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MATHEMATICAL MODELING OF MOUSE CELL'S CONDUCTIVITY IN PULSED ELECTRIC FIELD

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ABSTRACT

Based on the approximation by algebraic and transcendental functions, non-linear mathematical models were built for the experimentally obtained dependences of the conductivity of mouse's reproductive and embryonic cells on the strength of pulsed electric field applied. The approximation of experimental data was performed by the use of least squares method. The finding of extrema, inflection, intersection and curvature of approximating functions were carried out. To find out these characteristic points, the classical mathematical analysis methods were used. For this, the well-known methods of analytical geometry and the algebraic methods for solving equations were used. In addition, to calculate the coordinates of intersection points and curvature, the 1st and 2nd derivatives of non-linear functions were determined. The relationship was established between the calculated coordinates of characteristic points and the modes of membrane electroporation during the linear growth of electric field strength applied to a cell. The constructed mathematical models of experimental conductometric curves made it possible to calculate the strength of pulsed electric field, which is necessary and sufficient for the implementation of any electro-manipulation technologies based on the phenomenon of electroporation.

Keywords: Approximation, cell conductivity, electroporation, mathematical analysis, model

INTRODUCTION

In cell engineering there are several known methods based on the phenomenon of membrane electroporation in pulsed electric field (PEF). These methods are used *in vivo* and *in vitro* for the delivery of membrane-impermeable molecules, such as drugs, DNA, etc. as well as for cell electro-fusion and other applications in biomedicine and biotechnology (Pakhomov *et al.*, 2010; Chang *et al.*, 2012; Miklavcic, 2017). Electroporation has extensively been studied for long time (Abidor *et al.*, 1978), but the phenomenon is still of immense interest. The adequate models of single cell electroporation were developed and different mechanisms offered to; however, this not a simple biophysical phenomenon (Morshed *et al.*, 2013; Son *et al.*, 2014).

The simulation of different modes of cell membrane electroporation is an important addition to many experimental studies (Pakhomov *et al.*, 2010; Son *et al.*, 2014). In addition, some well-known concepts of membrane electroporation have periodically been revised; for example, chemical-thermo-dynamic and others (Weaver *et al.*, 1996; YiKuen *et al.*, 2012; Neumann and

Kakorinm, 2018). Also, the experimental results of single cells electroporation, including the use of microelectrodes, are modeled by various methods (Wang *et al.*, 2010; Shigimaga, 2013). A number of experiments and their theoretical analysis have shown close relationships between the parameters of electric pulse and the successful electroporation of cell membrane (Saulis *et al.*, 2013; Shigimaga *et al.*, 2013; Shigimaga, 2014*a*). New approaches to electroporation studies are applied in practice. One of them is conductometry of a single cell in PEF increasing in strength within a wide range (Shigimaga, 2014*a*; Shigimaga, 2019). At the same time, new problems appear requiring the explanation of results obtained and theoretical foundation of particular modes of electroporation. There is necessity to move from the phenomenal description of continuous development of the stages of cell electroporation in PEF increasing in strength to the mathematical modeling of the process. Based on appropriate algorithms and simple programmes, such modeling will allow to computerize the analysis of measurements and control important electroporation-based processes as electrofusion of cells used in reproductive biotechnology for cloning, hybridization, stimulation, etc. (Kolesnikiva *et al.*, 2013; Shigimaga, 2014*b*; Shigimaga, 2015).

Notwithstanding the fact that the main principles of the method of pulse conductometry of single cells of animals were first laid down long ago (Shigimaga, 2003), certain difficulties related to the methodology and measuring equipment did not allow the quick attainment of sufficiently accurate experimental results. Currently, the routine operations of experimental data processing have been computerized; digital measuring devices used and the methods improved (Shigimaga, 2014*a*; Shigimaga, 2015; Shigimaga, 2019). The results on pulse conductometry of reproductive cells (oocytes) and embryos is sufficient for their theoretical analysis for obtaining digital information on the membrane electroporation stages alternating continuously during the process of action upon a cell by PEF increasing in strength. The present study was aimed to develop mathematical models of experimental conductometric curves for calculating the intensity in a PEF with subsequent use in reproductive biotechnology for cloning, capacity measurement, cell stimulation, etc.

