UDK 533.9

THEORETICAL AND EXPERIMENTAL STUDY OF THE FACTORS OF STERILIZATION OF MEDICAL ARTICLES IN LOW PRESSURE GLOW DISCHARGE PLASMA

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In the report the results of experimental and theoretical studies of the parameters of direct current glow discharge plasma are presented. The efficiencies of its main sterilizing factors (charged particles, electrically neutral chemically active particles and ultraviolet radiation of the plasma) are also studied. The prospects of the use of such discharge for cold sterilization of medical instruments is estimated.

Introduction

In modern medical practice wide variety of heat sensitive instruments and materials is used which require cold sterilization techniques. Up to now sterilization of such articles was performed by means of toxic gases – pure ethylene oxide or its mixture with fluorochlorocarbons. This sterilization technique requires long (up to 24 hours) aeration process for processed articles and, the most essential, makes a serious danger for both servicing personnel health and environment. For these reasons the development of new cold sterilization techniques is essentially urgent problem. At present time one of the most serious alternatives for gas sterilization is represented by the use of gas discharge plasma as sterilizing agent. Main advantage of the plasma technique consists in fact that the plasma as chemically active medium is formed during the processes of excitation, dissociation and ionization of any gas or vapor medium, including non-toxic ones (even noble gases are suitable). Besides, in this case active particles exist only during the discharge glowing and disappear practically instantly after its turning off. These two circumstances provide complete solution of the problems of safety and ecology. In spite of fact that the use of gas discharge plasma for sterilization of medical articles was proposed yet in the 60-th years, up to now thorough investigations, which would allow objective estimations of efficiency and application range of this technique, were not accomplished. Particular aspects of this complex task were considered in [1-5]. In the present proceeding experimental and theoretical studies of physical processes in low pressure gas discharge plasma, which determine the efficiencies of sterilizing factors of the plasma, are performed. Respective medical-biological studies are performed as well. Obtained results give the answer to the question about the efficiency and application range of the plasma technique for the cases of using the most interesting (from viewpoint of the practice) gases: air, oxygen, hydrogen, carbon dioxide gas, nitrogen, argon.

Description of experimental setup and techniques of the measurements

Direct current glow discharge was used in the experiments for generation of the plasma. The discharge current was varied in range 0,05–0,7 A, the voltage – in range 400–600 V. The volume of work chamber, which served for placement of the articles to be sterilized, was varied in range 20–40 l. Prior to its filling with working gas (the gases mentioned above as well as their mixtures were used) the chamber was evacuated by means of forepump down to residual pressure of $3\cdot10^{-3}$ Torr. Pressure of working gases was varied in range $5\cdot10^{-2}$ – $25\cdot10^{-2}$ Torr. Measurements of plasma density and electron energy distribution functions (EDF) were performed by means of single and double Langmuire probes. It has been found that for pressure range given above the plasma density was practically independent on pressure and was determined only by introduced power. At variation of specific power W_d introduced into the discharge in range $3\cdot10^{-3}$–$30\cdot10^{-3}$ W/cm^3 the plasma density possessed practically linear growth from $7\cdot10^{8}$ up to $6\cdot10^{9}$ см$^{-3}$. At that the plasma inhomogeneity in main part of chamber volume did not exceed 25–30%. EDF measurements were accomplished for discharges on air, oxygen, nitrogen. In oxygen and air the dependence of EDF on energy possessed monotonous character. In nitrogen for particular discharge regimes inverted region on EDF in energy range ~ 2–4 eV was observed, which was due to vibrational excitation of N_2 molecules (curve 3 in Fig.1). It has been determined that for typical regimes of the discharge glowing ($P=10^{-1}$–$2\cdot10^{-2}$ Torr, $W_d = 3\cdot10^{-3}$–10^{-3} Вт/см^3) the electric field value in the plasma varies in range ≈0,1–1,0 V/cm.

Regimes of the discharge glowing were chosen in such way, that the temperature of sterilized test objects should not exceed 60°C, since it is required by the conditions of sterilization of articles made from heat sensitive materials.

