

Analysis of the Relationships Between the Parameters of the Electric Pulse Necessary for Cell Electroporation

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ABSTRACT

Theoretical relationships between the parameters of the electric pulse which is necessary for the poration of the cell have been obtained. Analysis has been made for the square wave and sine wave ac electric pulses. At first, the dependences of the fraction of electroporated cells, F_p , on the field strength E_0 at a fixed pulse length τ_i were calculated according for various pulse lengths. Then the relationship between the external electric field strength required to porate the cell, E_p , and the length of the square wave electric pulse, τ_i , was studied. It has been obtained that for long pulses the electric field strength required for the poration of cell membranes increases with decreasing pulse length though very slowly but for short pulses the electric field strength required increases rapidly. The relationships between E_p and the frequency of the applied ac field, ν , for various ac pulse lengths were calculated. Although the electric field strength for electroporation, E_p , is constant for frequencies $\tau_c < 10$ kHz, its value depends on the pulse duration: the longer the pulse the lower the value of E_p . At higher frequencies ($\tau_c > 10$ kHz) E_p is dependent on the frequency of the ac field. Theoretical dependencies are in agreement with experimental ones.

Keywords: Pulse duration, amplitude, frequency, electropermeabilization, erythrocytes

1 INTRODUCTION

The phenomenon of electroporation has the numerous applications in cell biology, biotechnology and medicine. A lot of theoretical studies are devoted to the analysis of the mechanism and kinetics of cell electroporation. However, no general equation that would allow the prediction of the effect of the exposure of a cell to a given electric field pulse was yet derived. Due to this experimental procedures are still optimized empirically only.

Although, on the basis of dye uptake studies in carrot, sugar beet, and wall-rocket protoplasts [1], proposed an equation relating the electric field strength E_0 and the pulse duration τ_i to the fraction of electroporated protoplasts, it was derived empirically by examination of experimental data without using any theoretical model. Thus theoretical analysis of the process of pore formation in a cell, which could provide qualitative relationship between the electric parameters, is still necessary.

Here, using the set of chosen parameters (the "standard cell"), theoretical relationships between the parameters of the electric pulse, which is necessary to porate the cell by square wave, exponential and sine wave ac electric pulses, are obtained. Analysis has been made assuming that pore formation is a random process [2].

2 THEORY

The formation of a metastable hydrophilic pore can be considered as a one-step process. In such a case, an equation correlating the fraction of electroporated cells, F_p , with the parameters of an electric treatment can be derived. In its simplest form, this expression can be written as [2]:

$$F_p(E_0, \tau_i) = 1 - \exp[-k_{f0}(E_0)\tau_i] \quad (1)$$

where E_0 is the electric field strength, τ_i is the duration of a square-wave electric pulse, and $k_{f0}(E_0)$ is the initial rate of pore formation in a cell. In the case of electroporation of a spherical cell, this rate can be calculated by [2]:

$$k_{f0}(E_0) = D \int_{-1}^1 \exp[G(1.5E_0ay - \Delta\Phi_0)^2] dy \quad (2)$$

where

$$D = \frac{2\pi va^2}{a_i} \exp\left[-\frac{\Delta W_f(0)}{k_B T}\right]$$

$$G = \pi C_m \frac{(\epsilon_w / \epsilon_m - 1)}{2k_B T} r_*^2 \quad (3)$$

$$y = \cos \theta.$$

Here a is the cell radius and $\Delta\Phi_0$ is the resting potential.

Equations (1)-(3) describe the following important characteristics of the cell electroporation process: (1) the dependence of the probability that a cell is porated, F_p , on the pulse duration τ_i at a fixed electric field strength E_0 (distribution function of cell poration times, $F_p(\tau_i)$) and (2) the dependence of F_p on the field strength E_0 at a fixed pulse length τ_i (distribution function of cell poration field strengths, $F_p(E_0)$).

Using Equations (2)-(3), which describe the rate of pore formation $k_{f0}(\Delta\Phi_m)$ in the spherical cell exposed to an

external electric field, one can obtain theoretical relationships between the parameters of the electric pulse as a result of the exposure to which a definite number of pores, n , has appeared in the cell:

$$\int_0^{\infty} k_{f0}(E_0, t) dt = n. \quad (4)$$

This equation is valid for any type of an electric treatment (exponential, square-wave, or other pulses). It can be expected that such a dependence represents, to some extent, the relationships between the parameters of the electric treatment resulting in cell electroporation. For example, the relationships between electric field strength, $E_{0.5}$, necessary for the poration of 50 % of the cells and the duration or frequency of electric field pulses, which are usually determined experimentally, could be estimated by using Eqn. (4).

