doi: 10.56761/EFRE2024.S4-O-023501

Voltage pulse generator with DC offset for drug delivery through model round window membranes

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Abstract. Existing methods of drug delivery to the inner ear, including the use of microneedles, micropumps and cochlea implants, are associated with a number of serious complications. It is assumed, due to the use of electric pulses with dc offset it is possible to promote enhanced penetration of drugs through the membrane of the human inner ear with its subsequent rapid recovery. The special voltage pulse generator was developed to study this delivery method. The model biological membranes were treated in a wide range of electrical parameters. The optimal range of currents, frequencies and pulse durations was established based on an assessment of the viability of membrane cells after electrical exposure through vital dye staining method. Preliminary results on the permeability of membranes for dexamethasone sodium phosphate were obtained using high-performance liquid chromatography.

Keywords: pulse generator, drug delivery, biological membranes, electroporation, ionophoresis, electrode cell, cell survival, permeability, chromatograms.

1. Introduction

Treatment of inner ear diseases with drugs is common in modern medicine, but its effectiveness relies on the drugs reaching the targeted cells. Traditional delivery methods have limitations, making the development of new methods that can overcome biological barriers a pressing need. [1, 2]. One non-invasive way to deliver drugs is to use an electric field to move ions. This approach involves ionophoresis and electroporation. Ionophoresis uses a weak, constant electric field to move small charged molecules, while electroporation increases the permeability of cell membranes to larger molecules. It is necessary to use less invasive approaches that combine membrane electropermeabilization, electroosmotic and electrophoretic movement of drugs [3, 4] to overcome biological barriers, such as the round window membrane of the cochlea. The goal of the work is the development of a special electrophysical setup providing electroporation and iontophoresis effects on the biological membrane. The development of such drug delivery methods could significantly improve the quality of life of patients with inner ear disorders.

2. Electrophysical experimental setup

An experimental electrophysical setup to treat the model round window membranes (MRWM) of the human inner ear was developed.

2.1. Experimental electrophysical setup

Thanks to the presence of two power sources, it is possible to control the voltage and current on two units of the setup: electroporation (voltage pulses) and ionophoresis (offset current).

The pulse generator is assembled in the Institute for Electrophysics and Electric Power of Russian Academy of Sciences using a half-bridge circuit with galvanic isolation [4]. Amplitude of the voltage pulses V_{PULSE} is determined by the GPR-730H10D power supply (GW Instek). The formation of high-voltage pulses occurs by switching the voltage from the power source to the top electrode of the electrode cell by the transistor *T1* (Fig. 1), while the other transistor *T2* is in the closed state. After the end of the pulse, the transistors *T1, T2* change their state, and the electrode

potential is compared with the DC offset potential V_{DC} (the voltage of the GPR-30H10D power supply). The control pulses are generated independently of each other. They determine duration and frequency of the output voltage pulses.

The GPR-30H10D power supply (GW Instek) is responsible for the ionophoresis and determines the bias current through the ballast resistor. It is possible to adjust the bias voltage of the V_{DC} pulses relative to zero potential.

The output of the generator was connected to the electrodes of the cell with the sample, filled with a buffer solution (Fig.1).