MATERIALS AND METHODS

The mathematical models for the experimentally obtained dependences of cell conductivity on pulsed field strength are based on their approximation by simple functions *viz.*, algebraic and transcendental. The approximation of experimental data was performed by the use of least squares method (Stigler, 1981). The finding of extrema, inflection and intersection points of approximating functions were carried out using elaborate or cite methodology (Chidume, 2019). In modeling we used our experimental data on pulse conductometry of mouse oocytes and two-cell embryos in a non-conducting solution (0.3 M mannitol or sucrose) (Shigimaga, 2003; Shigimaga, 2011*a*); the osmotic concentration and temperature of the solution (Shigimaga, 2011*b*). The solution osmotic concentrations were 0.1, 0.15 (except for embryos), 0.2, 0.3, 0.4 and 0.5 M. The solution temperatures were: 10, 17, 23, 25, 30 and 35°C for oocytes and 20, 22, 25, 27, 31 and 35°C for embryos. All the operations from plotting graphs to fitting of approximating equations were performed using a MS Excel program of Microsoft Office package (De Levie, 2004).

RESULTS AND DISCUSSION

The sets of concentration dependences of conductivity on PEF strength in mannitol aqueous solution (Sigma) having osmotic concentration of 0.1-0.5 M (Shigimaga, 2011*b*) at constant temperature of 22°C were experimentally obtained for mouse oocytes and two-cell embryos. Fig. 1

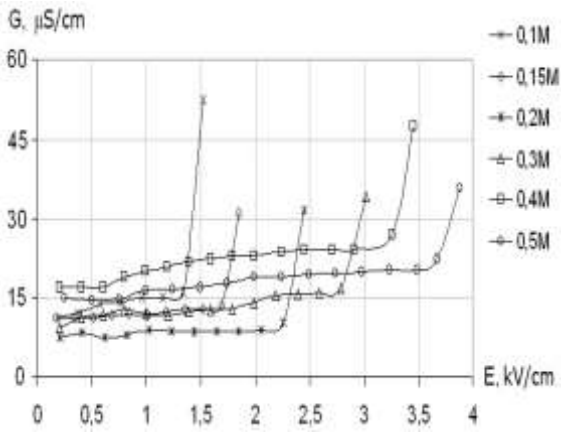


Fig. 1: The set of concentration dependences of oocytes conductivity on PEF strength

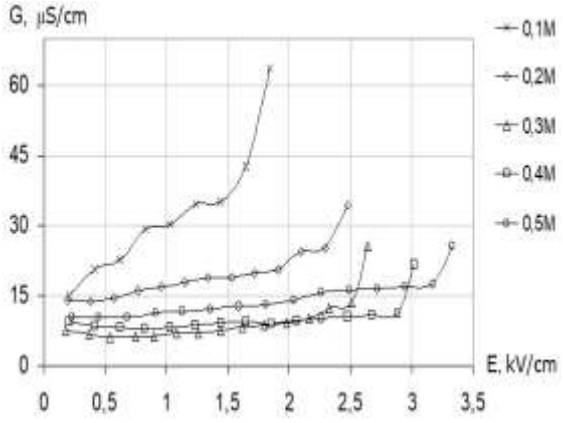


Fig. 2: The set of concentration dependences of embryos conductivity on PEF strength

and 2 show the set of dependences of conductivity of oocytes and embryos, respectively. It has been established that at a certain field strength an irreversible membrane breakdown occurred in all the cells (as indicated by the growth of conductivity) in all solutions, and with decreasing mannitol concentration the breakdown strength decreases. This effect may be explained by the osmotic reaction of cells to hypotonic or hypertonic conditions of the solution relative to isotonia (0.3 M).

