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OBTAINING AND PROPERTIES OF THE IMPLANT FILM MADE WITH CARBOXYMETHYLCELLULOSE CONTAINING SILVER NANOPARTICLES USED FOR TREATMENT OF BURN WOUNDS

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Abstract. *This article studies feasibilities of making an implant-film for treatment of burn wound, based on silver nanoparticles (AgNPs) stabilized by sodium-carboxymethylcellulose (Na-CMC) with degrees of substitution (DS) 0.65-0.90 and polymerization (DP) 200-600 synthesized using a photochemical method. The structural, physical and chemical, and physical-mechanical, burn wound healing properties as well as antimicrobial effects of implant-films containing the Na-CMC and AgNPs have been investigated. The shape, quantity, and size of the AgNPs embedded into the Na-CMC films were determined by UV-Vis spectroscopy, dynamic light scattering (DLS), atomic force microscopy (AFM) and transmission electron microscopy (TEM). It was found that the increase of silver nitrate concentration in solution of Na-CMC followed by photoirradiation leads to changes of AgNPs size and shape. It was found out that Na-CMC*

Introduction

One of the promising directions in the development of new chemical and pharmaceutical products is the design of medicinal biodegradable polymer films containing AgNPs. Such films exhibit prolonged therapeutic and bactericidal properties [1]. The bactericidal properties of AgNPs in correlation with particle size and a decrease of AgNPs size contribute to an increase of their antibacterial activity [2]. The synthesis of AgNPs with stable and defined shape and size is an important task to retain their high chemical and biological activity for long time [3]. AgNPs inhibit the activity of the enzyme

providing oxygen exchange in microbes, such as pathogenic bacteria, viruses, and fungi representing approximately 700 species of pathogenic flora and fauna [4].

AgNPs containing cellulose composites exhibited good biocompatibility and bactericidal properties. Therefore, such materials display broad application prospects in medicine, chemical catalysis, and other fields [5]. Na-CMC-AgNPs hydrogel was successfully prepared by a chemical method and showed high antibacterial activity against gram positive and gram negative bacteria which were considered as potential preparation for medical field [6].

CMC-AgNPs hydrogel was elaborated using microwave radiation and formed AgNPs of spherical shape with diameters ranging from 8 nm to 14 nm [7]. The size of AgNPs varied when different types of reducing agents were used; highest antimicrobial activity was linked with smaller size of AgNPs [8]. Stable AgNPs were obtained by reducing of chemicals with sodium borohydride at a low temperature in which Na-CMC was used as a stabilizer. Findings showed spherical AgNPs with unimodal distribution sizes in the range of 5 -80 nm. Both *S. aureus* and *E. coli* bacteria were inhibited by AgNPs [9].

Synthesis of CMC-AgNPs in aqueous solution of silver nitrate under UV irradiation evidenced that the reduction mechanism had mainly depended on the presence of the –COOH and –OH surface matrix of Na-CMC. The results confirmed that AgNPs formed with multiple individual spherical shapes and an average diameter was 15.5 nm [10].

CMC-AgNP hydrogels were synthesized via a UV-light photo-activated reaction, using a solution of $AgNO_3$ and AgNPs in the composite hydrogels, showed a size range of 50–200 nm and revealed high antimicrobial properties against different microorganisms such as *E. coli, S. aureus S. epidermidis, P. aeruginosa, C. albicans*. Moreover, the higher concentrations of silver were adapted to the external side of the wound dressing to improve the barrier effect of the device against infections [11].

films containing AgNPs in the size ranging from 5 to 35 nm had enhanced the microbicide effects and burn wound healing properties within 14 days.

Keywords: *silver nanoparticles, sodium-carboxymethylcellulose, degree of substitution, degree of polymerization, photo-irradiation.*

КУЙГАН ЯРАЛАРНИ ДАВОЛАШДА ТАРКИБИДА КУМУШ НАНОЗАРРАЛАРИ ТУТГАН КАРБОКСИМЕТИЛЦЕЛЛЮЛОЗА АСОСИДА ИМПЛАНТ-ПЛЁНКАЛАР ОЛИНИШИ ВА ХОССАЛАРИ

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Аннотация. *Мазкур мақолада алмашиниш даражаси (АД) 0,65-0,85 ва полимерланиш даражаси (ПД) 200-600 бўлган натрий-карбоксиметилцеллюлоза (Na-КМЦ) эритмасида барқарор кумуш нанозарралари (AgНЗ) фотокимёвий қайтарилиш усулида синтез қилинган. Таркибида AgНЗ ва Na-КМЦ тутган плёнкаларнинг физик-кимёвий, физик-механик ва куйган яраларни даволовчи хоссалари ҳамда бактерицид фаоллиги ўрганилган. Na-КМЦ плёнкаларида шаклланган AgНЗнинг ўлчами, шакли ва миқдори атом кучланишли микроскопия (АКМ), УБ-спектроскопия, динамик ёруғлик тарқалиши (DLS) ва трансмиссион электрон микроскопия (ТЭМ) усуллари орқали аниқланган. Na-КМЦ ва у асосида олинган имплант-плёнкаларда кумуш нитрат тузи концентрацияси ортиши билан фотокимёвий қайтариш натижасида шаклланаётган AgНЗ ўлчам ва шаклларининг ўзгариши аниқланди. Ўлчамлари 5–35 нм бўлган AgНЗ тутган Na-КМЦ плёнкалар юқори бактерицид фаоллик намоён қилганлиги ва 14 кун давомида куйган яраларни даволаш хусусиятига эга эканлиги аниқланди.*

Калит сўзлар: *кумуш нанозарралари, натрий-карбоксиметилцеллюлоза, алмашиниш даражаси, полимерланиш даражаси, имплант-плёнка, куйган яра, бактерицид хосса, фотолиз.*

ПОЛУЧЕНИЕ ИМПЛАНТ-ПЛЕНКИ НА ОСНОВЕ КАРБОКСИМЕТИЛЦЕЛЛЮЛОЗЫ, СОДЕРЖАЩЕЙ НАНОЧАСТИЦЫ СЕРЕБРА, И ЕЕ СВОЙСТВА ДЛЯ ЛЕЧЕНИЯ ОЖОГОВЫХ РАН

