy of tumors [7], etc.

All these applications are based on changes in transmembrane voltage which are induced by external electric fields. It is therefore evident that correct evaluation of the induced transmembrane voltage is of great importance in assessing the effects of applied electric fields on cells.

The analytical calculation of the induced transmembrane voltage $\Delta\Phi_m$ caused by a uniform direct electric field across a homogeneous membrane is based on the assumption that the cell is spherical. This postulation is incorrect for many kinds of cells, such as plated cells, cells in tissues, and rod-shaped bacteria; furthermore, the analytical calculations also do not apply in case of a charged membrane surface (in these cases, a numerical calculation is necessary; see also the comment in Section 4). For a spherical cell with no surface charge, $\Delta\Phi_m$ is calculated by solving the Laplace equation. Taking into

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account the geometric and material properties of the cell and the surrounding medium (Fig. 1), the position-dependent $\Delta\Phi_m$ follows the expression:

$$\Delta\Phi_m(t) = f_s ER\cos\theta\left[1-\exp\left(-\frac{t}{\tau}\right)\right] \tag{1}$$

where E is the strength of the electric field, R is the cell radius, θ is the polar angle measured with respect to the direction of the field, f_s is a function reflecting the electric and dimensional properties of the cell and the surrounding medium (for a course of derivation, contact the authors):

$$f_s = \frac{3\lambda_o\left[3dR^2\lambda_i + (3d^2R - d^3)(\lambda_m - \lambda_i)\right]}{2R^3(\lambda_m + 2\lambda_o)(\lambda_m + \frac{1}{2}\lambda_i) - 2(R-d)^3(\lambda_o - \lambda_m)(\lambda_i - \lambda_m)} \tag{2}$$

and τ is the time constant of the membrane which reads [8]:

$$\tau = \frac{RC_m}{\frac{2\lambda_o\lambda_i}{2\lambda_o + \lambda_i} + \frac{R}{d}\lambda_m} \tag{3}$$

Symbols in Eqs. (2) and (3) are defined in Fig. 1.

In the treatment of induced transmembrane voltage, two simplifications are usually made. Firstly, the membrane is considered to be absolutely insulating. This causes the function f_s to take the form of a constant:

$$\lambda_m = 0 \Rightarrow f_s = \tfrac{3}{2} \tag{4}$$

and Eq. (1) reduces to:

$$\Delta\Phi_m(t) = \tfrac{3}{2}ER\cos\theta\left[1-\exp\left(-\frac{t}{\tau}\right)\right] \tag{5}$$

Secondly, it is often assumed that the time constant of the cell membrane is much smaller than duration of the exposure to the field (i.e., pulse duration). In this case, the exponential time dependence approaches the static asymptotic value:

$$t \gg \tau \Rightarrow \left[1-\exp\left(-\frac{t}{\tau}\right)\right] \to 1 \tag{6}$$

which further simplifies Eq. (5) to yield the well known expression for the induced transmembrane voltage [9]:

$$\Delta\Phi_m = \tfrac{3}{2}ER\cos\theta \tag{7}$$

An experimental evaluation of the induced transmembrane voltage [10] gave significantly lower values of f_s than the theoretical estimation obtained from Eq. (7) (i.e., $f_s = 1.5$). The authors suggested that the derivation should be reexamined without neglecting the membrane conductivity. The same conclusion was also made in at least two other papers [11,12].

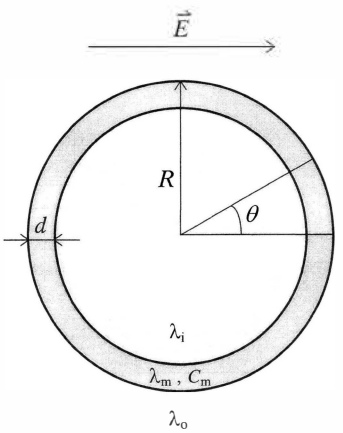

Fig. 1. The model on which the calculations were based. The cell is a sphere with radius of R. It is enclosed by a shell of uniform thickness d (the membrane). The external electric field is homogeneous, and E is the absolute value of the electric field strength vector. Specific conductivities are attributed to spaces occupied by cytoplasm (λ_i), membrane (λ_m) and extracellular medium (λ_o). Membrane capacitance is denoted by C_m.

