ELECTROPORATOR FOR LABORATORY TESTS

Vytautas Dumbrava, Vladas Juska, Darijus Pagodinas, Marius Gailius, Linas Sidaras

Signal processing department , Kaunas University of Technology
Studentu str. 50, LT-51368, Kaunas , Lithuania
T.+37037 300531; F.+37037753998; E. vytautas.dumbrava@ktu.lt

Abstract

In recent years the molecular therapy was successfully developed. Its aim is to achieve an active agent penetration into damaged cells only. This way the agent is getting more effective and causes less side effects. It is often that the membranes of damaged cells are closed and aggravates the active agent penetration inside the cells. It is find out that the use of electroporation methods enables us to solve this problem effectively.

Industry produces a lot of electroporators for clinical work. Laboratory equipment, however, has to have a broader range of parameters, to be able to optimize the electrical parameters and vary them in a relatively wide range.

The electroporator designed in Kaunas University of Technology, Signal processing department is intended for laboratory research purposes to find out more electroporation effects on cell membranes. This paper presents the already done electroporator with the enhanced facilities, its structure and technical parameters. The device has a personal computer (PC) to control parameters of electroporator and has the capabilities for data acquisition on PC and the subsequent digital processing. It also provides synchronization signals for electroporation with programmable temporal shift control.

1. INTRODUCTION

Since that time when the electroporation methods were invented it found more and more application for medical [1-3], and biotechnological [4-5] purposes. Elektroporation (EP) is one of possible methods in which DNA molecules can be delivered into cells by using a short electric pulses. Electrical breakdown of cell membranes can be either reversible or irreversible [3]. Each of them is widely used in a field medicine as well as in biotechnology. In medicine drug regimen traditionnally has been used in various ways: injection, infusions, inhalations, creams, and transdermal patches. In this case, the active agent is distributed throughout the body and this requires the introduction of much higher doses. Since at 1987 successfully was developed molecular therapy, during which an effort is made to reach the active agent only cells that are damaged. Then this agent is becomes more effective and cause fewer side effects. However, the cell membrane is closed and prevents the active agent to enter the cells, so the techniques used, allowing up to facilitate an active agent to enter the cells. It was found that the electroporation (EP) methods to perform duty in an effective way to overcome the cell membrane barriers.

The electroporation is also used to accelerate a green mass drying. [4]. Such a method enables us to save energy used during the drying process.

Electroporation is a new way for the mash treatment of vinification process. Electroporation the mash of red wine enables a fast extract the red pigments from the skin without remarkable heating of the mash. [5].

The aim of this article is to provide the structural scheme of created electroporator for medical purposes, describe the main parameters of the device and the present really output signals using the experimental time diagrams.

2. STRUCTURE

The industry has developed a whole range of industrial electroporators which are used in practice [6] and/or for scientific research in a field of biomedicine [7]. Such devices are rather expensive and are mostly used for standard techniques. The laboratory prototype of the electroporator was designed in Kaunas University of Technology, Signal Processing Department. The prototype has an enhanced facilities and it is made for scientific researches to find the optimal parameters. The quantity of energy absorbed into a cell depends direct from the electrical parameters and for each material it should be chosen and optimised separately [8].

Electroporation of cells in common theory are divided into the high (HV) (up to 1200V) and low amplitude (LV) (up to 100V) packets pulses with variable-frequency and then is observed the state of cell membrane barriers.

The common structure of given electroporator is shown in Fig. 1. The device can be controlled by local keyboard or using the virtual panel from PC. Using the local keyboard it is possible to control and change the electrical parameters in autonomic mode. All functions may also be accomplished by special PC program, which retains the values of operating parameters. In both cases the parameters are shown on local LCD placed within the device.

The values of HV and LV voltages are set up by microprocessor (CPU) and controlled by separate controller, which signals control the AC – DC converter.

Time settings are set by CPU, and the commutation is produced by HV and LV pulse generator. U and I sensors are engaged with the monitoring controller, which consequently informs the CPU about possible failure. Here as well the sequences of HV and LV pulses are summed up. The two dangerous situations of short circuit in the back stage and a failure of back switches can be displayed on the monitor. In both cases the HV voltage is switched off, and the control of switches is blocked. The power unit forms galvanically separated voltages for all of the electroporator units.

