Fast flow-through non-thermal pasteurization using constant radiofrequency electric fields

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Abstract: Pulsed Electric Field technologies have captured the attention of researchers on food pasteurization because of their non-thermal inactivation mechanism, which results in fresh-like products. Nevertheless, high voltage pulsing required by these technologies implies complex and costly generators. Here, as an alternative, it is proposed a method, partially inherited from research on cell electroporation for gene transfection, in which the liquid to be treated flows at high speed through a miniature chamber where the electric field is permanently applied. In particular, it is proposed that the constantly applied electric field consists of an AC signal (> 100 kHz) so that electrochemical by-products are minimized. The method, while being compatible with batch processing, will allow use of lower voltages and will avoid the pulsation requirement. The proposal is accompanied by a numerical study and an in vitro study which demonstrate its feasibility.

Keywords: Pulsed Electric Field; Electroporation; Flow-Through; Radiofrequency

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1.0 Introduction

Mainly due to their simplicity, thermal methods are the basis of most pasteurization technologies intended for food preservation. High temperatures, however, not only inactivate harmful microorganisms but also damage constituents of the medium under treatment, which may result in detrimental effects on nutrients, color, flavor and texture (Alwazeer, Delbeau, Divies, & Cachon, 2003; Lee & Coates, 2003; Polydera, Galanou, Stoforos, & Taoukis, 2004).

In the last decades, Pulsed Electric Field (PEF) technologies have captured the attention of researchers on food pasteurization (Raso & Heinz, 2006). This is in part a consequence of the growing demand for fresh-like foods; unlike thermal methods, PEF technologies do not cause a major impact on the organoleptic properties of treated foods as their cell killing mechanism is specifically targeted at cell membranes (Cserhalmi, Sass-Kiss, Tóth-Markus, & Lechner, 2006; Elez-Martínez, Soliva-Fortuny, & Martín-Belloso, 2006; Espachs-Barroso, Barbosa-Cánovas, & Martín-Belloso, 2003). Moreover, as an additional advantage, PEF technologies have the potential to minimize energy consumption in comparison with other pasteurization and extraction methods (Q. Zhang, Barbosa-Cánovas, & Swanson, 1995). Nowadays, in the context of renewable resources, this last aspect is particularly relevant for efficient oil extraction from microalgae for biofuel production (Sheng, Vannela, & Rittmann, 2011; Zbinden et al., 2013).

Nevertheless, it must be mentioned that, prior to current resurgence of interest in PEF technologies, there were a number of attempts to adopt PEF methods in the food industry at medium and large scale without much success (Allen & Soike, 1966; Beattie & Lewis, 1925). Failure to industrially adopt PEF processing was mostly attributed to the high cost of electrical pulse generators and, to a lesser extent, to their maintenance costs due to electrode erosion and, in fact, both aspects are still not satisfactorily resolved by current PEF technologies (Jeyamkondan, Jayas, & Holley, 1999; Toepfl, 2011).

Cell killing action in PEF technologies is based on the phenomenon known as irreversible electroporation (Palgan et al., 2012), which is very briefly described in the next paragraph.

When a cell is exposed to an external electric field, charge is accumulated on the cell membrane resulting in an artificial increase of the transmembrane potential (TMP). If such TMP increase is large enough, and sustained for long enough, cell membrane permeability to ions and macromolecules will

increase very significantly. The increase in permeability is, presumably, related to the formation of nano-scale defects or pores in the cell membrane; from which the term electro-“poration” stems. If the induced permeabilization is moderate, cell membranes will reseal and the cell will be fully viable minutes after field delivery. On the other hand, if the permeabilization is made excessive by delivering very high or prolonged fields, cells will end up dying. The former output is known as reversible electroporation and the later as irreversible electroporation. Both, reversible and irreversible electroporation modalities have important applications in biotechnology and medicine. For instance, reversible electroporation is now a routine technique in microbiology laboratories for *in vitro* gene transfection (Neumann, Sowers, & Jordan, 1989). In addition, during the last fifteen years, reversible electroporation has been applied experimentally and clinically to living tissues for *in vivo* gene therapy (electrogenetherapy) and for enhancing the penetration of anti-cancer drugs into undesirable cells (electrochemotherapy) (Marty et al., 2006). More recently, irreversible electroporation has been proposed and demonstrated as a minimally invasive tissue ablation method (Rubinsky, 2007). Typically, electric fields are not applied as a long single pulse but as a series of brief pulses (from fractions of microsecond to a few milliseconds) as this strategy has been shown to be far more effective in terms of permeabilization for the same field magnitude (Miller, Leor, & Rubinsky, 2005). Moreover, brief pulses of moderate field magnitude also offer a key advantage: Joule heating – always present when electric field pulses are applied – is minimized.

In current PEF technologies, the liquid to be treated is forced to flow at moderate speed through a relatively long chamber containing electrodes between which high voltage pulses, or bursts, are applied so that the required high electric fields for electroporation are produced. Typically, the delivered high voltage signals consist of either monopolar square pulses or bipolar square pulses. Bipolar pulses are more difficult to generate but minimize electrochemical reactions at the electrodes, which have detrimental effects on both the electrodes and the liquids. Within the chamber, each portion of liquid has to be subjected to a number of short pulses, or bursts, in order to achieve effective inactivation of microorganisms. This limits the speed at which fluids can flow through the chamber and, more importantly, it imposes severe constraints on the voltage generator. Among those constraints, the most demanding feature is short pulsation. To generate high voltage pulses of short duration (<1 μs) is technically feasible but it comes at an economic cost; particularly because it requires sophisticated high voltage and high-current switches. Spark gap switches – which show maintenance issues – were predominant in the past and now are being replaced by solid state switches (e.g. IGBTs), which are progressively achieving the required features in terms of speed and power. Costs associated with the generator would be greatly reduced if instead of requiring pulsated voltages it was possible to employ a

constant high-frequency AC voltage. The generator in this case would be similar to those employed in RF heating in food processing (Zhao, Flugstad, Kolbe, Park, & Wells, 2000).