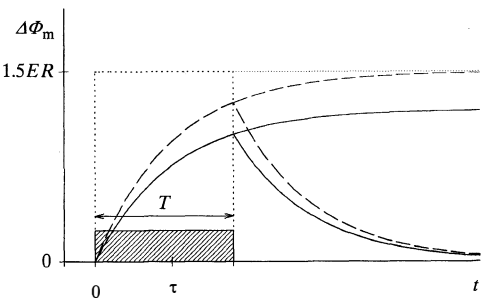

Fig. 2. Time course of the induced transmembrane voltage $\Delta\Phi_m$ at $\theta = 0$. According to the most simplified relation given by Eq. (7), $\Delta\Phi_m$ reaches its peak value immediately after the electric field has been turned on (dotted curve). If the time constant of the membrane is considered (Eq. 5), $\Delta\Phi_m$ keeps gradually approaching the peak value as long as the electric field remains turned on; after the field has been turned off, $\Delta\Phi_m$ gradually decreases (dashed curve, at pulse duration $T = 1.8\tau$). Furthermore, if the membrane is not regarded as absolutely insulating (Eq. 1), f_s is lower than 1.5, thus decreasing the peak value of $\Delta\Phi_m$ (solid curve, at $T = 1.8\tau$ and $f_s = 1.2$).

Eq. (2) shows that f_s is a function of λ_o, λ_m, λ_i, R and d. Also, τ given by Eq. (3) is a function of λ_o, λ_m, λ_i, R, d and C_m. For both f_s and τ, it is obvious that the justification of the described simplifications depends on the actual values of these parameters in a specific experiment (Fig. 2).

This study was designed to theoretically evaluate how strongly the two described simplifications affect the correctness of theoretical prediction of induced transmembrane voltage in different experimental situations.

2. Parameters and calculations

An average physiological value obtained from the literature was attributed to λ_o, λ_m, λ_i, R, d, and C_m (henceforth referred to as standard values of the parameters), as well as its lowest and highest values reported in the literature, defining the range of parameter variation in adequate parametric study (Table 1). In each parametric study, we varied the value of the analyzed parameter within its defined range, while other parameters were set at their standard values. The dependence of f_s and τ on λ_o, λ_m, λ_i, and R was analyzed, while d was kept at its standard value of 5 nm [13], and C_m was set at 0.01 Fm^{-2} [12,14]. Mathematica® software was used for numerical calculation.

For the physiological environment, the ranges of the values for the conductivity parameters are quite narrow, since the ionic composition of every compartment within the organism is well regulated. Thus, the ranges for λ_i and λ_m only reflect the differences between reported measurements (see Table 1). In the case of in vitro experiments, the choice of the extracellular medium is more arbitrary. For this reason, λ_o varies for several orders of magnitude between different experiments reported. The most extreme deviation from physiological conditions occurs when an extracellular medium with a significantly lower conductivity is used. This is often the case in experiments where cells are manipulated by intense

Table 1
Standard values and ranges used in parametric studies

Parameter	Denotation	Standard value	Lower limit	Upper limit
Extracellular medium conductivity	λ_o	2.0×10^{-1} S m^{-1} [a]	5.0×10^{-4} S m^{-1} [b]	2.0 S m^{-1} [c]
Membrane conductivity	λ_m	5.0×10^{-7} S m^{-1} [d,e]	1.0×10^{-8} S m^{-1} [f]	1.2×10^{-6} S m^{-1} [e]
Cytoplasmic conductivity	λ_i	2.0×10^{-1} S m^{-1} [g]	2.0×10^{-2} S m^{-1} [h]	1.0 S m^{-1} [i]
Cell radius	R	10 μm [j]	1 μm [j]	100 μm [j]
Membrane thickness	d	5 nm [j]	–	–
Membrane capacitance	C_m	1.0×10^{-2} F m^{-2} [f,i]	–	–

[a] Set at equal standard value as λ_i, as proposed by Lojewska et al. [11].
[b] Fuhr et al. [15].
[c] Set at 10 times the standard value.
[d] Gascoyne et al. [25].
[e] From Hu et al. [26], using the conversion by Arnold et al. [16].
[f] Hölzel and Lamprecht [14].
[g] Harris and Kell [27].
[h] Set at 1/10 of the standard value.
[i] Grosse and Schwan [12].
[j] Alberts et al. [13].

