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Production Under Plasma Radiation of a Long Living Complex That Decays to Peroxynitrite and Peroxynitrous Acid.

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ABSTRACT

The production of nitrogen compounds in water under spark discharge plasma radiation in air was investigated. Water in atmospheric pressure air contains dissolved oxygen and nitrogen. The maximum of the radiation spectrum was λ = 220 nm. A decrease in water pH was observed immediately after 20 min radiation treatment from pH 5.9 to pH 3.1. The pH decrease continued up to 14 days after treatment from pH 3.1 to pH 2.7. There was no further decrease in subsequent days. The ions NO_2^- and NO_3^- were stable products accumulated in water. Peroxynitrous acid and peroxynitrite were identified as intermediate species which appeared on the third day after irradiation. Their instantaneous concentrations were [ONOOH] ~ [$ONOO^-$] ~ 10^{-5} mol/l. The decrease in pH may be caused by peroxynitrous acid production and isomerisation of ONOOH to HNO_3. The yield of peroxynitrous acid during 20 min irradiation was (7.9 ± 0.1) 10^{-4} mol/l, and during next 14 days after treatment sum of the instantaneous yields was (1.19 ± 0.05) 10^{-3} mol/l. Appearing in water of $ONOOH/ONOO^-$, HNO_2 and HNO_3 can proceed through a stage of nitric oxide formation, although it was not directly identified. The appearance of products containing nitrogen may be a suggestion that $NO^\bullet$ is an intermediate species in many reactions.

Keywords: plasma radiation, nitric oxide, peroxynitrous acid, long living complex

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INTRODUCTION

Nitric oxide plays a significant role *in vivo*. In living systems $NO^{\bullet}$ can be produced by NO-synthases. In the case of incomplete (one electron) oxygen reduction the anion-radical $O_2^{\bullet-}$ is produced. Nitric oxide and superoxide interact at a high rate to produce peroxynitrite:

$$NO^{\bullet} + O_2^{\bullet-} + M \rightarrow ONOO^{-} + M, \quad k = 10^{9}\ l(mol\ s)^{-1} \qquad (1)$$

Under normal conditions the nitric oxide concentration in cell is about 10^{-8} mol/l, in pathology it can reach 10^{-5} mol/l. Peroxynitrous acid and peroxynitrite $ONOOH/ONOO^{-}$ have a dissociation constant, pK_a of 6.8. For a neutral solution in living tissue (pH = 7.4) the ratio of the concentrations $[ONOOH]/[ONOO^{-}]$ is 0.25, i.e. the dominant form is ionic peroxynitrite at 80%. The lifetime of peroxynitrite in neutral solution is ~ 1 s. Owing to its relatively long lifetime the peroxynitrite can diffuse appreciable distances from its place of production and can enter cells. As a strong oxidant, peroxynitrite is implicated in diseases such as Alzheimer's disease, sclerosis, and Parkinson's disease [1, 2]. When artificially produced and introduced into tissue samples peroxynitrite can be used for the investigation of these diseases, and study of the reaction mechanisms of peroxynitrite with biological structures.

Peroxynitrite produced in a chemical reaction, if placed in alkaline solution and at low temperature, can be kept for several days [2]. The peroxynitrite concentration of those samples is 10^{-3}–10^{-2} mol/l. In neutral solution peroxynitrite quickly decays.

It would be useful to generate peroxynitrite immediately in living tissue at neutral pH at the same concentrations as *in vivo* (~10^{-5} mol/l). This work concerns a source of plasma radiation which is able to generate in water (living tissue) a long-lived complex that decays to peroxynitrite and peroxynitrous acid. The goal of the work is the investigation of the kinetics of peroxynitrous acid and peroxynitrite production in water under plasma radiation of pulse discharge on air, and the post treatment effects, including the measurement of peroxynitrous acid and peroxynitrite concentrations immediately after irradiation and during the following 14 days.

MATERIALS AND METHODS

Materials and reagents

The stable free radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) and ethanol were purchased from Biowest, Russia. All reagents: $DPPH^{\bullet}$, ethanol, $NaNO_2$, HNO_3, metallic titanium and HCl were of pure grade. Twice distilled water at pH 5.9 was used. The water temperature was 20 ± 1 °C. Distilled water was stored in air for at least 24 hours before use to achieve a steady state concentration of dissolved gases (oxygen, nitrogen). The concentration of dissolved oxygen was measured by means of Clark's electrode and was $[O_2]$ = 6–7 mg/l. Light filters from window-glass, quartz glass and Teflon light absorber were used. The reagent $TiCl_3$ was produced by means of the dissolution of metallic Ti in strong hydrochloric acid.

Experimental setup

A sketch of the experimental setup is shown in Fig. 1. An SD-10 PILIMIN series spark discharge generator of pulse plasma radiation was used [3]. A glass vessel (position 3) is covered by a Teflon plate (position 2) with a hole of 5 cm diameter. The Petri dish (position 5) with the water sample can be covered when required experimentally by a simple window glass or quartz glass light filter (position 4). The volume of the water sample was 20 ml. The light path from the source to the sample can be stopped by means of a light absorber (stopper), which is in position 6. The distance from the electrodes (discharge plasma position) to the liquid surface was 3 cm. In the vessel there is no gas flow, and active species can't directed to treated sample, as in the case of gliding arc. All active species generated in the discharge region stay at the place of their production and all of them terminate in interactions with one another. In our experiment plasma radiation is the only active factor of the spark discharge.

