THE ANALYSIS OF ACTIVE PRODUCTS OF SPARK DISCHARGE PLASMA RADIATION DETERMINING BIOLOGICAL EFFECTS IN TISSUES

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The aim of the investigation is to analyze active products of spark discharge plasma radiation determining biological effects in tissues, estimate the radiation intensity, identify the resulting products and determine their concentrations.

Materials and Methods. The radiation intensity of discharge was estimated by means of detecting 1.5% solution of potassium iodine. The possibility of $OH^{\bullet}$ and $HO_2^{\bullet}$ formation was analyzed by oxalic acid and mohr's salt solutions. Hydrogen peroxide concentration was assessed by titanium ions test (λ=410 нм). And NO_3^- ion formation was studied by means of ion selective electrodes.

There was carried out chemical analytical and spectrophotometric analysis of products absorption over the range of λ=200–400 nm to identify the products forming and accumulating in liquid phase of discharge. The active products forming in gas phase of discharge were analyzed by Fourier infrared absorption spectra. pH was measured by device "Expert 001". All the solutions were treated in discharge optimal conditions: the capacity of pulse capacitor C=3.3 nanofarad, ballast resistance R=10 MOhm, power supply voltage U_{PS}=11 kV, pulse recurrence frequency — 10 Hz.

Results. Spark discharge plasma radiation initiates UV photon beam $(2.5\pm0.3)\cdot10^{15}$ $(cm^2\cdot s)^{-1}$, with energy density $(2.0\pm0.3)\cdot10^{-3}$ $J(cm^2\cdot s)^{-1}$, maximum radiation spectrum — 220 nm. Under discharge pH in water samples decreases, oxidizers and deoxidizers accumulate. There have been identified radicals $HO_2^{\bullet}$, initial yield — $(1.2\pm0.3)\cdot10^{-6}$ $mol(l\cdot s)^{-1}$. Initial yield of acid residues ($[H^+]$ increase) — $(5.8\pm1.6)\cdot10^{-7}$ $mol/(l\cdot s)^{-1}$, oxidizers $(3.3\pm1.0)\cdot10^{-6}$ mol-equiv./$(l\cdot s)^{-1}$, deoxidizers $(4.2\pm1.0)\cdot10^{-7}$ mol-equiv./$(l\cdot s)^{-1}$. The formation of nitro compounds containing CN, NH groups and organic compounds containing CH, CC groups has been proved. Nitrogen oxides and organic compounds make the main contribution to pH decrease.

Key words: radicals, reactive species, spark discharge plasma radiation, UV spectra, IR spectra.

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The studies of biological effects of low-temperature plasma are widely presented in foreign literature of the last decade [1, 2]. In contrast to plasma that comes in contact only with the surface, incoherent radiation of spark discharge plasma can penetrate inside an object. The researches show the effect of spark discharge plasma radiation on biological objects has unique features. In discharge zone in the process of plasma chemical reaction there are formed radical products causing biocidal and other biomedical effects [3]. Therefore, the estimation of the concentration of products formed both in gas and liquid phases, in generation of spark discharge plasma radiation is topical especially for biomedical researches as it helps to interpret the mechanisms of the effect of cells. The reactive species formation under electric discharge is paid much attention to. One of the most extensive reviews belongs to A. Fridman [4]. The radiation source of spark discharge is plasma thread, spectral range of plasma radiation being 200-800 nm, though UV range makes the main contribution to bactericidal effect and reactive oxygen species accumulation [5]. It has been stated earlier in studying electrolysis in glow discharge [6] that if the discharge occurs in gas phase, then all reactive species occur in gas phase too. There have been suggested the models of reactive species diffusion from gas phase into liquid [7]. When treated by electric discharge, organic compounds were seen to decompose and form new organic compounds [8]. In the air electric discharge (the combination of oxygen, nitrogen, carbon dioxide, and water vapours) is known to lead to nitrogen compounds synthesis [4]. A stable active compound formed in constant current in the air is hydrogen peroxide [9]. In flare corona electric discharge in the presence of water vapour there is observed the formation of a large number of reactive species, stable ones from them are nitrogen and hydrogen peroxide [10]. The works [10, 11] consider the mechanism of reactive species formation in electric discharge between an electrode and water surface. Plasma chemical processes in gas phase are shown to play the key role: reactive species form under electric discharge in the air in the presence of water vapour. There is the interaction between reactive species and dissolved substances in a thin surface layer, and interaction products spread inside liquid or a cell due to diffusion and under electric field developed by discharge current.

It should be noted that in gas discharge devices used as UV radiation sources (quartz lamps) electrodes are placed into quartz glass case. Quartz is radiotransparent but prevents the contact of active products formed in the discharge zone and a workpiece. In case of an open electric discharge, both the products formed in discharge filament, and UV radiation of discharge plasma have an effect on an object. In the Institute of Experimental Physics of Nuclear Centre (Russian Federal Nuclear Centre, All-Russian Research Institute of Experimental Physics, Sarov, Nizhny Novgorod region) there have been developed the devices with spark discharges in the air [12], and biomedical researches have been carried out. However, the detailed analysis of the products causing biological effects and the optimization of discharge parameters has not been performed.

The aim of the investigation is to analyze active products of spark discharge plasma radiation determining biological effects in tissues, estimate the radiation intensity, and to identify the resulting products and determine their concentrations.