For quantitative analysis of above effect, the mathematical processing of obtained concentration dependences was carried out using piecewise-linear approximation (Shigimaga, 2014*a*). The strength of irreversible electrical membrane breakdown for all mannitol concentrations and each cell was calculated by simultaneous solution of the system of two linear equations in general form using the known formulae of analytic geometry. The abscissa of the point of intersection of approximation straight lines was taken as the strength of electrical membrane breakdown. The obtained values of the strength of electrical breakdown depending upon the solution concentration for both oocytes and embryos were plotted in the same plot for comparison (Fig. 3). On the same plot the model functions which construct algorithms were explained further.

The mathematical model of the obtained concentration dependences of the strength of electrical breakdown of cells was built on the basis of following considerations. It is known that the degree of influence of solution osmotic concentration on a cell characterizes surface-volume relation W (Rozanov *et al.*, 1992); on the assumption of sphericity of cell it has the following form:

$$W = \frac{S}{V} = \frac{3 \times 4\pi R^2}{4\pi R^3} = \frac{3}{R} \qquad (1)$$

where S is the surface, V is the volume, and R is the radius of the spherical cell.

It is clear from the experiment (Rozanov *et al.*, 1992) that the rate of the change in above value is inversely proportional to the solution concentration and, thus we can write a simple differential equation:

$$\frac{dW}{dC} = \frac{1}{C} \qquad (2)$$

where C is the osmotic concentration of the solution.

On integrating the equation (2) with separable variables we have the solution in the form of logarithmic function:

$$W = \ln C + K \qquad (3)$$

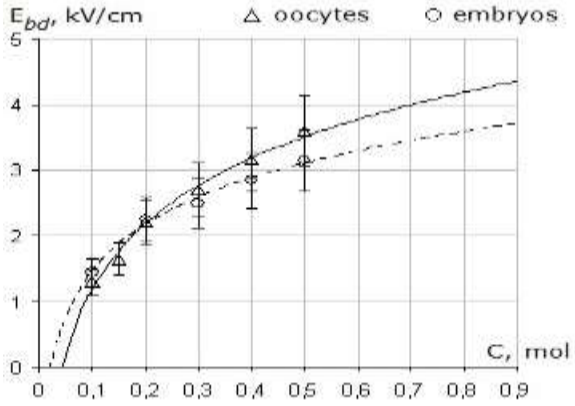


Fig. 3: The dependence of electrical membrane breakdown of mouse oocytes and two-cell embryos on the osmotic mannitol concentration

where K is the integration constant. Substituting expression (1) into (3) we have:

$$\frac{1}{R} = \frac{ln\, C}{3} + K_1 \qquad (4)$$

Further, the theory and practice of membrane electroporation of cells shows that the strength of electrical breakdown is inversely proportional to the cell radius i.e. $E_{bd} \sim R^{-1}$ (Morshed *et al.*, 2013; Neumann and Kakorinm, 2018). Consequently, using this fact, the expression (4) can be written as:

$$E_{bd} = A\, ln\, C + B \qquad (5)$$

where E_{bd} is the strength of electrical breakdown of a cell; A and B are the constants.

Thus, we obtain the mathematical model (5) of concentration dependence of the strength of electrical breakdown of cell. By approximating the model to the experimental points, the equations of model functions (with the determination coefficients) are obtained for oocytes and embryos (Fig. 3).