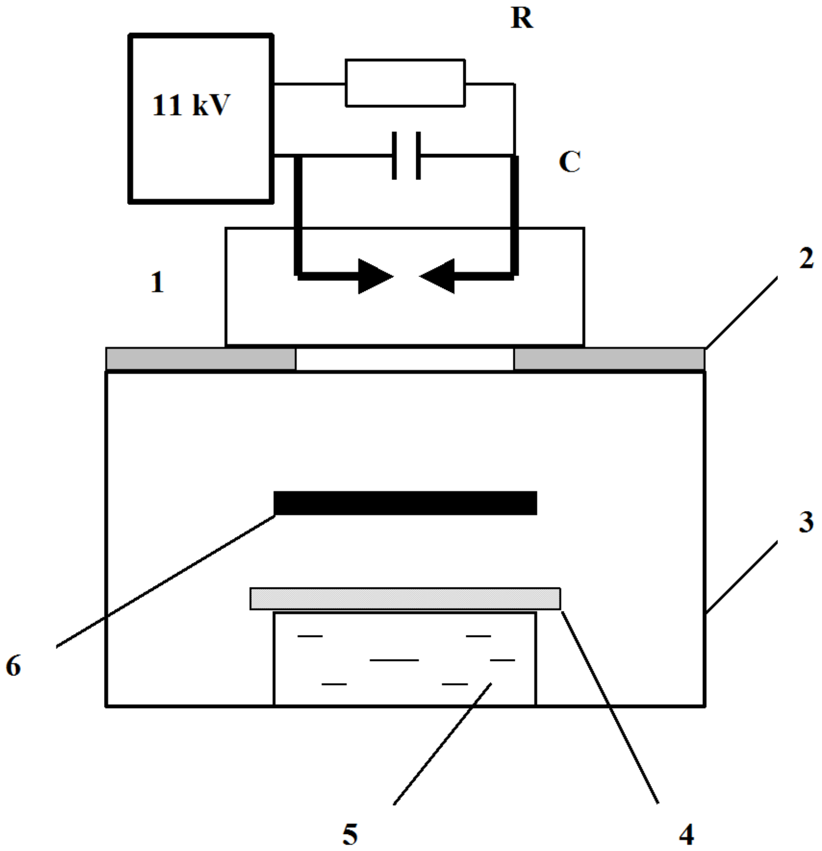

Figure 1: Sketch of experimental device and sample position. 1 – source of radiation, generator of spark discharge SD-10; 2 – Teflon cover; 3 – glass vessel; 4 – place for light filter; 5 – Petri dish with liquid sample; 6 – place for light absorber.

Source of radiation

We have used a very simple source of plasma radiation of the SD-10 PILIMIN series [3]. See Fig.1 for the scheme of the discharge circuit. A pulse ceramic capacitor C = 3.3 nf (V = 10 kV) was charged through a ballast resistor R = 10 MΩ from a high voltage power supply (U = 11 kV). The stainless steel electrodes had a diameter of 2 mm and a full length of no more than 15 mm. The gap between the electrodes was ~3 mm (breakdown voltage V_b ~6 kV). The electrodes and the capacitor were installed on a 5 mm thick Teflon plate, with the electrodes on one side and the capacitor on the other. The capacitor was connected to the electrodes by means of a thick aluminium bus through the Teflon plate. The ballast resistor was arranged in the high-voltage power supply. The power supply was connected with the radiation module by means of a high-voltage cable. When a high voltage was applied, a self-supporting discharge occurred. The pulse repetition rate was 10 Hz. The duration of the pulse front was 50 ns. The full pulse duration was 100 μs. The pulse energy was $W = \frac{CV_b^2}{2}$ = 5.9 x 10^{-2} J. The power of the high-voltage supply was 2 W. The density of energy flow for photons in the UVC range (200–280 nm in air) during the pulse (100 μs, 10 Hz) at a distance 3 cm from the electrodes (pulse power) was 2 J cm^{-2}, the middle energy density was (2 ± 0.3) x 10^{-3} J cm^{-2} s^{-1}. The density of photon flow in the UVC range was (2.5 ± 0.3) 10^{15} $(cm^2\ s)^{-1}$. The spectrum of UV-radiation was measured directly in work [3].

Solution of DPPH$^\bullet$

A solution of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) in 96% ethanol, 5 mg DPPH$^\bullet$ in 200 ml ethanol was used [4]. To study the reactions of species produced in water under plasma radiation, treated water was mixed with ethanol DPPH$^\bullet$ solution in a 1:1 volume ratio. The absorption spectrum at λ = 520 nm was measured 3 min after mixing. The control was ethanol solution DPPH$^\bullet$ mixed with non-treated distilled water 1:1 volume ratio. In the control sample the concentration was [DPPH$^\bullet$] = 3.15 10^{-5} mol/l. The instantaneous concentration of peroxynitrous acid was determined by reaction with the stable free radical DPPH$^\bullet$, which is reduced with hydrogen atoms.

Treatment of samples, UV-spectra and pH measurements

The treatment of liquid solutions by spark gas-discharge plasma radiation was carried out in a Teflon Petri dish 90 mm in diameter. The volume of liquid was 20 ml and the treatment time was up to 20 min. The distance from the liquid surface to the electrode (from the source of the radiation to the sample) was 30 mm. The Petri dish was inserted into a glass vessel with a capacity of 0.5 L. The glass was closed with a Teflon cover with a hole of 50 mm in diameter through which the electrode of the SD-10 generator could be introduced. The first measurements of pH and absorption spectra at wavelengths of 200–400 nm were made immediately after irradiation. Following this, daily measurements of pH value and absorption spectra were made for 14 days for all treated solutions. Absorption spectra were measured using a Fluorat-02 Panorama spectrophotometer (Lumex, St Petersburg, Russia). The thickness of the cuvette was 10 mm. The pH value was measured using an Expert 001 device (Econics Expert, Russia).

Identification of acid anions and peroxynitrite

Acid anions NO_2^- and NO_3^- were identified by means of ion-selective electrodes and absorption spectra: NO_2^-, λ = 360 nm (ε = 18 l mol^{-1} cm^{-1}) and NO_3^-, λ = 300 nm (ε = 7 l mol^{-1} cm^{-1}). Extinction coefficients were determined from absorption spectra of 10^{-2} M aqueous solutions of $NaNO_2$ and HNO_3 at λ = 360 and 300 nm. Peroxynitrite $ONOO^-$ has an absorption peak at λ = 301 nm (ε = 1670 l mol^{-1} cm^{-1}) [2], so the absorption peaks for nitric acid and peroxynitrite are the same. Nitric acid is a strong acid and in the case when concentration of nitric acid is dominant, $[HNO_3] > [HNO_2]$, the concentration of NO_3^- determines the pH value of the solution: $[NO_3^-] = [H^+] = 10^{-pH}$. The part of the optical density peak at 300 nm caused by nitric acid ions was $D = \varepsilon[NO_3^-] = \varepsilon 10^{-pH}$. For pH > 3 the part of the optical density caused by nitric acid ions was not greater than the value of experimental error and it can be ignored.

Determination of ozone and hydrogen peroxide

The evaluation of hydrogen peroxide in treated water was made by means of the reaction of H_2O_2 with fresh $TiCl_3$ solution [5]. In this reaction a complex is produced which has an absorption peak at λ = 410 nm. The $TiCl_3$ reagent was prepared immediately before measurement by means of metallic titanium dissolution in hydrochloric acid.

The evaluation of ozone in water treated by plasma radiation was performed by means of absorption spectrum measurement at λ = 260 nm. The extinction coefficient for ozone is ε = 3290 $(mol\ cm)^{-1}$ [6].

