# Determination of the Electroporation Threshold for Pulses from 95 ns to 20 µs

G. Saulis[*1], S. Balevicius[**], R. Saule*, V. Stankevic[**], and N. Zurauskiene[**]

[*]Department of Biology, Vytautas Magnus University
58 K. Donelaicio str., Kaunas 44248, Lithuania, [1]sg@kaunas.omnitel.net
[**]Laboratory of High Power Pulses, Institute of Semiconductor Physics
11 A. Gostauto str., Vilnius 01108, Lithuania

## ABSTRACT

The dependences of the fraction of electroporated mouse hepatoma MH-22A cells on the pulse intensity were obtained for the cells exposed to a single square-wave electric pulse with the duration from 95 ns to 20 µs. The amplitude of an electric pulse was varied from 0.4 to 12 kV/cm. Increasing the intensity of the electric field pulse increased the fraction of electroporated cells. The electric field, which lead to a given percentage of electroporation, decreased with increasing the pulse length. The dependence of the amplitude of the electric pulse required to electroporate 50 % of mouse hepatoma MH-22A cells on the pulse duration was also determined.

***Keywords***: potassium ions, potassium selective electrode, electropermeabilization, mouse hepatoma, nanosecond pulses

## 1 INTRODUCTION

The permeability of the cell membrane can be modified by exposing of cells to high-voltage electric pulses leading to the formation of nanometer-sized pores in the cell membrane (electroporation or nanoporation) [1]. This phenomenon is widely used in cell biology, biotechnology, and medicine [1-3]. For optimization of practical applications and comparison with theoretical modeling, it is important to know whether and how many of cells have become electroporated as a result of a particular electric treatment.

There are theoretical models allowing to obtain theoretical relationships between the parameters of the electric treatment resulting in cell electroporation for any type of an electric treatment [4,5]. However it is still difficult to predict individual responses of different cells to electric treatment [6,7]. This is because there are very few studies, in which the dependence of the electroporation threshold on the pulse duration would be determined [8,9]. Either the electric field parameters needed for the increase of the cell membrane permeability to a particular substance (electropermeabilization) [6] or cell viability [7] have been determined for several cell lines. As a result, the fraction of electroporated cells still needs to be determined empirically for each cell line [7].

Up to now, almost there are no studies in which the dependence of the amplitude of the electric pulse required to electroporate 50 % would be determined for the electric pulses with the durations shorter than 1 µs [8] and there is no study in which this would be done for pulses shorter than 320 ns. The main reason of this is, that it is difficult to detect the threshold of electroporation, because the pores created in the plasma membrane can be small [10-13]. Only recently, the electroporation threshold was compared for different cell lines [9]. However, this was done for the electric pulses in the range from 20 µs to 2 ms.

Here, the dependences of the fraction of electroporated mouse hepatoma MH-22A cells on the pulse intensity were obtained for the cells exposed to a single square-wave electric pulses with the durations from 95 ns to 20 µs.

## 2 MATERIALS AND METHODS

The culture medium consisted of Dulbecco's modified Eagle's medium (cat. no. D5546, Sigma-Aldrich Chemie, Steinheim, Germany) supplemented with 10 % fetal bovine serum (cat. no. F7524, Sigma-Aldrich Chemie), 1 % L-glutamine (cat. no. G7513, Sigma-Aldrich Chemie), 100 U/ml penicillin, and 100 µg/ml streptomycin (cat. no. P0781, Sigma-Aldrich Chemie). As an electroporation medium, the culture medium was used. Calibration solutions containing 0.2-100 mM KCl were prepared by diluting a stock solution of 100 mM KCl and adding 150 mM sodium chloride and 8 mM sodium benzoate [14]. The NaCl is added to keep sodium ion concentration close to that in electroporation medium.

The mouse hepatoma MH-22A cells were grown in monolayer cultures in 75-cm$^2$ flasks at 37 $^o$C and 5 % $CO_2$ in a water-jacketed incubator. When cells reached confluence they were trypsinazed, suspended in the culture medium at approximately 2-5×10$^7$ cells/ml, and kept for 60–70 min at room temperature (20–21 $^o$C) [15]. During this time, the cells restored the normal level of the intracellular concentration of potassium ions. Then the cells were electroporated within 15-20 min.

