12

The Pulse Intensity-Duration Dependency for Cell Membrane Electroporation

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12.1 Introduction

External electric field, which is applied to cells, can, under suitable field parameters, induce local distortions and structural rearrangements of lipid molecules in the cell membrane. Depending on the field parameters, the membrane hence becomes either transiently or permanently permeable even after the field has ceased, allowing molecules that are otherwise deprived of transport mechanisms to cross the membrane and reach the cytosol. This phenomenon is often referred to as electroporation or electropermeabilization.

The method is today successfully used in different applications, such as the introduction of molecules into cells (Rols and Teissié 1998, Neumann et al. 1999, Canatella et al. 2001, Maček Lebar and Miklavčič 2001), transdermal drug delivery (Prausnitz 1996, Denet et al. 2004, Pavšelj and Préat 2005), fusion of cells (Zimmerman 1982, Ogura et al. 1994, Ušaj et al. 2009), electroinsertion of proteins into membranes (Elouagari et al. 1995, Teissié 1998), sterilization (Knorr 1999, Rowan et al. 2000, Teissié et al. 2002, El Zakhem et al. 2007), and tissue ablation (Davalos et al. 2005, Lavee et al. 2007). The main clinical success of electroporation was achieved in the treatment of cutaneous and subcutaneous tumors, where chemotherapeutic drugs in combination with electric pulses were delivered to tumor cells (electrochemotherapy) (Heller et al. 1999, Mir and Orlowski 1999, Marty et al. 2006, Serša et al. 2008), while another application, a nonviral delivery of nucleic acids to cells (gene electrotransfection), is also gaining increasing interest (Jaroszeski et al. 1999, Šatkauskas et al. 2002, Golzio et al. 2007).

For each specific application of electroporation, the electric pulses applied to target cells have to be of appropriate amplitude, duration, number, and repetition frequency, and these parameters also need to be adjusted for the particular cell type, size, orientation, and density of cells. When molecules are to be transported into electroporated cells, the characteristics of these molecules must also be considered when adjusting the pulse parameters. Using numerous different parameters of electric pulses, the advantageous effects were reported for different applications of electroporation. Lack of systematic studies addressing the role of electric pulse parameters in the efficiency of electroporation is the reason that the relation between application effectiveness and certain combination of electric pulse parameters is still unclear. Such a relation can be extremely useful in electroporation-based treatment planning. Thus, we focus in this chapter on possible mathematical relation between the parameters of major importance—the amplitude of the electric field and the field duration. Therefore, the experimental data from successful electroporation-based applications are reviewed and possible mathematical relations between the amplitude of the electric field and the field duration are proposed.

12.2 Overview of the Pulse Parameters Used in Biomedical and Biotechnological Applications of Electroporation

To date, a vast number of different pulse parameters have been reported for various biomedical and biotechnological applications of electroporation. It is practically impossible to present all these parameters in one place; however, we tried to summarize some of the most typical parameters in Table 12.1, at least to illustrate their diversity. In the table, the amplitude of the pulses delivered to the electrodes is given as a voltage-to-distance ratio, which roughly equals to the value of the electric field if the field distribution between the electrodes is homogeneous. Depending on the recovery of cells after electroporation, the table was divided into two parts: (i) applications resulting in reversible electroporation, such as the uptake of small and large molecules, and nanoelectroporation, and (ii) applications resulting in irreversible electroporation, such as tissue ablation and sterilization.

From Table 12.1, it becomes clear that in different applications of electroporation, diverse parameters are used, but also, that these parameters can vary even for the same application. This can partly be attributed to the experimental setup (e.g., different cell lines, media, characteristics of molecules) and partly to the fact that different pulse parameters yield similar outcomes. The latter is the focus of our chapter and the description of these parameters is given in more detail below.