Measurements of IR spectra

Neutral, nonactive chemicals, which were produced in the discharge region and reached the sample surface, were studied by IR spectroscopy. The chemicals were adsorbed by KBr powder, which is transparent in the IR range. A Petri dish 40 mm in diameter with KBr powder (0.5 g) was placed in the same position as the liquid sample (position 5, Fig. 1). The time of treatment by the generator of spark discharge plasma radiation was 15 min. After treatment the KBr powder was pressed into tablets and absorbed spectra were measured by a FSM 1202 Fourier IR spectrometer (St. Petersburg, Russia).

RESULTS

Species produced in water

The pH value of water after treatment by spark discharge plasma radiation for 20 minutes decreased from 5.9 to 3.1. After the end of treatment the pH value continued to decrease and after 14 days the pH was 2.7. After longer periods than 14 days the pH value remained at 2.7. The concentrations of species found in water immediately after treatment and during the next 14 days are shown in Table 1. Ozone and hydrogen peroxide were not found in treated water: $[H_2O_2] < 10^{-4}$ M, $[O_3] < 10^{-6}$ M. The sum yield of nitric and nitrous acid (i.e. surely identified species) for a 20 min treatment time was determined on the basis of the pH value change at $(5.8 \pm 0.5)\ 10^{-9}$ mol$(cm^2\ s)^{-1}$.

The main stable species produced and identified in water immediately after treatment were nitric and nitrous acid ions. Peroxynitrous acid and peroxynitrite were not directly identified in the first two days, but nitrous and nitric acid production can take place via these species. This means the $ONOOH/ONOO^-$, produced at the time of radiation, is decayed. The concentration of nitrous acid decreased, and on the third day after treatment was not detected. The concentration of nitric acid continuously increased, and reached a maximum value on the fourteenth day. After 14 days the concentration of nitric acid did not vary further. On the third day after treatment peroxynitrous acid and peroxynitrite were detected, they disappeared on the fourteenth day. Peroxynitrous acid and peroxynitrite decay quickly, therefore the instantaneous concentration of these species is observed, which exists at a given point in time. If we suppose that the production of nitric and nitrous acids take place via peroxynitrous acid (in acid solution), then the yield of peroxynitrous acid for a 20 min irradiation is $(7.9 \pm 0.1)\ 10^{-4}$ mol/l, and during next 14 days after treatment when peroxynitrous acid is produced and decays, its yield is $(1.19 \pm 0.05)\ 10^{-3}$ mol/l. The sum yield of peroxynitrous acid, produced during irradiation and during the following 14 days is $(2 \pm 0.1)\ 10^{-3}$ mol/l.

Variation of the water pH was not observed during irradiation if the Petri dish (5) containing the water was covered by the window glass filter (4) (see Fig. 1). If the filter (4) was quartz glass, the water composition after treatment was the same as for treatment without a filter (see Table 1). This means that UVC radiation is the main active factor of spark plasma discharge in our case. UVC radiation freely passes through quartz glass and is fully absorbed by window glass. When the light path between the radiation source (discharge region) and the dish (5) was blocked by a non-transparent Teflon stopper (6), no variation in water pH was observed. This confirms that the main active factor is radiation, which cannot penetrate through the Teflon stopper. Species which are produced by the discharge itself can enter the surface of the sample by means of diffusion through the air, but they did not produce any chemical effects in water (according to variation in pH), as the pH value was stable.

UV absorbance spectra

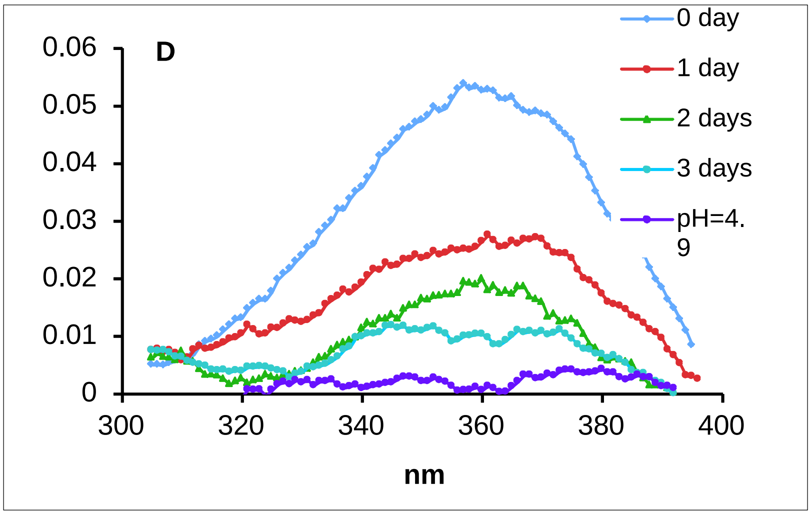

Figure 2: The optical density of water (pH_0=5.9) in region of the peak at λ = 360 nm immediately after treatment by spark discharge plasma radiation for 20 min (0 days), and on the subsequent three days after treatment. For comparison the optical density for a non-treated aqueous solution of nitric oxide (pH = 4.9) is shown.

The absorbance spectra of water in region of the peak at 360 nm immediately after treatment for 20 min and up to 3 days afterwards are shown in Fig. 2. In this region the absorbance is caused by NO_2^- ions. The optical density is determined relative to distilled water. The spectrum of non-treated water (low concentration solution of nitric acid, pH = 4.9) is shown in Fig. 2, and a peak at 360 nm is not observed. The highest value of optical density for the 360 nm peak, and consequently the highest concentration of NO_2^- ions, was observed immediately after treatment. Later the optical density decreased, on the third day being equal to the optical density of non-treated water (weak solution of nitric acid) within experimental error. It means that on the third day there are no NO_2^- ions in the water sample. In Table 1 the nitrous acid concentrations evaluated from spectra and measured by ion selective electrodes are shown. The concentration values obtained by these two methods are the same.