For electroporation, single square-wave pulses with the duration of 100 µs and 2 ms and the amplitude ranging from 0.2 to 2.4 kV/cm were used. A 50-µl droplet of cell suspension was placed between a pair of flat stainless-steel electrodes and was subjected to a single square-wave electric pulse.

When determining the fraction of electroporated cells, after the exposure to an electric pulse, the cell suspension was pipetted out of the chamber and immediately transferred to a chilled Eppendorf tube. To prevent pores from closing and to allow equilibration between intracellular and extracellular $K^+$ concentrations, the cells were kept on ice for 5-10 min and then kept for 30-40 min at 10-11 °C.

Then, the extracellular potassium concentration was measured by means of a mini $K^+$-selective electrode [10,15]. Mini $K^+$-selective and reference electrodes (cat. nos. 601 and 401, respectively, Diamond Electro-Tech) manufactured by Diamond Micro Sensors (Ann Arbor, MI, USA) were utilized. Potential measurements were made with pH-meter-millivoltmeter pH-150M (Gomel Factory of Measurement Instruments, Gomel, Byelorussia). All measurements were made at a temperature of 10-11 °C [10,15].

The fraction of electroporated cells was determined from [10,15]:

$$F = \{[K^+]_X - [K^+]_{0\%}\}/\{[K^+]_{100\%} - [K^+]_{0\%}\} \quad (1)$$

where $[K^+]_X$, $[K^+]_{0\%}$, and $[K^+]_{100\%}$ are the extracellular concentrations of potassium ions in the sample and solutions with intact and 100 % electroporated cells, respectively. An example of the obtained dependence of the extracellular potassium concentration of electroporated mouse hepatoma MH-A22 cell suspension on the pulse intensity is shown in Fig. 1.

The dependences of the fraction of electroporated cells, $F(E_0)$, on the pulse amplitude were fitted by a three-parameter sigmoid curve [15].

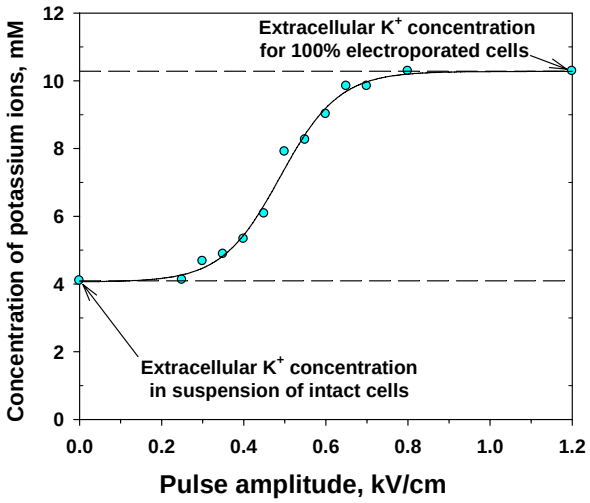


Figure 1: Example of obtained dependence of extracellular potassium concentration of electroporated mouse hepatoma MH-A22 cell suspension on the pulse intensity. The experimental points were fitted to four-parameter sigmoid curve [15].

## 3 RESULTS AND DISCUSSION

Experimental data show that 'threshold' electroporation of cells and liposomes can involve pores just large enough to let water and small ions through, but still impermeable to slightly larger molecules, such as mannitol (molecular weight $M_w$ = 182.17 Da), sucrose ($M_w$ =342.3 Da), or propidium iodide (radii of these molecules are in the range of 0.4–0.6 nm) [10-13]. The pores can even be small enough to discriminate between $K^+$, $Rb^+$ and $Na^+$ ions [10,16]. This creates difficuties when determining the cell electroporation.

When the cell membrane becomes electroporated, potassium ions leak out of the cells down their concentration gradient till the equilibrium between intracellular and extracellular concentrations has been established. So, the fraction of electroporated cells can be determined from the extracellular concentration of potassium ions [10,15,17]. In this study, we employed this approach of the determination of the electroporation threshold.

The dependences of the fraction of electroporated mouse hepatoma MH-22A cells on the pulse intensity were obtained for the cells exposed to single square-wave electric pulses with the durations from 95 ns to 20 µs. The amplitude of an electric pulse was varied from 0.4 to 12 kV/cm. The obtained dependences are presented in Fig. 2. It can be seen from this figure, that increasing the intensity of the electric field pulse increased the fraction of electroporated cells.