For efficient uptake of small molecules, such as lucifer yellow, propidium iodide, and bleomycin into cells, the electric fields for electroporation, which are usually given as voltage-to-distance ratio, are in the range of 1 kV/cm with durations extending from hundred microseconds to milliseconds (Mir et al. 1988, Wolf et al. 1994, Heller et al. 1996a, 1999, Kotnik et al. 2000, Maček Lebar and Miklavčič 2001, Marty et al. 2006, Serša 2006, Snoj et al. 2006). Such pulses were most often used in experiments involving electrochemotherapy, where small chemotherapeutic agents (bleomycin or cisplatin) were delivered into tumor cells by means of electric pulses.

Larger molecules are, especially due to their size, more difficult to introduce into electroporated cells. In general, four different pulse protocols were applied: (i) high electric field amplitudes from one to few kV/cm, lasting from few microseconds to hundred microseconds (Neumann et al. 1982, Potter et al. 1984, Heller et al. 1996b); (ii) low electric field amplitudes of few hundred volts per centimeter but longer durations ranging into tens of milliseconds (Suzuki et al. 1998, Mir et al 1999); (iii) a combination of high and low field amplitudes, with the former in the range of 1 kV/cm and duration of hundreds of milliseconds, and the latter with approximately 100 V/cm with duration from 10 ms to few hundred milliseconds (Sukharev et al. 1992, Bureau et al. 2000, Pavšelj and Préat 2005, Šatkauskas et al. 2005a, André et al. 2008, Kandušer et al. 2009, Villemejane and Mir 2009); and (iv) the most recent approach, where the pulse protocol described in (iii) was followed by a long train of 40 kV/cm pulses with nanosecond

TABLE 12.1 A Summary of Typical Pulse Parameters Used in Different Applications of Electroporation

	Applications	Pulse Parameters	References
Reversible electroporation	Uptake of small molecules	8 × 1300 V/cm, 100 µs 1–64 × (0.25–1 kV/cm), 20 µs to 1 ms 1–8 × (0.5–1.3 kV/cm), 100 µs to 1 ms 1–10 × 0.9 kV/cm, 1 ms 8 × 1.5 kV/cm, 100 µs	Mir et al. (1988), Heller et al. (1996a), Maček Lebar and Miklavčič (2001), Serša (2006), Marty et al. (2006), Snoj et al. (2006), Spugnini et al. (2009), Kotnik et al. (2000), and Wolf et al. (1994)
	Uptake of large molecules	(a) *HV and short* (1–3) × 4–8 kV/cm, 5 µs 6 × 1 kV/cm, 99 µs	(a) Neumann et al. (1982), Potter et al. (1984), Heller et al. (1996b), and Wolf et al. (1994)
		(b) *LV and long* 8 × 200 V/cm, 20 ms 8 × 250 V/cm, 50 ms	(b) Mir et al. (1999) and Suzuki et al. (1998)
		(c) *Combination of HV + LV* 1 × 1.5 kV/cm, 100 µs + 1 × 75 V/cm, 40 ms 4 × 1 kV/cm, 200 µs + 1 × 75 V/cm, 100 ms 1 × 0.8 kV/cm, 100 µs + 4 × 80 V/cm, 100–400 ms 1 × 1 kV/cm, 100 µs + 1 × 140 V/cm, 400 ms 1 × 0.7 kV/cm 100 µs + 200 V/cm, 400 ms 1 × 6 kV/cm, 10 µs + 1 × 200 V/cm, 10 ms	(c) Kandušer et al. (2009), Bureau et al. (2000), Šatkauskas et al. (2005a), André et al. (2008), Villemejane and Mir (2009), Pavšelj and Préat (2005), and Sukharev et al. (1992)
		(d) *Combination of HV + LV + nEP* HV + LV + 30,000 × 40 kV/cm, 10 ns	(d) Villemejane et al. (2009)
	Nanoporation	1 × 12 kV/cm, 60 ns	Pakhomov et al. (2007)
		1 × 26 kV/cm, 60 ns, 10 × 180 kV/cm, 10 ns; 1 × 65 kV/cm, 10 ns	Schoenbach et al. (2004)
		1 × 80 kV/cm, 4 ns	Vernier et al. (2008)
		3–5 pulses of 50 kV/cm, 60 ns	Schoenbach et al. (2001)
		10 × 25 kV/cm, 30 ns; 50 × 25 kV/cm, 7 ns	Vernier et al. (2003)
Irreversible electroporation	Tissue ablation	1 × 1000 V/cm, 20 ms	Edd et al. (2006)
		10 × 3800 V/cm, 100 µs	Maor et al. (2008)
		90 × 250 V/cm, 100 µs	Rubinsky et al. (2008)
		8, 16, 32 × 3800 V/cm, 100 µs	Lavee et al. (2007)
	Sterilization	>15 kV/cm, µs to ms	Knorr (1999)
		20 × 550 V/cm, 10 ms	Teissié et al. (2002)
		1000 × 5 kV/cm, 1 ms	El Zakhem et al. (2007)