$$1{,}45\, ln\, C + 4{,}52 = 0 \quad (R^2 = 0.99) \text{ for oocytes}$$

$$1{,}03\, ln\, C + 3{,}82 = 0 \quad (R^2 = 0.99) \text{ for embryos}$$

The extrapolation of approximating functions to zero strength of breakdown yields two values of concentration of mannitol of 0.044 M for oocytes and 0.025 for embryos. These values are obtained as a result of the solution of two corresponding equations which are obtained by setting equal to zero of approximating functions: $1{,}45\, ln\, C + 4{,}52 = 0$ and $1{,}03\, ln\, C + 3{,}82 = 0$. The derived solutions mean that at such osmotic concentrations of mannitol, there is no need to apply any external force in the form of a pulse for electrical breakdown of cells. In fact, in the experiment the cells did not withstand even when they were simply placed to mannitol with concentration < 0.1M – the membrane spontaneously ruptured a minute (in case with embryos – a little longer).

On the plot two approximating functions intersect at a certain point (Fig. 3). The point coordinates were determined by equating the functions for single cell and embryo, that gave the concentration C = 0.19 M. On substituting this value in any of the functions, the strength E_{bd} = 2.1 kV cm^{-1} was obtained. The point of the intersection of two approximating functions means the similarity of conducting properties of oocytes and embryos, which was reached at the osmotic concentration of 0.19 M, presumably due to the similar geometric structure of membranes. Hence, it follows that on the left from point C = 0.19 M, two-cell embryos can withstand larger hypotony than oocytes because the excess of the volume of perivitelline space was more than double. On contrary, on the right from the above point the strength of electrical breakdown of oocytes was larger than that of embryos, i.e. the latter were more vulnerable. It showed the extrapolation of curves to the largest possible (according to the solubility in water) osmotic concentration of mannitol C = 0.85 M. However, data even at the concentration of 0.6 M was complicated due to the methodic difficulties; the solution of these problems lead to the change in technological process and rendered the experiment more complicated.

The sets of temperature dependences of conductivity on field strength in the solution with isotonic concentration of mannitol were experimentally obtained for oocytes and two-cell embryos (Shigimaga, 2011*a*). Fig. 4 presents temperature dependences of oocyte and embryos conductivity. It

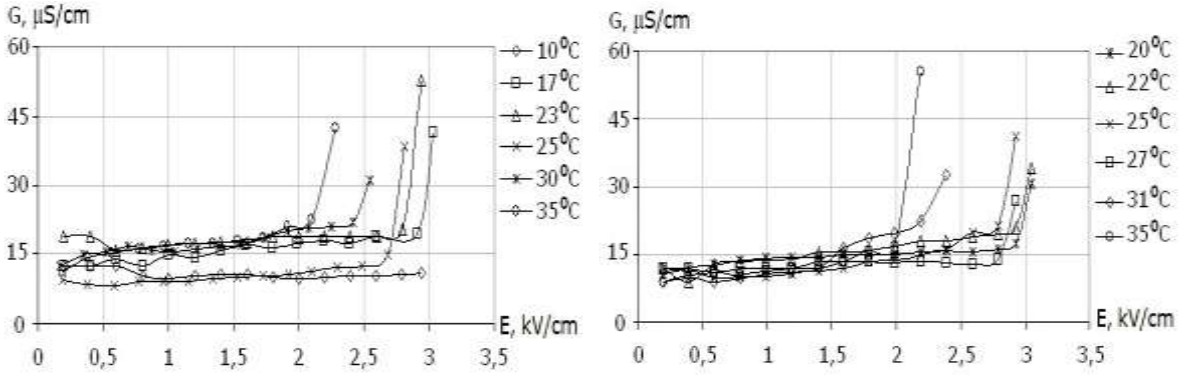


Fig. 4: The set of temperature dependences of oocyte (left side) and embryos (right side) conductivity on PEF strength

was established that in all cells the irreversible electrical breakdown of membrane occurred at certain field strength which systematically decreased with rise in solution temperature. At warmer temperature the cell membrane was a liquid-crystalline structure, the lipids of which developed at warmer temperature; however, on cooling to ≤15°C, they "freezed". Also, the quantitative analysis of the sets of temperature dependences were carried out using piecewise-linear approximation (Shigimaga, 2014*a*). For oocytes and embryos, the strength of electrical breakdown $E_{bd}(T)$ was calculated for the entire temperature range; and the points plotted in the same plot for comparison. Fig. 5 shows both dependences $E_{bd}(T)$ (black dots with errors), and they have an inflection point.