As mentioned, electroporation is not only employed for food processing but also for biomedical applications. In particular, electroporation is now routinely used in microbiology laboratories for gene transfection. Typically, the cells to be transfected and a medium containing DNA plasmids are poured into a square shaped cuvette containing two flat electrodes at opposite sides. Between those two electrodes a sequence of high voltage pulses is delivered by means of a desktop generator. As an alternative, in order to transfect large quantities of cells, it has been proposed to electroporate the cells by making them flow at high speed through a microfluidic chamber where the electric field is permanently applied (Geng, Zhan, Wang, & Lu, 2011; Movahed & Li, 2011). This concept is usually referred to as *flow through electroporation* and, besides its suitability for large-scale production, it displays at least two other significant advantages over the standard cuvette process. Namely: 1) it does not require high voltages because, in a chamber with small dimensions, the electric fields (i.e. voltage gradients) can be made very large with small voltages (Huang & Rubinsky, 2001; Huang & Rubinsky, 2003) and 2) it does not require voltage pulsation as an electric field pulse is virtually generated in each portion of fluid as it flows through the chamber. The aim of the study presented here was to assess whether such flow through electroporation concept is applicable to non-thermal pasteurization. In comparison to flow through electroporation for gene transfection, flow through electroporation for pasteurization will require much higher fields that must be applied for much shorter periods of time in order to avoid excessive heating. This, in turn, has an impact on the required fluid velocity. Precisely, a specific objective of the study was to assess whether the required fluid velocities for avoiding excessive heating would be achievable with reasonable chamber dimensions. In addition, and in contrast with systems proposed for flow through electroporation for gene transfection – in which DC voltages are applied between the electrodes within the microfluidic chamber –, here it is proposed to employ constant radiofrequency (>100 kHz) voltages so that electrochemical reactions at the electrodes are minimized or even completely avoided (Morren, Roodenburg, & de Haan, 2003). This feature will be crucial in terms of electrodes maintenance and liquid integrity. In comparison to pulsed systems, the use of a constant radiofrequency signal will greatly simplify the design of the generators

Figure 1 shows a realizable implementation of the flow through electroporation concept for industrial pasteurization: the fluid to be treated would flow through orifices in a three-layer structure consisting of a thick metallic electrode, a thin insulator and a second thick metallic electrode. The electrodes would be energized by a moderate AC voltage so that each microscopic portion of fluid would experience a

high electric field AC burst during passage through the insulator layer. As displayed at the bottom of the figure, it would be possible to concatenate a number of those stages so that a series of bursts would be delivered and the inactivation would be more effective. What follows can be considered an analysis – and a proof-of-concept – of one of the conduits (i.e. orifices) in the three layer structure.

2.0 Materials and methods

2.1 Numerical study

A geometrical model (Fig. 2) was designed for simulating, by using numerical methods, a single generic conduit of the three-layer structure presented above (Fig. 1). It consists of a metallic tube (length = 20 mm; inner diameter = 0.514 mm; thickness = 0.025 mm) followed by an insulator tube (length = 0.45 mm, inner diameter = 0.514 mm; thickness = 0.025 mm) which is followed by a second metallic tube (same geometrical features than first metallic tube). The model was implemented, and simulated, in COMSOL Multiphysics 4.2a (COMSOL AB, Stockholm, Sweden), which is a Finite Element Method (FEM) software platform able to concurrently model diverse physics. In this case, three physics were modeled: DC electrical conduction, turbulent flow and heat transfer. As indicated in Fig. 2, the problem was solved in COMSOL Multiphysics using 2D axial symmetry in order to minimize computation time. The mesh used in the FEM simulation was composed by 238304 triangular elements.

Numerical values for the dimensions and other parameters were selected as to be consistent with the *in vitro* study later described.

Three materials are present in the implemented model: the electrodes material (stainless steel), the insulator material (PTFE) and the fluid to be treated (slightly conductive water). Values selected for main parameters related to these thee materials are given in Table 1. These values – with the exception of the mild electrical conductivity for water, which is later discussed – correspond to default values from the *Material Library* provided in COMSOL Multiphysics.

The DC voltage applied between the high voltage electrode and the ground electrode was 570 V which corresponds to the RMS value of the applied bipolar square pulses in the *in vitro* study. By performing a DC electrical conduction simulation applying this voltage, simulated heating effects are equivalent to those that would be obtained with a transient electrical simulation modeling the periodic bipolar signal but at the benefit of much shorter computation time.

Significant heat is generated within the water portion between the electrodes due to the Joule effect. As it is described later, such heat production is particularly high at the boundaries between the electrodes and the insulator because of electric field edge effects. Resulting temperature increase, however, is rapidly spread out through thermal conduction and turbulent flow mixing and it is later, downstream, taken out by natural convection cooling. Such convection cooling on the surface of the steel electrode is modeled here by simple heat flux conduction in which the heat transfer coefficient, h, is 5 W m^{-2} K^{-1} and the external temperature is 25ºC.

Fluid flow was assumed to be turbulent and it was modeled by the so called standard k-epsilon model which belongs to the category of the so called two equation turbulence models for performing computational fluid dynamics (CFD). Water input speed was imposed to be 5 m/s and outlet pressure was imposed to be 1 atm. Other parameter values related to this fluid flow model were taken from the Material Library provided in COMSOL Multiphysics.

2.2 In vitro study

A proof of concept system was built for demonstrating the feasibility of the fast flow-through non-thermal pasteurization technique proposed here (Fig. 3). The system consists of a high voltage generator, a treatment chamber and a syringe pump.

The high voltage generator is able to deliver to the chamber a bipolar square signal at 100 kHz with a peak to peak amplitude of up to 1280 V (Fig 4b). Its general architecture is represented in Fig.4a. Three main parts are distinguishable. First, the high voltage DC supply – which consists in a symmetrical Cockcroft-Walton's circuit powered from the mains (He, Zhang, Liu, Zhang, & Zhang, 2011) – generates approximately ± 325 V DC. Second, the H-bridge transforms the DC voltage provided by the Cockcroft-Walton's circuit into the bipolar square signal by switching the polarity of the connections. Such switching is performed by means of four solid state switches consisting of MOSFET transistors (FQPF2N80 by Fairchild Semiconductor) which are activated by optically isolated drivers. Third, control of those switches is performed by means of two synchronized square signals externally produced by a function generator (AFG 3022B by Tektronix). Duty cycle of those signals was set to 40% in order to produce a dead time between commutations at the H bridge.