The optical density of treated water sample in region of the peak at 300 nm, observed on the third, fourth, eighth and fourteenth days after treatment is shown in Figure 3. In this spectral region the absorption is caused by NO_3^- and $ONOO^-$ ions [2]. Peroxynitrite is experimentally observed by the peak in the absorption spectrum at $\lambda = 301$ nm. In the same part of the spectrum there is an absorption peak of NO_3^- ions at $\lambda = 300$ nm. The optical density of NO_3^- ions can be separated from peroxynitrite, as the concentration of ions NO_3^- can be evaluated on the basis of the pH value and by means of an ion selective electrode, and we have a measured value for the extinction coefficient for nitric acid ($\varepsilon = 7$ l mol^{-1} cm^{-1}). The calculated optical density of nitric acid peak at pH = 3 is D = 0.007, within the limit of experimental error, which is equal to 0.005. Therefore the optical density of the peak caused by NO_3^- ions can be seen at pH < 3. At pH = 2.7 which was observed on the fourteenth day after plasma radiation treatment, the calculated optical density of nitric acid peak was D = 0.013. This value is approximately equal to the experimental value of optical density D = 0.015 ± 0.005 for the peak at 300 nm (see Fig. 3) in treated water. It means that on the fourteenth day only nitric acid remained in the water, the concentration remaining the same on subsequent days as the pH value did not vary. During the first days, when pH > 3 , the optical density of the nitric oxide peak was not more than D (< 0.007), and within the limit of experimental error. Therefore in our experiment all of the optical density of peak at 300 nm for pH > 3 can be attributed to peroxynitrite.

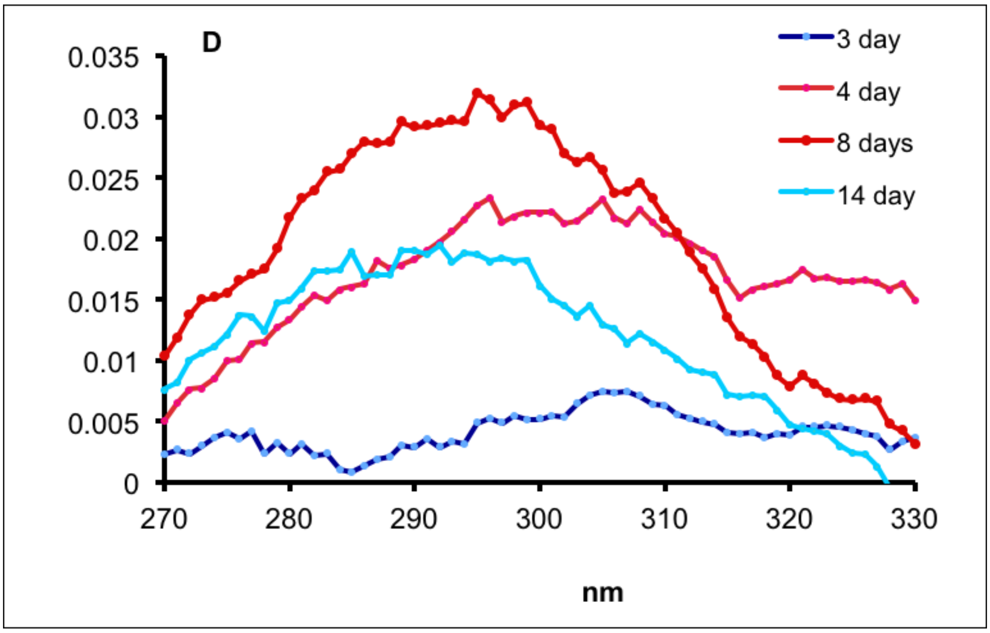

Figure 3: The optical density D for water samples ($pH_0 = 5.9$) in the region of the peak at 300 nm treated by plasma radiation for 20 min and measured on the third, fourth, eighth and fourteenth days after treatment.

There was no peak at 300 nm immediately after treatment or for the next two days beyond the limits of experimental error. A peak at 300 nm appeared on the third day, reaching a maximum value on the eighth day and decreasing to 0.015 ± 0.005 (relative to the base line) on the fourteenth day. On subsequent days the optical density in the 300 nm region remained the same. Immediately after treatment, on the first and second days this spectral region is obscured by the 360 nm peak, therefore in Fig. 3 this spectra not shown. The results in Table 1 concerning peroxynitrite concentration are obtained on the basis of spectral data and nitric acid concentration measured with an ion selective electrode.

IR absorption spectra

Stable chemicals having a red-brown colour, are produced in discharge itself, settling on the walls of the glass vessel and they may reach the surface of treated water. These products are neutral; they have no chemical effect in water. To detect these products, KBr powder spread on the Petri dish was treated with plasma radiation generator in the same way as the water samples. After treatment the powder was pressed into tablets and an IR absorption spectrum was measured. This spectrum is shown in Figure 4. The main absorption peak is in the range of wave numbers 1390–1350 cm^{-1}. With increasing time of treatment the absorption of this peak is strongly increased. There are other weak peaks, but their absorbance is small. The absorbance in region 1390–1350 cm^{-1} is correlated with the vibration of the functional group N=C-O [7, 8].

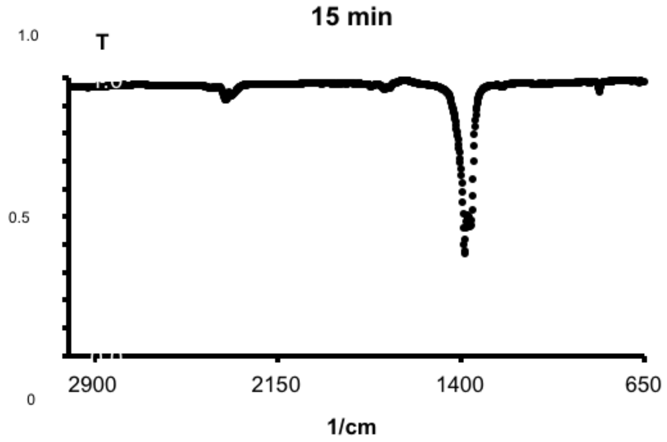

Figure 4: IR spectrum of sediment (T – transmittance), collected on KBr crystal during 15 min of treatment by the SD-10 generator.