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Аннотация. *В данной статье стабильные наночастицы серебра (НЧАg) были синтезированы фотохимическим методом в растворе натрий-карбоксиметилцеллюлозы (Na-КМЦ) со степенью замещения (СЗ) 0,65-0,85 и степенью полимеризации (СП) 200-600. Изучены структура, физико-химические, физико-механические и противоожоговые свойства, а также бактерицидная активность пленок Na-КМЦ, содержащих НЧАg. Методами атомно-силовой микроскопии (АСМ), УФ-спектроскопии, динамического рассеяния света (DLS) и просвечивающей электронной микроскопии (ПЭМ) определены форма и размеры НЧАg, присутствующие в пленках Na-КМЦ. Установлено, что с увеличением концентрации нитрата серебра в растворах Na-КМЦ в процессе фотооблучения происходит изменение размера и формы НЧАg. Экспериментально доказано, что пленки Na-КМЦ, содержащие НЧАg размером от 5–35 нм, обладают высокой бактерицидной активностью и ранозаживляющими свойствами при ожоговых ранах в течение 14 суток.*

Ключевые слова: *наночастицы серебра, натрий-карбоксиметилцеллюлоза, степень замещения, степень полимеризации, фотооблучение.*

Composites of Na-CMC of different degrees of substitution (DS) and molecular weight (Mw) containing stabilized AgNPs were green synthesized from the reduction of Ag^+ into aqueous solutions of the polysaccharide by using the chemical method. Experimental evidence suggests that particle size distribution and morphology of AgNPs change according to the quantity of silver nitrate added to the reaction, as well as the DS and Mw of Na-CMC used for composites preparation [12].

The transition of Ag^+ from the ionic state to metallic ones Ag^0 reduces its toxicity with respect to cells without suppression of the antimicrobial activity against the pathogenic microflora. Na-CMC is water-soluble film forming biodegradable polymer that is widely used for fabrication of oral pharmaceuticals in particular to increase viscosity of ointments, in production of hydrogel-based pastes, and as drug carriers [13].

The aim of this study is to prepare Na-CMC stabilized AgNPs of different sizes and shapes embedded into polymer films as well as to investigate their structure, physical-chemical properties, antimicrobial effect and assess the burn wound healing properties in rat models.

Experimental section

The Synthesis of AgNPs with Na-CMC hydrogels

Purified samples of Na-CMC with the degrees of substitution (DS 0.62-0.85) and polymerization (DP 210-610) were obtained from the cotton cellulose and used as polymer matrices [14]. Various concentrations of aqueous $AgNO_3$ were used in order to prepare AgNPs within the films of Na-CMC. Mainly 2-4 wt. % aqueous solutions of purified Na-CMC samples with various DS and DP were applied as film forming matrix after removal of gel fractions. The calculated amounts of 0.1-0.001 M aqueous solutions of $AgNO_3$ and 0.1-0.5 wt. % of glycerol, which played the role of a plasticizer, were added to the gel free Na-CMC solutions, and stirred until homogeneous Ag^+CMC^- hydrogels had formed. The photochemical reduction of Ag^+ immobilized within the Na-CMC was performed at T 25°C by irradiation with a DRSH-250 high-pressure

mercury vapor lamp wattage = 35 Wt and wavelength λ=365 nm. The dispersions of AgNPs in the matrix of Na-CMC hydrogels were prepared by ultrasonic treatment with the help of UZDN-1 and U-4.2 ultrasonic dispersers. AgNPs contained Na-CMC films were obtained by casting the preformed hydrogel on the degreased glass plate and then drying it at a temperature range 25-40°C.

Physical and chemical methods of investigation

The mechanical properties of films (40-120 μm thickness, 20 mm width and 200 mm length) were determined in a uniaxial tension mode using the Zwick-1445 tensile testing machine (Germany). The morphology of surface layers of the AgNPs embedded into Na-CMC film was examined on AFM-5500 (Austria). The size and shape of the AgNPs in the Na-CMC films were determined using a transmission electron microscope (TEM-100, Ukraine) and ZETASIZER Nano ZS. The average size of the AgNPs replaced on the film surface was found by processing the corresponding micrographs with the help of Mathcad soft. Optical absorption spectra of the films (thickness is 40-45 μm) were recorded on a Specord M210 instrument at wavelengths between 200 and 900 nm.

Determination of the bactericidal effect. The bacterium *Staphylococcus epidermidis* and the yeast fungus *Candida albicans*– human and animal pathogens were used as test cultures to assess the bacterial effectiveness of the films [15]. The following samples were added to test-tubes containing thioglycollic medium (for *Staphylococcus epidermidis*) and Saburo (for *Candida albicans*):

№	Samples name
1	Na-CMC film
2	Na-CMC film containing Ag^{+}= 0.025 wt.%
3	Na-CMC film containing Ag^{0}= 0.025 wt.%
4	Na-CMC film containing Ag^{0}= 0.25 wt.%
5	Na-CMC film containing Ag^{0}= 2.5 wt.%

10 wt. % of NaCl solution was used as a test medium. The final concentration of 150 cells/mL test culture was added to each test-tube within six hours. Samples were incubated at 34 °C during 48 (for *Staphylococcus epidermidis*) and 72 h (for *Candida albicans*).

Burn wound healing activity

In vivo the tests were performed on white rats (180–220 g) that had been kept in standard cages at a controlled temperature (25 °C) with a light/dark cycle of 12 h, with free access to water and food. Rats had been fasted (solids) for 18 h, and acclimatized to the test environment 2 h before each experiment. The animals were randomly divided into different groups. The animals were anesthetized with a combination of ketamine hydrochloride and xylazine hydrochloride (50 and 5 mg/kg^{-1}, intramuscular injection, respectively). After the experimental procedures, the animals were euthanized by sodium thiopental (100 mg/kg^{-1}, intraperitoneal injection). All test protocols were approved by the Uzbekistan Animal Ethics Committee).

Two groups of rats (n = 5 per group) were divided into control and experimental groups treated with one dose per wound. In the same animal, two 62 mm incisions (surgical wounds) were performed. One wound (left side) received the Na-CMC film base, and the other (right side) received the test formulation (Na-CMC film contained AgNP). The procedures were performed in aseptic environment, with all autoclaved surgical materials. First, a manual trichotomy was performed in the middle region of the dorsum. For wound induction, a circular metal punch 52 mm in diameter was used in the cervical dorsum region of each animal.