The treatment chamber was made from a stainless steel hypodermic needle (gauge 21; nominal outer diameter= 0.812 mm, nominal inner diameter = 0.514 mm). The needle was cross cut into two sections and a dielectric epoxy conduit with a length of approximately 0.5 mm was created between both

sections so that a structure equivalent to that of Fig. 2 was created. Fabrication of the dielectric conduit was performed as follows: a PTFE (Teflon) cable with a diameter of 0.5 mm was introduced through the two needle sections, then, the two needle sections were separated at the specified distance (0.5 mm) and a drop of epoxy was poured in between the two sections. Once the epoxy was cured, the PTFE cable was pulled out and the whole structure was secured to a breadboard structure in which electrical connections to terminals were performed by means of silver epoxy (Fig. 5). Finally, the conduit integrity and its length were verified with an optical imaging profiler (PLu neox by Sensofar, Terrassa, Spain)

The custom made syringe pump pushes the plunger of a 20 ml syringe producing water velocities through the treatment chamber from about to 5 m/s to 14 m/s. In all the experiments reported here, the voltage applied to the pump was set so that the water velocity was 5 m/s.

The sample to be treated in this proof-of-concept study was commercial mineral water (table 2) intentionally contaminated with Eschericia coli bacteria. The following paragraphs detail how the *in vitro* study was performed.

Lyophilized pellets of Escherichia coli (strain ATCC # 25922) were acquired from Microbiologics (St. Cloud, Minnesota, USA). After reviving the cultures, these were allowed to grow by incubating them in Luria-Bertani (LB) broth at 37 ºC. Passages were frequently performed in the same conditions as to harvest samples for the experiments with an incubation time from a minimum of 24 hours and up to 48 hours. At each experimental treatment, a bacterial suspension sample was diluted in a volume of the commercial mineral water at proportion 1/20 and thoroughly mixed by means of a vortex mixer. At this stage, the electrical conductivity of the mixture was approximately 0.15 S/m and the concentration of bacteria was about 3×10^8 CFU/ml. Bacterial counting (before and after treatment) was performed measuring viable plate counts after 48 hours incubation on MacConkey's agar (catalogue number M7408 by Sigma-Aldrich, St. Louis, Missouri, USA).

Treatments were performed as follows: first, a sterile syringe was loaded with the contaminated water and, once inserted into the pump, the pump was activated and about a second later the high voltage generator was switched on; the water that went through the chamber during this time lapse was dismissed (not treated). Then, a sterile flask was put in place to collect the effectively treated water which ran for about 15 seconds. And, finally, before the syringe ran out of water, the flask was removed and the high voltage generator and the pump were switched off. In order to emulate concatenated

chambers (Fig. 1), experiments were performed in which the treated water samples were treated again up to three times more.

3.0 Results and discussion

3.1 Numerical study

The simulated current flowing through the chamber is 30 mA (RMS) which implies that the chamber resistance is 19 kΩ. The simulated electric field magnitude (RMS) at the treatment chamber is displayed in Fig. 6. The edge effect is quite noticeable: close to the tube inner surface, the fluid will experience two very significant electric field peaks and the overall field will be significantly larger than the field experienced at the center of the chamber (r=0). In particular, in this case the maximum electric field magnitude experienced at the center will be about 9 kV/cm (RMS) whereas the electric field close to the surface reaches peak values well above 15 kV/cm (RMS). Nevertheless, this electric field heterogeneity is not relevant provided that all fluid portions are sufficiently treated (regarding exposure time and field magnitude) and that no fluid portion is excessively heated for too long.

Simulated fluid temperature increase due to Joule heating is displayed in Fig. 7a. Because of lower fluid velocity in the vicinity of the tube wall (Fig 7b) and because of the field edge effects, which cause heating spots, temperature increase is particularly significant on the tube inner surface. Nevertheless, such local temperature increase is below 9ºC and it is rapidly diluted downstream. The average temperature increase at the exit of the treatment chamber is 3.4ºC.

3.2 In vitro study

Fig. 8 displays the cell counting results for three sets of experiments in which three voltage amplitudes were tried: 0 V_{pp}, 640 V_{pp} (285 V_{RMS}) and 1280 V_{pp} (570 V_{RMS}). For the 0 V condition two series (repetitions) were performed and for the 640 V_{pp} and the 1280 V_{pp} conditions three repetitions were performed. For each condition the average value is plotted; the error bars correspond to the maximum and minimum measured values. CFU counts after treatment are expressed relatively to the CFU counts in each sample before treatment.

Average CFU reduction when applying four consecutive treatments is 0.56-log when 640 Vpp were delivered and 0.81-log when 1280 V_{pp} were delivered.

Fluid temperature was measured after treatment when 1280 V_{pp} were delivered and it resulted in an average temperature increase of 4.5ºC. By performing dummy treatments (0 V_{pp}) the average temperature increase was about 0.5ºC. That is, of the 4.5ºC increased during treatment about 0.5ºC correspond to friction rather than to the electric field.

Under the assumption that the chamber resistance was 19 kΩ, it can be computed that the delivered electrical power was 4.3 W for the 640 V_{pp} condition and 17.1 W for the 1280 V_{pp} condition. Taking into account that water velocity was 5 m/s and that the inner diameter of the chamber was 0.5 mm, and under the assumption that water density was 1000 kg/m^3, then it can be estimated that the energy consumption per unit of mass was 17.4 kJ/kg for the 1280 V_{pp} condition. Under the assumption of no heat losses, such energy density would imply a temperature increase of 4.16ºC (water C_p = 4183 J·kg^{-1}·K^{-1}), which is slightly higher that the experimentally obtained temperature increase (4 ºC) and the simulated temperature increase (3.4ºC).