Absorbance spectra of DPPH$^\bullet$

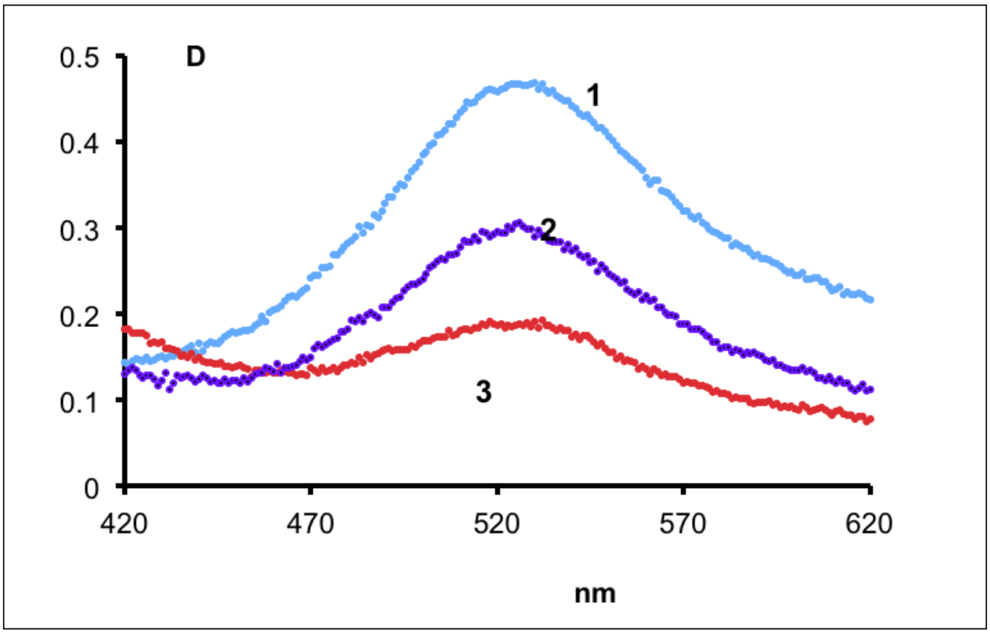

Figure 5: The absorption spectra of a (1:1) mix of DPPH$^\bullet$ in ethanol solution with treated water samples in the region of the peak at 520 nm: 1 – non-treated water; 2 – water on the third day after treatment; 3 – water immediately after treatment by plasma radiation.

The stable free radical DPPH$^\bullet$ is identified by a UV spectrum peak at λ = 520 nm. Optical density decrease of this peak indicates decreasing radical concentration. The DPPH$^\bullet$ radical structure prevents its interaction with radicals of complicated structure. It is a bad scavenger of reactive oxygen species [4, 9]. The radical DPPH$^\bullet$ can freely interact only with the most mobile radical, which is the hydrogen atom. Therefore the decrease of optical density in the peak at 520 nm is caused first of all by presence of reductant (atom H$^\bullet$). Among the species produced under plasma radiation of spark discharge with reduction properties are nitrous and peroxynitrous acids.

Absorption spectra of DPPH$^\bullet$ ethanol solution mixed with treated water (1:1) are shown in Figure 5. It can be seen from Fig. 5 that the maximal value of optical density of the peak at 520 nm is observed for DPPH$^\bullet$ mixture with non-treated water. Immediately after treatment by plasma radiation the optical density shows a strong decrease. On the third day the optical density is increased. The dependence of ratio D/D_0 on time, where D is the optical density for the mixture of DPPH$^\bullet$ ethanol solution with water at t days after treatment and D_0 is optical density with non-treated water is shown in Figure 6.

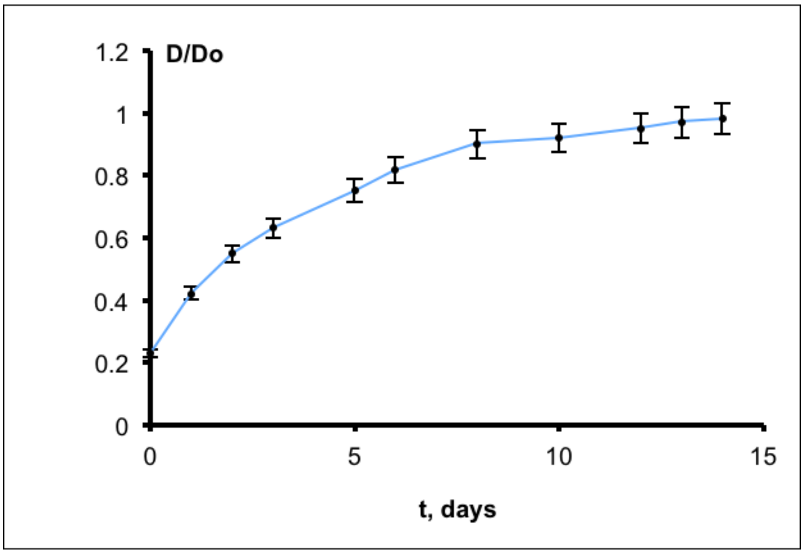

Figure 6. Ratio of optical densities D/D_0 for the peak at 520 nm in a 1:1 mixture of DPPH$^\bullet$ ethanol solution with water samples immediately after treatment (t = 0) and for water sample t days after treatment where D is the optical density for the mixture of DPPH$^\bullet$ ethanol solution with water at t days after treatment and D_0 is the optical density of a 1:1 mixture of DPPH$^\bullet$ ethanol solution with water sample before treatment.

Table 1: Values of pH and concentrations of species produced in water after treatment by spark discharge plasma radiation for 20 min. The initial water acidity was $pH_0 = 5.9 \pm 0.1$.

Time after treatment, days	pH	$[NO_2^-]$ mol/l	$[NO_3^-]$ mol/l	Instantaneous concentration, mol/l	
				$[ONOO^-]$ *)	[ONOOH]
0	3.1 ± 0.05	0.005 ± 0.0015	$(8 \pm 0.05)\ 10^{-4}$	$<6\ 10^{-6}$	–
1	3.05 ± 0.05	–	–	–	–
2	3.0 ± 0.05	0.001 ± 0.0005	$(9 \pm 0.05)\ 10^{-4}$	$<6\ 10^{-6}$	–
3	3.0 ± 0.05	$<6\ 10^{-4}$	$(1 \pm 0.05)\ 10^{-3}$	$(1.2 \pm 0.6)\ 10^{-5}$	$(1.4 \pm 0.7)\ 10^{-5}$
4	3.0 ± 0.05	–	–	–	–
8	2.95 ± 0.05	$<6\ 10^{-4}$	$(1.15 \pm 0.05)\ 10^{-3}$	$(1.8 \pm 0.5)\ 10^{-5}$	$(1.3 \pm 0.7)\ 10^{-5}$
10	2.9 ± 0.05	$<6\ 10^{-4}$	$(1.25 \pm 0.05)\ 10^{-3}$	$(1.8 \pm 0.5)\ 10^{-5}$	$(1.1 \pm 0.7)\ 10^{-5}$
14	2.7 ± 0.05	$<6\ 10^{-4}$	$(2 \pm 0.05)\ 10^{-3}$	$<6\ 10^{-6}$	$< 0.5\ 10^{-5}$
*) NO_3^- contribution was subtracted					

Table 2: Yields of active species on 100 eV consumed energy.