Materials and Methods. The experiments were carried out according to the following scheme (Fig. 1). Spark arrester electrodes *1* were placed in fluoroplastic case *2*, the inside diameter being 50 mm, and height – 30 mm. In a number of experiments, between gas discharge interspace and a sample there was placed a fluoroplastic plate *3* or light filters *4*, radiotransparent for waves of defined length. Fluid samples in reaction vessel *6* were place into a vessel (reaction vessel) *5*, 90 mm in diameter. The sample volume was chosen for particular experiments. There were used bidistilled water (pH — 6.2–6.5) and analytical reagents.

To assess the intensity of UV radiation of discharge plasma, 1.5% solution of potassium iodine (KI) [10, 13] was used as detecting fluid. UV radiation free path in solution was studied as the dependence of total number of absorbed photons per 1 cm^2 of surface (solution titer) depending on liquid layer height. KI solution was treated in glass vessels. The distance from liquid surface to an arrester was 2 cm. the radiation was performed in the open air. Molecular iodine formed under spark discharge radiation was titrated by 0.02 n of sodium thiosulfate in acid medium. The number of absorbed UV photons was equated to the number of formed atoms I, and quantum yield of the process was taken equal to 1. The sample cycle time was 900 s. Liquid layer thickness ranged from 0.5 to 20 cm.

The concentration of the formed oxidizers was estimated analytically, 2 ml of KI solution, with concentration of 17 g/l, and 2 ml of diluted (1:4) sulfuric acid were added into water sample, 50 ml; and then it was titrated by 0.02 n solution of sodium thiosulfate (5 g/l). To assess the deoxidizers' concentration, 2 ml of diluted sulfuric acid were added into sample, 50 ml, heated it to 60° C and titrated by 0.05 n solution of potassium hypermanganate (1.58 g/l) [14]. pH was measured by "Expert 001" (Econix Company, Moscow, Russia). Absorption spectra of pretreated water in UV

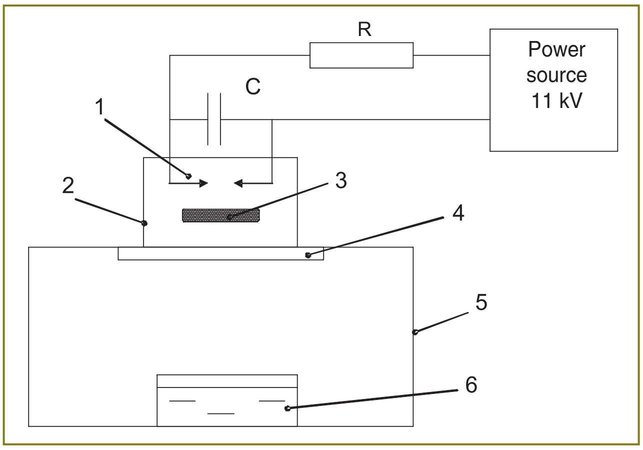

Fig. 1. Structural experimental design: *1* — spark arrester; *2* — arrester frame; *3* — fluoroplastic plate location; *4* — glass filters mounting location; *5* — reaction chamber; *6* — cell with liquid

band were estimated by spectrofluorometer "Fluorat-02 Panorama" (Lumex, S-Petersburg, Russia).

To assess the possibility of $OH^{\bullet}$ и $HO_2^{\bullet}$ radical formation, 4 ml of water sample was being treated within 10 minutes, the water sample having been pretreated by oxalic acid (~10^{-3} mol/l) or mohr's salt (~10^{-3} mol/l) [14]. The concentration of preparations was chosen in such a way that in case they are spent on oxidation by reactive species, the expected reagent concentration change will not exceed the measurement error. On the other hand, the concentration of these substances should be much more than the concentration of radicals to be revealed. If the condition is fulfilled, primary radicals are not spent when interacting among themselves, but according to mass action law they are absorbed by the reagents added.

Hydrogen peroxide concentration was studied by the reaction with titanum ions resulting in specific complex formation giving yellow and orange colour (λ=410 nm) [15].

NO_3^- ion formation was studied by means of ion-selective electrodes (ЭМ-NO3-01 "Tbilpribor", NPO "Analitpribor", Tbilisi, Georgia). NH_4^+ ion concentration was studied by spectrophotometer using Nessler's reagent (λ~400–425 nm).

Additionally, chemical and spectrophotometric analysis was carried out to identify the products which form and accumulate in liquid phase after spark discharge plasma radiation. All the solutions were treated in optimal conditions of discharge (capacity of pulse capacitor C=3.3 nF, ballast resistance R=10 MOhm, power supply voltage U_{PS}=11 kV), pulse recurrence frequency — 10 Hz.

Active products formed in gas phase were studied by infrared absorption spectra (IF spectra) [16]. The products were absorbed by potassium bromide KBr (0.5 g) transparent in IR spectrum. The cell, 40 mm in diameter, with KBr powder (See Fig. 1, *6*) was placed immediately opposite the spark interspace at the same distance as liquid samples (20 mm) inside the reaction vessel *5*. Measurements were taken in two conditions: 1) in the presence of water; 2) in the presence of water (15 ml) in the vessel *6*. Then KBr treated by the discharge was pressed into tablets and absorption spectra were analyzed by Fourier IR spectrometer ФСМ-1202 (Saint Petersburg, Russia).