However, when using Eqn. (1)-(4), the time dependences of the transmembrane potential has to be taken into account. The time-dependence of the transmembrane potential generated by a square-wave electric pulse across the non-conducting membrane of a spherical cell is (Fig. 3A) [3-5]:

$$\Delta\Phi_g(t) = \frac{3}{2} E_0 a \left[1 - \exp\left(-\frac{t}{\tau_c}\right) \right], \quad (5)$$

Here τ_c is the time constant of the membrane charging process, which can be expressed as [4,6]:

$$\tau_c = \frac{aC_m}{\frac{2\lambda_o\lambda_i}{2\lambda_o + \lambda_i} + \frac{a}{h}\lambda_m}, \quad (6)$$

where C_m is the specific membrane capacitance, λ_o and λ_i are the specific conductivities of extra- and intracellular media respectively, and h is the membrane thickness.

In the case of a sine-wave electric field, the transmembrane potential has to be calculated by [7]:

$$\Delta\Phi_g = \frac{3}{2} E_0 a \cos \theta / \sqrt{1 + (2\pi\nu\tau_c)^2}, \quad (7)$$

where ν is the frequency of the ac field.

Here, Eqns (1)-(7) are used to get theoretical relationships between the parameters of the electric treatment, which is needed to electroporate the cell. For the most calculations, the following set of parameters (the "standard cell") was used: $\nu = 10^{11} \text{ s}^{-1}$, $a_i = 0.6 \text{ nm}^2$, $\Delta W_f(0) = 45 k_B T$, $r_* = 0.3 \text{ nm}$ [8], $a = 3.5 \text{ }\mu\text{m}$, $C_m = 1 \text{ }\mu\text{F/cm}^2$, $\epsilon_w = 81$, $\epsilon_m = 2$, $T = 295 \text{ K}$, $\Delta\Phi_0 = 25 \text{ mV}$.

3 RESULTS AND DISCUSSION

First, the dependences of the fraction of electroporated cells, F_p , on the field strength E_0 at a fixed pulse length τ_i were calculated according to Eqns. (1)-(3) for various pulse lengths with $\Delta W_f(0) = 40 k_B T$, $r_* = 0.34 \text{ nm}$. The results are shown in Figure 1. Experimental dependences obtained for human erythrocytes [9] are also presented in this figure. It can be seen that increasing either the pulse amplitude or the pulse duration increases the fraction of electroporated cells.

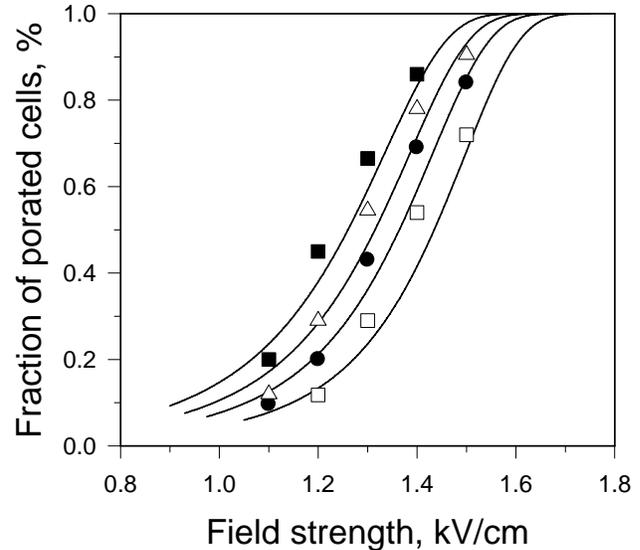


Figure 1: Theoretical dependences (solid lines) of the fraction of electroporated cells on the electric field strength E_0 calculated from Eqns. (1)-(3) at pulse lengths: \square 150 μs , \bullet 250 μs , Δ 350 μs , \blacksquare 500 μs . Experimental points are the data for human erythrocytes. Theoretical curves are calculated for $\Delta W_f(0) = 40 k_B T$ and $r_* = 0.34 \text{ nm}$.

Reproduced from [4].

Then, the relationship between the external electric field strength required to porate cell, E_p , and the length of the square wave pulse, τ_i , was studied. The theoretical dependence calculated for the "standard cell" according to Eqn. (4) is shown in Fig. 2A. The same relationships obtained for human erythrocytes by Kinoshita and Tsong [10] is plotted in Figure 2B.

It has been obtained that the cell poration time depends on the pulse intensity: the shorter the pulse length, the higher the field strength should be (Fig. 2). These dependences are much more pronounced for short pulses ($\tau_i < 10 \tau_m$). For long pulses ($\tau_i > 10 \tau_m$) the electric field strength required for the poration of cell membranes increases significantly slower with decreasing the pulse length (Fig. 2).