At temperature >30°C, the closing of membrane electropores accelerates after reversible break-down due to the membrane thermolability (Pakhomov *et al.*, 2010; Chang *et al.*, 2012). Therefore, despite a decrease in electrical breakdown strength with the rise in temperature, the electropores close faster than form. When the temperature decreases the situation is reversed. It means that there is a peculiar competition of above processes, which is displayed on the plot where the point of their equilibrium is the inflection point. On this basis, Verhulst-Pearl logistic function was chosen for building the model of experimental dependence $E_{bd}(T)$. It has an inflection point and is bounded from above and below by asymptotes which have physical justification in this case. At temperature <15°C the membrane 'freezes' and electrical breakdown strength is maximal; while at ≥40°C its thermal destruction takes place and electrical breakdown strength is minimal; therefore:

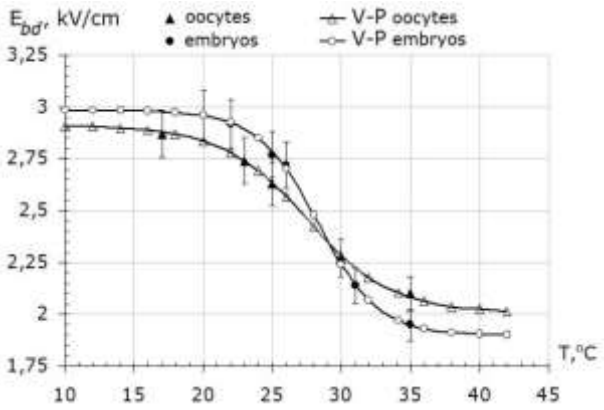


Fig. 5: The temperature dependence of electric breakdown strength E_{bd} for oocytes and embryos (black dots with errors) and its approximation by Verhulst-Pearl logistic function (white dots)

Table 1: The parameters of the model Verhulst-Pearl function for oocytes and embryos

parameter	Cells	
	Oocytes	embryos
A	2.910	2.986
B	0.901	1.087
C	0.327	0.455
D	27.51	28.29
R^2	0.999	0.9999

where *a, b, c, d* are the model parameters (6)

$$E_{bd} = a - \frac{b}{1+exp[-c(T-d)]} \qquad (6)$$

where *a, b, c, d* are model parameters, *T* is temperature, and E_{bd} is electrical breakdown strength.

The calculated model parameters and coefficients of determination R^2 (the experiment-model goodness of fit) are given in Table 1.

In accordance with the required condition $\frac{d^2E_{bd}}{dT^2}(T) = 0$ of the inflection point, at its substitution in (6) the abscissae of temperature points were calculated: for oocytes T = 27.5°C and for embryos T = 28.3°C. For embryos, this temperature was somewhat higher which may be attributed to the lipid composition of their membrane containing apparently large amount of high-melting lipids.

The sets of conductivity dependences on field strength were experimentally obtained for oocytes and two-cell embryos in normal conditions (0.3 M sucrose, T = 20-22°C). Fig. 6 shows the typical curve for individual cells.