The treatments were applied immediately after surgery and daily thereafter at the same time. Both the base gel and the formulations were applied to the wounds of the animals on days 0, 1, 4, 7, and 14 days after surgery, and the healing process was evaluated. All of the animals were examined daily for macroscopic evaluation of the wound, observing the presence or absence of hemorrhage, exudates, and crust, and the data were recorded in individual files. The wounds were photographed at days 0, 1, 4, 7 and 14 of the experimental protocol, and the area of the wound area was measured. At the end of the chronic treatments, the animals

were euthanized with anesthetic overdose (thiopental sodium, 100 mg/kg^{-1}

Findings and discussion

The most important physicochemical characteristics of Na-CMC that determine their possible application are the solubility and the degree of purity. For investigation we selected both water-soluble and water-insoluble fractions of purified Na-CMC with DS=0.62-0.85 and DP=210-610.

Table 1

Effects of the DS and DP of Na-CMC samples on the quantity and composition of water-soluble and water-insoluble fractions

Indexes of purified Na-CMC samples			Indexes of Na-CMC samples after their centrifugation					
Samples of Na-CMC from CC	DS	DP	Soluble fractions, %	DS	DP	Gel fractions, %	DS	DP
1	0.62	210	71	0.65	200	29	0.11	230
2	0.67	320	75	0.69	300	25	0.12	380
3	0.82	520	98	0.85	510	3	0.15	600
4	0.85	610	99	0.90	600	1	0.17	700

As seen from Table 1, with increasing of DS, the water-soluble fraction of the Na-CMC increases, while the content of the insoluble gel fraction decreases. With increasing of DS, the amount of gel fraction of Na-CMC in water decreases. This effect probably occurs due to destruction of hydrogen bonds between macromolecules and increasing of the DS in Na-CMC. Moreover, the composition of the Na-CMC gel fraction depends on the type of cellulosic raw material and methods of treatment.

It was found that the content of gel fraction in Na-CMC samples of cotton pulp for the whole DP and DS intervals was bigger than that of Na-CMC pristine sample 4 (Table 1).

This result could be explained by high DS of Na-CMC.

Further the behavior of Na-CMC (DS=0.65-0.90 and DP=200-600) with incorporated silver ions and nanoparticles was studied (Table 2). Aqueous solutions of Na-CMC exhibits the polyelectrolyte character [17].

The conditions of replacement of sodium ions in Na-CMC by silver ions were also studied. It was found that when Na^+-ions in Na-CMC with DS = 0.65; 0.69; 0.85; and 0.90 are replaced by 0.35; 0.4; 0.5 and 0.6 mol.% Ag^+ ions, the stable Ag^+CMC^- hydrogel complexes with poor water-solubility is formed. Probably, the carboxyl groups of Na-CMC are able to form complexes [18] with silver ions. The critical concentration of Ag^+ that causes hydrogel formation of Na-CMC is presented in Table 2.

Table 2

Physicochemical characteristics of Na-CMC containing Ag^+

Samples of Na-CMC	Characteristic of Na-CMC		Content of Ag^+, mol. %	Solubility in water, %	Relative viscosity η_{rel}
	DP	DS			
1	0.65	200	-	71.0	1.223
			0.05	63.6	1.721
			0.10	44.2	2.342
			0.25	29.7	2.983
			0.35	18.1	Hydrogel
2	0.69	300	-	75.0	1.470
			0.05	69.8	1.841
			0.10	58.5	2.985
			0.30	34.7	3.543
			0.40	26.2	Hydrogel
3	0.85	510	-	98.0	2.128
			0.10	89.3	2.651
			0.20	56.0	3.426
			0.40	30.8	4.576
			0.50	14.9	Hydrogel
4	0.90	600	-	99.0	1.894
			0.15	92.0	1.912
			0.20	64.7	2.732
			0.45	27.5	3.871
			0.60	19.0	Hydrogel

Higher DS concentration of Ag^+ that is essential for forming of poor water-soluble Ag^+CMC^- causes an increase in hydrogel complex.

Numerous studies show that the AgNPs containing materials possess good antimicrobial and wound healing properties [19]. Therefore, the conditions of forming of Ag^+CMC^- hydrogels containing stabilized AgNPs were studied using photochemical methods [20]. Comparison of different methods [21] of Ag^+ reduction to nanometallic (Ag^0) state shows that photochemical reduction is the most effective tool enabling to control the size of nanoparticles avoiding formation of by-products.

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Stable nanoparticles synthesized by photochemical methods have a narrow particle size distribution and high stability in aqueous media in comparison with colloidal systems obtained by conventional method [22]. In the course of photochemical reduction, optically generated electrons migrate and combine with electron sinks at the embedded interface and near the surface. The negatively charged electrons attract the interstitial Ag^+ ions present in thermodynamic equilibrium, which move towards the trapped electron. This is the first step in the sequence of electron and interstitial atom trapping and development of silver clusters and nanoparticles as the basis of latent images.

Analyzing the spectroscopic data of AgNPs and Na-CMC nanocomposites, one can assume that the negative charges of the carboxymethyl groups "trap" the positively charged Ag^+ [23, 24]. Then the reaction sequence according to the Mott-Gurney mechanisms can be shown as follows:

$$R\text{-}COO^- + (Ag^+)_n \xrightarrow{h\nu} R\text{-}COO^\bullet : Ag^0, \quad (1)$$

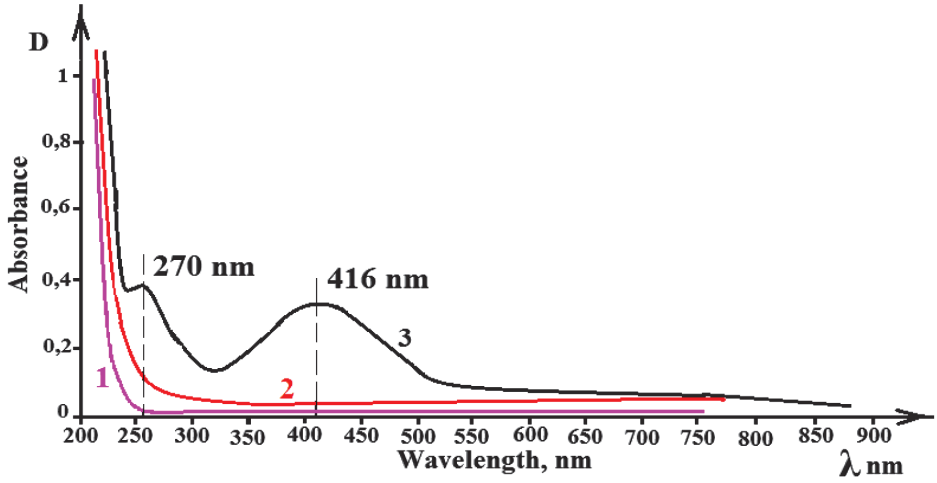
$$R\text{-}COO^\bullet : Ag^0 + e^- \longrightarrow R\text{-}COO^- : Ag^0, \quad (2)$$

$$R\text{-}COO^-: Ag^0 + Ag^+ \xrightarrow{h\nu} R\text{-}COO^\bullet: Ag_2^0. \quad (3)$$

$$R\text{-}COO^-: Ag_2^0 \xrightarrow{h\nu} R\text{-}COO^- : (Ag^0)_n. \quad (n)$$

Thus, the photostimulated development of AgNPs in the Ag^+CMC^- hydrogel can be considered as electron-stimulated nuclear process based on Mott-Gurney theory [23] as in case of photography process.