Process	Primary species	Yield of species on 100 eV
Pulse UVC radiation	$HO_2^{\bullet}$, $O^{\bullet}$, N_2O	28
Radiation-chemical, (electron beam) [16]	e_{aq}^{-}, $H^{\bullet}$, H_2 reductants $OH^{\bullet}$, H_2O_2, $O^{\bullet}$ oxidants	~ 3.5 ~ 3.5
Dielectric barrier discharge (DBD) [17]	$OH^{\bullet}$, O_3	0.1 – 1

The decrease in optical density indicates the presence of reductants in the water. In the first two days after the treatment the reductants are nitrous and peroxynitrous acids. The concentration of nitrous acid is high and it is difficult to separate the reduction effect of nitrous acid from the reduction effect of peroxynitrous acid. On the third day nitrous acid is fully decayed and the main reduction effect is due to peroxynitrous acid. Therefore we assume that starting on the third day the decrease in optical density at the 520 nm peak is determined by peroxynitrous acid. Results concerning the peroxynitrous acid concentration in Table 1 are obtained on the basis of this assumption. On the fourteenth day after treatment the optical density of the $DPPH^{\bullet}$ solution mixture with treated water was equal to the optical density of the mixture with non-treated water within the limits of experimental error. Therefore on the fourteenth day peroxynitrous acid was no longer present in the treated water.

The instantaneous concentration of peroxynitrous acid is observed at any given time, the same is true for the concentration of peroxynitrite. Peroxynitrous acid and peroxynitrite are unstable and quickly decay. The appearance of reducing properties in treated water and the observation of a peak in the absorption spectrum connected with peroxynitrite, means that a compound exists, which is produced during irradiation and decays forming peroxynitrous acid and peroxynitrite over several days.

DISCUSSION

Production of active species

In dielectric barrier and gliding arc discharges the energy in the region of the discharge gap is high and the power of the discharge is 0.1 – 1 kW, therefore primary active species produced in wet air are highly active radicals $NO^{\bullet}$, $OH^{\bullet}$, atoms of nitrogen, oxygen and hydrogen [10]. Reactions with these primary active species leads to the production of secondary species. Secondary species in a gliding arc are carried away from the discharge region by airflow and contact the surface of a treated sample.

In contrast to all kinds of discharges where plasma is in contact with the sample, we have used a plasma radiation source in which plasma has no sample contact. Active species produced in the discharge region can't reach the surface of treated sample and they terminate at the place of their production. From active species in the discharge region, stable compounds are formed, which as vapour can leave the discharge region and settle on surfaces. It was found that these compounds have no chemical activity and they not lead to water pH variation. By means of IR spectroscopy it was found that these compounds include functional N=C-O groups.

The spark plasma generator has essentially less power than the gliding arc type, only ~ 0.6 W (used power supply was 2 W). But at the time of the radiation pulse a high instantaneous density of primary species is created, providing the possibility of qualitatively new processes. A relatively low power radiation source has some advantages. At high power of discharge and high yield of active species there is a high probability of species terminating in interactions one another, and with decreasing discharge power the probability of their termination is decreased.

It should be emphasised that the active species (radicals $R^{\bullet}$) produced by the physical action (discharge) in the solution can interact with dissolved molecules, B, at the rate $w_1 = k_1[R^{\bullet}][B]$ and terminate at the rate $w_2 = k_2[R^{\bullet}][R^{\bullet}]$; that is, they uselessly disappear. We are primarily interested in chemical effects, therefore the rate of reaction with molecules w_1 must essentially be more than the rate of termination radicals w_2, i.e. $w_2 << w_1$. Hence, for the maximum chemical effect:

$$\left[R^{\bullet}\right] << \frac{k_1}{k_2}[B] \qquad (2)$$

That is, the concentration of the generated radicals must be small enough [11]. If the optimal conditions are found, both a decrease and increase in the power of physical action (in our case, the power of electric discharge, i.e. the discharge capacitor C value, Fig. 1) equally lead to a decrease in the chemical effect. This case was realised in our work. It was found that the optimal chemical effect (decreasing pH) in liquid under the action of plasma radiation is achieved at a discharge capacitor value of 3.3 nf [3]. The chemical effect decreases with both decreases and increases of the discharge capacitor value.

It can be proposed, keeping in mind the analogy with the above modes of discharge (DBD and gliding arc), that the action of UVC radiation in water must produce hydroxyl radicals. A possible mechanism of hydroxyl radical creation could be the process $H_2O + h\nu \rightarrow OH^{\bullet} + H^{\bullet}$. The bond dissociation energy of the water molecule is 116 kcal/mol [12]. This photon has an energy of 5.03 eV, when $\lambda \sim 246$ nm. It means that under UVC radiation with $\lambda < 246$ nm the break-up of water molecules with the production of hydroxyl radicals is possible. However, the probability of this process is small [13]. Hydroxyl radicals can be produced with appreciable probability at $\lambda < 180$ nm.

At high instantaneous radiation density, arising at the time of the discharge pulse, the following mechanisms of active species production can exist under a flash of UVC radiation through exited states of water molecule [14]:

$H_2O + \gamma \rightarrow H_2O^*$ (3)
$H_2O^* + H_2O^* \rightarrow HO_2^{\bullet} + H_2 + H^{\bullet}$ (4)
$H^{\bullet} + O_2 + M \rightarrow HO_2^{\bullet} + M$ (M – third particle) (5)
$H^{\bullet} + H^{\bullet} + M \rightarrow H_2 + M$ (6)

Under a photon flash excited states of water molecules are produced (reaction 3). Excited molecules in view of its their high instantaneous density can interact with one another (reaction 4). In this case the energy consumed in one interaction with the creation of active species (reaction 4) is essentially more than the energy, which is consumed by the action of one photon. For the case of reaction 4, two photons participate in the process of active species production. For a continuous photon beam this requirement is not realised. The possibility of interaction of excited water molecules H_2O^* with one another will be small because of their low instantaneous concentration, and the yield of reactions 4, 5, 6 in the case of a continuous photon beam is small. This conclusion is confirmed by the data of work [15] in which the yield of $HO_2^{\bullet}$ radicals in water under spark plasma radiation, as used in this work, is compared with the radiation of a mercury UV lamp ($\lambda = 253.7$ nm). The intensity of UVC radiation from the mercury lamp is 430 times more, than the intensity of spark plasma radiation, but the yield of $HO_2^{\bullet}$ radicals in both cases is the same.