Samples quadruple treated at 1280 V_{pp} were tested for iron ion contents by means of test strips (“Iron Check”, Sensafe by Industrial Test Systems, Inc.), which have a detection sensitivity of 0.1 ppm, with negative results.

4.0 Discussion

The reported *in vitro* study demonstrates that the proposed method is indeed capable of performing bacteria inactivation in fluids without producing excessive heating. It could be fairly argued, however, that the achieved inactivation rates (Fig. 8) would not be acceptable for any industrial application. Hence it is convenient to disclose that such poor result was caused by an excess of optimism by the authors regarding the inactivation capabilities of a 10 kV/cm AC signal (100 kHz). We wrongly anticipated that about 10 cycles – roughly equivalent to the transient time through the chamber – of a 10 kV/cm signal would suffice for killing most of the bacteria. Obviously this is not the case and a larger voltage magnitude should have been employed. Unfortunately, at the time of the *in vitro* study, the generator had already been built and it was decided not to modify it for creating larger voltages. Nevertheless, it is worth noting that it would had been relatively easy to modify it for creating much larger sinusoidal voltages by adding a resonant network (Dieckerhoff, Ruan, & De Doncker, 1999). As a matter of fact, we anticipate that this will be the sort of circuit topology to be used in industrial applications.

As stated in the previous section, it can be estimated that each 1280 V_{pp} treatment had an energy consumption of about 17.4 kJ/kg. Therefore, four consecutive treatments (as assayed here), would imply an energy consumption of about 69.6 kJ/kg which lays within the 50 kJ/kg - 200 kJ/kg range for preservation of liquid media by current PEF technologies (Toepfl, 2011). Nevertheless, in comparison to current PEF technologies, the full treatment described here, consuming 69.6 kJ/kg, was not able to achieve a sufficiently high inactivation rate for industrial preservation. This may indicate that the 100 kHz bursts employed here are not as energetically efficient as the pulses currently employed in PEF technologies.

Inactivation rate has a strong dependency on the electric field magnitude and on the number of pulses. Extrapolating experimental data by Zgalin et al (Zgalin, Hodzic, Rebersek, & Kanduser, 2012) on E. Coli inactivation with 100 µs square pulses, we now anticipate that, by doubling the voltage magnitude and by doubling the number of consecutive treatments, 4-log inactivation rates could be achieved.

By doubling the electric field the average temperature increase would be quadrupled (Joule heating is proportional to the square of the electric field) which implies that, in the configuration presented here, temperature increases of about 15 ºC would be produced, which are still acceptable. Moreover, it would also be possible to use higher fluid velocities and to concatenate more treatment stages to compensate for loss of exposure time. Note that the developed syringe pump is able to make the fluid flow up to 14 m/s and in this study the fluid velocity was only 5 m/s.

Regarding Joule heating it is important to note that it is proportional to the medium conductivity. In the case presented here, original mineral water would have experienced a very low temperature increase as its conductivity is about 0.028 S/m. By adding the bacteria suspension sample, however, its conductivity was increased to 0.15 S/m. This is in fact quite fortunate for gauging industrial applicability because such conductivity is in the same order than that of other liquids in which bacterial inactivation is of interest. For instance, tap water conductivity is about 0.005 to 0.08 S/m, lemonade conductivity is about 0.12 S/m and milk conductivity is about 0.45 S/m (H. Zhang, 2007).

To increase the number of concatenated treatment chambers should be not problematic. Although each treatment chamber implies fluidic resistance and, thus, a pressure loss, it will be possible to easily intercalate pumps in between stacks of treatment chambers if pressure loss is excessive so as to prevent driving multiple chambers with a single pump.

Turbulent flow was assumed for the numerical study because the Reynolds number (Re) for this problem is 2900 and laminar flow occurs when the Reynolds number is below 2300. It could be argued that 2900 is within the transition flow region between pure laminar flow (Re< 2300) and the pure turbulent flow (Re>4000) and, therefore, use of the turbulent flow model could be questioned. However, as discussed, it is likely that, in practical systems using the proposed method, fluid velocities quite larger than 5 m/s will be employed. Hence it was decided to use a turbulent flow model rather than a laminar flow model. As a matter of fact, laminar flows were simulated (although not reported here) and it was observed that heating was much more problematic: fluid velocity on the liquid-tube interface is virtually zero and no mixing occurs. Therefore, forcing turbulent flow (by increasing velocity and enlarging the diameter of the chamber) is highly advisable in order to prevent localized excessive heating.

Finally as a cautionary remark, it is important to note that the induced transmembrane potential by the alternating electric field will decrease at very high frequencies (> 1 MHz for cells with a diameter of 10 μm) and, therefore, permeabilization – and inactivation efficacy – will decrease (Kotnik & Miklavčič, 2006). Thus, high frequencies (>10 kHz) are recommended in order to minimize electrochemical effects (Morren et al., 2003) but very high frequencies (> 10 MHz) should not be used as these wont be effective.

5.0 Conclusions

As an alternative to standard Pulsed Electric Field (PEF) technologies, here it has been proposed and demonstrated a method in which the liquid to be treated flows at high speed through a miniature chamber where an alternating electric field is permanently applied. Although the proof of concept *in vitro* results were not completely satisfactory in terms of inactivation rate because of the use of a too low voltage magnitude, no major obstacles were identified that could prevent successful use of the method in industrial scenarios.

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Figure 1 Realizable implementation of the fast flow through electroporation concept for pasteurization. Top: parts of a single stage treatment chamber. Bottom: Concatenation of treatment stages and representation of electric field magnitude at each section along fluid path.

Figure 2 Main parts of the model employed to numerically simulate a single conduit.

Figure 3 Implemented proof of concept system. (The treatment chamber is out of scale).

Figure 4. (a) Implemented generator able to deliver a high-voltage bipolar square signal by means of an H bridge configuration.(b) The generated signal when applied to the treatment chamber.

Figure 5 Picture of the whole proof of concept chamber and a magnification of the insulator section.

Figure 6 Simulated electric field magnitude within the treatment chamber (kV/cm). (a) Color map and isolines. (b) Electric field across fluid flow axis (z) for three depths.

