

## Sterilization of dielectric containers using a fore-vacuum pressure plasma-cathode electron source

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**Abstract.** We describe our work on sterilization of 10 ml glass and 60 ml plastic cylindrical containers using a fore-vacuum pressure, plasma-cathode, electron beam source. Beam plasma is formed inside the vessel by injection of a low-energy electron beam at 3 - 6 keV energy and current of 50 mA, at a working gas (air) pressure of 8 Pa. The gas composition was tracked by a quadrupole gas analyzer type RGA-100. As a test biological object for sterilization we used *E. coli* ATCC 25922 bacteria, the inner surface of each vessel was inoculated with a bacterial suspension. We find a smooth dependence of the degree of sterilization on the total energy density injected into the vessel. The efficacy of sterilization of container inner surfaces using a fore-vacuum pressure, plasma-cathode e-beam source of relatively low energy (a few keV) electrons is thus demonstrated.

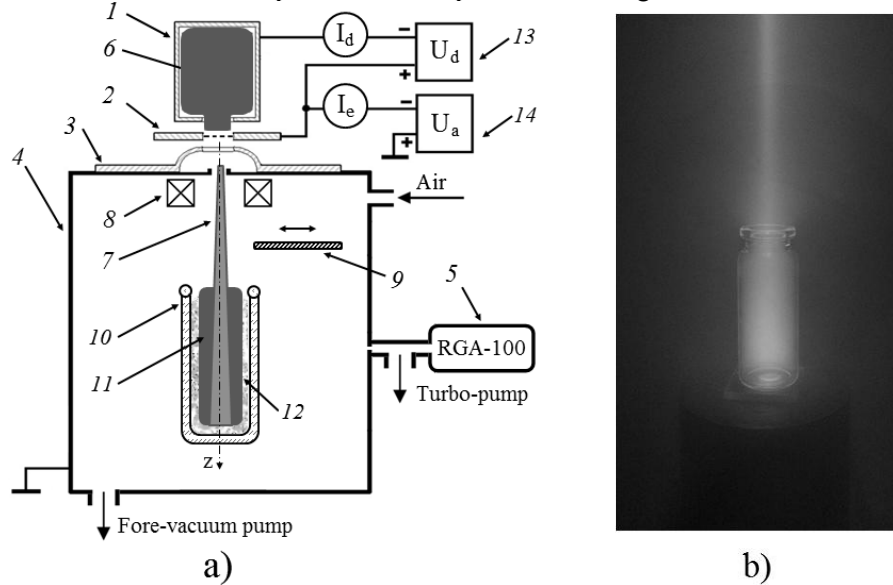
### 1. Introduction

Sterilization is a concern and of great importance in the pharmaceutical, food, chemical industries and in medicine. Sterilization technologies need to provide high bactericidal effect with great reliability and performance. In recent years, in addition to traditional sterilization methods (such as dry and moisture heating, and chemical exposure) low-temperature plasma sterilization techniques have been actively developed. Effective plasma sterilization technologies have been demonstrated for heat- and chemical-sensitive products at atmospheric pressure [1] and under vacuum conditions (at a gas pressure of 1-100 Pa [2]). To date, plasma sterilization of products made of dielectric material (glass, plastic) has been carried out using various kinds of gas plasmas such as barrier discharge [1,3] and microwave plasma [2]. However the generation of plasma by a dielectric barrier discharge and its variations allows sterilizing only flat objects [4]. RF discharge sterilization technology is well-developed. An RF discharge allows formation of relatively dense ( $10^{10}$ - $10^{11}$  cm<sup>-3</sup>) spatially uniform plasma for sterilization of three-dimensional objects [4] and a variety kinds of containers [5]. At the same time, RF-formed plasmas have some disadvantages, such as low efficiency of energy transfer from the power supply to the plasma and the problem of matching the RF generator to the plasma [6]. Some novel possibilities for plasma generation are opened by application of electron beams [7]. Fore-vacuum pressure, plasma-cathode electron beam sources can produce relatively dense plasma not only in metal vacuum chambers but also within dielectric vessels by injection of a low-energy electron beam (2 - 8 keV) through the vessel opening [8]. We describe here our work in developing this approach for sterilizing the inner surfaces of glass and plastic vessels by plasma formation by an electron beam at fore-vacuum pressures.



## 2. Experimental

A simplified schematic of our experimental setup is shown in figure 1.