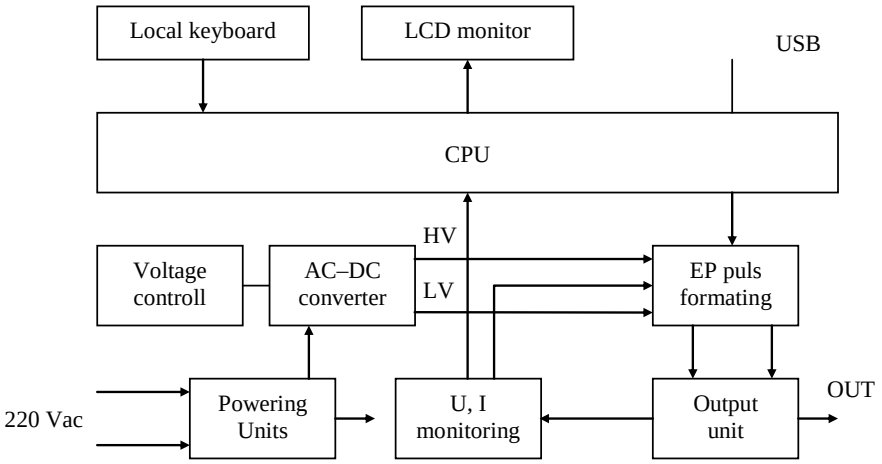

Figure 1. Structure scheme

EP is controlled by three Atmega 64 CPU units. Atmega 16 performs voltage control, while Attiny 13 serves for U and I monitoring. Such a function division between different CPU units made the main CPU software (which performs communication between PC and keyboard, arranges and indicates the parameters) to be much simpler. It also allows to start the EP pulse sequence by using an external pedal, and to indicate the parameter values on the PC monitor and LCD screen at the same time.

For the EP we use a special solution which has sufficiently high electrical conductivity in comparison with 1.5% salt solution used before. When the volume resistance equals around 80 Ohms and HV pulse amplitude of 1000 V as a result we get 12,5 kW pulse power. For that reason the Corner Dubilier capacitor with small parasitic inductance L_P was chosen. The equivalent experimental scheme showed in Fig.2. Apart from the above mentioned values the experimental scheme includes circuit capacitance C_M and the capacitance between electrodes and solution C_S.

The augmentation rate of pulse current can be defined as follows:

$$I(t) = [C_M + C_S]\frac{du_c}{dt}. \quad (1)$$

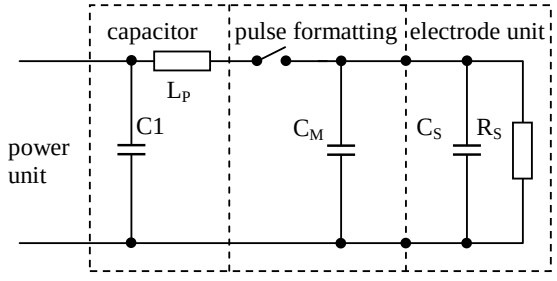

Figure 2. Equivalent scheme of electroporator output

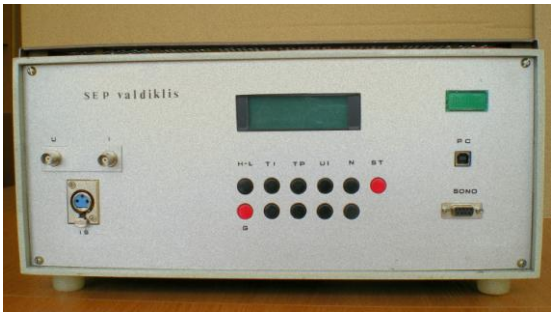

Figure 3. View of the electroporator

The common view of electroporator is shown in Fig. 3. The main parameters of EP device are shown in Table 1.

3. EXPERIMENTAL RESULTS

The oscillograms of an output signal are represented in Fig. 4. It is obvious that HV and LV signals can be supplied to the load simultaneously. The number of positive HV pulses in a sequence may vary from 1 to 9 and their amplitude is in a range between 100 and 1100 V., while duration varies between 1 and 1000 µs. LV voltage may be changed in its polarity up to max. 100 V. It means that LV pulses are at about 10% of HV amplitude value. The detail electrical parameters of the EP device are shown in Table 1.