Figure 7. (a) Simulated temperature increase at the treatment chamber. (b) Simulated fluid velocity inside the treatment chamber.

Figure 8. Experimentally obtained E. coli inactivation for three voltage magnitudes and up to four consecutive treatments.

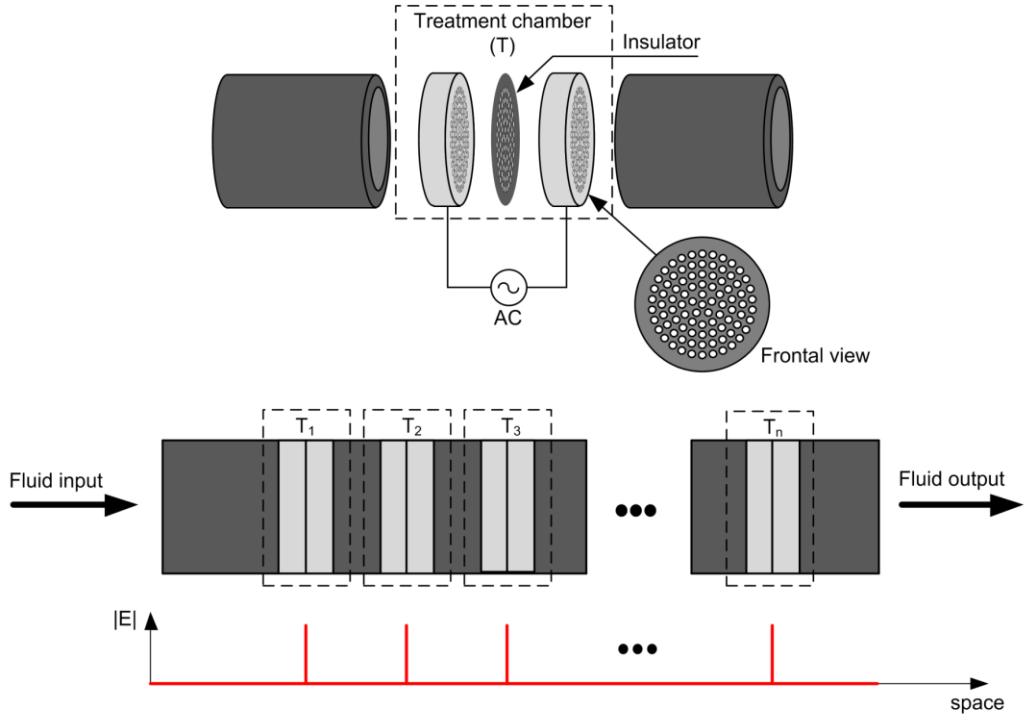


Fig. 1

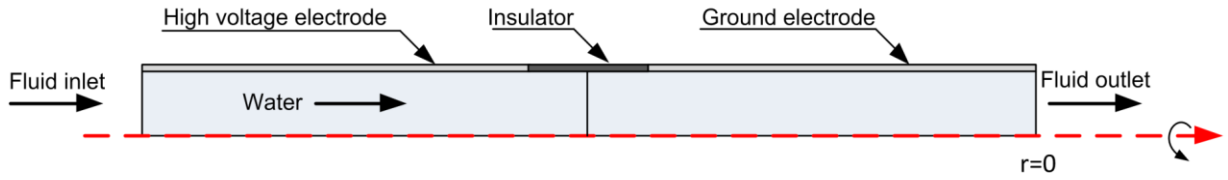
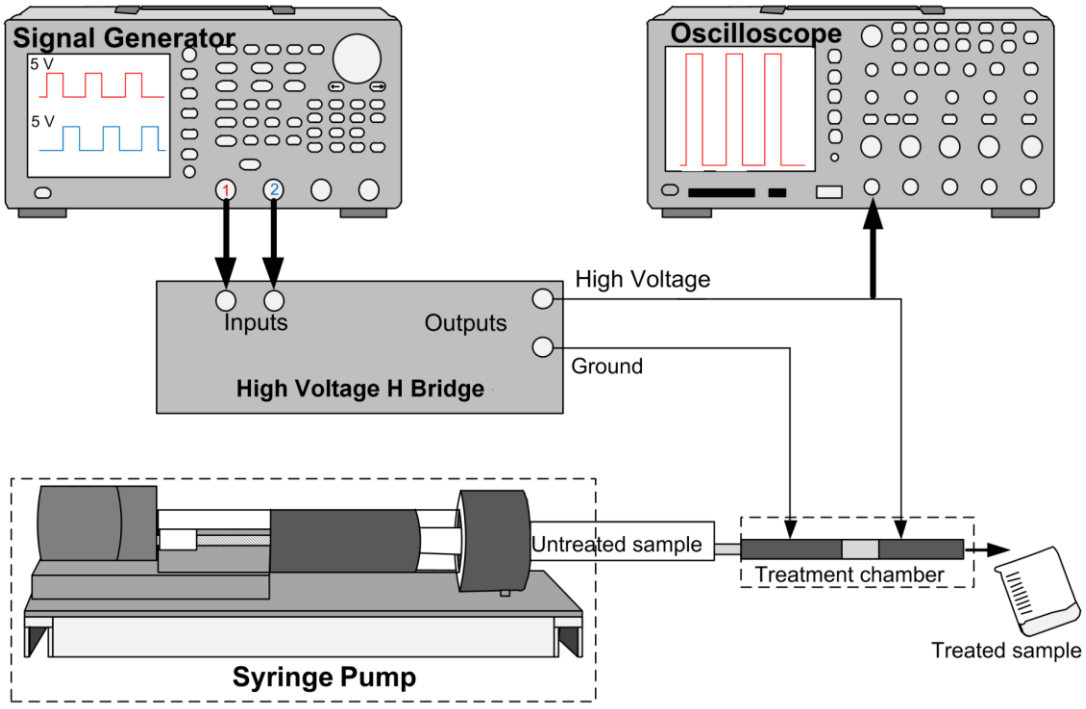