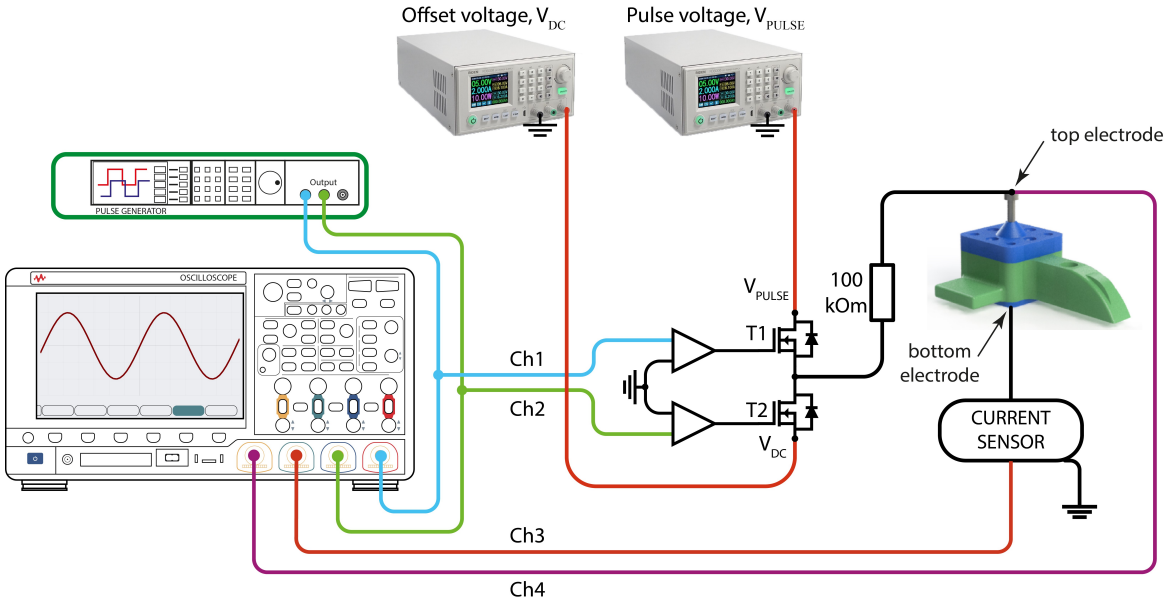

Fig. 1. Experimental electrophysical installation for combined electroporative-iontophoretic effect.

Figs. 2 and 3 show typical positive and negative waveforms of the current through the cell and the voltage across it.

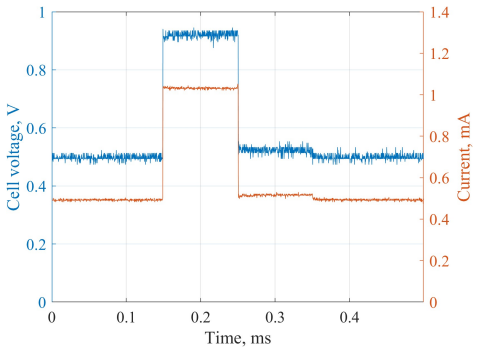

Fig. 2 Typical waveforms of the current through the cell and the voltage across it (positive polarity).

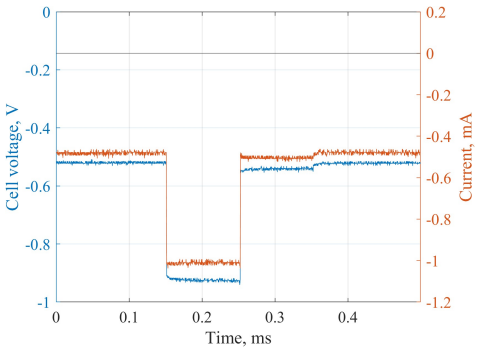

Fig. 3. Typical waveforms of the current through the cell and the voltage across it (negative polarity).

2.2. Cell design

The electrode cell for experiments with MRWM was developed [5]. Cross section of the electrode cell is shown in Fig. 4. The cell is made of biocompatible ABS plastic on a 3D printer. The electrodes are silver chloride electrodes ordinary used for electrocardiography. The top electrode is mounted in the cover that closes the cell with the sample. The bottom electrode is inserted in the bottom part of the cell. The cell is filled with 0.9 % NaCl buffer solution. The sample

membrane is placed on the bottom cell part, filled with the buffer solution (Fig. 5) A drug solution is applied through the holes in the cover. A siphon device equalizes pressure during filling and sampling. There is a special tube for sampling from the side.

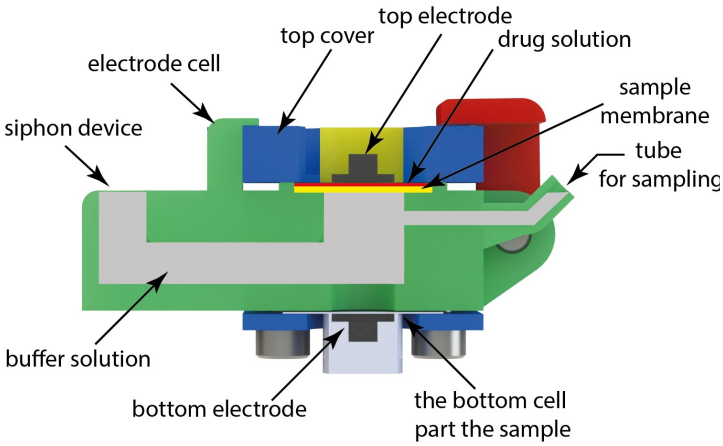

Fig. 4 Electrode cell cross section.