The discharge was form in the following way. Pulse capacitor C (working voltage 10 kV) was charged through ballast resistance R from electric power supply, U_{PS}=11 kV. Ballast resistance ranged over 10–20 MOhm (9–18 resistors МЛТ-2 of 1.1 MOhm). Polarity of electric power supply was of no importance. Stainless electrodes, 2 mm in diameter, had the total length up to a maximum 15 mm. the spacing was set in such a way that breakdown voltage of the interspace was 6 kV (the distance between the electrodes was ~3 mm). In high voltage charging there was spark discharge. The pulse-recurrence frequency was changed depending on R and C values in the range of 0.5–10 Hz. There was used capacitor battery КВИ-3, initial capacity — 3.3 nF (one capacitor), maximum capacity — 66 nF (20 capacitors). The capacitors were fixed on aluminum tires, 6 mm in thickness. The minimum distance from a capacitor to an arrester (when one capacitor was used) was 45 mm. There was studied the reactive species yield dependence of capacity value C, when R=const and of resistance value R, when C=const. Capacitor charge energy — $CU^2/2$, pulse-recurrence frequency of discharge $f \sim (2\pi RC)^{-1}$. The energy released in discharge in unit time $W \sim U^2(4\pi R)^{-1}$ does not depend on C value. Therefore, when C value is changed (R, U_{PS}=const), pulse-recurrence frequency is changed, though average power supply current and average energy released in spark gap in unit time remain constant. If R is changed (C, U_{PS}=const), the pulse-recurrence frequency and the capacity in spark gap (capacity increases in R decrease) vary. If power supply voltage U_{PS} is changed, then there change both the pulse-recurrence frequency of discharge changes too, as the time of achievement of spark gap discharge voltage changes, and average power intake (charged in the discharge). In voltage rise, the frequency and power increase, if voltage decreases – they decrease.

To choose the parameters of discharge circuit, there was measured pH after the treatment of water sample, 4 ml, within 10 min. The water sample was 20 mm away from the spark zone. In the experiments the values of capacity C and resistance R were changed in the range of $3.3 \leqslant C \leqslant 66$ nF, $10 \leqslant R \leqslant 20$ MOhm. Minimum pH value (maximum chemical effect) was stated to be reached in C=3.3 nF d and R=10 MOhm (pulse-recurrence frequency ~10 Hz). Maximum yield of oxidizers and deoxidizers determined by sodium thiosulfate and potassium hypermanganate titration was reached in the same parameters of discharge circuit. Therefore, all further measurements were performed in C=3.3 nF and R=10 MOhm (pulse-recurrence frequency ~10 Hz), U_{PS}=11 kV.

Results and Discussion.

The estimation of intensity and penetration distance of UV radiation of spark discharge plasma. Active phases of discharge, when reactive species can be generated, can be divided into two parts. The 1st stage is the leading edge of discharge. At this time electric field intensity in the interspace between the electrodes is maximal. The voltage difference between the electrodes is about 6 kV. Chances are high that highly excited particles (radicals) will occur. The 2nd stage is the master pulse advancing. At this stage the spark channel represents a heated conductor, ionized gas — plasma in spark channel – starts radiating in UV, in visible and red range. The voltage difference between the electrodes is about 100 V. Electric field in discharge filament is minimal. Formed reactive species due to their high concentration are consumed when interacting among themselves [17]. So, in the first stage, the spark channel is the source of chemically active particles (radicals), and in the second – the source of radiation including UV spectrum (hot black body), and stable products of chemical transformations.

When in electric discharge in the interspace between electrodes high electric field intensity is constantly persisting, e.g. in corona discharge, the discharge in the air in the presence of water vapour results in reactive species accumulation: nitrogen and hydrogen peroxide. In this situation nitrogen compounds are formed but their role is insignificant [10]. In spark discharge the situation is different. High electric field intensity persists only in leading edge passing. At that moment chances are high that highly excited particles will form. When the leading edge ends, discharge current reaches its maximum, field intensity is low, though the spark channel runs hot. The air including nitrogen, oxygen, carbon dioxide, water vapours is ionized passing into plasma. At that moment between the ions and the radicals formed in the leading edge of a pulse there are various plasma chemical reactions resulting in both degradation of the existing molecules and the synthesis of new substances. As the discharge channel cools down, stable compounds are formed that can have increased chemical activity. When the capacitor C (Fig. 1) is completely discharged, the current will stop and it will start charging from power supply through resistance R. The field intensity in the space between the electrodes starts growing slowly. At that time there is no ionization in the gas as the field intensity is low, and when it reaches its breakdown value, then again a discharge occurs and the process is repeated. Therefore, during the spark discharge the compounds are formed which have all the chemical elements the gas contains.

In the present experiment there were assessed spectral characteristics of the formed radiation. In electric discharge in the air no emission lines in UV spectrum are formed, so glow in this part of spectrum is the radiation of black body heated to a high temperature.

Let us consider the relations between the energy deposit into plasma column and plasma temperature. Let η be the conversion of efficiency coefficient of electric energy into plasma heating, $W_0=CU^2/2$ – capacitor charge energy. Then

$$\eta W_0 = mc_V \cdot \Delta T = \rho \cdot \pi \cdot r^2 \cdot \ell \cdot c_v \cdot \Delta T.$$

Where $\rho=1.29\cdot10^{-3}$ g/cm^3 — air density; r — plasma column radius; — discharge gap length; $c_v=0.72$ J/(g·deg) — air heat capacity in constant volume.