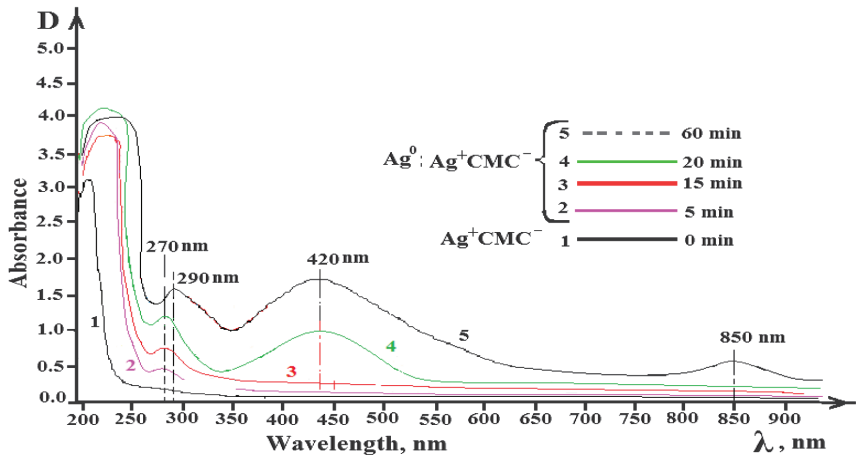
To confirm this statement, UV-Vis spectra of Na-CMC, Ag^+CMC^-, and Ag^0CMC were registered and compared. After UV – irradiation of Na-CMC solutions containing Ag^+ a stable colloidal system of nanosilver (pale – yellow colour) is formed with absorbance maximum at λ_{max} = 416 nm and size of AgNPs 5-25 nm (Fig. 1, curve 3).



The UV-irradiation duration for the samples was equal to 25 minutes, [Na-CMC] = 8×10^{-3}mol/L (2 wt.%); [$AgNO_3$] = 3·10^{-5}mol/L (0.25 wt.%).

Figure 1. UV-Vis absorption spectra of: (1) Na-CMC, (2) Ag^+CMC^-, (3) Ag^0CMC

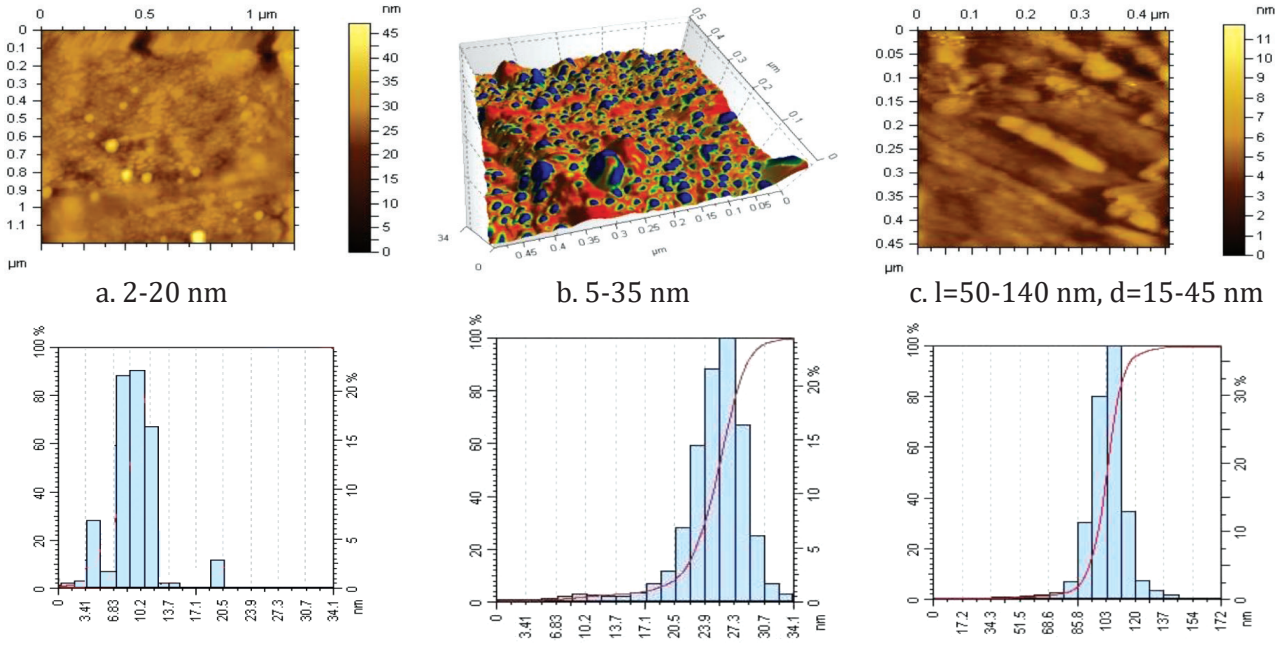
No changes were observed for initial Na-CMC solutions (Fig. 1, curve – 1) and unreduced Ag^+ in Ag^+ CMC^- (Fig. 1, curve – 2) in the spectral region of 250-900 nm. With the growth of photolysis time, the color of the solutions changed from pale yellow to brown. According to literature data [25, 26] such changes are probably caused by increased quantity and size of AgNPs. To confirm this assumption, absorption spectra of Ag^+CMC^- were obtained at different irradiation time of Ag^+CMC^- system composed of 8×10^{-3} mol/L of Na-CMC (2 wt.%) and 3·10^{-5}mol/L of silver nitrate. Fig. 2 shows the UV-Vis spectra of Na-CMC hydrogels containing AgNPs after different photo-irradiation time. Appearance of the shoulder at λ_{max} = 270 nm after 5 minutes photo-irradiation can be attributed to development of stable silver clusters [27], probably Ag_8^{2+} (Fig. 2, curve - 2).