Notes: The pulse parameters are given as no. of pulses × electric field, pulse duration. The electric field is given as voltage-to-distance ratio. HV, high voltage pulse; LV, low voltage pulse; nEP, nanosecond pulse.

duration, minutes later (Villemejane et al. 2009). These four protocols were typically used for transporting fragments of DNA into cells *in vitro* or *in vivo* for efficient gene electrotransfer.

While pulse protocols described above were mostly targeted to the plasma cell membrane, very strong fields of short, nanosecond duration were reported to affect also membranes of cell organelles. For this purpose, pulses exceeding 10 kV/cm (sometimes even 100 kV/cm) were used with durations from few nanoseconds to tens of nanoseconds (Schoenbach et al. 2001, 2004, Vernier et al. 2003, 2008, Pakhomov et al. 2007).

In tissue ablation, cells were irreversibly electroporated with either (i) high number of pulses with a low amplitude (a few hundred volts per centimeter) and hundred microsecond durations (Rubinsky et al. 2008); (ii) low number of pulses with few kV/cm lasting hundred microseconds (Lavee et al. 2007, Maor et al. 2008); or (iii) with pulses of 1 kV/cm and long duration of tens of milliseconds (Edd et al. 2006).

For sterilization, electric fields larger than 15 kV/cm and lasting from microseconds to milliseconds were delivered to irreversibly electroporate and destruct the membranes of microorganisms (Knorr 1999, Teissié et al. 2002). Alternatively, lower fields from few hundred volts per centimeter to few kV/cm and durations of milliseconds to tens of milliseconds were used for water sterilization (Teissié et al. 2002, El Zakhem et al. 2007).

In general, from the pulse parameters summarized in Table 12.1, it follows that the values of the field amplitudes and durations needed for efficient electroporation of cells (either for the successful uptake of molecules or destruction of cells) are inversely proportional. Namely, with higher pulse amplitudes, the pulse duration causing a similar effect can be shorter and vice versa. This observation is not new and was reported by many authors before. From Table 12.1, it can also be seen that the change in the value of a certain pulse parameter can be compensated by carefully adjusting the values of other parameters, e.g., reduction in the pulse amplitude can be compensated by increasing the pulse duration or number of pulses. This observation can be extremely useful in applications, where the limitations of the electrical devices, such as pulse generator (or electroporator), have to be taken into account.