If the voltage in which there is the breakdown of discharge gap, U=6 kV, then for C=3.3 nF, discharge energy will be $5.9\cdot10^{-2}$ J. If discharge gap length = 3 мм and plasma column diameter ~0.7 mm (the column diameter was assessed visually by spark photography), plasma column heating temperature will be ~$1.3\cdot10^4$ °K. In case of black body radiation according to Wien's displacement law, maximum radiation spectrum (λ) is related to body temperature (T): λ_{max}(м)= 2.9 10^{-3}/T (°K). If T~$1.3\cdot10^4$ °K λ_{max}=220 nm. The radiation will pass through air and come into liquid, so the choice of capacity C=3.3 nF is justified. If the capacity and discharge voltage increase, maximum radiation is shifted to the area of vacuum ultraviolet, $\lambda<180$ nm. Radiation $\lambda<180$ nm is absorbed by the air, does not reach the surface of the treated object. As the spark channel cools down, maximum radiation spectrum will be shifted to longer waves and pass through the area of 180-300 nm and cause chemical and biological effects. Though due to radiation loss, $\lambda<180$ nm, radiant efficiency decreases.

Currently for UV radiation dosimetry there can be used photometric devices with photomultiplier tube. However, when studying intensive bursts of radiation, there can be overloading in photoelectronic devices, therefore intense radiant flux is to be determined by means of chemical dosimeters (actinometers). For radiation dosimetry in our research we used wide-spread substance – potassium iodine (KI). In UV photon absorption the following process takes place: $2KI+2h\rightarrow2K+I_2$. Potassium iodine has 326, 261 и 234 nm absorption bands [13]. In our experiment plasma radiation spectrum is continuous, has the character of black body radiation heated to a definite temperature, i.e. continuous spectrum with the maximum defined by plasma temperature. The value of maximum of spectrum in radiator cooling down is shifted from ultraviolet to visible light, passing through these bands, that allows suggesting complete absorption of UV radiation by KI solution. Therefore, in rather thick liquid in the solution there will be absorbed all UV radiation that can have an effect on a treated object.

The assessment of the intensity of spark discharge plasma UV radiation showed the solution titer increases and reaches plateau as the liquid layer increases. If water volume is small not all the radiation is absorbed. If the selected liquid layer height exceeds the UV radiation track length, the latter is absorbed completely and the titer of entire solution remains constant even in the increase of liquid volume and height. The measurements showed the layer height for complete radiation absorption by detecting liquid is 3.0±0.5 cm. Thus, under spark discharge plasma UV radiation KI solution is stained uniformly about 3 cm depthward.

An average titer in plateau area corresponded to photon flux $(2.5\pm0.5)\cdot10^{15}(cm^2\cdot s)^{-1}$ or $(4.0\pm0.5)\cdot10^{-9}$ (photons mol)$(cm^2\cdot s)^{-1}$. If average photon energy is 5 eV, then energy flux will be $1.25\cdot10^{16}eV(cm^2\cdot s)^{-1}$ or $(2.0\pm0.5)\cdot10^{-3}J(cm^2\cdot s)^{-1}$. The energy released in the discharge with interspace sparkover voltage being 6 kV, capacity — 3.3 nF, and pulse-recurrence frequency about 10 Hz, is ~0.6 J/s. Taking into account the space angle (the distance from the arrester to liquid surface is 2 cm) UV radiation efficiency is ~20%.

The analysis of chemical effects in liquid. pH value under a spark discharge in treated water, physiological saline, cell suspension, and biological liquids, is known to decrease twice or so within 10 min [5], oxidizers and deoxidizers accumulate [4]. The mechanism of pH decrease is not defined. Therefore in the present investigation there was studied the effect of various areas of spark discharge plasma UV radiation and other related factors on the formation of reactive species and hydrogen ion in bidistilled water. 4 ml of water, the layer thickness being 5 mm, was treated in enclosed volume within 10 min. The radiator was covered by the following filters by turns: 1) a glass filter with pass band of 278 nm and more; 2) quartz glass with pass band of 185 nm and more; 3) with no filter, and the pass band was determined by air layer; 4) fluoroplastic plate, 1 mm in thickness, radiopaque, but the gas bubbles above the water and in the discharge zone were freely communicating.

During the treatment no reactive species were found to form under long-wave radiation discharge with $\lambda>278$ nm (case 1) (concentration not above 10^{-7} mol-equiv./l), pH value does not change.

If the water is treated by an open discharge with no filter or with a filter of $\lambda>185$ nm (cases 2, 3), there is pH decrease up to 2.8–3.0. Oxidizers and deoxidizers will form. However, deoxidizers concentration titrated by $KMnO_4$ using quartz filter decreases tenfold. It follows that the considerate part of deoxidizers forms in the electric discharge itself, and then comes from gas phase into liquid.

In cases 3 and 4, when light radiation discharge area came into water or was not covered by fluoroplastic plate, and gas bubbles of the discharge and above the water were communicating, there was observed no difference in pH value and titration of oxidizers and deoxidizers.

Fig. 2 shows the change in reactive species concentration C (mol-equiv./l) depending on treatment time. There were studied hydrogen ions concentration $[H^+]=10^{-pH1}-10^{-pH0}$, where pH0 и pH1 — acidity of initial and treated by the discharge water. The concentration of oxidizers and deoxidizers was calculated based on titration findings. Initial yield of deoxidizers (Curve 1) equals to $(4.2\pm1.0)\cdot10^{-7}$ mol-equiv./(l·s), hydrogen ions (acid residual) — $(5.8\pm1.6)\cdot10^{-7}$ mol/(л·с) and oxidizers $(3.3\pm1.0)10^{-6}$ mol-equiv./(l·s). Fig. 3 shows the dependence of reactive species concentration on treated water volume ($60\leqslant V\leqslant350$ ml) treatment period of each point is an hour.