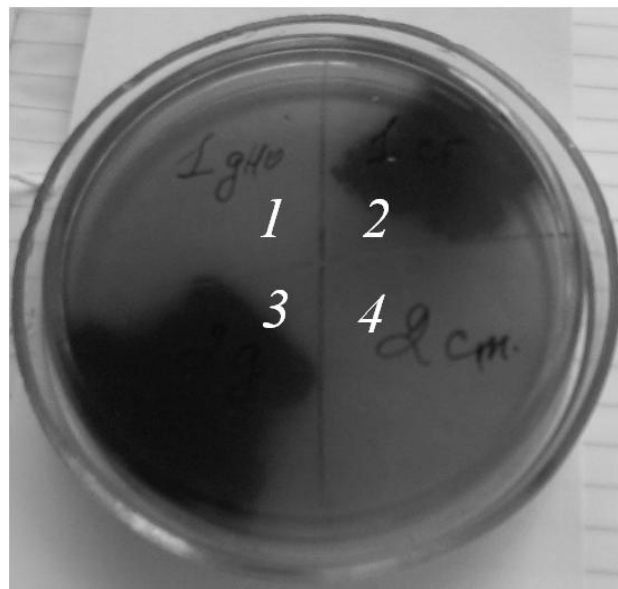


**Figure 1.** (a) Experimental set-up: 1 – hollow cathode, 2 – anode, 3 – extractor, 4 – vacuum chamber, 5 – residual gas analyzer RGA-100, 6 – discharge plasma, 7 – electron beam, 8 – focusing system, 9 – shutter, 10 – vessel, 11 – beam plasma, 12 – bacteria suspension, 13 – discharge power supply; 14 – acceleration voltage power supply. (b) Photo of the beam plasma during the sterilization process.

We used a steady-state fore-vacuum pressure plasma-cathode electron source [7] (1-3) with hollow cathode. In this source, a glow discharge was established between the hollow cathode 1 and anode 2. The discharge current  $I_d$  was 140 - 160 mA with a discharge voltage  $U_d$  of 400 - 420 V. The working gas was air at a pressure of 8 Pa. Pressure in the vacuum chamber 4 was regulated by varying the flow rate of air flowing into the chamber, which was pumped only by a mechanical fore-vacuum spiral pump type ISP-1000C. The working gas composition was monitored using a quadrupole residual gas analyzer 5, an RGA-100 [9, 10]. The analyzer volume was additionally pumped by a turbo-molecular pump to pressures of about  $10^{-2}$  Pa. When an electron-accelerating voltage  $U_a$  of from 3 to 6 kV is applied between the anode 2 and the accelerating electrode 3, a continuous, cylindrical electron beam 7 is extracted from the discharge plasma 6 through the anode grid. The value of electron beam current was measured as the current  $I_e$  in the accelerating voltage supply circuit and was set to 50 mA. The e-beam diameter was about 5-10 mm. The magnetic system 8 was intended to focus the e-beam. Above the dielectric vessel 10, a shutter 9 was mounted. This shutter defines the beginning and end of the e-beam exposure session; in the closed position the shutter prevents penetration of the electron beam into the vessel. The duration  $\tau$  of each exposure session was 5 s; during experiments, we made from 1 to 18 exposure sessions. When the electron source is operating appropriately, the shutter is opened and the sterilization process proceeds for a predetermined number of sessions. Thus the total exposure time is determined by the number of sessions, each session being of 5 seconds duration. The electron beam penetrates into the cylindrical vessel and produces inside it beam plasma 11. For our experiments we used two types of vessels – a 10 ml container made of glass, and a 60 ml container made of plastic (polypropylene). The glass vessels had an inner diameter  $d_1 = 20$  mm and height  $h_1 = 55$  mm, and the polypropylene vessels had dimensions  $d_2 = 36$  mm and  $h_2 = 62$  mm. Each vessel, when it was placed

in the vacuum chamber, was co-axial with the axis of the electron source. After 5 s of exposure, the shutter closes, and the accelerating voltage is switched off.