Furthermore, there have been many contradicting reports on the threshold value of the electric field required for electroporation of cells. Electroporation of the cell membrane is usually associated with an increase of the electric field–induced transmembrane voltage above a certain threshold, which is estimated to be between 200 mV and 1 V (Tsong 1991, Teissié and Rols 1993, Towhidi et al. 2008). Therefore, a threshold value of the electric field that must be exceeded in order to load specific molecules into the cell or to affect the cell viability is expected. However, many theoretical studies, especially those, which were based on the theory of electroporation (Neumann et al. 1982), predicted that electroporation is not a threshold phenomenon. According to theoretical studies, the presence of the electric field only increased the probability of the occurrence of water pores in the membrane (hence the term electroporation). The discrepancy between experiments and theory can be explained by the fact that in experiments, electroporation of the cell membrane was determined by the detection of the internalized molecules, and this can be limited by the sensitivity of the experimental setup and the characteristics of these molecules. For example, several authors reported that structural changes in the membrane and the related increased permeability of the membrane became detectable within a few microseconds after the onset of the electric field (Benz et al. 1979, Kinosita and Tsong 1979, Hibino et al. 1993, Griese et al. 2002, Kakorin and Neumann 2002, Pavlin et al. 2005), but the occurrence of these changes did not coincide with the detection of the transmembrane transport of molecules, which was detected milliseconds or seconds after the pulse (Dimitrov and Sowers 1990, Tekle et al. 1990, 1994, Gabriel and Teissié 1999). The transport of single-atom ions and molecules continues for seconds or even minutes after electroporation, until the cell membrane completely recovers (reseals) or until the equilibrium in concentration of ions and molecules is obtained.

TABLE 12.2 Most Frequently Used Molecules for Studying Electroporation

Molecule	Molecular Weight (Da)	The Number of Molecules Inside the Cell Relative to the Outside (%)
Lucifer yellow	457	100
Propidium iodide	660	90
Bleomycin	1,500	30
Fragments of oligonucleotides	3,000	20
Ribosome inactivating protein PAP	30,000	10
FITC dextran	70,000	<1
Antibodies	150,000	0.1

Source: Maček Lebar et al., *Med. Razgl.*, 37, 339, 1998.

Note: The relative number of molecules was determined for the optimal electroporation parameters.

In contrast, membrane conductivity, which is elevated during the pulse, returns close to the initial value much faster, milliseconds after the exposure (Kinosita and Tsong 1979, Hibino et al. 1993, Schmeer et al. 2004). To a certain extent, these differences can be attributed to the size and the charge of molecules, which hinders the permeation of molecules through an electroporated cell membrane. Table 12.2 demonstrates the influence of the size of the molecules (molecular weight) on the transport into electroporated cells.

12.3 Mathematical Description of the Relation between the Field Strength and the Field Duration

In most studies, cell viability or the uptake of molecules into cells was measured only for a very limited range of pulse parameters. Furthermore, the mathematical relation between the parameters of major importance, field strength and pulse duration, was addressed only by few authors.

Krassowska et al. (2003) tested a train of six pulses with 10 different pulse durations (between 50 µs and 16 ms) and 9–14 different field strengths to determine the magnitude of the electric field required to kill 50% of the cells (E_{50}). When plotted in logarithmic scale, the relationship between E_{50} and pulse duration (T) was

$$E_{50} = kT^{A}. \tag{12.1}$$

On the basis of their experimental results, they reported a value of A as being very close to −1, indicating that E_{50} is inversely proportional to T. The relation proposed by Equation 12.1 does not have a threshold value for extremely long pulse durations; i.e., the limit of E_{50} as T approaches infinity is 0.

A similar relation was found by Butterwick et al. (2007) who studied the threshold for tissue damage as a function of pulse duration. They used balanced biphasic pulses of seven different pulse durations ranging from 6 µs to 6 ms. The authors reported that for a single pulse, the damage threshold current density j, scaled with pulse duration (T), can be approximated by $1/T^{0.5}$.

He et al. (2007) performed more than 2000 single-cell measurements on five molecules of different sizes to describe a functional dependence between the threshold electric field for loading molecules into cells, E_{EP}, and pulse duration T. Thirteen pulse durations ranging from 400 µs to 15 ms were used and corresponding threshold electric field E_{EP} for each molecular size was determined. They demonstrated that if the electric field is smaller than the threshold value E_0, cell electroporation cannot be detected, not even by extending the pulse duration. They concluded that the values of the threshold electric field, E_{EP}, for loading different molecules formed a three-parameter exponential function of pulse duration T:

$$E_{EP} = E_0 + E_1 e^{-T/\tau}, \tag{12.2}$$

where

E_0 is the threshold value of the electric field at the longest duration
E_1 is the difference of threshold electric field at longest duration and shortest duration
τ is the time constant of exponential decay curve

The limit of E_{EP} as T approaches infinity is E_0; meaning that the proposed E_0 is a threshold value of the electric field that must be exceeded in order to load the specific molecule into cells. The larger the size of the molecule (molecular weight), the higher the value of E_0 is.