Fig. 2



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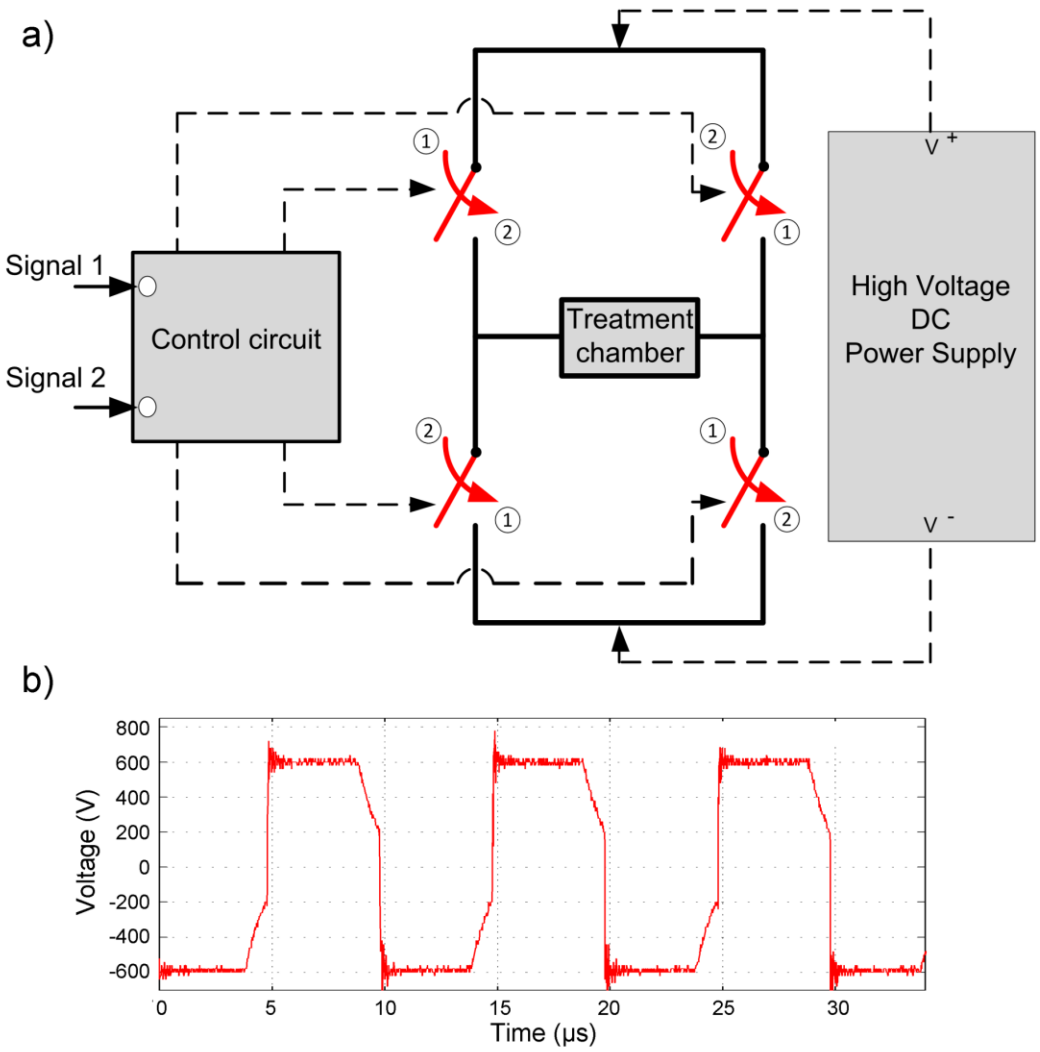
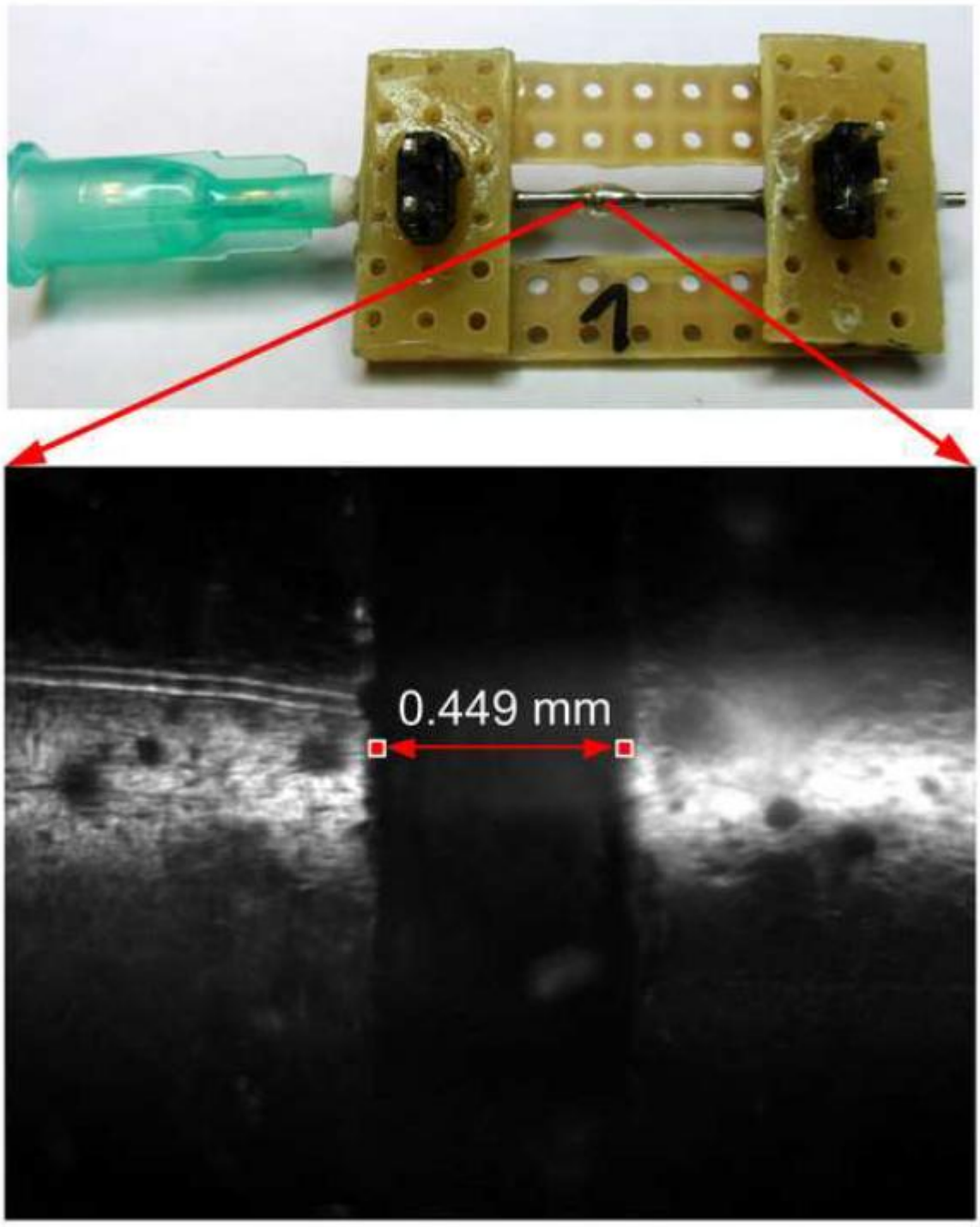