electric fields (e.g., electroporation, electrofusion, electrorotation, dielectrophoresis), as the use of a low conductivity medium reduces the electric current and thus the heating of the suspension. In measurements of the electrorotational spectra, the use of an extracellular medium with conductivity as low as 5×10^{-4} S m^{-1} was reported [15], which is approximately four hundred times lower in comparison to the physiological extracellular solution. In most similar experiments, conductivity of extracellular medium was in range between 10^{-3} and 10^{-2} S m^{-1} [14,16–21].

3. Results and discussion

3.1. Function f_s

In the first part of the study we tested the sensitivity of f_s to variations in conductivity parameters (λ_o, λ_m, λ_i), and the dimension of the cell (R). For this, the electric pulse duration was assumed to have lasted long enough to justify the simplification given by Eq. (6). Since f_s is proportional to the induced transmembrane voltage $\Delta\Phi_m$, deviation of f_s provided by Eq. (2) from the simplified value of $f_s = 1.5$ indicated the error margin in the evaluation of $\Delta\Phi_m$ by using Eq. (7).

We found that in the case of parameter values which correspond to the physiological conditions, Eqs. (1) and (7) yield very similar values of the induced transmembrane voltage, i.e., f_s evaluated by using Eq. (2) is sufficiently close to the simplified value of $f_s = 1.5$ (the deviation never exceeds the 5% limit). However, if the cells are exposed to the field in a low conductivity medium, f_s is significantly reduced, leading to a considerably lower value of $\Delta\Phi_m$ compared to Eq. (7) (Fig. 3a). With respect to these results, the sensitivity of f_s to other parameters (λ_m, λ_i, and R) was studied at a typical physiological value of the extracellular medium conductivity ($\lambda_o = 2 \times 10^{-1}$ S m^{-1}) and at two values characteristic for a low conductivity medium ($\lambda_o = 5 \times 10^{-3}$ and 1×10^{-3} S m^{-1}).

Fig. 3b to Fig. 3d show the dependence of f_s upon these parameters. The value of f_s generally decreases with increasing membrane conductivity, but the effect is much more distinctive in a low conductivity medium (Fig. 3b). With changes in cytoplasm conductivity within the defined range, f_s practically retains a constant value (under 5% change), however, the use of a low conductivity medium again causes a considerable decrease of f_s (Fig. 3c).

Eqs. (4) and (7) predict that both f_s and $\Delta\Phi_m$ are independent from cytoplasmic conductivity, membrane conductivity and extracellular conductivity, as well as from membrane thickness. This is not the case with the cell radius, as according to