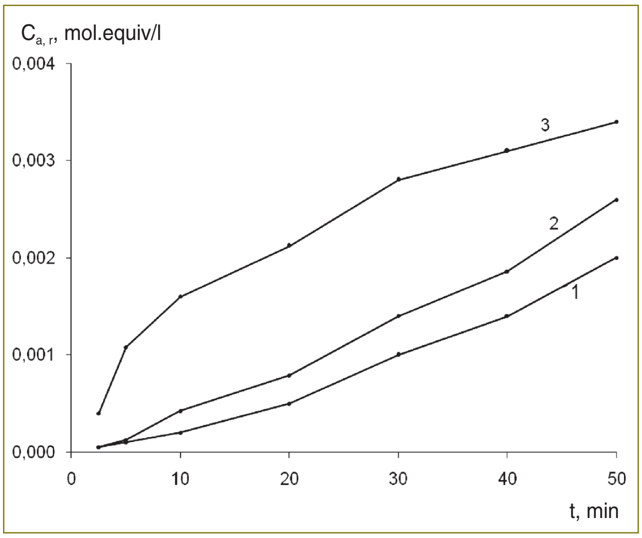

Fig. 2. Reactive species accumulation in 150 ml of water: *1* — overall deoxidizers level, $KMnO_4$ titration; *2* — $[H^+]$ concentration, pH-metry; *3* — overall oxidizers level, titration by sodium thiosulphate. Car — concentration mol-equiv./l

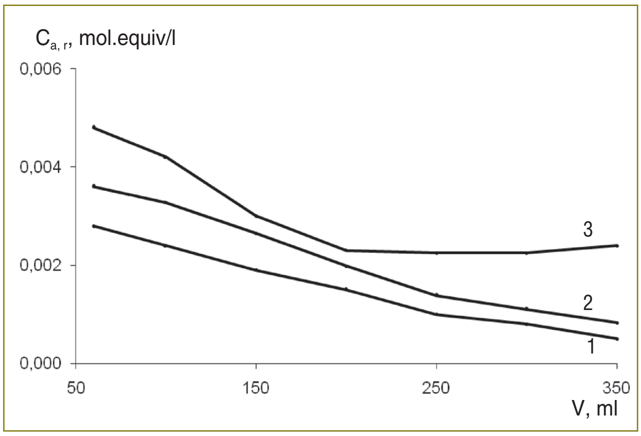

Fig. 3. The dependence of the active species Car (mol.equiv/l) concentration on the treated water volume; 1 — overall deoxidizers level, $KMnO_4$ titration; 2 — $[H^+]$ concentration, pH-metry; 3 — overall oxidizers level, I^- oxidation (titration by sodium thiosulphate)

The dependences of deoxidizers concentration and $[H^+]$ on time are similar and close to linear one. Their initial yields are little different from each other. Initial yield of deoxidizers is much more, though in 10 min or so the yield decreases up to the level of oxidizers and $[H^+]$.

It can be related to deoxidizers' concentration growth in gas phase. Evidently, when the concentration of the products accumulated in gas phase reaches the critical value, they start sedimentating on the reaction vessel walls, and the rate of their absorption by liquid decreases. There is really the deposition of russet sediments on the reaction vessel walls. The dependences of products concentration on the reaction vessel volume are much different. The concentrations of deoxidizers and $[H^+]$ are increasing in water volume decrease, so that the amount of the products absorbed by is about the same. For oxidizers the situation is different. If the water volume is from 60 to 200 ml, the amount of the substance absorbed by water is about the same, while in the volume of 200–350 ml the amount of oxidizers grows in proportion to the volume. The observation of the treated solutions within 14 days showed pH value to persist (~3) during the observation period, and the concentration of oxidizers and deoxidizers in 4 days drops to zero.

Identification of primary reactive species in liquid. Oxalic acid and mohr's salt were added to water to identify primary reactive species $OH^\bullet$ и $HO_2^\bullet$. In long-term treatment reactive products accumulate, though at the beginning of the process the particles concentration resulted from the external action is low (the yield of particles per a second). Therefore, when adding the substance with concentration of 10^{-3} mol/l, i.e. much more than the concentration of any primary particles, these primary particles will interact with the substance first. In this case there will be no accumulation of after-products. And observing the use of the substance one can assess the type and the yield of primary reactive species.

Oxalic acid can be oxidized at a significant rate by hydroxyl radicals only [10, 18]. Radicals $HO_2^\bullet$ do not interact with oxalic acid. Initially the volume of $KMnO_4$ solution necessary for oxalic acid titration was measured. After being treated by spark discharge, $KMnO_4$ consumption for sample titration practically does not change. It means that primary reactive species do not interact with oxalic acid, and stationary concentration of hydroxyl radicals that can form in the process of further transformations does not exceed 10^{-8} mol/l, i.e. primary reactive species do not form.

The process $H_2O\rightarrow OH^\bullet + H^\bullet$ could be the possible mechanism of hydroxyl radical formation. Molecule breaking energy is 116 kkal/mol [19]. It corresponds to quantum energy 5.03 eV, wave length $\lambda\sim246$ nm, i.e. under UV radiation with $\lambda<246$ nm molecular decomposition with hydroxyl radical formation is possible, though such a process is unlikely [20]. Hydroxyl radicals form in λ<180 nm with a remarkable probability.