For mathematical modeling and subsequent analysis of the above curves it was found convenient to use the piecewise-linear approximation by partitioning the entire curve into 2-3 segments and polynomial fitting to them. The partition into the segments is conventional and is made so that the fitted polynomials can fully describe the experiment (in accordance with the criterion $R^2 > 0.95$) and they should not have higher degrees than 5 or 6 (for simplifying the calculations). Fig. 7(1) presents the example of the fitting of embryo conductivity

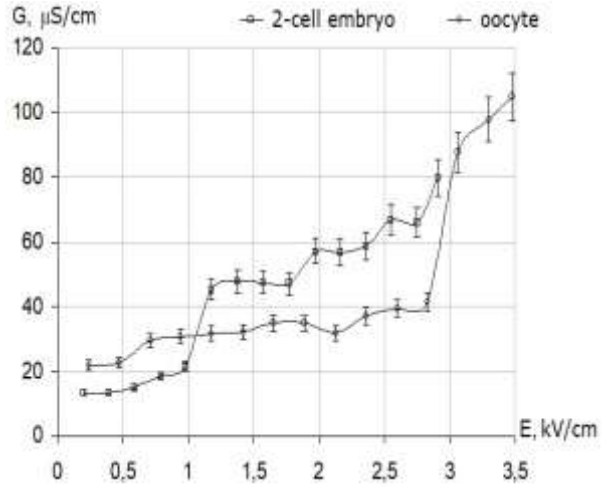
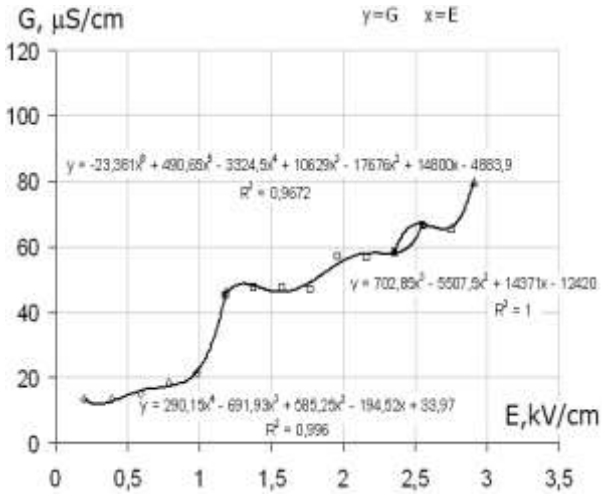


Fig. 6: The conductivity dependence of mouse oocyte and two-cell embryo on PEF strength in 0.3 M sucrose

curve. The fitting of the curve partitioned into three segments gave polynomial equations shown on the plot. Then, using the classical mathematical analysis the polynomial extrema and inflection points were determined. It revealed a particular mode of membrane electroporation. The coordinates of the points of reversible electroporation and irreversible electrical breakdown of cell membrane can be easily found by the curvature of the approximating the polynomial:

$$\boldsymbol{K = \frac{G''}{[1+(G')^2]^{3/2}} = f(E)} \qquad \textbf{(7)}$$

where *G'*, *G''* are the first and second derivatives of conductivity polynomial, *E* is the filed strength. The condition of extrema of curvature function is $\boldsymbol{K' = f'(E) = 0,}$ which



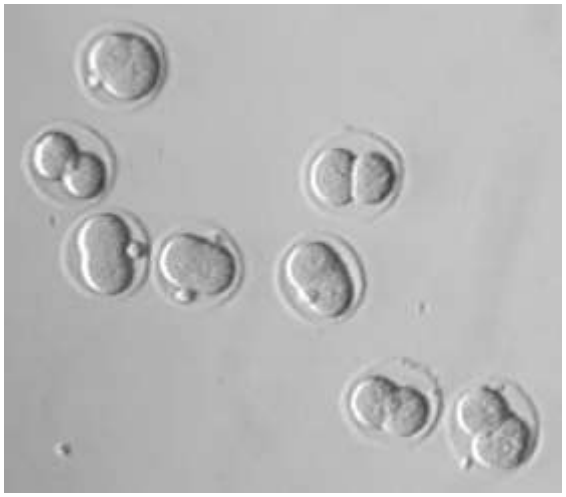


Fig. 7: The piecewise-nonlinear approximation of conductivity curve of a two-cell embryo by three polynomials (left side); The group of two-cell embryos at different stages of fusion (right side)

after the differentiation of equation (7) and its simplifications has the following form:

$$\boldsymbol{\frac{d^3G}{dE^3}\left[1+\left(\frac{dG}{dE}\right)^2\right] - 3\left(\frac{d^2G}{dE^2}\right)^2\frac{dG}{dE} = 0} \qquad (8)$$