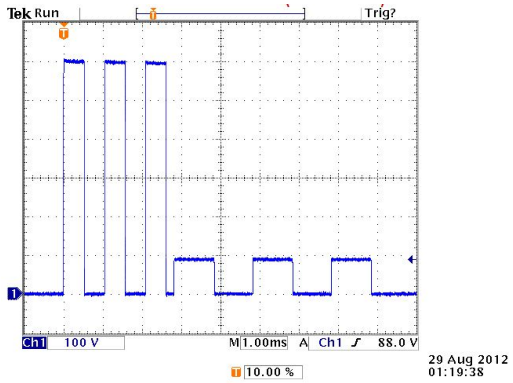

Table 1. Electrical parameters of electroporator"

No.	Parameter	Value
1	HV pulse amplitude	(100 – 1100) V
2	HV pulse duration	(1 – 1000) µs
3	HV pulse number	(1 – 9)
4	HV pulse polarity	positive
4	LV pulse amplitude	(4 – 100) V
5	LV pulse duration	(1 – 1000) ms
6	LV pulse number	(1 – 9)
7	LV pulse polarity	positive or negative
7	Delay between HV ir LV sequence	(5 µs – 1 s)
8	Protectors activation rate	< 30 µs
9	Emergency HV voltage shutdown speed	< 5 ms

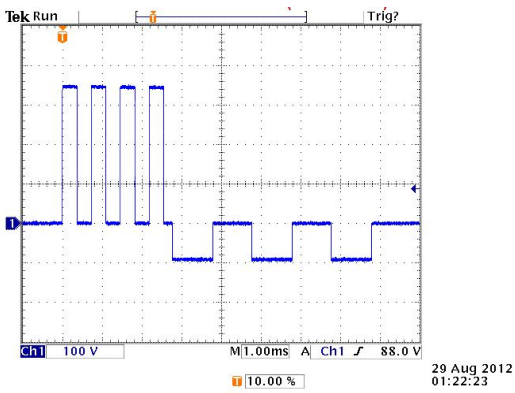

Figure 4. Experimental time diagramm

The control of electrical parameters is performed on virtual PC display (Fig. 5).

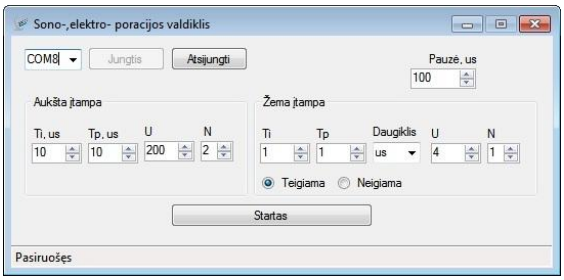

Figure 5. Virtual panel

It is possible here to set all needed output signal combinations. Management of the multiprocessor system is used with typical peer communication channels SPI and USART interfaces. All system components are galvanically separated, because initial tests showed an absolute necessity of these methods. Bandwidth is achieved using a 10 Mbps optical separation. On the final stages are used IGBTs field transistors with maximal breakdown voltage 1700 V.

Due to a low reliability were abandoned standard driver's stages and used schemes with discrete transistors.

5. CONCLUSION

The structural scheme of the electroporator used the methods and developed electroporator parameters, experimental results of output signals are presented. Laboratory model of electroporator has a wider possibilities and used for medical applications

References

[1] A. J. H. Sale and W. A. Hamilton, "Effects of high electric fields on microorganisms: I: Killing of bacteria and yeasts", *Biochim. Biophys. Acta,* Volume 148, 1967 pp. 781–788.

[2] Sukhendu B. Dev, Dietmar P. Rabussay, Georg Widera, and Gunter A. Hofmann," Medical Applications of Electroporation", *IEEE Transactions on Plasma Science,* Volume 28, No. 1, February, 2000, pp.207-223.

[3] T. Kotnik, P. Kramar, G. Pucihar, D. Miklavčič, M. Tarek," Cell Membrane Electroporation—Part 1: The Phenomenon", *IEEE Electrical Insulation Magazine,* Volume 28, No. 5, pp.14-23.

[4] M.Sack, C. Eing, L. Buth, Th. Berghöfer, W. Frey, and H. Bluhm," Electroporation as an optimizing step in drying of green biomass", Pulsed Power Conference, 16th IEEE International ,Volume 1, 2007, pp.756-759.

[5] M. Sack, G. Müller: "Optimisation of an Electroporation Device for Mash". *Proc. OPTIM2008*, Brasov, Romania, May 2008, pp. 113-118.

[6] "Cliniporator EPSo2. Technical sheet", *IGEA S.p.A. Via Parmenide 10/A,* Carpi, Modena, Italy,pp.1-4.

[7] Congo Tak-Shing Chinga, Tai-Ping Suna, Wei-Ti and etc. **Huanga** "A circuit design of a low-cost, portable and programmable electroporation device for biomedical applications". *Sensors and actuators B: Chemical, 2012, pp. 292-300.*

[8] S. Rodamporn. "Optimal Parameters of Electroporation for Gene and Tissue", Biomedical Engineering International Conference (BMEiCON-2011), pp. 279-282.