The following mechanism of reactive species formation through excited water molecules state can be suggested:

$$H_2O^*+H_2O^*\rightarrow HO_2^\bullet+H_2+H^\bullet;$$
$$H^\bullet+O_2+M\rightarrow HO_2^\bullet+M \text{ (M — the third particle)};$$
$$HO_2^\bullet+HO_2^\bullet\rightarrow H_2O_2+O_2,\ k=8.3\cdot10^5 \text{ l/(mol}\cdot\text{s)}.$$

In this case two photons participate in the act of a reactive particle formation. We suppose that radical $HO_2^\bullet$ is primary reactive particle. Radicals $HO_2^\bullet$ formation is possible using mohr's salt. Ferrous form in mohr's salt can be oxidized by both hydroxyl radicals, k_{OH}=1.5·10^6 (mol·s)$^{-1}$, and $HO_2^\bullet$ radicals, k_{HO2}=8·10^5 (mol·s)$^{-1}$. Ferrous form can also be oxidized by hydrogen peroxide, though slowly, $k_{Fe,H2O2}$=56 l/(mol·s). Before the treatment $KMnO_4$ volume necessary for Fe^{2+} titration in mohr's salt was determined. Immediately after the treatment, the solution titer increased by the value approximately equal to water titer after the treatment. In 15 minutes or so the solution titer decreased by the same value it had increased compared to the initial level (before the treatment). The result can be explained as follows. In the process of treatment by spark discharge, $HO_2^\bullet$ radicals form, and they react both with each other, and with ferrum mohr's salt

$$HO_2^\bullet+HO_2^\bullet\rightarrow H_2O_2+O_2,\ k=8.3\cdot10^5 \text{ l/(mol}\cdot\text{s)};$$
$$HO_2^\bullet+Fe^{2+}\rightarrow Fe^{3+}+HO_2^-,\ k=8.0\cdot10^5 \text{ l/(mol}\cdot\text{s)}.$$

The first reaction is unlikely, as the radical concentration is low. In the second reaction with ferrum that mohr's salt contains, there forms the product of hydrogen peroxide HO_2^- dissociating, i.e. both reactions result in hydrogen peroxide formation. The solution titer grows due to peroxide accumulation. Hydrogen peroxide further reacts with the ferrum remaining in mohr's salt:

$$H_2O_2+Fe^{2+}\rightarrow Fe^{3+}+OH^\bullet+OH^-,\ k=56 \text{ l/(mol}\cdot\text{s)}.$$

In this reaction both newly formed hydrogen peroxide and ferrous iron in mohr's salt are spent. The reaction is slow, so it continues after the treatment and ends after the formed hydrogen peroxide is spent.

To assess the role of the reaction $H^\bullet+O_2+M\rightarrow HO_2^\bullet+M$ (when M — the third particle) the water with low oxygen concentration was bubbled by nitrogen. In zero-oxygen conditions, $HO_2^\bullet$ radicals yield must have decreased twofold as the reaction $H^\bullet+O_2+M\rightarrow HO_2^\bullet$ + M will not contribute to $HO_2^\bullet$ formation. In fact it decreased by 30%. It can be considered natural as it is hardly possible to free the solution coming in contact with air from dissolved oxygen completely. Thus, primary reactive species – $HO_2^\bullet$ radicals are stated to form in water under the studied spark discharge. According to measurements, $HO_2^\bullet$ radicals formation rate is (1.2±0.3)·10^{-6} mol(l·s)$^{-1}$.

Let us estimate the possibility of hydrogen peroxide decomposition by discharge UV radiation. Under UV radiation beam, hydrogen peroxide decomposes in the following way:

$$2H_2O_2+2h\nu=2OH^\bullet+H_2O_2.$$

$OH^\bullet$ radicals will in their turn interact with peroxide:

$$H_2O_2+OH^\bullet\rightarrow H_2O+HO_2^\bullet,\ k=3\cdot10^7 \text{ l/(mol}\cdot\text{s)}.$$

The rate of hydrogen peroxide decomposition by UV radiation beam:

$$\frac{d[H_2O_2]}{dt}=I_0(1-\exp(-2{,}3\cdot\varepsilon\cdot1\cdot[H_2O_2])).$$

Where: I_0 — UV photons beam intensity; ε — extinction coefficient, ε=18 l/(mol·cm); l — peroxide layer thickness, cm; $[H_2O_2]$ — peroxide concentration, mol/l.

If liquid layer thickness is 0.5 см and $[H_2O_2]$ — 0.5·10^{-3} mol/l (maximum concentration accumulated by the end of the treatment), less than 3% of photons are spent on peroxide decomposition. With that hydroxyl radicals appear, their concentration being equal to zero at the beginning of the treatment and starting increasing in the concentration growth of accumulated peroxide. Though their yield is low and do not they play an important role.

The process of water pH decrease under spark discharge plasma radiation. Under electric discharge in the air and in water nitrogen compounds can form. Molecular decomposition $N_2\rightarrow N^\bullet+N^\bullet$ и $O_2\rightarrow O^\bullet+O^\bullet$ and further reactions of these radicals can be their formation mechanism in discharge zone. Under UV radiation the process $N_2+O_2+\gamma\rightarrow N_2O+O^\bullet$ is possible.