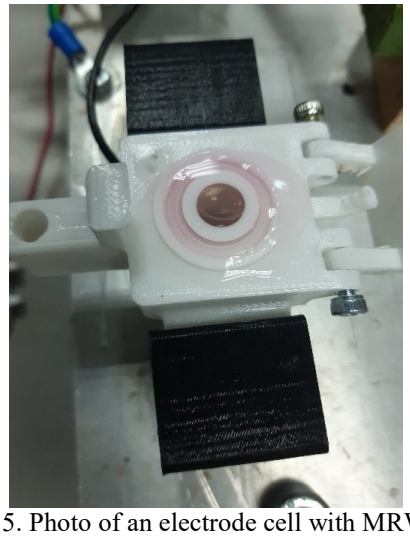

Fig. 5. Photo of an electrode cell with MRWM.

It is assumed that, under the influence of electrical pulses, a certain amount of the drug will pass through the membrane and enter the buffer solution. The entire structure of the electrode cell is securely fixed on the table in order to prevent its shaking and the penetration of the drug into the cell during and after the experiment.

2.3. Biological membranes

The MRWMs of the human inner ear, grown at the Koltzov Institute of Developmental Biology of Russian Academy of Sciences, served as biological membrane samples [7]. Membranes of thickness 200-500 μm were grown on a collagen basis produced by the biotechnological company "IMTEK"". Firstly, human fibroblasts were applied to the surface – 50000 cells per insert in 100 μl of medium. After 1 day, the medium was removed, and HaCaT cells were applied, 100000 cells per insert in 100 μl of medium. Then the resulting MRWMs were cultivated in a nutrient medium [8]. Cultivation was carried out in an incubator with 5% CO_2 under saturated humidity conditions.

3. Measurement and diagnostics methods

To ensure the accuracy of the results, several dozen electrode cells were produced, which were sterilized in advance. To obtain reliable and distinguishable results, the experimental series were repeated from 2 to 5 times, followed by statistical processing.

To assess the viability of MRWM cells the samples were stained with calcein green vital dye. [9]. The vitality of the MRWM cells was determined by the fluorescence intensity of the green color, obtained with the use of ImageJ free Software. The amount of drug passed through the membrane in the selected samples was assessed using gas chromatography at an installation at the Nesmeyanov Institute of Organoelement Compounds of Russian Academy of Sciences.

The measuring elements were represented by two Keysight N2842A 300 MHz probes: the first one was connected to the cell to measure the voltage drop across it, the second one was connected to a 500 Ohm resistive low-inductance sensor. Both probes were connected to a Keysight DSOX2024A 200 MHz 2 GS/s digital oscilloscope, and their signals were recorded in CSV format for further processing on a PC. The general view of the experimental stand is shown in Fig. 6.

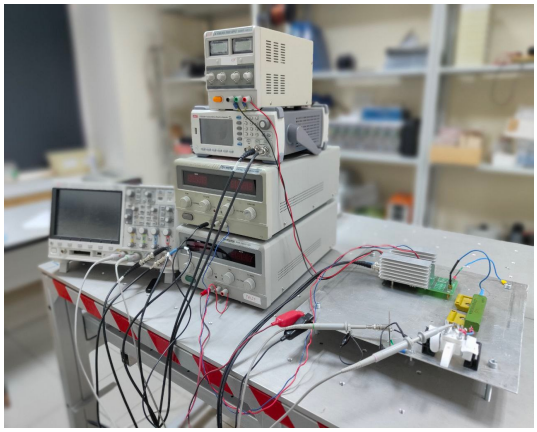

Fig. 6. General view of the experimental stand.

4. Experimental part

The experiments were carried out using the experimental electrophysical setup to determine the viability of membrane cells under pulsed and direct currents, and to determine the effects of polarity and frequency on the MRWM cells viability. After the experiment, the membrane permeability was determined using chromatograms of the treated solution.

4.1. Cell viability under DC current

The first series of experiments was carried out at DC current values of up to 1.5 mA in the absence of superimposed pulses. The value of charge passed through the membrane was 0.5 C, which was ensured by different exposure times for all samples. Based on the processing results, the dependences of the green dye activity on the current value were obtained (Fig. 7). Taking into account the errors, it can be stated that in the range of DC currents from 0.2 mA to 1.5 mA there is no noticeable change in cell viability.