Metal and glass Petri dishes with internal square surface of about 10 cm^2 were used as test objects. Medical-biological researches were performed with microorganisms in vegetative, spore and virus forms, however, the results presented below were obtained with the use of spores Bac.subtilis and Bac.stearothermophilus, which appeared to be the most resistant to the action of sterilizing factors of the plasma. For contamination of the test objects aqueous spores suspension was used, which was homogeneously deposited onto internal surface of Petri dishes. Initial amount of the spores on the test objects was varied in range $10^5 - 10^8$ (that is, average surface density comprised $10^4 - 10^8$ spores/cm^2). After incubation of the test objects processed in the plasma (incubation duration 48-72 hours) their sterility check was performed by means of immediate colony count technique. After that the survival curves were built, that is, the dependencies of the number of survived microorganisms on the sterilization time.

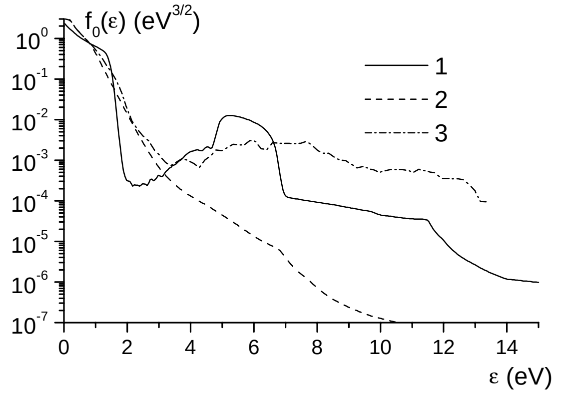

Fig.1. Typical shape of electron energy distribution function: 1 – theoretical calculation for nitrogen; 2 – calculation for oxygen; 3 – experimental results for nitrogen

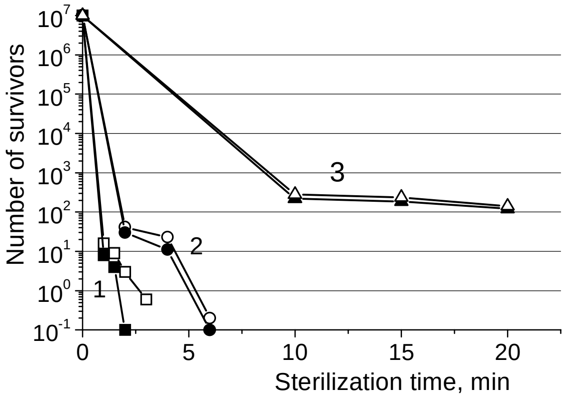

Fig.2. Survival curves for spores Bac.subtilis, obtained by colony count technique at sterilization by integral action of the plasma (●) and just by UV radiation of the plasma (■) for various working gases: 1 –oxygen; 2 –air; 3– nitrogen. P=$2\cdot10^{-2}$ Torr, $W_d = 3\cdot10^{-3}$ W/cm^3. Initial microbial load – 10^7 spores

Experimental results

In earlier proceedings of the authors [3-5], devoted to determining of main regularities of the sterilization by low pressure glow discharge plasma, it has been determined that:

1. Sterilization time for all used gases is practically independent on the gas pressure in range of its variation ($8\cdot10^{-2}$–$25\cdot10^{-2}$ Torr), however, it decreases with the growth of specific power introduced into the discharge. Thus, the sterilization efficiency for each kind of the gas is determined by the plasma density.
2. The most efficient working medium is oxygen subsequently followed by air, carbon dioxide gas, hydrogen, argon, nitrogen.
3. Plasma sterilization efficiency decreases with the growth of initial density of the spores on the test objects from 10^4 to 10^7 spores/cm^2. The reason for such effect is due to diminishing of penetration of sterilizing agents of the plasma to the spores due to their aggregation and forming the bundles at the density of 10^7 spores/cm^2. It is obvious that such peculiarity is inherent to all kinds of the plasma sterilizers.
4. Charged particles of the plasma do not play essential role in plasma sterilization and thus the main sterilizing factors are represented by UV radiation and electrically neutral chemically active particles of the plasma.