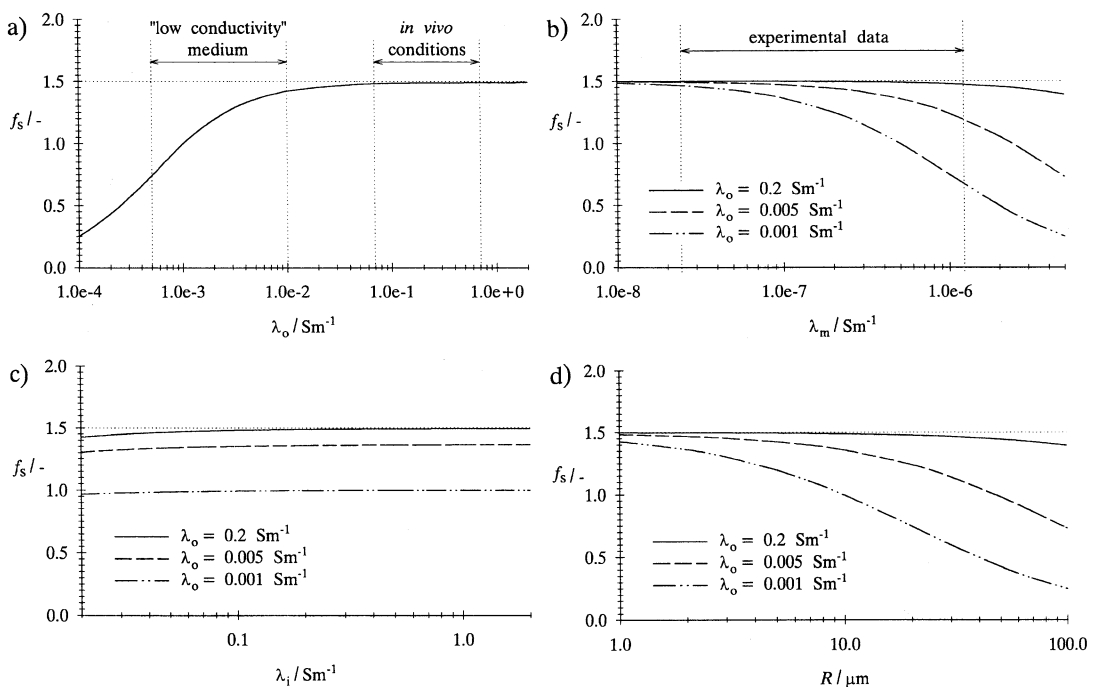

Fig. 3. Dependence of function f_s on electric and dimensional properties of the cell and the surrounding medium. (a) Influence of extracellular medium conductivity upon the value of f_s (at $\lambda_i = 2.0 \times 10^{-1}$ S m^{-1}; $\lambda_m = 5.0 \times 10^{-7}$ S m^{-1}; $R = 10$ μm; $d = 5$ nm). (b) Influence of membrane conductivity upon f_s (at $\lambda_i = 2.0 \times 10^{-1}$ S m^{-1}; $R = 10$ μm; $d = 5$ nm). (c) Influence of cytoplasmic conductivity upon f_s (at $\lambda_m = 5.0 \times 10^{-7}$ S m^{-1}; $R = 10$ μm; $d = 5$ nm). (d) Influence of cell radius upon f_s (at $\lambda_i = 2.0 \times 10^{-1}$ S m^{-1}; $\lambda_m = 5.0 \times 10^{-7}$ S m^{-1}; $d = 5$ nm).

these equations $\Delta\Phi_m$ is proportional to R, whereas f_s itself does not depend upon R. Eq. (2) suggests that the latter presumption is incorrect, since R appears in the expression describing f_s (Fig. 3d). Thus, the linear relationship between transmembrane voltage and cell radius also becomes invalid.

3.2. Time constant (τ)

The second part of the study focused on the sensitivity of the time constant τ to the same parameters (λ_o, λ_m, λ_i, and R). Analysis showed that τ decreases with increasing conductivity of the extracellular medium, indicating that the induced transmembrane voltage follows the change of an external electric field faster if the cell is exposed in a physiological medium. At a pulse duration exceeding $T = 3\tau$, $\Delta\Phi_m$ comes within the 5% range from a static asymptotic value, thus practically justifying the assumption made in Eq. (6).

When all parameter values correspond to physiological conditions, τ is in the microsecond range (Fig. 4a). In this case, pulses lasting over 10 μs assure correctness of a static evaluation of $\Delta\Phi_m$. Again, the situation changes when a "low conductivity" medium is used, as the necessary pulse duration comes into the range 0.1 ms to 1 ms (Fig. 4a).Similar to the f_s analysis, the dependence of τ on λ_m, λ_i, and R was studied at a typical value of a physiological medium and at two "low conductivity" values (Fig. 4b to Fig. 4d). The time constant generally decreases with increasing membrane conductivity (Fig. 4b), as well as with increasing cytoplasm conductivity (Fig. 4c), but in both cases, an extracellular medium with low conductivity shifts the range of τ up at least one order of magnitude. Eq. (3) shows that the time constant is not entirely proportional to the cell radius, since R also appears in the denominator of the expression. For a physiological extracellular medium, deviation from the linear relationship is very small. In a low conductivity medium, the non-linearity becomes much more obvious, while the range of τ again shifts upwards significantly (Fig. 4d).

To make results of the analysis applicable for the design of an electrical protocol for cell electroporation, we plotted curves that indicate the electric field intensity needed to induce 250 mV of change in the transmembrane voltage for different pulse durations of the exposure field. The curves were calculated for four cell radii that cover the dimension range of biological cells. The calculations were performed for exposure in physiological (Fig. 5a) and low conductivity medium (Fig. 5b). In a physiological solution, the required field for inducement of a certain transmembrane voltage is substantially lower than in a low conductive medium and, in general, practically independent on the pulse length. The only situation when a field significantly stronger than predicted by the simplified solution should be used is in the attempt to electroporate a very large cell (e.g., 50 μm) with very short electric pulses (e.g., 1 μs duration).