Šatkauskas et al. (2005b) electroporated the tumors *in vivo* with eight square wave electric pulses of variable durations (0.1, 0.25, 0.5, and 1 ms) delivered at 1 Hz. Using tumor doubling time as a criteria, they observed three-parameter exponential relation between pulse strength (defining the same tumor

doubling time and consequently the same efficiency of electrochemotherapy) and pulse duration, similar to what was reported by He et al. (Equation 12.2).

According to the studies mentioned above, we can summarize that a threshold value of electric field needed for irreversible electroporation with extremely long pulse durations does not exist. On the other hand, a threshold value of electric field needed for reversible electroporation with extremely long pulse durations depends on the size of molecules; the smaller the size of the molecule, the lower the threshold value of the electric field is. For the realistic limiting case of molecular size, namely, single-atom ions, a threshold value of electric field at extremely long pulse durations should be very low or, hypothetically, even disappear. To investigate this, a threshold value of the electric field needed to load calcium ions into cells with a single pulse of different pulse durations was studied.

12.4 Materials and Methods

12.4.1 Cells

Chinese hamster ovary cells (CHO-K1) were plated in Lab-Tek II chambers (Nalge Nunc International, United States) at 2×10^5 cells/chamber in the culture medium HAM-F12 supplemented with 8% fetal calf serum, 0.15 mg/mL L-glutamine (all three from Sigma-Aldrich, Steinheim, Germany), 200 units/mL benzylpenicillin (penicillin G), and 16 mg/mL gentamicin and incubated in 5% CO_2 at 37°C. The experiments were performed 18–24 h after plating, when most cells were firmly attached to the surface of the chamber (see Figure 12.1) and most of them did not yet divide.

12.4.2 Monitoring Electroporation

To monitor electroporation of cells, a fluorescent calcium indicator Fura-2AM was used (Grynkiewicz et al. 1985). Fura2-AM enters the cell through an intact membrane, and is transformed in the cytosol into Fura2, the active and membrane-nonpermeant ratiometric dye (excitation 340/380 nm, emission 540 nm). Electroporation results in the entry of Ca^{2+} ions into the cells, where their binding to Fura2 causes the change in the fluorescence of the dye. Under moderate pulse parameters, the cell recovers after electroporation, stores the excess Ca^{2+} into the intracellular reservoirs or excludes the Ca^{2+} from the cytoplasm. The fluorescence thus decreases with time back to the initial value, allowing for another repetition of the experiment on the same cells.

The procedure for staining the cells with Fura was already described elsewhere (Towhidi et al. 2008). Briefly, prior to experiments, the cells were washed with Spinner's Minimum Essential Medium (SMEM, Gibco, United States; a calcium-depleted modification of EMEM), left at room temperature for 25 min in SMEM containing 2 μM Fura2-AM, and subsequently washed again with SMEM to remove the dye from the extracellular medium. Finally, SMEM was replaced with culture medium HAM-F12, which