On having solved the corresponding exponential equation obtained after substituting the approximating polynomial into deferential equation (8), we obtain the coordinates of all points of curvature; the maximal value of curvature corresponded to the irreversible breakdown, and the intermediate values were different degrees of electroporation. Conventionally, the zero (weak) electroporation at 0.36 kV cm^{-1} has mainly a stimulating character and can be used, for example, for electro-transfection of substances which normally cannot penetrate through membrane. At PEF strength of 1.12 kV cm^{-1} (the point of inflection), the first reversible breakdown or electroporation of the place of contact of membranes of two embryo blastomeres took place. This is the electrofusion mode. When the further action of field on embryo is stopped, its blastomeres fuse after a while. Fig. 7(2) presents the group of such fusing embryos after PEF action. With the growth

of field strength, the other two reversible electroporation occurred at two points 1.74 and 2.17 kV cm^{-1} [Fig. 7(1)]. In this case, electrofusion was still possible but the viability of fused object significantly decreased because the energy used for the two latter electroporation was 2.4 and 3.8 times larger than that for the first electroporation, i.e. the danger to damage the cell organelles was great. When the field strength grew to 2.75 kV cm^{-1}, the irreversible breakdown of membrane of one blastomere, as a rule, took place on the side of anode and, thus the fusion becomes impossible. Consequently, for maximally effective electro-fusion the field strength in the range of 1.0-1.3 kV cm^{-1} is required and sufficient.

Conclusions: The mathematical models built do not exclude any other approaches to modeling intended for more complete explanation of electroporation process in PEF with increasing strength. Modeling provide not only computerization of analysis of measurements, but also and, secondly, the individual approach to each cell, which is lost at the standard statistic treatment of data obtained for the group of cells. The offered approach to modeling and analysis of cell conductometry data is rather promising, since it gives an opportunity to realize the process of differential pulsed conductometry which allows the approximation of cell conductivity by polynomial and automatic calculation of polynomial curvature in all points of both reversible and irreversible membrane electroporation. The coordinates of curvature points (at reversible electroporation) can be used for realization of different modes of electro-manipulation with living cell, i.e. electro-transfection of molecules through membrane, electrofusion (cloning, stimulation) - for purposeful lysis of a cell with the lost mechanism of apoptosis, for example, malignant cells. The mathematical models of experimental conductometric curves allow calculating the PEF strength required and sufficient for the realization of any technologies of electro-manipulation based on the electroporation phenomenon.

REFERENCES

Abidor, I.G., Chernomordic, L.V. and Chizmadzhev, Y.A. 1978. Electrical breakdown two-layer lipid membranes. *DAN USSR,* **240**(3): 1239-1244.

Chang, D.C., Chassy, B.M., Saunders, J.A. and Sowers, A.E. 2012. *Guide to Electroporation and Electrofusion.* Academy Press, New York, USA.

Chidume, O.C. 2019. *Foundations of Mathematical Real Analysis: Computer Science Mathematical Analysis.* Ibadan University Press, Ibadan, Nigeria.

De Levie, R. 2004. *Advanced Excel for Scientific Data Analysis*. Oxford University Press, New York, USA.

Kolesnikova, A.A., Shigimaga, V.A. and Smolyaninova, E.I. 2013. Effect of maturation stimulation on electrical conductivity and fertilization of mouse oocytes. *Fundamental Research,* **4(**4): 896-899.

Miklavcic, D. 2017. *Handbook of Electroporation.* Springer International Publishing, Cham, the Switzerland.