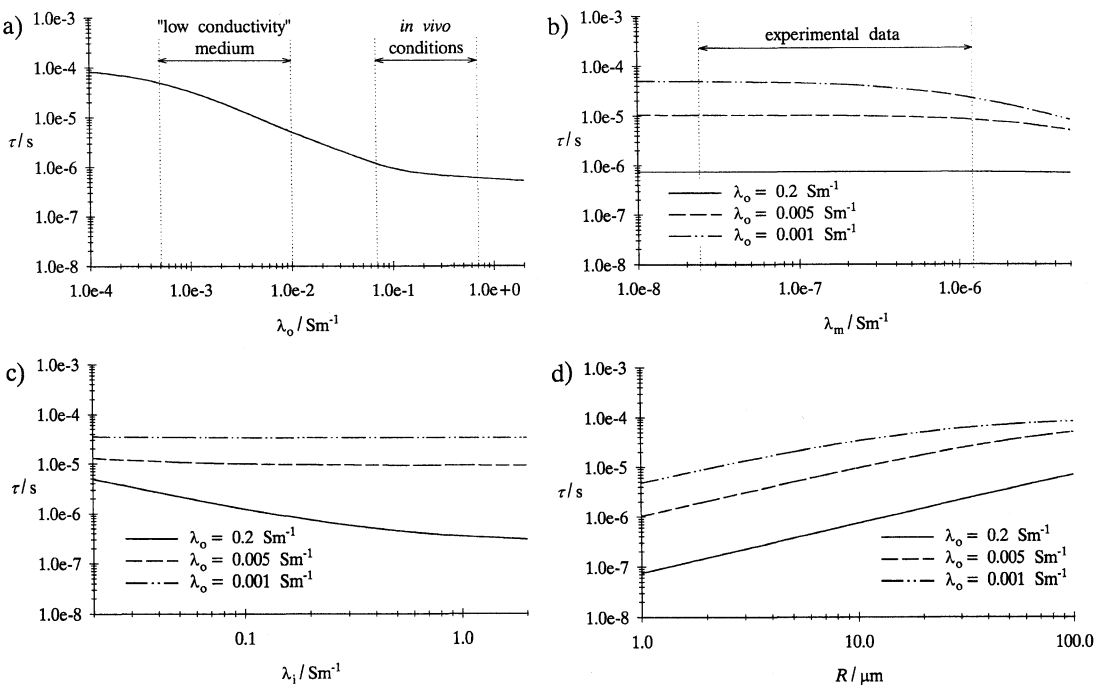

Fig. 4. Dependence of τ on electric and dimensional properties of the cell and the surrounding medium. (a) Influence of extracellular medium conductivity upon the time constant of the cell membrane τ (at $\lambda_i = 2.0\times10^{-1}$ S m^{-1}; $\lambda_m = 5.0\times10^{-7}$ S m^{-1}; $R = 10$ μm; $d = 5$ nm; $C_m = 0.01$ F m^{-2}). (b) Influence of membrane conductivity upon τ (at $\lambda_i = 2.0\times10^{-1}$ S m^{-1}; $R = 10$ μm; $d = 5$ nm; $C_m = 0.01$ F m^{-2}). (c) Influence of cytoplasmic conductivity upon τ (at $\lambda_m = 5.0\times10^{-7}$ S m^{-1}; $R = 10$ μm; $d = 5$ nm; $C_m = 0.01$ F m^{-2}). (d) Influence of cell radius upon τ (at $\lambda_i = 2.0\times10^{-1}$ S m^{-1}; $\lambda_m = 5.0\times10^{-7}$ S m^{-1}; $d = 5$ nm; $C_m = 0.01$ F m^{-2}).