Current proceeding is devoted to study of efficiency of the sterilization by UV radiation and electrically neutral chemically active particles of the plasma. For determining of relative contribution of plasma ultraviolet radiation the experiments have been performed, in which one group of the test objects was opened during the sterilization, whereas another one was placed under the filter made from either lithium fluorine (LiF) or quartz glass of KU-1 type with 3 mm thickness. Thus, sterilization of the first group of the test objects was performed by integral action of the plasma (first of all, by UV radiation and action of chemically active electrically neutral particles), and that of the second group was provided by means of just UV radiation with wavelength $\lambda \geq 120$ nm in case of filter made from LiF and with wavelength $\lambda \geq 160$ nm in case of quartz filter. In Fig.2 the survival curves are presented for the sterilization by integral plasma action and that by ultraviolet radiation of the plasma for the cases of oxygen, air and nitrogen use. One can see from the figure that the curves obtained with and without filter use are practically identical for these gaseous media. Analogous results were obtained in all working pressure ranges and specific powers W_d for all used working media with the use of both KU-1 filters and LiF ones. Thus, it is possible to conclude that sterilization of opened surfaces is determined mainly by ultraviolet radiation of the plasma. Measurements have also shown that sterilization is mainly performed by UV radiation generated in wavelength region $\approx$160-220 nm. It should be noted that the efficiency of sterilization by UV radiation of the plasma is essentially higher than that by UV radiation of mercury lamps commonly used in medical practice. Particularly, time of sterilization by plasma radiation with $W_S \approx 100$ μW/cm^2 is approximately five times shorter than that by UV radiation of lamp BUV-30 with essentially higher intensity $W_S = 1500$ μW/cm^2 (see Fig.3). It should be also noted that sterilization by UV radiation of the plasma possesses one more important advantage – here the effect of shadowing is significantly absent, because radiating plasma wraps around sterilized articles, like a liquid. Naturally, it is valid only for articles which do not have the holes smaller than Debye radius of the plasma electrons. Considering sterilization of articles with complex shape, that is, those having slits and holes smaller than Debye radius of the electrons, it will be determined by less efficient factor – electrically neutral chemically active particles of the plasma, rather than by its UV radiation. For this reason it is very important to separate the efficiency of sterilization by these particles. For that we have developed the technique which enabled separation of the action provided by electrically neutral particles from more powerful background presented by UV radiation of the plasma. The idea of the technique consisted in the use of small size mesh grid (with mesh size smaller than Debye radius of the electrons) for reflection of charged plasma particles, and the shield which is placed behind the grid for reflection and absorption of UV radiation of the plasma. For comparison of sterilization efficiencies by UV radiation of the plasma and active electrically neutral particles of the plasma in Fig.4 corresponding survival curves are presented for the use of oxygen and air as working gases. One can see from the figure that the sterilization time in oxygen due to electrically neutral chemically active particles is just 2 times longer than that due to UV radiation of the plasma. In case of air use these times differ more essentially – by factor of 5-6.

Numerical simulation

As it follows from the experiments described above, main sterilizing role in case of opened surfaces is performed by UV radiation. Charged plasma components do not participate in the sterilization (it will be shown below that the plasma flow onto sterilized surface is essentially less than flows of UV quanta and electrically neutral chemically active particles).

In case when the cavities with complex shape are present main sterilizing factor is represented by neutral particles. In oxygen the following particles can be basically considered as mentioned ones: atomic oxygen, ozone, excited atoms and molecules; in nitrogen these particles are its excited atoms and molecules.