4.2. Cell viability after pulse treatment

This experiment was conducted at the DC offset, corresponding to an average current through the cell of 1 mA. The voltage 100 μs pulses of different amplitude at frequency of 1 kHz were imposed on the sample, what provided the pulse current from 0 to 2 mA. The value of charge passed through the membrane was 0.5 C. Based on the results, the dependences of the calcein green activity on the current value were obtained (Fig. 8). It was found that with an increase in pulse current above 1.3 mA, the number of living cells significantly decreases.

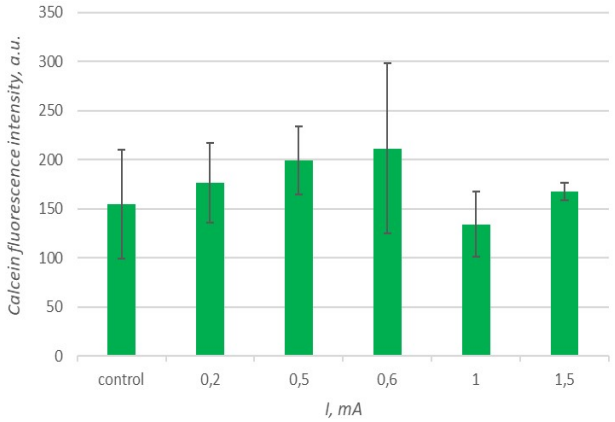

Fig.7. Dependence of calcein fluorescence intensity on the DC current value

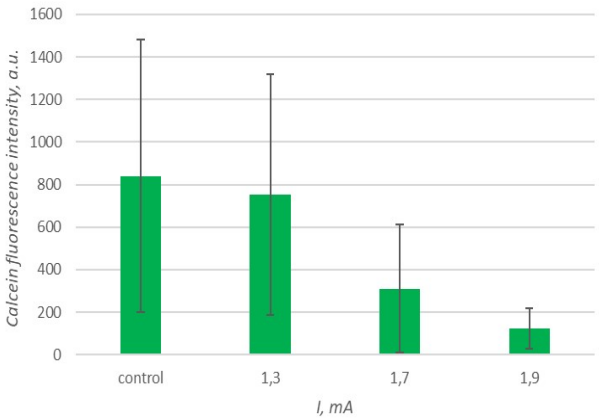

Fig.8. Dependence of calcein fluorescence intensity on the value of pulse current

4.3. Cell viability at different polarity and frequency

Experiments were carried out at a constant average current of 0.5 mA, a pulse current of 1 mA, and a pulse duration of 100 μs. The passed charge was set to 450 mC. The objective of the experiment was to determine the effect of pulse repetition frequency of positive and negative polarity on the viability of MRWMs. Three repetition frequencies were set as reference: 1 Hz, 10 Hz and 100 Hz. Based on the processing results, the dependence of the calcein green activity on the frequency value was obtained (Figs. 9, 10). Based on the results obtained, there is a slight decrease in the number of living cells closer to 100 Hz frequency for positive polarities. But still, we can say that the pulse repetition frequency in the range of 1 – 100 Hz at a constant current of 0.5 mA and a pulsed current of 1 mA has practically no effect on cells despite of the voltage pulses polarity.

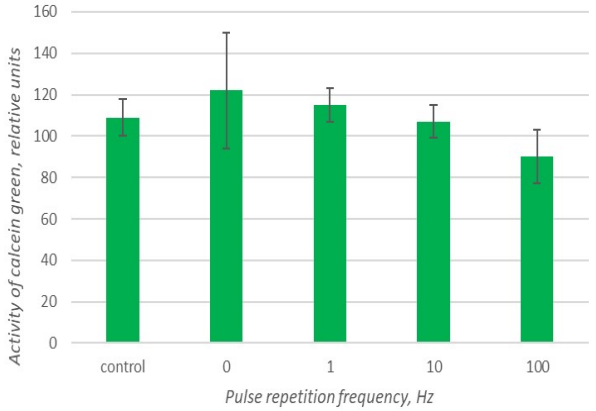

Fig. 9. Dependence of calcein fluorescence intensity on the value of pulse current (positive polarity).