[Na-CMC] = 8×10^{-3}mol/L (2 wt. %); and [AgNO$_3$] = 3·10^{-5}mol/L (0.25 wt.%). UV-irradiation time is 0 (1), 5 (2), 15 (3), 20 (4), and 60 min (5)

Figure 2. UV-Vis absorption spectra of photochemically reduced of Ag⁺ in Ag⁺CMC⁻

After photolysis of Ag⁺CMC⁻ during 15-20 min the intensity of absorption band at λ_{max} = 270 nm increases indicating on the formation of smaller stabilized silver clusters with sizes 2-8 nm (Fig. 2, curves – 2,3). The absorption band at λ_{max} = 420 nm is attributed to the larger AgNPs [27] with sizes 5-35 nm (Fig. 2, curve – 4). Further irradiation of Ag⁺CMC⁻ system during 60 minutes leads to appearance of absorption bands with maxima at λ_{max} = 290 nm and λ_{max} = 420 nm. This is due to increasing of the numbers of larger silver clusters [28] sized 5-35 nm (Fig. 2, curve – 5). In addition, a weak maximum at λ_{max} = 850 nm was observed in the near-IR region owing to exclusively rod-shaped [29] AgNPs (Fig. 2, curve – 5).



a. 2-20 nm b. 5-35 nm c. l=50-140 nm, d=15-45 nm

Concentration of [Na-CMC] = 8×10^{-3}mol/L; UV–irradiation time is 30 min.

Figure 3. AFM microphotographs and histograms of Na-CMC films containing 0.025 (a), 0.25 (b), and 2.5 wt.% (c) of AgNPs

In the following step the Na-CMC films prepared with 2 wt. % of Na-CMC solution (DS-0.90, DP- 600, pH = 8.5) containing 0.025-2.50 wt. % of $AgNO_3$ were photo-irradiated. The

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UV-irradiation induced the change of Na-CMC color solution from pale yellow to brown. It is likely due to increasing of the amount of AgNPs of different sizes. However, pure Na-CMC solution did not change the color and remained transparent after UV-irradiation. To confirm the formation of AgNPs and to determine the forms and sizes of AgNPs in the composition of Na-CMC the AFM investigations of Na-CMC films were performed. Figure 3 shows the electron micrographs of Na-CMC films formed under UV – irradiation containing 0.025-2.5 wt. % silver nitrate.

As seen from Fig. 3a, during the photo-irradiation of 0.025 wt. % of $AgNO_3$, the silver clusters and nanoparticles with sizes of 2-30 nm are formed within the structure of Na-CMC films. Increased concentration of $AgNO_3$ 10 times (0.25 wt. %) in Na-CMC films leads to development of spherical AgNPs with sizes 5-35 nm (Fig. 3b). Higher silver nitrate concentration in the Na-CMC structure up to 2.5 wt.% induces the growth of the number of AgNPs sized from 5 to 35 nm and formation of rod-shaped AgNPs [30] with lengths 50-140 nm and widths 15-45 nm (Fig. 3c). Therefore, high Ag^+ content in Na-CMC films leads to the development of relatively narrow size distribution of spherical and rod shaped AgNPs.

It was reported [31] that the shapes and sizes of AgNPs in various polymer films may be determined by UV – spectroscopy. UV – spectra of Na-CMC films prepared from the solutions with 0.025-2.50 wt. % of silver nitrate are shown in Fig. 4.

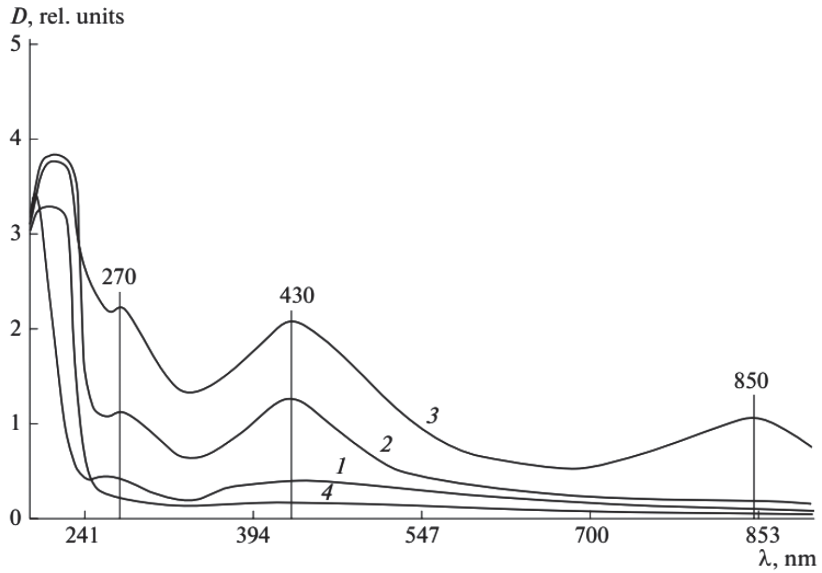
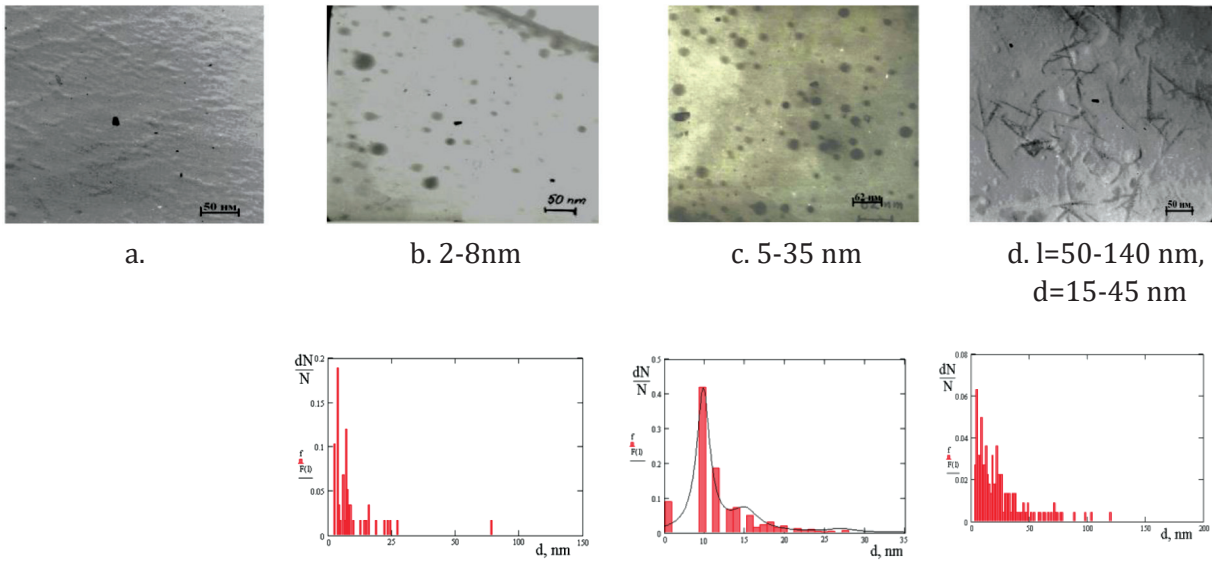