Stable products of nitrogen-containing radical transformation are ions NO_2^- and NO_3^-. The formation of NO_3^- (ions of hydrogen nitrate) is identified by means of ion-selective electrodes. The concentration of the ions NO_3^- revealed by this technique relates to pH decrease approximately by 90%. Ions $[H^+]$ concentration was calculated by the correlation $[H^+]=10^{-pH}$ — 10^{-pH0}, where pH and pH0 are the values for treated and initial water respectively. If pH << pH0 it is believed that $[H^+]=10^{-pH}$. We can take approximately that $[H^+]=[NO_3^-]$. If water volume increased, pH of solution grew, i.e. NO_3^-concentration dropped (See Fig. 3, Curve *2*). The dependence of $[H^+]=10^{-pH}-10^{-pH0}$ on treatment period for water volume of 5 ml is given in Fig. 2 (Curve *2*).

To estimate the products composition formed under spark discharge the following procedure was carried out. 220 ml of water was being treated by spark discharge for 2 hours. Then 110 ml of the sample was used for analysis of pH (pH=2.26), oxidizers (4.5±1.0)·10^{-3} mol/l and deoxidizers (2.5±0.5) 10^{-3} mol/l. The remaining water (110 ml) was being treated by flare corona discharge in oxygen within an hour. All organic compounds are known [10] to dissolve if treated in such a way. After being treated by corona discharge, the water sample was kept in reactor with high voltage off for 24 h for the formed nitrogen to dissolve. Next day after the treatment pH=2.46, oxidizers and deoxidizers (less than 10^{-7} mol/l) were not revealed. It means that the basic oxidizers and deoxidizers accumulated in water are organic compounds. They can contribute about 100% to pH value. But the same contribution to pH is made by nonidentified ions NO_2^- oxidized by flare corona discharge to NO_3^- [6].

Identification of oxidizers and deoxidizers. When the water is treated by spark discharge, oxidizers and deoxidizers accumulate in it. It would appear reasonable that iodine I^- is oxidized by nitrogen, and the basic oxidizer reacting with $KMnO_4$ is hydrogen peroxide that contributes to iodine oxidation as well. But our experiments show neither ozone nor hydrogen peroxide in water after the treatment of spark discharge radiation. First, there is no ozone smell in sparking. Secondly, no peroxide is found in the reaction with titanium ions. Specific complex staining the solution yellow-orange is known to form when hydrogen peroxide interacts with titanium ions (λ=410 nm) [15]. No solution staining when titanium ions are added results in upper limit of hydrogen peroxide concentration $5 \cdot 30^{-5}$ mol/l, while the concentration of deoxidizers titrated by $KMnO_4$ is ~10^{-3} mol/l.

It was observed in the experiment that red-brown residuum accumulated on the walls of an arrester and a reaction chamber. There was no visual water colour change. The water analysis using Nessler's reagent demonstrated ions NH_4^+ to be present in water. After 10 minutes of treatment their concentration was ~ 100 mg/l. NH_4^+ formation rate was $(1.7 \pm 0.5) \cdot 10^{-10}$ mol/(l·s).

The composition of products accumulating in water was assessed by UV- and infrared spectrometry techniques. The UV spectrum form of the treated water (Fig. 4) is typical of nitrosamines [21]. The concentration of these products being treated within 10 and 20 min grew, and if the treatment period increased up to 30 min, the concentration dropped. It can be expected that nitrosamines are spent when interacting with other products.

There were studied infrared spectra of products formed under spark discharge in the air per 60, 30, 15, 5 and 1 min. Fig. 5 presents infrared spectra after treatment by crystal KBr radiation within 60 and 1 min.

The list of all the revealed lines and the correlation of spectral IR peaks with the data from literature are given in the Table. The Table demonstrates that in gas phase there form nitro compounds containing CN and NH groups and organic compounds with CH- and CC-groups. Nitrogen containing compounds are known to play a significant role in treating various diseases [22]. Therefore, the use of spark discharge in medicine has great perspectives.

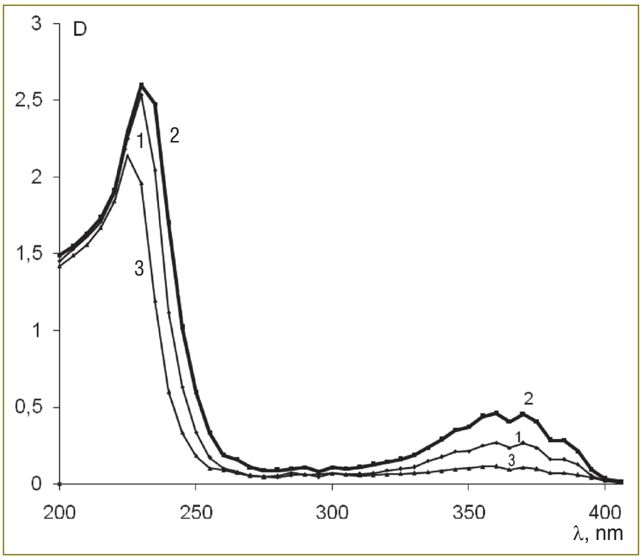

Fig. 4. UV spectra of water treated by spark discharge plasma radiation within 10–30 min: *1* — 10 min; *2* — 20 min; *3* — 30 min