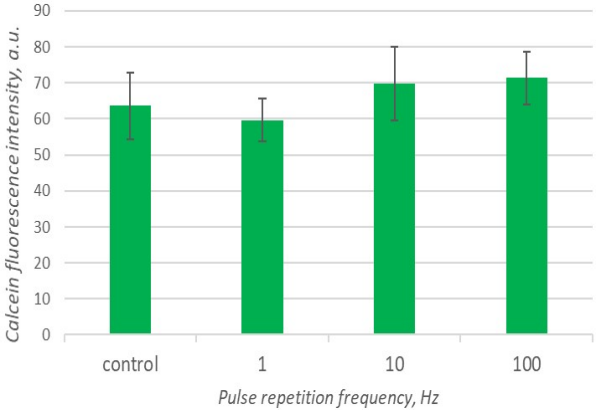

Fig. 10. Dependence of calcein fluorescence intensity on the value of pulse current (negative polarity).

4.4. Membrane permeability

The experiment was conducted at the DC offset, corresponding an average current through the cell of 1 mA. The voltage 100 μs pulses at frequency of 100 Hz were imposed on the sample, what provides the pulse current of 2 mA. The exposure time was 8 minutes. Solution of dexamethasone sodium sulfate 4 mg/ml was used as a drug.

The efficiency of dexamethasone passage was determined by chromatography. Absorption signal areas corresponding to the original solution and the treated one are shown in Figs. 11 and 12.

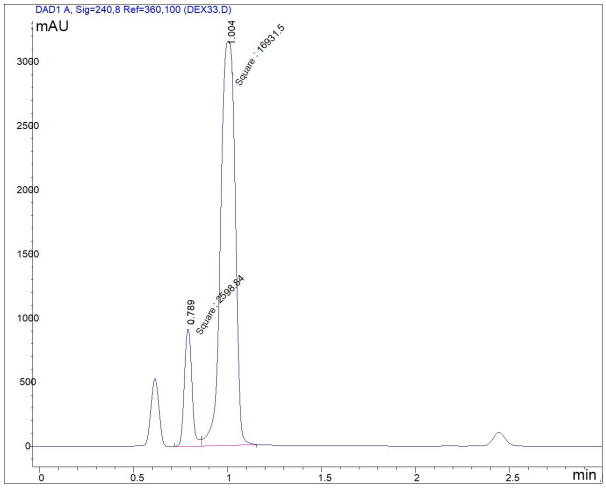

Fig. 11. Control chromatogram of dexamethasone (right peak).

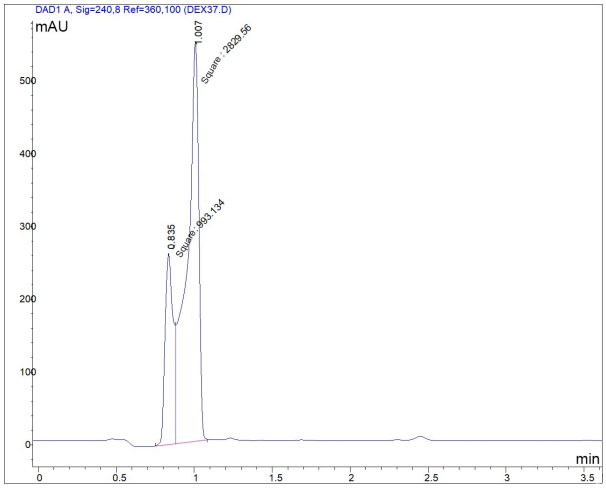

Fig. 12. Chromatogram of a sample for the presence of dexamethasone (right peak) after the experiment.

Based on the ratio of the areas of dexamethasone activity signal 16931.5/2829.56, the amount of drug passed was about 16%.

5. Conclusion

Thus, a number of important results were obtained.

A tendency towards deterioration in the viability of MRWM cells with increasing amplitude of the pulse current was revealed, which made it possible to select its limiting value of 1.3 mA for further experiments. It was found that DC current up to 1.5 mA, frequency range from 0 to 100 Hz and polarity do not significantly affect the viability of the membranes.

The effectiveness of the combined electroporation-ionophoresis method for drug delivery was confirmed on the example of the dexamethasone sodium sulfate.

Acknowledgements

This work was carried out with the support of Moscow Center for Technology Development in Healthcare, Agreement No. 2012-1/22.

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