Figure 4. Absorption spectra of photochemically reduced Na-CMC films containing 0.025(1), 0.25(2), and 2.5 wt. % (3) $AgNO_3$. Curve 4 represents the pristine Na-CMC film

The appearance of a maximum at λ=270 nm at 0.025 wt.% silver nitrate in Na-CMC film (Fig.4, curve – 1), is caused by the development of silver clusters generated through dimerization of AgNPs. In the spectrum of the Na-CMC film containing 0.25 wt.% silver nitrate (Fig.4, curve – 2), a new absorption band with a maximum at λ=430 nm is observed owing to the presence of AgNPs [32] sized 5 to 35 nm. The intensity of the absorption band with a maximum at λ=430 nm is highest at the concentration of silver nitrate 2.5 wt. % (Fig.4, curve –3). This is caused by increased numbers of AgNPs with sizes 5-35 nm. Appearance of the broad peak with maximum at λ=850 nm is attributed to exclusively rod shaped AgNPs.

In the next step we studied the impact of Ag^+ concentration on the size and development

of AgNPs in Na-CMC films. Transmission electron microscopy investigation of Na-CMC film contained AgNPs was carried out and the results are presented in Fig. 5.



a. b. 2-8nm c. 5-35 nm d. l=50-140 nm, d=15-45 nm

(a) pure Na-CMC film, (b) Na-CMC contained Ag^+= 0.025 wt.%, (c) Na-CMC contained Ag^+ 0.25 wt.%, (d) Na-CMC contained Ag^+ = 2.5 wt.%, Time of UV - irradiations 20 min.

Figure 5. TEM microphotograph of Na-CMC film contained AgNP (a), (b), (c) (d) and their histograms

TEM images show development of spherical AgNPs with sizes of 2-8 nm (Fig. 5. b) and 5-35 nm (Fig. 5 c) at low concentrations of Ag^+ (0.025 wt.% and 0.25 wt.%) after photochemical reduction. With increased Ag^+ concentration in Na-CMC film up to 2.5 wt.%, nanoparticles become rod-shape with l=50-140 nm and d=15-45 nm (Fig. 5 d).

The mechanism of rod-like shaped of AgNPs development in these conditions still remains unclear [33]. Thus, the size and shape of AgNPs formed in Na-CMC film by photochemical reduction of Ag^+ depend on DS, concentration of Na-CMC, concentration of Ag^+, and time of photochemical irradiation. In the presence of Na-CMC the macro anions effectively bind Ag^+, and the UV – reduction led to the successive development of clusters and nanosilver depending on the concentration of Ag^+.

The microbicidal effects of the Na-CMC films samples containing silver cations and nanoparticles were examined with respect to test cultures of the opportunistic pathogens *Staphylococcus epidermidis* and *Candida albicans*.

The concentration-dependent antimicrobial efficacy of the AgNPs of different sizes and shapes on the above mentioned organisms is shown in Table 3.

It is clear that Na-CMC film containing AgNPs sized 5-35 nm completely inhibits the growth of *Staphylococcus epidermidis* and *Candida albicans* and, thus, is the most active film.

The samples of Na-CMC films containing relatively large (length is 50-140 nm and width15-45 nm) rod shaped nanoparticles are less active than the spherical nanoparticles (5-35 nm). This circumstance may be explained by small surface areas of the rod-shaped [34] AgNPs.

The Na-CMC film containing AgNPs with spherical structures and sizes of 5-35 nm showed the highest activity against *Staphylococcus epidermidis* and *Candida albicans* due to the large surface area of the nanoparticles and their ability to penetrate into the cell nucleus.

Table 3

Comparative findings of the antimicrobial effects of Na-CMC films containing AgNPs of different sizes and shapes

№	$AgNO_3$ content in the film, wt %	AgNPs content in the film, wt %	Shape and size of AgNPs	Strains	
				Staphylococcus epidermidis	Candida albicans
0	Control	Na-CMC	-	$5*10^{12}$ CFU mL^{-1}	$1*10^{7}$ CFU mL^{-1}
1	2,5	2,41	Rod-shaped 50-140 nm long and 15-45 nm wide	$2*10^{7}$ CFU mL^{-1}	$1*10^{2}$ CFU mL^{-1}
2	0,25	0,24	Spherical 5-35 nm	Absent	Absent
3	0,025	0,023	Spherical 2-20nm	$1*10^{6}$CFU mL^{-1}	Absent

Note: *CFU mL^{-1} is the number of colony-forming units per mL.*

The Na-CMC film containing rod-shaped AgNPs with l=50-140 nm and d=15-40 nm was less active than films containing AgNPs with spherical structures, but more active than the Na-CMC film containing Ag^+.

In addition, Na-CMC film containing AgNPs sized 2-20 nm (0.023%) was proved to be less active than other films. This result may be explained by the fact that the total content of AgNPs in such Na-CMC films turned out to be almost an order of magnitude less than that in the samples of films containing 5-35 nm (0.23%) AgNPs. Because the concentration of Ag^+ in the Na-CMC hydrogels utilized for the film development was low (0.023%), they were almost completely associated with carboxylate anions of Na-CMC. This limited mobility of Ag^+ that is responsible for the lower rate of AgNPs formation, which that seems to occur only in a "nanoreactor" [35] structure.