In order to assess the sterilizing effect, the inner surface of the vessel (both bottom end and side walls) was inoculated by *E. coli* ATCC 25922 -containing suspension with bacterial concentration of about 1-2 McFarland turbidity standards [11], which is approximately equal to  $1 \times 10^9$  colony forming units per ml (cfu/ml) of *E. coli*. Preparation of the bacterial suspension for inoculation of the vessel surface and the control Petri dish was performed using following standard methods. An *E. coli* culture was grown on a simple agar plate for 24 h. After 24 h of culture growth, the *E. coli* suspension of 1-2 McFarland turbidity standards was prepared using 0.9% NaCl solution. This suspension was spread on the bottom and sidewalls of the vessel using a sterile swab, and the vessel placed in the vacuum chamber for plasma and e-beam processing. After exposure, the vessel was removed from the chamber and sent for bacteriological examination. To prevent the unwanted contamination, processed vessels were placed in a closed container, and in such “packed form” were delivered to bacteriological lab located on the other side of the city. Swabs were taken from the inner surface (separately from the bottom and from the sidewalls) in 2 ml of 0.9% NaCl solution, and incubated at 37 °C for 1-2 days in selective Endo medium in a separate Petri dish. Each experimental result was compared with non-exposed seeding of *E. coli* in a control Petri dish with Endo medium. If the sample from the container surface from which the swab was made was not sterile, the *E. coli* culture growth was observed in the corresponding Petri dish sector (Figure 2).



**Figure 2.** Petri dish containing swabs from sterile (1, 4) and non-sterile (2, 3) sites of the vessels.

As primary experimental exposure parameter we chose the total energy  $E_{\Sigma}$  that is injected into the container over the complete exposure time, as calculated using

$$E_{\Sigma} = I_e U_a \cdot n \cdot \tau, \quad (1)$$

where  $n$  is the number of exposure sessions, selected so that for different beam energies  $U_a$  the value of  $E_{\Sigma}$  was roughly constant,  $I_e$  is the e-beam current, and  $\tau$  is the duration time of each exposure (5 seconds).

To assess the sterilization effectiveness, we calculated the total energy density  $D_E$  (in J/mm<sup>2</sup>), which we define as the ratio of the total energy given by equation (1) to the vessel inner surface area  $S$ :

$$D_E = \frac{E_\Sigma}{S} \quad (2)$$

The experiments were carried out at a constant gas pressure  $p = 8$  Pa and with beam current  $I_e = 50$  mA for three different beam energies (3, 4.5 and 6 keV). The sterilization degree was determined as the ratio of the number of sterile samples from the vessel surface to the total number of samples in each series.

### 3. Results and discussion

The experimental results for sterilization of glass and polypropylene vessels are summarized in table 1 and table 2 respectively.

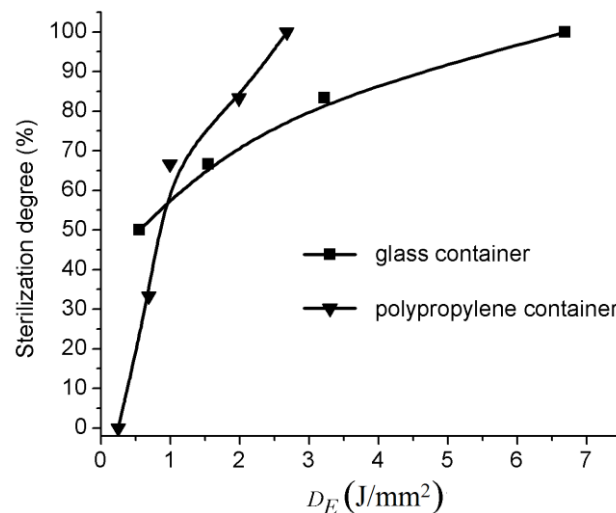
**Table 1.** Glass vessel sterilization results

| Test No. | $U_a$ (keV) | Number of sessions, $n$ | $D_E$ (J/mm <sup>2</sup> ) | Result      |             | Sterilization degree (%) |
|----------|-------------|-------------------------|----------------------------|-------------|-------------|--------------------------|
|          |             |                         |                            | Bottom end  | Sidewalls   |                          |
| 1        | 3           | 1                       | 0.56                       | Sterile     | Non-sterile | 50                       |
|          | 4.5         | 1                       |                            | Non-sterile | Sterile     |                          |
|          | 6           | 1                       |                            | Sterile     | Non-sterile |                          |
| 2        | 3           | 4                       | 1.55                       | Sterile     | Sterile     | 66.7                     |
|          | 4.5         | 3                       |                            | Sterile     | Non-sterile |                          |
|          | 6           | 2                       |                            | Sterile     | Non-sterile |                          |
| 3        | 3           | 9                       | 3.22                       | Sterile     | Sterile     | 83.3                     |
|          | 4.5         | 6                       |                            | Sterile     | Sterile     |                          |
|          | 6           | 4                       |                            | Sterile     | Non-sterile |                          |
| 4        | 3           | 18                      | 6.69                       | Sterile     | Sterile     | 100                      |
|          | 4.5         | 12                      |                            | Sterile     | Sterile     |                          |
|          | 6           | 8                       |                            | Sterile     | Sterile     |                          |

