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FORMATION OF SILVER NANOPARTICLES IN PVA-PEG HYDROGEL UNDER ELECTRON IRRADIATION

The formation of silver nanoparticles in a hydrogel on the basis of polyvinyl alcohol and polyethylene glycol at its crosslinking under the electron irradiation has been studied using the optical spectroscopy and scanning electron microscopy methods. The growth of nanoparticles 40–70 nm in size and their clustering into aggregates about a few hundred nanometers in diameter are demonstrated. The total concentration of nanoparticles and their size correlate with the concentration of ionic silver in the initial solution and the electron irradiation dose. The formation of nanoparticles is interpreted as a result of the radiation-induced chemical reduction of silver in the solution that is spatially confined in the cells of a 3D microstructure in the crosslinked hydrogel. The radiation-crosslinked hydrogel demonstrates an antiseptic effect for 7 of 8 tested microorganisms at silver concentrations of 0.001–0.003 wt.%, which is at least an order of magnitude lower than effective concentrations of ionic and colloidal silvers.

K e y w o r d s: silver nanoparticles, hydrogel, electron irradiation, antiseptic.

1. Introduction

Radiation-crosslinked polymer hydrogels prepared on the basis of the polyvinyl alcohol (PVA)–polyethylene glycol (PEG) system are classified as "soft matter". Their consistency is similar to that of elastic jelly, and their water content can reach up to 95%. Structurally, those hydrogels are a three-dimensional (3D) network of chain polymer macromolecules, which are connected with one another by means of radical links (crosslinks). The cell size in such a network is of an order of 0.1–1.0 μm. Radicals for the crosslinking are formed due to a water radiolysis under the high-energy irradiation, in particular, with relativistic electrons [1].

The network cells are filled with water, whose molecules are retained in the network due to their polar interaction with inhomogeneities of the electric field created by the polymer chains. Such materials are more and more used in medicine as dressing materials for the treatment of open wounds and burns [2]. They are sterile, do not stick to the wound, and maintain a moist environment in the latter, which promotes the acceleration of the healing process. Furthermore, they can contain therapeutic agents.

However, there is a problem to provide bactericidal properties to medical dressings fabricated from radiation-crosslinked polymer hydrogels. This problem arose because ionizing radiation that is used for the crosslinking destructively affects most of pharmacological agents located in the hydrogel. For example, antibiotics lose their biological activity after the irradiation by more than a half.

An effective antiseptic, which should evidently be weakly sensitive to radiation, is metallic silver nanoparticles [3]. The latter react with the cell membrane of the pathogens of infectious diseases. This membrane is a structure of special proteins (peptidoglycanes). The nanoparticles block their ability to transport oxygen into the bacterium cell. As a result, the microorganism dies. In this case, the silver action is specific with respect to the cellular structure rather than the infectious DNA (as in the case of antibiotics). This circumstance is connected with the fact that cells of higher organisms have membranes of a completely different type (they do not contain peptidoglycanes). Therefore, silver nanoparticles do not affect the cells of such organisms, including human ones [4–6].

Recently, it was shown that silver nanoparticles can be formed immediately in the bulk of a polyacryl-based polymer hydrogel as a result of the radiation-induced chemical reaction of ionic silver reduction by gamma irradiation [7]. The aim of this work was to analyze a possibility to form silver nanoparticles in the bulk of a more biologically compatible hydrogel based on the PVA-PEG system in the course of its crosslinking under the electron irradiation. We will also intend to estimate the antiseptic ability of silver nanoparticles with respect to microorganisms that are the most challenging in modern medicine.

2. Experimental Part

The process of silver nanoparticle formation in the PVA-PEG hydrogel is studied. The properties of the latter were described in detail in work [1]. For its preparation, an aqueous solution of polyvinyl alcohol PVA-11/2 and polyethylene glycol PEG-6000. As a polymer solvent, silver nitrate $AgNO_3$ solutions in distilled water with the concentrations $C = 0$, 10, 20, 30, 60, 120, and 240 mg/l (i.e. within an interval of 0.001–0.024 wt.%) were used.

The experimental specimens were hermetically sealed in 4×6-cm^2 polyethylene bags, each containing 5 cm^3 of the hydrogel with a certain $AgNO_3$ concentration. If a bag was arranged on a horizontal surface, the liquid gel in it formed a homogeneous layer about 0.2 cm in thickness. Five such hydrogel specimens were produced and studied for each $AgNO_3$ concentration.