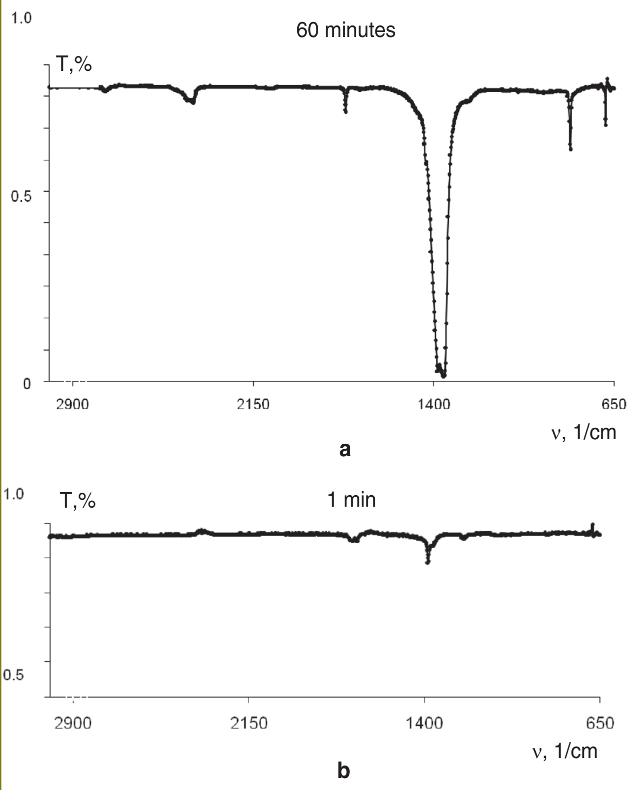

Fig. 5. UV spectra of products formed in spark discharge generation in the air for 60 min (a), for 1 min (b); T — transmission,%; ν — wave number, 1/cm

Conclusion. Spark discharge plasma radiation is found to initiate UV photons flux $(2.5 \pm 0.3) \cdot 10^{15}$ $(cm^2 \cdot s)^{-1}$, energy density being $(2.0 \pm 0.3) \cdot 10^{-3}$ J $(cm^2 \cdot s)^{-1}$, radiation spectrum maximum — 220 nm. Under the discharge in water samples pH decreases, oxidizers and deoxidizers accumulate. Radicals $HO_2^\bullet$ are identified, initial yield — $(1.2 \pm 0.3) \cdot 10^{-6}$ mol $(l \cdot s)^{-1}$. Initial yield of acid residues ($[H^+]$ increase) — $(5.8 \pm 1.6) \cdot 10^{-7}$ mol-equiv. $(l \cdot s)^{-1}$, oxidizers — $(3.3 \pm 1.0) \cdot 10^{-6}$ mol-equiv. $(l \cdot s)^{-1}$, deoxidizers — $(4.2 \pm 1.0) \cdot 10^{-7}$ mol-equiv. $(l \cdot s)^{-1}$.

The main contribution to pH decrease up to 2.8–3.0 level and the accumulation of oxidizers and deoxidizers initiates ultraviolet radiation with wave length being λ>185 nm. Radiation penetration into liquid 3.0±0.5 cm. The concentration of NH_4^+ ions when treated by discharge radiation within 10 min is about 100 mg/l. NH_4^+ ions production rate is $(1.7 \pm 0.5) \cdot 10^{-10}$ mol/(l·s). Hydrogen peroxide content in liquid does not exceed $5 \cdot 10^{-5}$ mol/l.

There has been proved the formation of nitro compounds containing CN, NH groups, and organic compounds containing CH, CC groups. Nitrogen oxides (90%) and organic compounds (10%) make the main contribution to solution pH decrease.

The position of maximum products in infrared spectra formed in spark discharge generation in the air, and their correlation with molecular structures according to literature data

Treatment time	No	Position (from-to) 1/cm	Maximum, 1/cm	Peak height, relative unit	Correlation of experimental infrared spectra peaks and molecular structures [16]
60 min	1	658–675	667	0.011	C≡C-H deformation
	2	683–696	687	0.118	C≡C-H deformation CH≡CH deformation cis-
	3	804–854	833	0.185	(R)O-N=O nitrates CN aromatic nitro compounds CN aliphatic nitro compounds
	4	1211–1569	1350 1359 1390	0.81 0.902 0.816	C-H deformation OH deformation NH_4^+ amino acid band 2 -N-N=O nitrosamines C-C alkyl residue valent (R)-N-O_2 aliphatic nitro compounds (R)ONO_2 covalent nitrates C-N amines, C-N amides
	5	1749–1782	1776	0.079	R'R''C=O carbonyl compounds COOH 6-amino acids (RC=O-)2O acid anhydrides
	6	2374–2487	2424	0.04	$(R)_3$ NH^+
1 min	1	671–680	678	0.027	C≡C-H deformation
	2	690–719	700 713	0.009 0.007	C≡C-H deformation
	3	1216–1232	1232	0.009	C-C groups
	4	1328–1500	1384	0.079	C-H, OH, NH_4^+ -N-N=O nitrosamines (R)-N-O_2 nitro compounds (R)ONO_2 nitrates
	5	1676-1745	1689 1708 1735	0.016 0.016 0.003	(R)O-N=O nitrates Trans- RHC= CHR Cis- RHC= CHR R'R''C=N-R R'R''C=O carbonyl compounds -COOH α-amino acids (RC=O-)2O acid anhydrides RC=ONH2 amides RC=OOR'' esters RC=OOH carboxylic acids

Active products formed in liquid and gas phases of discharge are certain to form inside a cell as well. Nitrogen oxides and radical products take part in many metabolic processes, particularly in apoptosis and proliferation of cells. Therefore, the investigation of cells and the whole body condition in response to spark discharge plasma radiation is of great importance for biomedical researches.

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