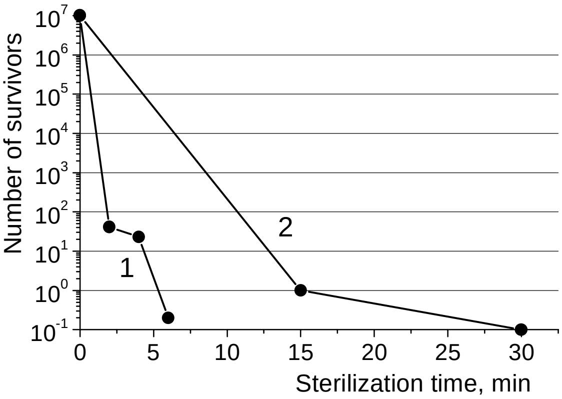

Fig.3. Survival curves for spores Bac. subtilis obtained by colony count technique at sterilization by UV radiation of air plasma, $W_S \approx 100\ \mu W/cm^2$, $\lambda \approx 160\text{-}220$ nm (curve 1) and UV radiation of lamp BUV-30, $W_S = 1500\ \mu W/cm^2$, $\lambda \approx 254$ nm (curve 2)

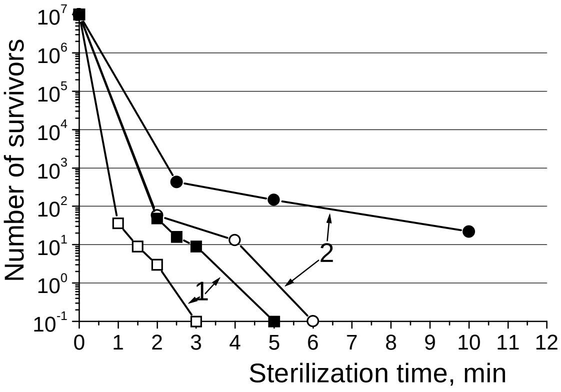

Fig.4. Survival curves for spores Bac.subtilis, obtained by colony count technique at sterilization of opened surfaces by electrically neutral active particles of the plasma (filled symbols) and UV radiation of the plasma (hollow symbols) for the cases of use of various working gases: 1 – oxygen; 2 – air. $P = \cdot 10^{-1}$ Torr, $W_d = 3\cdot 10^{-3}$ W/cm^3, initial microbial load 10^7 spores

For determining quantitative and qualitative composition of the plasma, first of all, chemically active neutral components and radiation, numerical simulations of glow discharge in nitrogen and oxygen were performed for the conditions of plasma sterilizer operation.

In numerical simulation we followed from the system of kinetic equations for neutral and charged components of the mixture:

$$\frac{dN_i}{dt} = \sum_i k_i N_i + \sum_{i,j(i\leq j)} k_{ij} N_i N_j + \ldots$$

Here the first term in right side describes the processes linear on the concentrations of mixture components N_i, the second term describes pair collisions, etc. Rate constants k_{ej} for pair collisions with participation of electrons were determined from Boltzman equation which was solved together with the system of kinetic equations. The equations were solved with the use of numerical techniques which were used and approved earlier in [7] with assumption of uniform distribution of concentrations of all mixture components.

It was assumed that gas ionization in the sterilizer is accomplished by the beam of fast electrons with energy~ 450 eV, since, as it was shown by the measurements of potential in glow discharge, practically whole applied voltage ~ 450 V falls on the near-cathode layer having thickness~ 1 cm. Electric field in main region of the discharge is close to uniform one and has a value of ~ 0,1 V/cm at pressure ~ 0,1 Torr, which ensures drift character of electrons escaping from the volume onto the anode. Death of electrons due to electron recombination is not determining factor in electrons balance due to low concentration of the plasma. In calculations of UV radiation only transitions from lower excited state to vibrationally excited levels of ground state were taken in consideration. In case of nitrogen those are Laiman-Birdge-Golfild bands.