The relatively high antimicrobial activity of AgNPs compared with Ag^+ may be caused by the following:

The inability of AgNPs to form chemical bonds with the functional groups on the surface of *Staphylococcus epidermidis* and *Candida albicans*cells, and, probably, by their ability to penetrate into the nucleus of cells and inhibit cell growth and activity;

Decreased AgNPs sizes led to an increase in the total surface area and acceleration of their contact with cells and penetration into cell nuclei of both microbe strains;

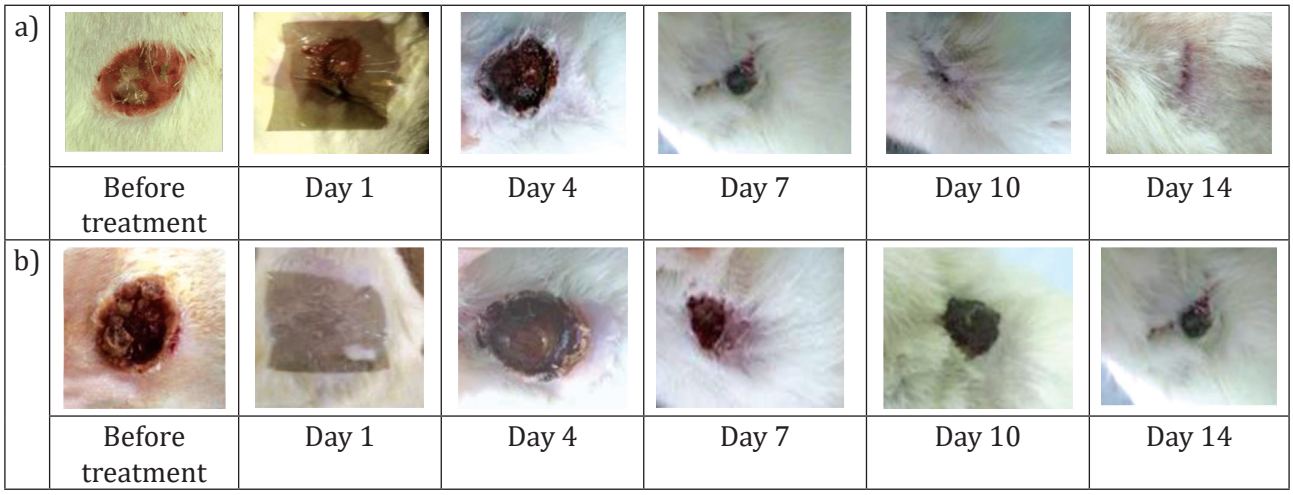
As the nanoparticle sizes increased and their shapes changed from spherical to rod-shaped, total surface area, readily decreased thereby leading to a limited ability of the resulting nanoparticles to enter the cell nuclei of the *Staphylococcus epidermidis* and *Candida albicans* strains.

In the next step we studied impact of Na-CMC film contained 0.23% AgNPs with sizes 5-35 nm to wound healing process and the results are presented in figure

This analysis showed that over the first of one postoperative days, there was no significant difference between the means of contraction rates of the wounds during using of the Na-CMC-AgNPs group (47 ± 3.2 mm^2) when compared to Na-CMC film group (62 ± 2.8 mm^2). At 4 day after surgery the wound contraction index was significantly higher in the group treated with the of Na-CMC film contained 0.23% AgNPs with sizes 5-35 nm (43 ± 3.0 mm^2, $p < 0.05$) when compared to that in the group with Na-CMC film (52 ± 3.9 mm^2). At seven days after surgery, a significant difference was observed between the mean contraction rates of the wounds of the group treated with the Na-CMC film contained 0.23% AgNPs with sizes 5-35 nm (16 ± 3.1 mm^2, $p < 0.05$), when compared to that in groups Na-CMC (31 ± 4.7 mm^2).

Within 14 days after surgery, there was no significant difference between the mean contraction rates of the wounds of the animals containing the Na-CMC film contained 0.23%

AgNPs with sizes 5-35 nm (3 ± 0.5 mm^2) when compared to Na-CMC group (11 ± 1.6 mm^2). However, the mean rate of wound contraction was significantly higher in the group treated with Na-CMC film contained 0.23% AgNPs with sizes 5-35 nm when compared to that in the Na-CMC group.



a) Using Na-CMC film contained 0.23 % silver nanoparticles with sizes 5-35 nm.
b) Using Na-CMC as control

Figure 6. Representative images of the burn wound healing process caused by Na-CMC film, contained silver nanoparticles on the back of rate models

In the analysis of wound contraction, there was a progressive decrease in the lesion area in all groups and no wound presented a greater area than the initial one, and the area decreased owing to the mechanism of tissue contraction. It was also observed that the percentages of wound contractions were higher within seven days; this was expected, since the fibroplasia phase of healing occurs within 7 to 14 days, causing the presence of fibroblasts and myofbroblasts.

After 14 days of treatment, it was observed that in the group which used Na-CMC film contained 0.23% AgNPs with 5-35 nm of size, the healing was superior compared to that with Na-CMC.

Conclusion

Optimal conditions for the development of AgNPs with different shapes and sizes in the structure Na-CMC films with different DS and DP have been identified. It was established that the silver cations within Na-CMC macromolecules can play the role of «nanoreactors» where according to the theory of Mott-Gurney the carboxylic groups of Na-CMC «trap» the silver cations and promote the photostimulated development of AgNPs. The form and size of AgNPs within Na-CMC hydrogels and films were controlled and evaluated by UV-Vis spectroscopic, AFM and TEM methods. Depending on concentration of the polymeric substrate, Ag^+ and UV – irradiations conditions, the spherical and rod-like AgNPs are formed and stabilized by Na-CMC. The correlation between the size and form of AgNPs immobilized within the Na-CMC films and their biological activity was stablished. It showd that the smaller the AgNPs in size the more enhanced their bactericidal effect. It was also observed that the percentages of wound contractions were higher in seven days and this had been expected, since the fibroplasia phase of healing occurs between 7 and 14 days, causing the presence of oblasts and myofbroblasts. The prepared biodegradable Na-CMC films containing AgNPs are of interest as bactericidal and bacteriostatic coatings for treatment of burns and trophic ulcers.

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REFERENCES

1. Zhubanov B.A., Batyrbekov E.O., Iskhakov R.M. Polymer Materials with Therapeutic Effect. Kompleks, Almaty, 2000, 220 p.

2. Morones J.R., Elechiguerra J.L., Camacho A., Holt K., Kouri J.B., Ramirez J.T., Yacaman M.J. The bactericidal effect of silver nanoparticles. Nanotechnology, 2005, no. 16, pp. 2346-2353.

3. Shcherbakov A.B. and other. Preparations of silver in the past, present and future. Pharmaceutical journal, 2006, no. 5, pp. 45-57.

4. Soni I., Salopek-Bondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J. Colloid Interface Sci., 2004, no. 275 (1), pp.177-182.

5. Xu Y., Li S., Yue X., Lu W. Review of Silver Nanoparticles (AgNPs)-Cellulose Antibacterial Composites. BioResources, 2017, no. 13 (1), pp. 2150-2170.