From the relationships of the fraction of electroporated cells on the pulse amplitude obtained at different pulse durations, the pulse amplitude inducing electroporation of

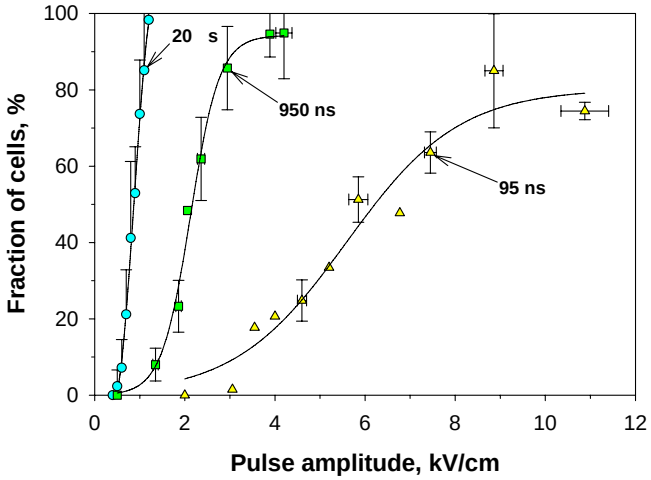


Figure 2: The dependences of the fraction of electroporated mouse hepatoma MH-22A cells on the amplitude of a single square-wave electric field with the duration of 95 ns, 950 ns and 20 µs. The fraction of electroporated cells was determined from the amount of potassium ions released from the cells [15].

50 % of cells, $\Delta E_{50\%}$, can be estimated for each pulse length. The obtained dependence of $\Delta E_{50\%}$ on the pulse duration is shown in Fig. 3.

The transmembrane potential generated by the external electric field depends on the cell radius [18]. It can be calculated from [18]

$$\Delta\Phi_m = 1.5E_0a, \quad (1)$$

where $E_0$ is the electric field strength and $a$ is the cell radius, which was 7.7 μm for mouse hepatoma MH-22A.

The dependence of $\Delta\Phi_{m50\%}$ on the pulse duration obtained for mouse hepatoma MH-22A cells from the data presented in Fig. 2 is shown in Fig. 3. It can be seen, that for shorter pulses much stronger electric fields have to be used to electroporate the cells.

From Eq. (1) we get the values of the transmembrane potential in the range of 1.0-7.3 V. These values are significantly smaller than the ones obtained in the case of electroporation of human erythrocytes, Chinese hamster ovary, and rat glioma C6 cells with longer pulses. For the electric pulses with the duration in the range of 50 μs-2 ms, the values of the transmembrane potential in the range of 480-930 mV were obtained [9].

## 4 CONCLUSION

Increasing the intensity of the electric field pulse increased the fraction of electroporated mouse hepatoma MH-22A cells. The transmembrane potential required to electroporate the cells with a square-wave electric pulse decreases with increasing the pulse duration. It is in the range of 1.0-7.3 V for pulses with the duration from 95 ns to 20 μs.

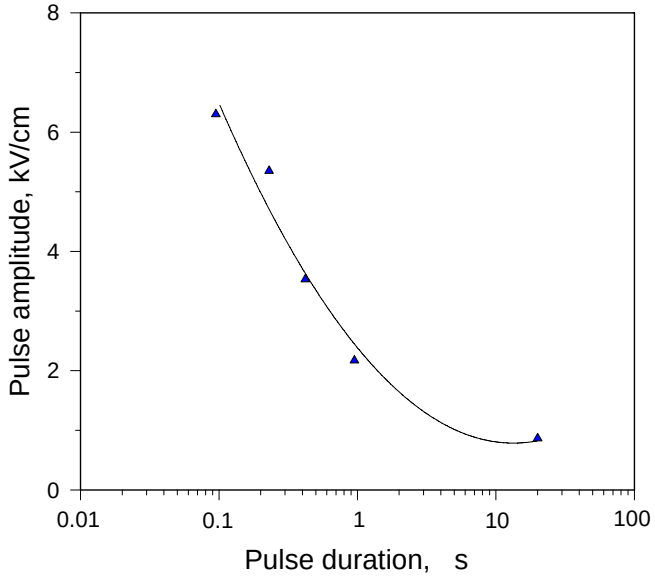


Figure 3: The dependence of the maximal transmembrane potential (at the poles of the cell) required to electroporate 50% of cells on the pulse duration, obtained for mouse hepatoma MH-22A cells. Data were taken from Fig. 2.