Results of numerical simulation and their comparison with the experiment

In Fig.1. typical shapes of electron energy distribution function (EDF) in nitrogen and oxygen (curves 1 and 2, respectively) are presented. In case of nitrogen on the EDF in 2–4 eV energy range inverted region ($\frac{df_0}{d\varepsilon} > 0$) is observed, which is due to vibrational excitation of N_2 molecules. Presence of inverted region is confirmed by experimental measurements (curve 3). In case of oxygen the dependence of EDF on energy possesses monotonous behavior, since the cross section value for vibrational excitation of O_2 molecules is essentially less than that of N_2, and also due to cutoff of EDF on electron excitation $O_2(^1\Delta_g)$ with low threshold energy, which is also in agreement with the experiment. It should be noted that essential condition of the presence of inverted region on EDF in nitrogen consists in requirement for electric field in the discharge to be small, which is inherent for low pressure ($p \le 0{,}1$ Torr) glow discharge. EDF in this situation is analogous to the distribution of electrons in disintegrating plasma in certain moment after turning off electric field [6]. In Figs.5 and 6 the dependencies of concentrations of main plasma and mixture components on pressure for nitrogen and oxygen are presented. One can see from Fig.5 that concentrations of the components which are primary products of electron-molecular reactions (concentrations of plasma, atomic nitrogen and oxygen, excited molecules N_2 and O_2) are practically independent on pressure. It is due to fact that at pressure increase the cutoff of EDF tail is enhanced at energies of dissociation, excitation and ionization. Rate constants k_{ej} of dissociation, excitation and ionization decrease inversely proportionally to gas concentration. Overall rates of formation respective plasma and mixture components, which are determined as $k_{ej}N_{N_2,O_2}$ products, remain at that practically constant.

It should be noted that concentrations of such chemically active components, as atomic oxygen and excited oxygen molecules $O_2(^1\Delta_g)$ reach big enough values $\sim 10^{12}$ cm^{-3}. Significantly higher value of $O_2(^1\Delta_g)$ concentration, as compared to that of N_2^* (difference by three orders of magnitude), is due to low excitation energy ($\varepsilon = 0{,}95$ eV) for $^1\Delta_g$ level in oxygen. Concentrations of secondary products of electron-molecular reactions (Fig.6), including those of UV quanta $N_{\hbar\omega_2}, N_{\hbar\omega_3}$, are small ($\sim 10^3$ cm^{-3}). However, it should be taken into account that sterilization is determined by flows of respective biologically active components onto substrate, penetrability and level of their action on the spores, rather than the concentrations. Flow of UV quanta $N_{\hbar\omega_2,\hbar\omega_3} \cdot c$ comprises $3\cdot 10^{13}$ cm^{-2}s^{-1}, which is more than one order of magnitude higher than the plasma flow ($\sim 10^{12}$ cm^{-2}s^{-1}) due to low rate of its ambipolar diffusion. Calculated values of UV quanta flow and plasma concentration are in good agreement with experimental data. Flows of atomic oxygen and $O_2(^1\Delta_g)$, $O_2(b^1\Sigma_g^+)$ ($\sim 10^{15}$–10^{16} cm^{-2}s^{-1}) have the highest values among neutral active particles due to high concentrations of mentioned species. Considering ozone, as one can see from Fig.6, its concentration (unlike concentrations of the other active components) grows up with pressure increase. Since the sterilization efficiency does not depend on pressure, it can be undoubtedly stated that ozone does not play essential role in the process of sterilization of opened surfaces of the instruments. It is most likely due to its low concentration ($\sim 10^7$ cm^{-3}) and, respectively, to its low flow ($\sim 10^{11}$ cm^{-2}s^{-1}). It should be noted that high sterilization efficiency of UV radiation is most likely due to peculiarities of its interaction with the spores.

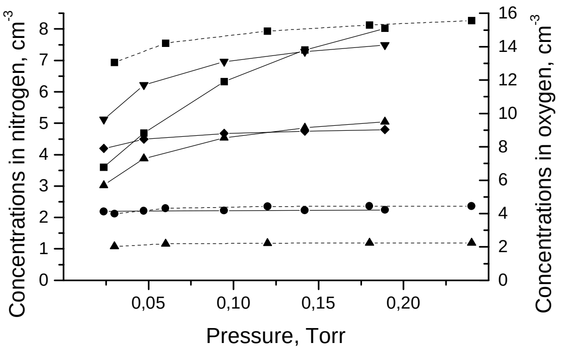

Fig.5. Dependencies of concentrations of the plasma components on pressure: dashed curves for nitrogen (■ – $n_e \times 10^8$; ● – $N_{N_2^*} \times 2\cdot 10^9$; ▲ – $N_N \times 10^{12}$); solid curves for oxygen (■ – $n_e \times 10^8$; ● – $N_O \times 10^{12}$; ▲ – $N_{O_2(^1\Delta_g)} \times 10^{11}$; ▼ – $N_{O_2(b^1\Sigma_g^+)} \times 10^{10}$; ◆ – $N_{O_2^*} \times 10^8$)