**Table 2.** Polypropylene vessel sterilization results

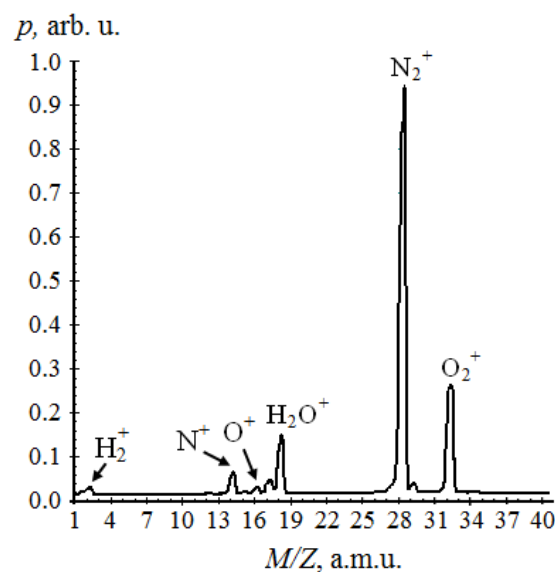
| Test No. | $U_a$ (keV) | Number of sessions, $n$ | $D_E$ (J/mm <sup>2</sup> ) | Result      |             | Sterilization degree (%) |
|----------|-------------|-------------------------|----------------------------|-------------|-------------|--------------------------|
|          |             |                         |                            | Bottom end  | Sidewalls   |                          |
| 1        | 3           | 1                       | 0.25                       | Non-sterile | Non-sterile | 0                        |
|          | 4.5         | 1                       |                            | Non-sterile | Non-sterile |                          |
|          | 6           | 1                       |                            | Non-sterile | Non-sterile |                          |
| 2        | 3           | 4                       | 0.69                       | Sterile     | Sterile     | 33.33                    |
|          | 4.5         | 3                       |                            | Non-sterile | Non-sterile |                          |
|          | 6           | 2                       |                            | Non-sterile | Non-sterile |                          |
| 3        | 3           | 6                       | 0.99                       | Non-sterile | Sterile     | 66.67                    |
|          | 4.5         | 4                       |                            | Sterile     | Non-sterile |                          |
|          | 6           | 3                       |                            | Sterile     | Sterile     |                          |
| 4        | 3           | 6                       | 1.99                       | Non-sterile | Sterile     | 83.33                    |
|          | 4.5         | 8                       |                            | Sterile     | Sterile     |                          |
|          | 6           | 12                      |                            | Sterile     | Sterile     |                          |
| 5        | 3           | 8                       | 2.68                       | Sterile     | Sterile     | 100                      |
|          | 4.5         | 11                      |                            | Sterile     | Sterile     |                          |
|          | 6           | 16                      |                            | Sterile     | Sterile     |                          |

As shown in the tables, the sterilization degree increases with the number of exposure sessions for all electron beam energies. With increasing total energy density  $D_E$ , the sterilization degree reaches 100% (figure 3). Note that the bottom ends, as well as the sidewalls of the vessels, are completely sterilized. It should also be noted that there were no visible surface deformations of polypropylene vessels at a beam energy of 3 keV for any number of exposure sessions. In all cases the *E. coli* culture seeded in a control non-exposed Petri dish demonstrated marked growth.



**Figure 3.** Sterilization degree as a function of total energy density injected into the vessel.

The working gas mass spectrum (figure 4) indicates the presence in the gas composition of a considerable amount of molecular nitrogen and oxygen along with a smaller amount of atomic nitrogen and oxygen, the latter of which determine the bactericidal properties of the plasma, as has been reported [12]. With increasing number of sessions and hence total exposure time of the active plasma components on the microbial cells, the sterilizing effect increases.



**Figure 4.** Mass spectrum of working gas obtained using RGA-100, at a gas pressure of 8 Pa.

#### 4. Conclusion

We have demonstrated the sterilization of the inner surfaces of glass and polypropylene vessels by means of a fore-vacuum pressure, plasma-cathode e-beam source of low energy (a few keV) electrons, without using toxic gases or other chemicals and extremely high heating. The degree of sterilization depends on the total energy density injected into the vessel, suggesting the existence of experimental regimes for effective sterilization of glass and heat-sensitive plastic containers. More detail understanding of the mechanisms responsible for successful sterilization by fore-vacuum electron sources requires further experiments, and these issues are the subject of our on-going research.

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