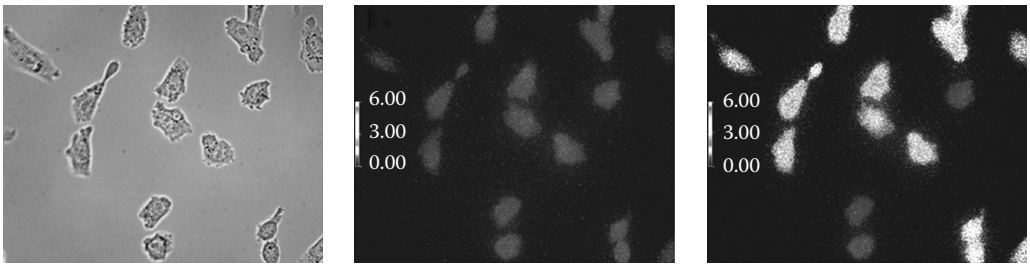

FIGURE 12.1 (A) CHO cells: bright field. (B) Fluorescence of cells in control (nonporated cells). (C) Cells 1 min after electroporation with a 100 V (250 V/cm), 10 ms pulse. Brighter cells were electroporated. Field direction was from top to bottom.

contains Ca^{2+} ions (app 1 mM). Thus, the calcium ions were present in the extracellular medium but were nearly absent from the cytosol, as they do not readily cross the nonporated cell membrane.

12.4.3 Pulse Delivery

Single rectangular electric pulse with duration of 0.03, 0.1, 1, 10, or 50 ms was generated with a Cliniporator™ device (IGEA s.r.l., Carpi, Modena, Italy). For a given pulse duration, the pulse amplitude was increased stepwise with each consecutive pulse (a 10 V step) until approximately 70% of the cells were electroporated (Figure 12.1). The second pulse with higher amplitude was delivered with at least 5 min delay to enable cell recovery (verified in a separate experiment, see below), except if cells already became fluorescent (electroporated). In this case, the next pulse was delivered 5 min after the fluorescence of cells returned to the initial value. To speed up the resealing, the cells were kept at 37°C.

The pulses were delivered to a pair of parallel Pt/Ir wire electrodes with 0.8 mm diameter and 4 mm distance between them, which were positioned at the bottom of the chamber. Cells were monitored under a fluorescence microscope (40× objective, AxioVert 200, Zeiss, Germany), equipped with a CCD camera and a monochromator (both Visitron, Germany). The presence of calcium was determined ratiometrically using MetaFluor 5.1 software (Molecular Devices, GB), with the excitation wavelengths set at 340 and 380 nm, and the emission measured at 540 nm for both excitation wavelengths.

12.4.4 Verification of Cell Recovery after Electroporation

To test if cells completely recovered 5 minutes after electroporation, we performed an additional experiment. Cells were prepared and incubated with the dye as described above. However, they were not electroporated in the culture medium but in SMEM, which does not contain Ca^{2+} ions. The pulse with the amplitude leading to 70% of electroporated cells was delivered (see above) and 5 min later the Ca^{2+} ions were added to SMEM to obtain a 1 mM Ca^{2+} concentration in the chamber.

Since the fluorescence of the cells remained low and unchanged, Ca^{2+} ions apparently did not enter the cells, meaning that the cells fully recovered after electroporation. The same pulse was applied again, and since the Ca^{2+} was now present in the extracellular solution, the fluorescence of the cells increased, confirming that the pulse was indeed electroporating the cells. Similar results were obtained for all investigated pulse durations.

12.4.5 Numerical Modeling of the Transport of Small Molecules into Electroporated Cells

Optimization of the pulse parameters for efficient electroporation is usually related to time-consuming experiments. An alternative is to use an appropriate model for this phenomenon and calculate the influence of different parameters on the transport. The models are based on the assumption that the electric field increases the probability of the occurrence of structural changes in the lipid bilayer of the membrane (water pores). Currently, the models are able to predict the influence of only a few pulse parameters (e.g., amplitude, duration) on pore formation and closure (DeBruin and Krassowska 1999, Neu and Krassowska 1999, Neumann et al. 1999, Joshi et al. 2002, Gowrishankar and Weaver 2003), while a descriptive model of the electroporation-mediated molecular transport is missing. We recently developed a *dynamic* model of the transport of molecules into electroporated cells of arbitrary shapes, which is able to predict the efficiency of electroporation for different pulse parameters. The model (Miklavčič and Towhidi 2008) is described in detail in one of the chapters of this book; below, we only give its brief description.