Fig. 4



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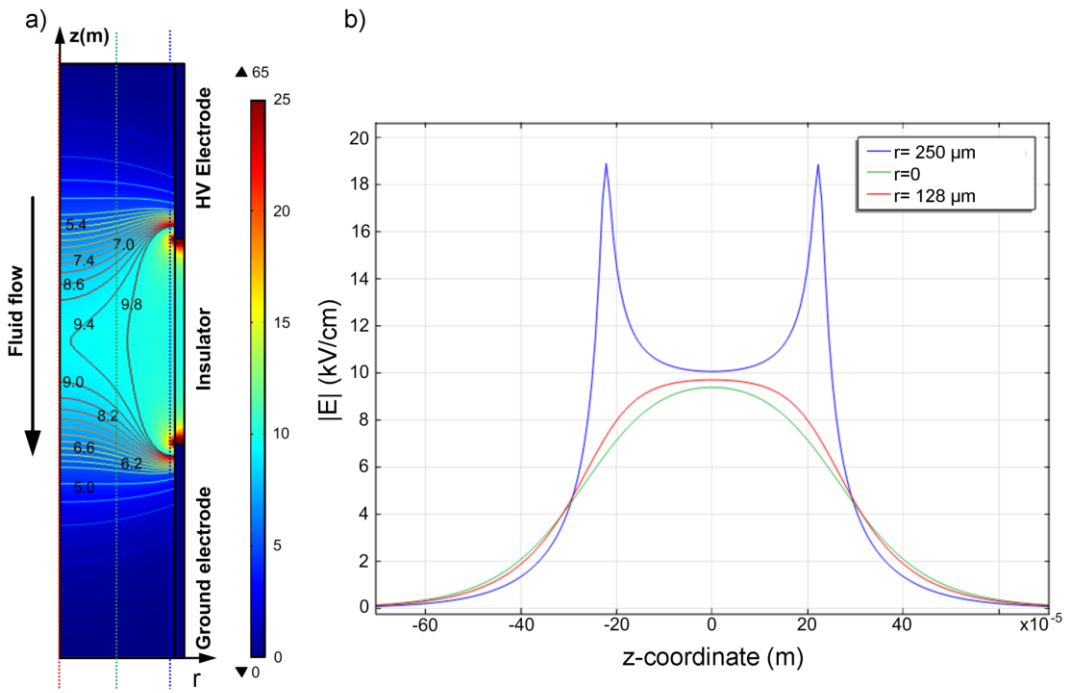

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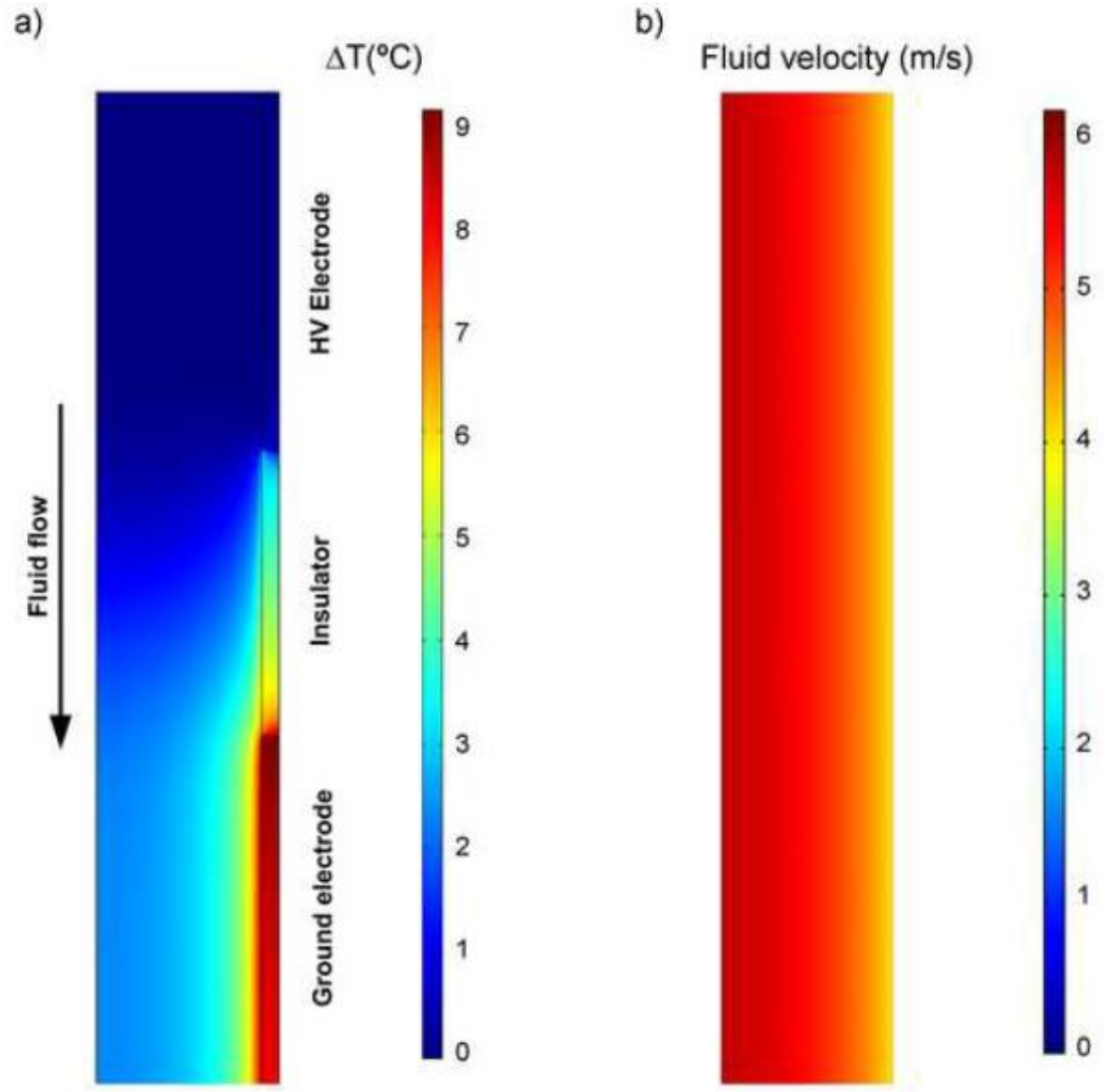