The radiation-crosslinking of hydrogel was performed at room temperature making use of 4-MeV electrons to doses of 25–140 kGy. A linear electron accelerator "Elektronika" was used as a radiation source. As a result of the crosslinking, the hydrogel in the specimens was transformed from the liquid state into a state of elastic rubber-like jelly, which was capable of the approximately twofold elastic stretching before its breaking.

Radiation-crosslinked hydrogels with various $AgNO_3$ concentrations were used to measure their optical absorption spectra in a spectral interval of 200–600 nm (a SHIMAZDU UV-2450 spectrometer), to study the surface morphology of dehydrated hydrogels using the scanning electron microscopy method (a JSM-35 microscope), and to analyze the bactericidal activity of hydrogel with respect to 8 species of bacteria (by the inhibition of their growth zone).

3. Results and Their Discussion

The presence of $AgNO_3$ with the indicated concentrations in the hydrogel was found not to significantly affect the spectrum of the latter before its electron irradiation. But after the irradiation to the doses required for the radiation-crosslinking in the hydrogel to take place, there appeared a broad band with a maximum at 400 nm in the hydrogel absorbance spectrum. By all its attributes, this band corresponds to the plasmon absorption in metallic nanoparticles [8]. The amplitude and location of the band maximum correlate with the $AgNO_3$ concentrations in the initial gels. This relation is illustrated in Fig. 1, which demonstrates the absorption spectra of hydrogel specimens with various $AgNO_3$ concentrations subjected to the same irradiation dose D.

The band associated with the plasmonic absorption appeared only in the specimens containing $AgNO_3$ and subjected to the irradiation. It is evidently connected with the excitation of plasmons in silver nanoparticles that arise owing to the radiation-induced reduction of metallic silver. In this case, the mechanism of silver nanoparticle formation can be described as follows. The dissolution of $AgNO_3$ in water results in the appearance of Ag^+ and NO_3^- ions. Under normal conditions, electrostatic repulsion does not allow ionized silver atoms to approach one another and form a cluster. Under the irradiation with high-energy electrons, the solution be-

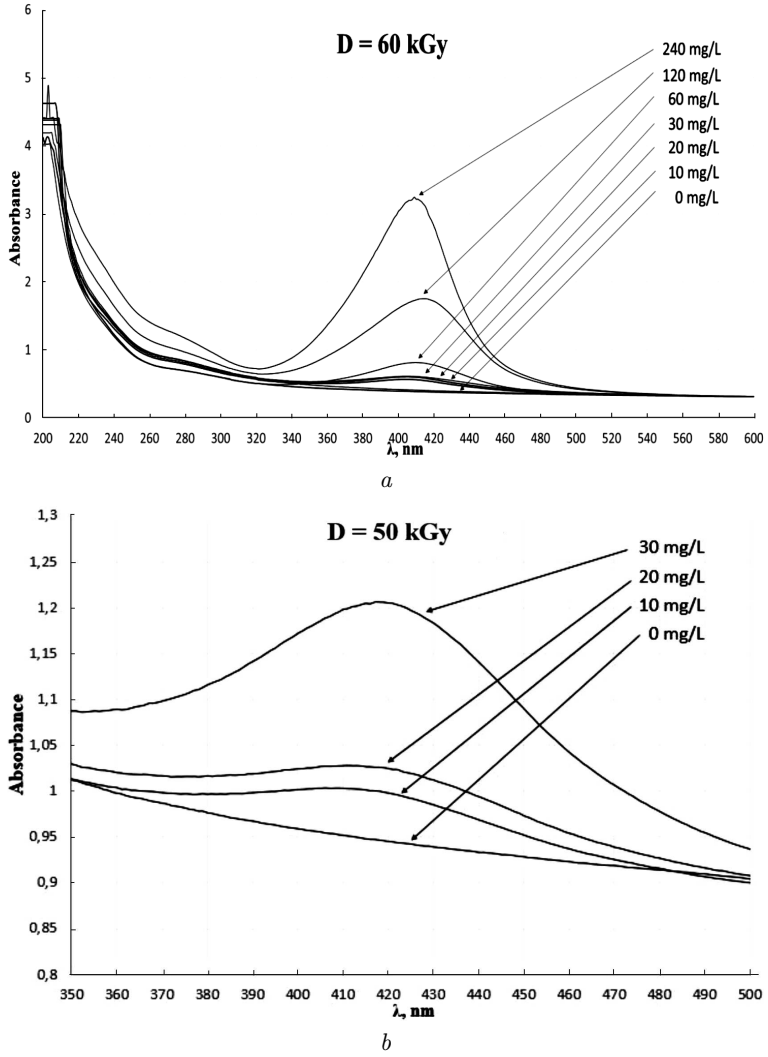


Fig. 1. Optical absorbance spectra of hydrogel with various $AgNO_3$ concentrations after the electron irradiation: the general appearance of spectra (a), spectral section with the plasmonic absorption for the specimens with the lowest $AgNO_3$ concentrations (b)