## 5 ACKNOWLEDGEMENT

This work was in part supported by grant T-39/09 from the Lithuanian State Science and Studies Foundation.

## REFERENCES

[1] J. Gehl, "Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research", Acta Physiol. Scand., 177, 437-447, 2003.

[2] M. J. Jaroszeski, R. Heller, and R. Gilbert "Electrochemotherapy, Electrogenetherapy, and Transdermal Drug Delivery", Humana Press Inc., Totowa, NJ, 2000.

[3] B. Rubinsky, "Irreversible electroporation in medicine", Technol. Cancer Res. Treat., 6, 255-260, 2007.

[4] G. Saulis, P.C. Wouters, Probable Mechanism of Microorganism Inactivation by Pulsed Electric Fields, in: H.L.M. Lelieveld, S. Notermans, S.W.H. De Haan (Eds.), Food Preservation by Pulsed Electric Fields: From Research to Application, Woodhead Publishing Limited, Cambridge, 2007, pp. 138-155.

[5] G. Saulis, M.S. Venslauskas, "Cell electroporation. Part 1. Theoretical simulation of the process of pore formation in the cell", Bioelectrochem. Bioenerg., 32, 221-235, 1993.

[6] M. Cemazar, T. Jarm, D. Miklavcic, A.M. Lebar, A. Ihan, N.A. Kopitar, G. Sersa, "Effect of electric-field intensity on electropermeabilization and electrosensitivity of various tumor-cell lines *in vitro*", Electro- and Magnetobiology, 17, 263-272, 1998.

[7] M.J. O'Hare, M.G. Ormerod, P.R. Imrie, J.H. Peacock, W. Asche, Electropermeabilization and Electrosensitivity of Different Types of Mammalian Cells, in: M.J. Jaroszeski, R. Heller, R. Gilbert (Eds.), Electrochemotherapy, Electrogenetherapy, and Transdermal Drug Delivery, Humana Press Inc., Totowa, NJ, 2000, pp. 319-330.

[8] K. Kinosita, T.Y. Tsong, "Voltage-induced pore formation and hemolysis of human erythrocytes", Biochim. Biophys. Acta, 471, 227-242, 1977.

[9] G. Saulis, R. Saule, "Comparison of electroporation of different cell lines *in vitro*", Acta Phys. Pol. A, 115, 1056-1058, 2009.

[10] G. Saulis, R. Praneviciute, "Determination of cell electroporation in small volume samples by using a mini potassium-selective electrode", Anal. Biochem., 345, 340-342, 2005.

[11] A.G. Pakhomov, R. Shevin, J.A. White, J.F. Kolb, O.N. Pakhomova, R.P. Joshi, K.H. Schoenbach, "Membrane permeabilization and cell damage by ultrashort electric field shocks", Arch. Biochem. Biophys., 465, 109-118, 2007.
[12] K. Kinosita, T.Y. Tsong, "Formation and resealing of pores of controlled sizes in human erythrocyte membrane", Nature, 268, 438-441, 1977.
[13] B. Deuticke, K. Schwister, Leaks Induced by Electric Breakdown in the Erythrocyte Membrane, in: E. Neumann, A.E. Sowers, C.A. Jordan (Eds.), Electroporation and Electrofusion in Cell Biology, Plenum Press, New York, 1989, pp. 127-148.
[14] "Instructions for Use 601 Mini Potassium Electrode", Diamond Micro Sensors, L.L.C. (Michigan, USA), 2004.
[15] G. Saulis, S. Satkauskas, R. Praneviciute, "Determination of cell electroporation from the release of intracellular potassium ions", Anal. Biochem., 360, 273-281, 2007.
[16] E.M. El-Mashak, T.Y. Tsong, "Ion selectivity of temperature-induced and electric field induced pores in dipalmitoylphosphatidylcholine vesicles", Biochemistry, 24, 2884-2888, 1985.
[17] F. Riemann, U. Zimmermann, G. Pilwat, "Release and uptake of haemoglobin and ions in red blood cells induced by dielectric breakdown", Biochim. Biophys. Acta, 394, 449-462, 1975.
[18] H.P. Schwan, "Electrical properties of tissue and cell suspensions", Adv. Biol. Med. Phys., 5, 147-209, 1957.