The model is based on previously suggested (Neumann et al. 1998) and recently confirmed (Böckmann et al. 2008) model, where the occurrence of structural changes in the membrane due to the external electric field is described with a transition from the closed/initial lipid state (C) to the open or porous

state (P_2), as depicted in Equation 12.3. The transition occurs with two intermediate states, C_1, where lipid head groups are tilted, and P_1, which is a prepore or a transient pore state.

$$C \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} P_1 \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} P_2. \tag{12.3}$$

The permeability of the membrane in P_1 state is negligibly small so P_2 is predominantly responsible for the molecular transport. Pore formation and closure are denoted by the rate coefficients k_1, k_2, and k_3, which were considered as being equal ($k_1 = k_2 = k_3 = k_p$) (Neumann et al. 1998) but field dependent. This is in contrast to previous studies, where the average k was considered (Neumann et al. 1996, 1998, 1999), but allowed the calculation of the spatial and temporal distribution of pores on the membrane by this model.

The model was supplemented with equations describing transport to determine the molecular uptake into a single cell. The total uptake was computed with integration of transported molecules through the cell membrane over the time and the cell surface. Considering the geometry of the cell of interest, the solutions of self-consistent equations of suggested model were calculated using COMSOL 3.3 package based on finite-element method.

12.5 Results and Discussion

Figure 12.2 shows the experimentally determined electric field (E) needed to electroporate 70% of cells (filled circles) at different pulse durations (T). An exponential function with three constants (Equation 12.2; dotted line), proposed by He et al., and function proposed by Krassowska et al. (Equation 12.1; dash-dotted line) were fitted to the data using SigmaPlot 8.0. It can be seen that the mathematical relation described by Equation 12.1 fits the data much better ($R^2 = 0.9983$) than the relation described by Equation 12.2 ($R^2 = 0.9908$). The value of parameter A in mathematical relation described by Equation 12.1, which fits best the experimental data, is −0.1661. This is roughly six times smaller than the value of A given by Krassowska. A smaller value of A was expected, because we were reversibly electroporating cells in contrast to Krassowska, which performed irreversible electroporation, and also, we were loading smaller ions into cells.

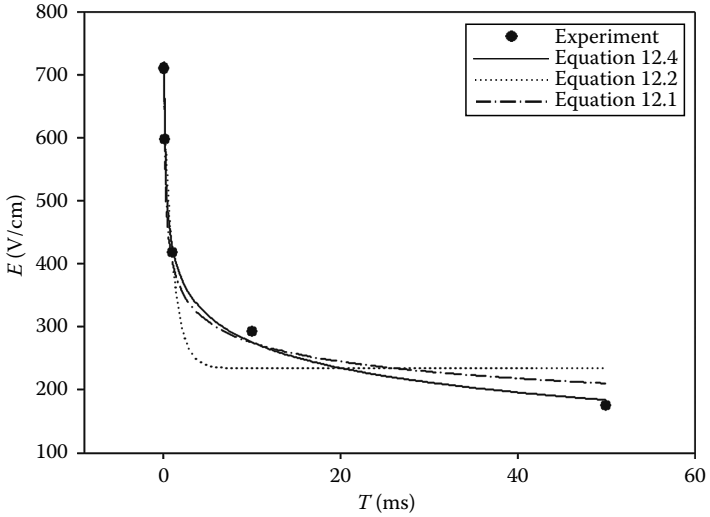

FIGURE 12.2 Experimentally determined electric field (E) needed to load calcium ions into 70% of cells at different pulse durations (T). Mathematical relations described by Equations 12.1, 12.2, and 12.4 were fitted to the data (filled circles) and are presented by dash-dotted line, dotted line, and solid line, respectively.