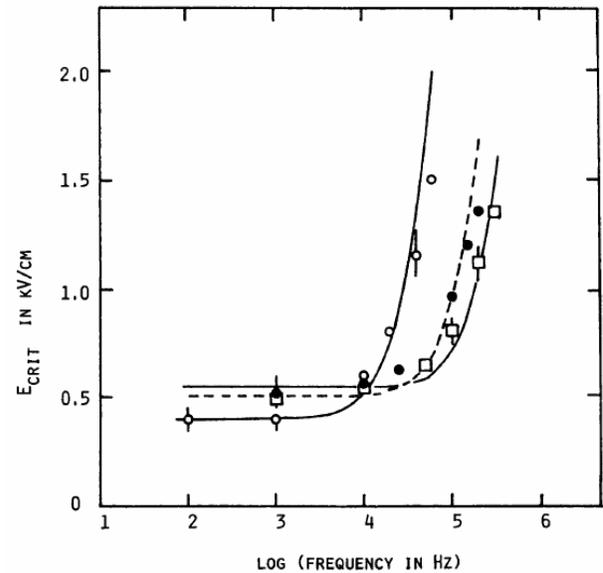
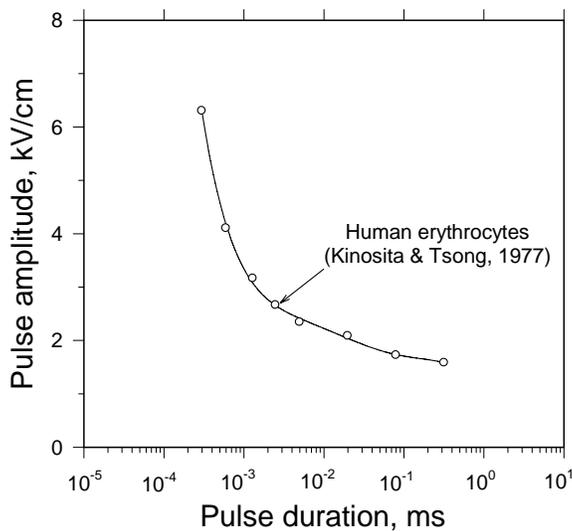
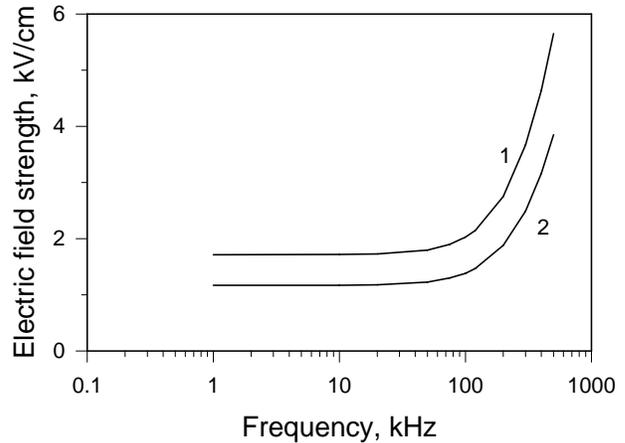
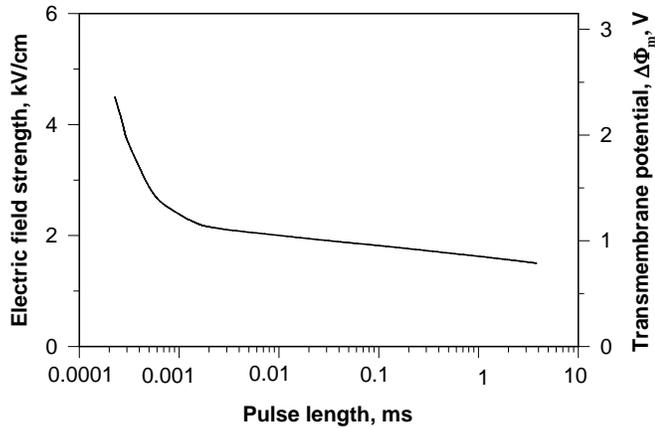


Figure 2: (A) Theoretical dependence of the electric field strength necessary for electroporation, E_p , on the pulse duration calculated for the square wave electric pulse. (B) The dependence of the amplitude of a square-wave electric pulse required to electroporate 50 % of human erythrocytes on the pulse duration, taken from [5].

Figure 3: Theoretical dependences of the electric field strength necessary for electroporation, E_p , on the pulse duration calculated for the square wave (curve 1) and exponentially decaying (curve 2) electric pulses. (B) The strength of an ac field pulse with the duration of 200 ms as a function of the ac frequency, obtained for murine melanoma cell line (Tib9). Data are taken from Marszalek et al. [7].

In addition, taking into account that the generated transmembrane potential depends on the frequency of the ac electric field (Eqn. 7) [4], the relationships between E_p and the frequency of the applied ac field, f , were calculated. It has been obtained that although the electric field strength for electroporation, E_p , is constant for frequencies less than 10 kHz but its value depends on the pulse duration and decreases with increasing τ_i (Fig. 3A). At higher frequencies ($f > 1/2 \pi \tau_m$) E_p is dependent on the frequency of the ac field.

In Fig. 3B, the dependence of the strength of an ac field pulse with the duration of 200 ms, required to electropore the cells to propidium iodide, on the ac frequency are shown. Data are taken from Marszalek et al. [11].

From the analysis presented here, it is seen that the dependences obtained theoretically are in accordance with experimental observations [10,11].

4 CONCLUSION

Theoretical equations, which were derived assuming that the formation of a metastable hydrophilic pore is a random one-step process, are in agreement with the experimental dependencies obtained with different forms of electric treatment and on different cell lines.

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