Fig. 7

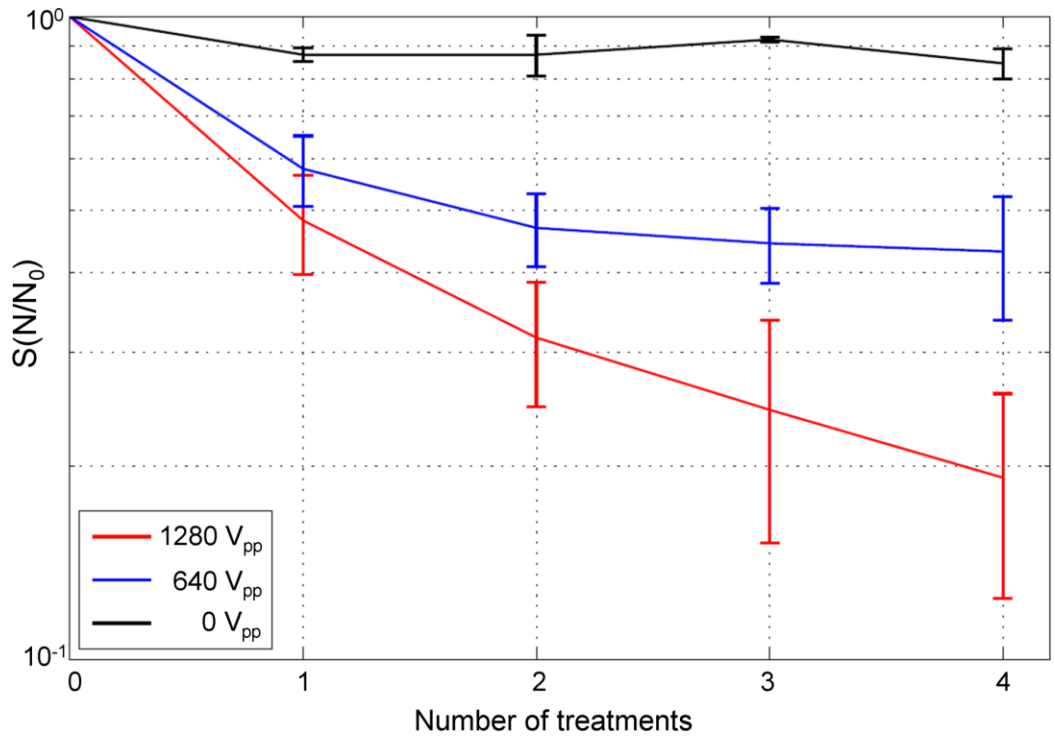

Fig. 8

Table 1 Material parameter values used in the numerical study.

	Stainless steel	PTFE	Conductive water
Thermal conductivity, k ($W \cdot m^{-1} \cdot K^{-1}$)	44.5	0.24	0.602
Mass density, ρ ($kg \cdot m^{-3}$)	7850	2200	998
Heat capacity, C_p ($J \cdot kg^{-1} \cdot K^{-1}$)	475	1050	4183
Electrical conductivity, σ ($S \cdot m^{-1}$)	4.032×10^{6}	1×10^{-25}	0.15
Dynamic viscosity, μ(Pa·s)	–	–	0.89

Table 2 Chemical composition of the mineral water used for electroporation experiments as provided by the bottling company (Aigua de Ribes, Ribes de Freser, Spain).

Analyte	Quantity
Solids	207 mg/l
Bicarbonates	163.5 mg/l
Sulfates	30.4 mg/l
Chlorides	3.6 mg/l
Calcium	54.4 mg/l
Magnesium	7.5 mg/l
Sodium	4.9 mg/l
Potassium	0.6 mg/l
Nitrates	6.7 mg/l
Fluorine	0.1 mg/l
Silica	8.3 mg/l

- Highlights

- Novel method for non-thermal treatment of fluids.
- Liquid flows at high speed through a chamber where an AC field is constantly applied.
- No need for pulsating high-voltage generators.
- Simulation: no significant heating occurs using feasible dimensions and velocities.
- In vitro study: E. Coli inactivation in water is demonstrated.

Industrial Relevance

This paper describes an electroporation based method for non-thermal pasteurization of liquids that, in comparison to existing Pulsed Electric Field technologies, does not require high voltage pulsed generators. The method consists in circulating the liquid at high speed through a miniature chamber where an AC electric field of moderate magnitude is permanently applied. By combining several miniature chambers in parallel and in series batch processing will be possible. Here it is analyzed and demonstrated the performance of a single miniature chamber.