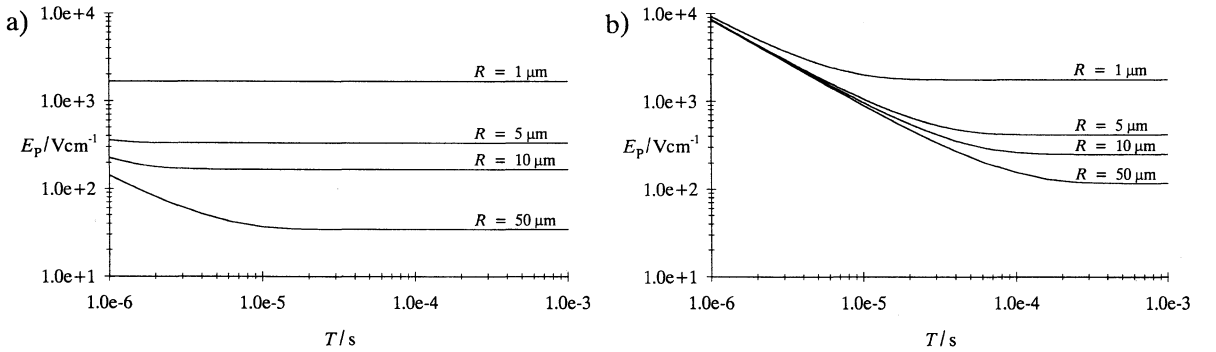

Fig. 5. Minimum electric field strength E_P needed for electroporation as function of pulse duration T (at breakdown transmembrane voltage of 250 mV). (a) In a typical physiological extracellular medium, $\lambda_o = 2.0\times10^{-1}$ S m^{-1} (at $\lambda_i = 2.0\times10^{-1}$ S m^{-1}; $\lambda_m = 5.0\times10^{-7}$ S m^{-1}; $d = 5$ nm). (b) In a low conductivity extracellular medium, $\lambda_o = 1.0\times10^{-3}$ S m^{-1} (at $\lambda_i = 2.0\times10^{-1}$ S m^{-1}; $\lambda_m = 5.0\times10^{-7}$ S m^{-1}; $d = 5$ nm).

In a low conductivity medium, the accurate estimation of the required electrical parameters for electroporation is more complex. Fig. 5b shows that for larger cells electroporated with short pulses or in a high frequency field, the required field intensity could be up to two orders of magnitude higher comparing to the simplified prediction. The results indicate that if pulses shorter than 20 μs are used in a low conductive medium, calculations should be performed without simplification (i.e., calculations should be based on Eq. (1)).

4. Conclusion

The results related to the influence of the extracellular medium conductivity on the induced transmembrane voltage could explain some of the reported differences between the theoretical values referring to Eq. (7) and the experimental results. Thus, in cases where cells are surrounded by a medium of low conductivity ($\lambda_o < 10^{-2}$ S m^{-1}), the approach to the calculation of the transmembrane voltage should be based on the more complex Eq. (1). Electrorotation and dielectrophoresis are certainly among those cases, because a low conductivity medium is required for the attainment of adequate circumstances in order to use these measuring methods. Reduction of the transmembrane voltage in a low conductivity medium is most apparent when large cells (e.g., oocytes) are used (Fig. 3d).

The calculations also show that the use of a low conductivity medium increases the charging time (i.e., time constant) of the cell membrane (Fig. 4a). Therefore, with a low conductivity medium, in order to induce electroporation with microsecond pulses, an amplitude of ~ 10 kV cm^{-1} must be applied (see Fig. 5b). If the pulse duration is in the range of 100 μs–1 ms, a much lower pulse amplitude (e.g., 500 V cm^{-1}) can be used. Besides medium conductivity, cell size is again a relevant factor to be considered in the determination of the necessary pulse duration, as the time constant of the membrane rises almost proportionally to the cell radius (Fig. 4d).

The final part of the study also showed that in a low conductivity medium, the extracellular conductivity, as well as cell the radius, have to be taken into account in order to choose the optimal pulse duration and amplitude for electroporation.

Among other possible reasons for experimentally observed lower values of the induced transmembrane voltage which are not discussed in this paper, the charged cell surface has also been reported to cause major deviations of the transmembrane voltage from values predicted by Eq. (7) [12,22]. It has also been shown that a non-spherical shape of the cell deforms the cosine distribution of the induced transmembrane voltage [23], thus changing the effective area of electroporation [24].

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