There are dissolved gases in water: nitrogen and oxygen. The energy of UVC photons with $\lambda < 250$ nm is enough to realise the following processes:

$N_2 + O_2 + h\nu \rightarrow N_2O + O^{\bullet}$ (7)
$O_2 + h\nu \rightarrow 2O^{\bullet}$ (8)

Thus we suppose that the primary active species produced in water under UVC radiation are $HO_2^{\bullet}$ radicals, $O^{\bullet}$ atoms and N_2O molecules.

The production of $HO_2^{\bullet}$ radicals, and the absence of hydroxyl radicals and hydrogen peroxide were directly shown in previous work [5]. $HO_2^{\bullet}$ radicals exist in acid solution, in neutral and alkali solution they exist as $O_2^{\bullet-}$. The equilibrium takes place as follows:

$HO_2^{\bullet} \rightarrow O_2^{\bullet-} + H^+$, $pK_a = 4.8$ (9)

Under spark discharge plasma radiation the pH of water is quickly decreased (see Table 1), therefore one species directly found in [3] was the radical $HO_2^{\bullet}$. All primary and secondary species via their interactions finally led to the production of definitively identified products: nitrous and nitric acid. The yield of acid ions was $(5.8 \pm 0.5)\ 10^{-9}$ mol(cm^2 s)$^{-1}$. The energy flow of UVC radiation was 2 10^{-3} J(cm^2 s)$^{-1}$ or 1.24 10^{16} eV(cm^2 s)$^{-1}$ [3]. Hence the yield of the final species produced under spark plasma radiation was ~ 28 active species per 100 eV energy of radiation. The yield of the primary species, from which final species are produced, must be not less than the yield of final species. Therefore we assume that the yield of the primary species is equal to the yield of the final species. A comparison of active species yields, which were generated under various conditions, is shown in Table 2. The optimisation of the discharge conditions for the generator of the spark plasma radiation provided a high yield of active species per unit of consumed energy. The yield of species per 100 eV of consumed energy under plasma radiation is more than under electron beam and dielectric barrier discharge (DBD). For gliding arc generators, analogous data are absent.

Under treatment by spark discharge plasma radiation hydroxyl radicals, hydrogen peroxide and ozone can be generated in secondary reactions with active species [3, 14]. For the detection of ozone and hydrogen peroxide as reaction products we used the same methods as in Ref. [5, 6], where these products were determined as products of secondary reactions in gliding arc plasma. But in our experiment we found that under spark plasma radiation the concentrations of hydroxyl radicals, hydrogen peroxide and ozone were small, not more than threshold of sensitivity of the detection methods used. The absence among secondary species of hydroxyl radicals, hydrogen peroxide and ozone can be explained by their termination in reactions with each other.

Among the secondary active species the more interesting ones are nitric oxide $NO^{\bullet}$, nitrous and peroxynitrous acid, and peroxynitrite. The mechanism of nitric oxide production can be the reaction:

$N_2O + O^{\bullet} \rightarrow 2NO^{\bullet}$ (10)

The production of nitrous acid can be by the following reactions [18]:

$NO^{\bullet} + O^{\bullet} + M \rightarrow NO_2 + M$ (11)
$NO^{\bullet} + NO_2 + M \rightarrow N_2O_3 + M$ (12)
$N_2O_3 + H_2O \rightarrow 2HNO_2$ (13)

Directly produced nitric oxide as a product of spark plasma radiation in water was not found in our experiment, but confirmation of nitric oxide production is present among the products peroxynitrite, peroxynitrous, nitrous and nitric acids, which are formed in reactions with nitric oxide. The main mechanisms of peroxynitrite and peroxynitrous acid production in the case of our experiment are the reactions [2]:

In neutral and alkali solution:

$O_2^{\bullet-} + NO^{\bullet} + M \rightarrow ONOO^- + M$ (14)

In acid solution:

$HO_2^{\bullet} + NO^{\bullet} + M \rightarrow ONOOH + M$ (15)

Peroxynitrous acid (peroxynitrite) is a strong one electron and two-electron oxidant. Peroxynitrous acid is the main form of $ONOOH/ONOO^-$ ($pK_a = 6.8$) in acid solution. Acid solution is quickly achieved in water under spark discharge plasma radiation. The oxidative properties of peroxynitrous acid are realised in the case of decay through the channels [10]:

$ONOOH + H^+ + e^- \rightarrow NO_2^{\bullet} + H_2O$ ($E = 2$ V) (16)
$ONOOH + 2H^+ + 2e^- \rightarrow HNO_2 + H_2O$ ($E = 1.67$ V) (17)

In these processes the oxidative power of nitrogen does not change, nitrogen remains three valent (oxidation power 3+). Isomerisation of peroxynitrous acid leads to the production of nitric acid:

$$ONOOH \rightarrow HNO_3 \qquad (18)$$

In the course of isomerisation the nitrogen atom oxidises by atom transfer from state 3+ to state 5+. Nitrites (state 3+) which are produced in processes 16 and 17, can be oxidants or reductants, in acid solution they are oxidised to nitrate (state 5+), in alkali solution nitrite is reduced to nitric oxide $NO^{\bullet}$ (2+). In the course of nitrogen oxidation 3+ → 5+ two-electron transfer occurs. These electrons cause the reduction processes. So, peroxynitrous acid can be both oxidant and reductant.

The decrease of the water pH in the course of irradiation by spark plasma can be caused by peroxynitrous acid production and its decay to nitric acid. The protonated form of peroxynitrite, peroxynitrous acid ONOOH ($pK_a = 6.8$), has a lifetime ~ 1.3 s [19]. The absorption spectrum of peroxynitrous acid has a weak broad peak at $\lambda = 355 – 360$ nm ($\varepsilon = 100$ mol^{-1} cm^{-1}) and the optical density slowly increases with decreasing of wavelength. Therefore to directly detect the peroxynitrous acid in our experiment is impossible. However, identification of peroxynitrous acid is possible on the basis of its reductive properties.

The decrease in pH of treated water, which continues up to 14 days after treatment, is likely caused by the production and decay of peroxynitrous acid. Immediately after treatment in water the pH becomes acidic, and in this condition only peroxynitrous acid (not peroxynitrite) can exist. The peak of peroxynitrite (300 nm) in absorption spectra during the first two days in treated water was not observed. Peroxynitrite appeared on the third day after treatment.