comes ionized, and there emerge a large number of free electrons. Some of them are captured by positive ions Ag^+ and neutralize the latter. The electrostatic repulsion between silver atoms disappears, they aggregate into clusters, and the latter eventually form metallic nanoparticles of silver. This model explains the correlation between the initial $AgNO_3$ concentration and not only the amplitude of the plasmon absorption band, but also the position of its maximum, which depends on the nanoparticle size [8]. Table 1 illustrates the effect of the initial $AgNO_3$ concentration on the position $\lambda_{\max}$ of the plasmon band maximum and the dominating nanoparticle size d in accordance with the following empirical expression obtained for silver nanoparticles in water [9]:

$$d = \sqrt{24 + 100(\lambda_{\max} - 385)} + 4.9.$$

It is evident that higher $AgNO_3$ concentrations give rise to a significant growth of the nanoparticle size.

Figure 2 demonstrates a modification of the hydrogel optical spectrum with an increase of the elec-

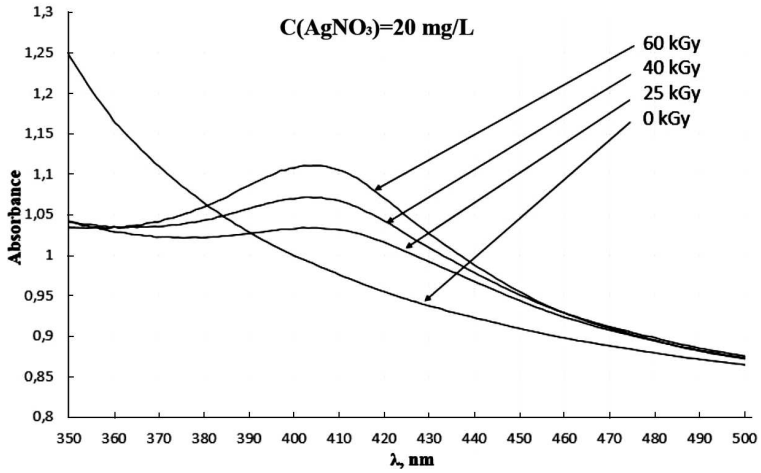


Fig. 2. Optical absorbance spectra of hydrogel subjected to various electron irradiation doses

tron irradiation dose for the specimens with the same initial $AgNO_3$ concentration, namely, 20 mg/l. The amplitude growth and the displacement of the plasmon absorption band peak are clearly observed. In Table 2, the wavelengths λ_{max} of the peak maximum and the corresponding estimates of the dominating nanoparticle size within a dose interval from 25 to 60 kGy are quoted.

The data in Fig. 2 and Table 2 testify that an increase in the irradiation dose leads to an increase in the number and size of Ag nanoparticles provided that the $AgNO_3$ concentration is fixed. This behavior may mean that, at the doses that are optimal for the hydrogel crosslinking (30–40 kGy), a large amount of silver still remains in the ionic state. On the other hand, one should bear in mind that the light refractive index in the radiation-crosslinked hydrogel can differ from the corresponding value for pure water; in particular, it can increase with the radiation dose. According to the results of work [10], this effect can change the dependence of the the plasmonic band maximum position on the nanoparticle size and bring about the overesitmation of the actual size values.