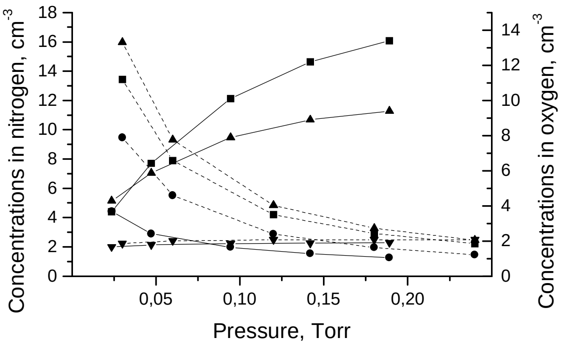

Fig.6. Dependencies of concentrations of the plasma components on pressure: dashed curves for nitrogen (■ – $N_{N^+} \times 10^4$; ● – $N_{N^*} \times 100$; ▲ – $N_{\hbar\omega_1}$; ▼ – $N_{\hbar\omega_2} \times 10^3$); solid curves for oxygen (■ – $N_{O_3} \times 10^6$; ● – $N_{O^+} \times 10^6$; ▲ – $N_{O^-} \times 10^6$; ▼ – $N_{\hbar\omega_3} \times 10^3$)

Distribution of N_2 and O_2 molecules on vibrational states is analogous to [8]. Here we do not concentrate attention on vibrationally excited molecules $N_2(v)$ and $O_2(v)$, since they have the same valence as N_2 and O_2 in ground state. And the last do not provide sterilizing action on studied biological objects at any concentration.

Densities of the components, which may provide sterilizing action (UV quanta, O, $O_2(^1\Delta_g)$, $O_2(b^1\Sigma_g^+)$), possess linear growth with increase of the discharge current. It is in a good agreement with the measurements of sterilization efficiency, which grows up with the increase of discharge power (current). It should be noted that dependence of the plasma concentration on the discharge current agrees with experimentally measured one.

In conclusion of the present section, on a basis of data given above, we would note that in oxygen and air atmospheres main particles, which determine the sterilization efficiency, are atomic oxygen and excited molecules $O_2(^1\Delta_g)$, $O_2(b^1\Sigma_g^+)$. Concentrations of active components in N_2 are significantly less than that in O_2, which explains the reason for increase of sterilization time in nitrogen, as compared to those in oxygen.

Brief conclusions

On the basis of accomplished studies it is possible to conclude the following:

1. Main role in plasma sterilization of opened surfaces is performed by UV radiation of the plasma in wavelength range ≈ 160–220 nm.
2. Efficiency of sterilization by UV radiation of the plasma is essentially higher than that in case of UV radiation sources commonly used in medical practice.
3. Sterilization of the instruments with complex shape is mainly determined by the action of electrically neutral chemically active plasma particles.
4. At the use of oxygen and air as working medium the time of sterilization of opened surfaces by active electrically neutral plasma particles is 2–6 times longer than that in case of action of UV radiation.
5. In result of numerical simulations it is shown that in oxygen plasma the highest concentrations, among all active electrically neutral particles, are possessed by oxygen atoms and oxygen molecules excited to electron levels with energies 0,98 eV and 1,64 eV, which determine the sterilization efficiency for the instruments with complex shape.
6. Calculated values of the plasma concentration, electron energy distribution function, density of UV radiation flow, and also dependencies of plasma sterilizing components on the discharge parameters are in good agreement with experimental data.

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This proceeding is supported by grant # 57 of Science and Technology Center in Ukraine.