An even better fit (R^2 = 0.9996; solid line) is obtained with modified version of Equation 12.1 written as

$$E = \left[c_1 + c_2 \ln(T)\right] T^{\mathrm{m}}. \tag{12.4}$$

Equation 12.4 is the solution of the second-order Euler–Cauchy equation:

$$T^2 \frac{d^2E}{dT^2} + aT\frac{dE}{dT} + bE = 0, \tag{12.5}$$

for the case, when characteristic equation has just one real repeated root m; i.e., $a = 1–2m$ and $b = m^2$. The fit to experimental data gives $m = 0.0345$.

Similar results are obtained if the predictions of the numerical model are taken into consideration (Figure 12.3). Mathematical relations described by Equations 12.1, 12.2, and 12.4 were fitted to the numerical data (filled circles) and are presented by dash-dotted line, dotted line, and solid line, respectively. Again, Equation 12.4 gives the mathematical relation that fits the data best ($R^2 = 0.9991$), while the mathematical relation given by Equation 12.2 is the least adequate ($R^2 = 0.9911$).

Electroporation–based technologies and medical applications have already shown their laboratory and clinical relevance. *In vitro* electroporation is becoming a standard tool in biotechnology, and medical applications are in progress. Although the number of successful applications is increasing, several questions concerning the optimization of pulse parameters for specific application are still open. Among them is determination of appropriate amplitude, duration, number, and repetition frequency of electric pulses that assure successful application or treatment with minimal possible side effects. A review of the studies related to pulse parameters used in biomedical and biotechnological applications shows a palette of efficient pulse parameters combinations. It is apparent, that the change in the value of a certain pulse parameter can be compensated by carefully selected values of the other parameters. This observation can be extremely useful in the process of electroporation-based treatment planning, where limitations of the electrical devices have to be taken into account.

Systematic studies aimed at addressing the role of electric pulse parameters in efficiency of electroporation applications are scarce. Even scarcer are mathematical relations describing the pulse parameters.

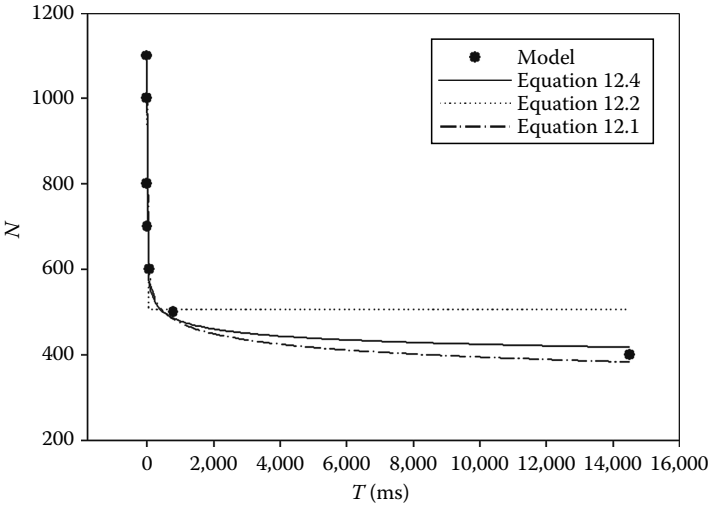

FIGURE 12.3 The calculated uptake of small molecules (N) at different pulse durations (T). Mathematical relations described by Equations 12.1, 12.2, and 12.4 were fitted to the data (filled circles) and are presented by dash-dotted line, dotted line, and solid line, respectively.

In this chapter, we focused on the mathematical relation between the electric field strength and pulse duration that assure loading of small ions into cells. The experimental results demonstrated that a threshold value of electric field needed to load small ions into the cells with extremely long pulse durations does not exist. The mathematical relationship proposed (Equation 12.4) agreed well with the experimental data and predictions of the model for a wide range of pulse durations (from μs to s). According to this relationship, we can select different pairs of electric field amplitude/duration that assure similar effectiveness of electroporation, which can represent an improvement in treatment planning.

Acknowledgment

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