Table 1. **Effect of the initial $AgNO_3$ concentration on the position λ_{max} of the plasmon band maximum and the dominating nanoparticle size *d***

$AgNO_3$ concentration, mg/l	λ, nm	Nanoparticle size, nm
10	406	51
20	411	56
30	417	62

Table 2. **Effect of the irradiation dose on the position of the plasmonicband maximum λ and on the dominant size *d* of nanoparticles**

Irradiation dose, kGy	λ, nm	Nanoparticle size, nm
25	402	46
40	403	48
60	405	50

Direct scanning electron microscopy (SEM) researches of radiation-crosslinked hydrogels is a complicated task, because it is impossible to obtain a required vacuum in the microscope owing to the intense evaporation of water, which the hydrogel mainly consists of. However, some information on the presence and the size of metal particles in the hydrogel can be obtained with the help of SEM for specimens dehydrated during a long drying procedure. After this procedure, the specimen volume decreased by almost an order of magnitude, the concentration of Ag particles in it increased, and some of those particles coagulated into clusters. Nevertheless, all that did not hamper the detection of metal particles 40–70 nm in diameter and their diffusive clusters about a few hundred nanometers in dimensions emerging in such a "dried" hydrogel. When performing SEM researches, a thin

(30–50 nm) carbon layer was deposited onto the material surface, because the dried specimens had a low electrical conductivity and became charged under the electron irradiation.

Figure 3 demonstrates the SEM image of the surface of a hydrogel specimen irradiated in the low-energy secondary electron regime. The size of the smallest spots equals 40–45 nm, whereas large spots 100–300 nm in diameter look like aggregates of smaller ones. The correspondence of those spots to accumulation sites of metal silver was verified by additional researches in the "compo" mode. In this mode, the signal registered by a microscope is formed by high-energy backward reflected electrons. The number of the latter is mainly determined by the average atomic number of the object and weakly depends on the surface topography. In our studies, it was objects giving a noticeable signal in the "compo" mode that were considered to be silver nanoparticles.

Figure 4 exhibits electron microscopic images of the same area of the hydrogel surface obtained in the low-energy secondary electron (a) and backward reflected electron (b) modes. In the former case, besides bright spots of silver nanoparticles, other details of the specimen surface can also be observed. At the same time, the image obtained in the backward reflected electron mode allows one to observe only silver nanoparticles and their aggregates 50–150 nm in dimensions.

Attention should be paid that, under the irradiation, the processes of silver reduction, silver nanoparticle formation, and radiation-crosslinking of polymer take place simultaneously, which results in the appearance of a 3D network of polymer macromolecules. The size of a cell in this network is about a few hundred nanometers. Therefore, this nanostructuring of hydrogel can become a barrier to the drift of silver nanoparticles, their coagulation into larger aggregates, and transformation into ordinary colloidal silver. Perhaps, this is a mechanism that is responsible for a relatively small change of the dominant nanoparticle size with the growth of the $AgNO_3$ concentration and the irradiation dose. It is important to emphasize that, in the considered intervals of silver concentrations and electron irradiation doses, the basic radiation-crosslinked hydrogel retains its physicochemical properties required for its medical application [1].

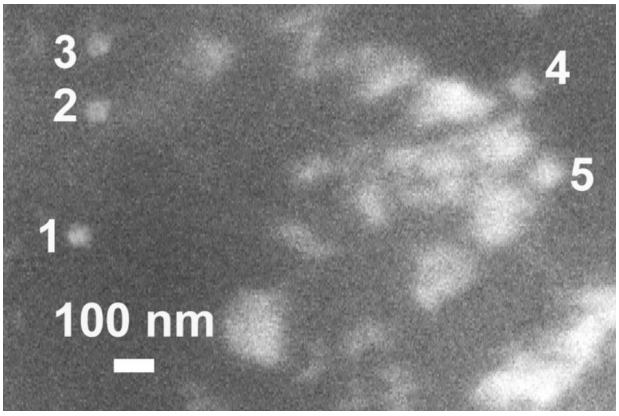
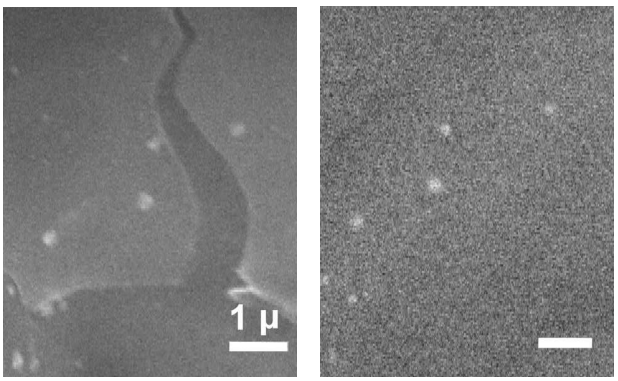