Morshed, B.I., Shams, M. and Mussivand, T. 2013. Deriving an electric circuit equivalent model of cell membrane pores in electroporation. *Biophysical Reviews and Letters,* **8**(1): 21-32.

Neumann, E. and Kakorinm, S. 2018. Membrane electroporation: Chemical thermodynamics and flux kinetics revisited and refined. *European Biophysics Journal,* **47**(4): 373-387.

Pakhomov, A.G., Miklavčič, D. and Markov, M.S. 2010. *Advanced Electroporation Techniques in Biology and Medicine*. CRC Press, New York, USA.

Rozanov, L.F. and Smolyaninova, E.I. 1992. Osmotic behavior of mouse oocytes and embryos. *Problems of Cryobiology and Cryomedicine,* **4**: 26-32.

Saulis, G., Saule, R., Bitinaite, A., Zurauskiene, N., Stankevic, V. and Balevicius, S. 2013. Theoretical analysis and experimental determination of the relationships between the parameters of the electric field pulse required to electroporate the cells. *IEEE Transactions Plasma Science,* **41**(10): 2913-2919.

Shigimaga, V.A. 2003. Determination of conductivity of animal embryo cells. *Problemy Bioniki,* **59**: 60-64.

Shigimaga, V.A. and Yu. Ye. Megel 2011*a*. Method for determination of conductivity of oocytes and embryos in different conditions of dielectric medium. **In**: *Vestnik NTU «KhPI» (Sb. trudov «Novye resheniya v sovremennykh tekhnologiyakh»)*, Kharkov: NTU (KhPI)., № 9: 140-144.

Shigimaga, V.A. and Yu. Ye. Megel. 2011*b*. Investigation of conductivity of cell at varying osmotic concentration of medium. *Eastern-European Journal of Enterprise Technologies,* **N 2/5**(50): 53-55.

Shigimaga, V.A., Levkin, D.A. and Megel, Yu. E. 2012. Mathematical modeling of membrane in connection with cell conductivity in various solutions. *Eastern-European Journal of Enterprise Technologies,* **4**(4): 32-35.

Shigimaga, V.A. 2013. Nonlinear electrical model of conductivity biological cell. *Technical electrodynamics*, **6**: 30-35.

Shigimaga, V.A. 2014*a*. *Biotechnical Complex for Pulse Conductometry and Animal Cells Electro-manipulations*. Thesis for Doctor of Technical Science degree. Kharkov, Ukraine. p. 25.

Shigimaga, V.A. 2014*b*. Measurements of the capacitance of a biological cell by a pulse method. *Measurement Techniques*, **57**(2): 213-217.

Shigimaga, V.A. 2015. Pulsed conductometry in a variable electric field: outlook for the development of measurements. *Measurement Techniques,* **57**(10): 1213-1218.

Shigimaga, V.A. 2019. Conductometry in pulsed electric field with rising strength: Bioelectro-chemical applications. *Analytical and Bioanalytical Electrochemistry,* **11**(5): 598-609.

Son, R.S., Smith, K.C., Gowrishankar, T.R., Vernier, P.T. and Weaver, J.C. 2014. Basic features of a cell electroporation model: Illustrative behaviour for two very different pulses. *Journal of Membrane Biology,* **247**: 1209-1228.

Stigler, S.M. 1981. Gauss and the invention of least squares. *Annals of Statistics,* **9**(3): 465-474.

Wang, M., Orwar, O., Olofsson, J. and Weber, S.G. 2010. Single-cell electroporation. *Analytical and Bioanalytical Chemistry,* **397**(8): 3235-3248.

Weaver, J.C. and Chizmadzhev, Y. 1996. Theory of electroporation: A review. *Bioelectrochemistry and Bioenergetic*, **41**: 135-160.

YiKuen, Lee and Deng PeiGang 2012. Review of micro/nano technologies and theories for electroporation of biological cells. *Science China Physics, Mechanics and Astronomy,* **55**(6): 996-1003.