Peroxynitrite is experimentally observed in the peak in the absorption spectrum at $\lambda \sim 300$ nm. In the same place there is an absorption peak of NO_3^- ions, but the optical density of NO_3^- ions ($\varepsilon = 7$ l mol^{-1} cm^{-1}) can be seen at pH < 3. For pH = 3 the optical density of nitric acid ions, D = 0.007. Such a value of optical density is within the limits of experimental error (± 0.005), therefore at pH > 3 nitric acid makes no contribution to the optical density in the 300 nm peak. All optical density of the 300 nm peak at pH > 3 can therefore be assigned to peroxynitrite. At pH = 2.7, which is achieved on the fourteenth day after plasma radiation treatment, the optical density value for nitric acid calculated on the basis of its concentration and pH value $[H^+] = [NO_3^-] = 10^{-pH}$ is D = 0.015 ± 0.005. At pH = 2.7 the optical density of treated water on the fourteenth day in the region of the 300 nm peak is D = 0.013 ± 0.005. On subsequent days (after the fourteenth day) the pH value does not change. It means that on the fourteenth day only nitric acid remains in the treated water sample.

Distilled water was exposed to air for at least one day before the experiment, to achieve a steady state concentration of dissolved gases contained in air: oxygen, nitrogen, carbon dioxide. Let us evaluate the role of carbon dioxide absorbed from air. Carbonic acid exists in water solution in equilibrium with carbon dioxide hydrate:

$$CO_2 \cdot H_2O \leftrightarrow H_2CO_3 \qquad (19)$$

The equilibrium constant is 1.7 10^{-3} and the rate of direct reaction is 0.039 s^{-1}; the rate of the reverse reaction is 23 s^{-1}. In acid solution the equilibrium is strongly shifted leftwards, and at pH ~ 3 or lower carbonic acid decays (does not exist). Therefore absorption of carbon dioxide by water samples with pH ≤ 3 does not take place and the decrease in pH of water samples after treatment is not due to carbonic acid.

The dissolution of alkali metal salts, which are contained in glass is possible. In this case the pH value could increase. However, it is well known that chemical glass is stable in acidic solution. An additional experiment was made to evaluate the role of external factors in pH value variation when the sample is kept for a long time. The solution of nitric acid at pH 3 was kept for 14 days in the same conditions as the water sample treated by plasma radiation. No variation of the pH value was observed.

Active species are produced in water under the action of background radiation which is determined by both cosmic radiation and the radiation of construction elements (walls etc.) [20]. The chemical reactions initiated by these species could affect the results. The steady state concentration of active species produced under background radiation in fresh distilled water is minimal and is achieved after about one day [20]. Therefore we kept distilled water not less than one day to guarantee a stable water composition.

Complex production

The peroxynitrite become observable on the third day after plasma radiation treatment, when a peak at 300 nm appeared which could not be attributed to nitric acid as the pH was greater than three. The reductive properties of water treated by plasma radiation were observed immediately after treatment. These reductive properties can be attributed to nitrous and peroxynitrous acid. On the third day after treatment the nitrous acid had decayed and the reductive properties of the sample can be attributed to peroxynitrous acid. The sample had reductive properties for 14 days.

On the third day a 300 nm peak appeared, which can be attributed to peroxynitrite. The component of the optical density in the 300 nm peak attributable to nitric acid immediately after treatment (Table 1) at pH = 3.1 is within the limits of experimental error. With decreasing pH the optical density attributable to nitric acid increased and reached 100% at pH = 2.7 on the fourteenth day. If the sample is kept more than 14 days the reductive properties of the sample are no longer observed and further pH decreases do not occur. The concentration of peroxynitrous acid, evaluated on the basis of its reductive properties, and peroxynitrite concentration, evaluated on the basis of optical density in the peak at 300 nm for the time from three up to 14 days after plasma radiation treatment are approximately the same: $[ONOO^-] \sim [ONOOH] \sim 10^{-5}$ mol/l. It may be proposed that this is the instantaneous concentration, observed in the course of the decay of a complex X', which can be produced at the time of plasma irradiation. During further storage of the sample complex X' undergoes the transformation:

$$X' \rightarrow X \rightarrow ONOO^- + ONOOH \qquad (20)$$

Initially complex X' can include peroxynitrous acid, as it is one of the species produced immediately under pulse plasma radiation in acid solution. The complex can start to decay immediately after formation, but there is no peroxynitrite in the decay products. In the first two days the reductive properties of peroxynitrous acid cannot be separated from those of nitrous acid. We can propose that in structure of the X' complex during two days regeneration can take place, and as a result the peroxynitrite appears among the products of complex decay on the third day.

Production of a long-living chemical compound, which decays to peroxynitrite and peroxynitrous acid, could be the cause of the strong sporicidal effect after treatment of micromycets spores with spark gas-discharge plasma radiation (generator SD-10), which is observed in [21, 22]. Micromycets spores are covered with an opaque peptidoglycan layer, through which UV-radiation as well as short-lived species (radicals) cannot penetrate easily. The species which persist from ~1 s up to 14 days can enter into the spore, where they decay to peroxynitrite and peroxynitrous acid, causing irreversible damage to DNA molecules [23]. As a result the spores do not grow out and a 100% sporicidal effect is achieved. An evaluation of cytotoxic effect mechanisms of gas-discharge plasma radiation was made in [24].

Let us compare the processes under UVC radiation and gliding arc radiation. The energy of UVC photons in air is in the range 4.4–6.2 eV. The energy of ions in a gliding arc is 6–9 eV [25]. Therefore the gliding arc can produce more active species such as $OH^\bullet$ and $NO^\bullet$. Devices constructed on the basis of gliding arc radiation can be used for the inactivation of bacteria and wastewater purification [26].

The source of gas-discharge plasma radiation used in our work creates active species on the surface of the treated object in an area of about ten square centimetres with the same concentration as gliding arc, as the pH value after treatment in both processes is the same. Therefore this source of gas-discharge plasma radiation can be used in biomedical investigations and in view of its simplicity may find a broad range of applications.

CONCLUSIONS

- A gas-discharge source of plasma radiation generated active species in a treated object, which were finally transformed into nitric acid. Nitrogen compounds can cause the decreasing pH.
- The primary active species are $HO_2^{\bullet}$, $O^{\bullet}$ and N_2O.
- An intermediate active species is peroxynitrous acid, which decays to nitrous and nitric acids. Its concentration is [ONOOH] ~ 10^{-3} mol/l.
- Under pulse plasma radiation of spark gas-discharge in air a complex is produced which decays to peroxynitrite and peroxynitrous acid. The instantaneous concentration of the complex decay products is [$ONOO^-$] ~ [ONOOH] ~ 10^{-5} mol/l.

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