Fig. 3. Surface of a specimen with an initial $AgNO_3$ concentration of 30 mg/l and irradiated to a dose of 50 kGy. The figures denote silver nanoparticles with the following characteristic sizes: 40–45 nm (*1–3*), 55 nm (*4*), and 70 nm (*5*)



a *b*

Fig. 4. Electron microscopic images of the dried hydrogel surface obtained in the low-energy secondary electron (a) and backward reflected electron (b) modes

Table 3. **Results of bacteriological analysis**

Microorganisms	$AgNO_3$ concentration, mg/l							
	0	10	20	30	50	60	120	240
Pseudomonas aeruginosa	0	0	0	10	12	15	17	20
Acinetobacter baumannii	0	0	0	0	0	0	0	0
Proteus mirabilis	0	0	0	10	10	10	10	10
Klebsiella pneumoniae	0	14	15	17	20	20	20	20
E.coli	0	0	10	15	15	15	15	15
St. aureus	0	0	0	15	20	20	20	20
Enterococcus faecalis	0	0	0	0	0	25	25	25
Candida albicans	0	0	0	10	10	10	10	10

To estimate the bactericidal properties of the specimens of radiation-crosslinked hydrogel with silver nanoparticles, the width of the growth inhibition zone around the specimens of crosslinked hydrogel with eight different silver concentrations was measured for eight species of microorganisms. Eight species of microorganisms (the laboratory strains) were placed in Petri dishes with the Mueller Hinton II Agar medium (Liofilchem, Italy). Bacterial suspensions with a concentration of 0.5 Mac Farland for bacteria and 2 Mac Farland for fungi were used. The turbidity was measured with the help of a Densimat device (bioMerieux, France). Sterile specimens of examined hydrogel in the form of 10×10-mm^2 squares and 3 mm in thickness with various silver concentrations were put on cinoculated surface. Hydrogel specimens with eight initial $AgNO_3$ concentrations indicated above (from 0 to 240 mg/l) were used. After the thermostating at 37°C for 24 h, the width of the microorganism growth inhibition zone around the hydrogel specimens was measured.

The species of microorganisms and the $AgNO_3$ concentration values in the initial specimens are quoted in Table 3 (the left column and the upper row, respectively). The tabulated values demonstrate the measurement results for the growth inhibition zone width (in millimeter units). The electron irradiation dose was the same (60 kGy) for all specimens. The tabulated data testify to the appearance and increase of the bacterial growth inhibition zone with the growth of the silver concentration in the specimens for 7 of 8 microorganism species. For most of them, the effect manifested itself already at concentrations of 10–30 mg/l, i.e. 0.001–0.003 wt.%, which is significantly lower than the active concentrations of ionic and colloidal silvers [11, 12]. The result obtained testifies to the bactericidal effect of silver just in the nanoparticle state.

4. Conclusions

A possibility to form silver nanoparticles in a biocompatible hydrogel prepared on the basis of PVA-PEG system in the process of its crosslinking under the high-energy electron irradiation is shown. Two independent methods are applied to detect the influence of the initial ionic silver concentration and the electron irradiation dose on the dominant size of nanoparticles in the radiation-crosslinked hydrogel. The bactericidal efficiency of the radiation-crosslinked PVG-PEG hydrogel with silver is shown to be determined by the presence of silver nanoparticles.

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ФОРМУВАННЯ НАНОЧАСТИНОК СРІБЛА У ГІДРОГЕЛІ ПВС-ПЕГ ПІД ДІЄЮ ОПРОМІНЕННЯ ЕЛЕКТРОНАМИ

Р е з ю м е

Методами оптичної спектроскопії та растрової електронної мікроскопії досліджено формування наночастинок срібла в процесі радіаційної зшивки електронним опроміненням гідрогелю на основі системи полівиниловий спирт–поліетиленгліколь. Показано формування наночастинок розміром 40–70 нм та їх скупчень масштабу сотень нанометрів. Загальна концентрація і розмір наночастинок корелюють з концентрацією іонного срібла у вихідному гелі та з дозою електронного опромінення. Утворення наночастинок інтерпретовано як результат радіаційно-хімічного відновлення срібла в умовах розчину, просторово обмеженого комірками 3-d мікроструктури зшитого гідрогелю. Отриманий радіаційно-зшитий гідрогель демонструє антисептичну дію на 7 із 8 випробуваних видів мікроорганізмів вже при концентраціях атомів срібла $(1-3)\cdot 10^{-5}$, що принаймні на порядок менше аналогічно діючих концентрацій іонного та колоїдного срібла.