6. Hebeish A., Hashem M., El-Hady M.M.A., Sharaf S. Development of CMC hydrogels loaded with silver nanoparticles for medical applications. Carbohydr Polym., 2013, no. 92 (1), pp. 407-413.

7. Alshehri S.M., Aldalbahi A., Al-hajji Abdullah Baker, Chaudhary A.A., Panhuis M., Alhokbany N., Ahamad T. Development of carboxymethyl cellulose-based hydrogel and nanosilver composite as antimicrobial agents for UTI pathogens. Carbohydr Polym., 2016, no. 138, pp. 229-236.

8. Krutagn P., Bhavesh B., Tulsi M., Tejal S., Atindra Sh., Suhagia B.N. Role of stabilizing agents in the formation of stable silver nanoparticles in aqueous solution: Characterization and stability study. Journal of Dispersion Science and Technology, 2017, no. 38 (5), pp. 626-631.

9. Pedroza-Toscano M.A., López-Cuenca S., Rabelero-Velasco M., Moreno-Medrano E.D., Mendizabal-Ruiz A.P., Salazar-Peña R. Silver Nanoparticles Obtained by Semicontinuous Chemical Reduction Using Carboxymethyl Cellulose as a Stabilizing Agent and Its Antibacterial Capacity. Journal of Nanomaterials, 2017, pp. 1-7.

10. Basuny M., Ali I.O., El-Gawad A.A., Bakr M.F., Salama T.M. A fast green synthesis of Ag nanoparticles in carboxymethylcellulose through UV irradiation technique for antibacterial applications. Journal of Sol-Gel Science and Technology, 2015, no. 75 (3), pp. 530-540.

11. Raho R., Nguyen N.Y., Zhang N., Jiang W., Sannino A., Liu H., Paladini F. Photo-assisted green synthesis of silver doped silk fibroin carboxymethyl cellulose nanocomposite hydrogels for biomedical applications. Materials Science and Engineering, 2020, no. 107, pp. 110219-110232.

12. Martínez-Rodríguez M.A., Madla-Cruz E., Urrutia-Baca V.H., De la Garza-Ramos M.A., González-González V.A., Garza-Navarro M.A. Influence of Polysaccharides' Molecular Structure on the Antibacterial Activity and Cytotoxicity of Green Synthesized Composites Based on Silver Nanoparticles and Carboxymethyl-Cellulose. Nanomaterials, 2020, no. 10 (6), pp. 1164-1182.

13. Sarymsakov A.A., Kholturaev B., Yunusov Kh.E, Oksengendler B.L., Nurgaliev I.N., Letfullin R.R. The Role of Electron Structure of Polymers at Their Biodegradation in Living Organism. Drug Des Dev Deliv, 2020, no. 1, pp. 110-118.

14. Yuldoshov Sh.A., Sarymsakov A.A., Goyipov I.Sh. Purified Carboxymethylcellulose for the Pharmaceutical Industry. International J. of Advanced Science and Technology, 2020, no. 29 (9s), pp. 4733-4737.

15. Mirzoeva A.E. Methods for General Bacteriology. Moscow, 2002, 62 p.

16. Shahverdi A.R., Fakhimi A., Shahverdi H.R., Minaian S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. Nanomedicine, 2007, no. 3 (2), pp. 168-171.

17. Carlos G.L., Ralph H.C., Peter G., Joao T.C. Viscosity and scaling of semiflexible polyelectrolyte Na-CMC in aqueous salt solutions. Macromolecules, 2016, no. 50 (1), pp. 332-338.

18. Garza-Navarro M.A., Aguirre-Rosales J.A., Llanas-Vázquez E.E., Moreno-Cortez I.E., Torres-Castro A., González-González V. Totally Ecofriendly Synthesis of Silver Nanoparticles from Aqueous Dissolutions of Polysaccharides. International Journal of Polymer Science, 2013, pp. 1-8.

19. Bardania H., Mahmoudi R., Bagheri H. et al. Facile preparation of a novel biogenic silver-loaded Nanofilm with intrinsic anti-bacterial and oxidant scavenging activities for wound healing. Sci Rep., 2020, no. 10, pp. 6129-6143.

20. Lilia C.C., Flávia R.O., Silva L.G. A simple method to synthesize silver nanoparticles by photo-reduction. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2007, no. 305 (1), pp. 54-57.

21. Loginov A.V., Gorbunova V.V., Boysova T.B. Methods for preparation of metal colloids. Journal general chemistry, 1997, no. 67 (2), pp. 189-200.

22. Yunusov K.E., Sarymsakov A.A., Atakhanov A.A., Ashurov N.Sh., Rashidova S.Sh. Physicochemical studies of cotton cellulose and its derivatives containing silver nanoparticles. Chemistry of Natural Compounds, 2011, no. 47, pp. 370-373.

23. Mott N.F., Gurney R.W. Electronic processes in ionic crystals. New York, Oxford University Press, 1940, 172 p.

24. Mittelman A.M., Fortner J.D., Pennell K.D. Effects of ultraviolet light on silver nanoparticle mobility and dissolution. Environmental Science: Nano, 2015, no. 2 (6), pp. 683-691.

25. Khagvajava L., Yaşab I., Çelikc E., Koizhaiganovaa M., Saria Ö. Antimicrobial activity of colloidal silver nanoparticles prepared by sol-gel method. Digest Journal of Nanomaterials and Biostructures, 2011, no. 6 (1), pp. 149-154.

26. Yunusov Kh.E., Rashidova S.Sh., Sarymsakov A.A. Problems and prospects application of silver nanoparticles in medical practice. Nanoscience and nanotechnology an Indian journal (NSNTAIJ), 2016, no. 10 (2), pp. 83-97.

27. Kreibig U., Vollmer M. Optical Properties of Metal Clusters. Springer, Berlin, 1995, 532 p.

28. Yunusov Kh.E., Mullajonova S.V., Sarymsakov A.A., Jalilov J.Z., Turakulov F.M. et al. Antibacterial effect of cotton fabric treated with silver nanoparticles of different sizes and shapes. Nanomater Nanotechnol Nanomed, 2019, no. 5 (2), pp. 016-023.

29. Sang H.L., Bong-Hyun J. Silver Nanoparticles: Synthesis and Application for Nanomedicine. Mol Sci., 2019, no. 20 (4), p. 865.

30. Yunusov K.E., Sarymsakov A.A., Rashidova S.Sh. Structure and properties of biodegradable carboxymethyl cellulose films containing silver nanoparticles. Polymer Science: Series A, 2014, no. 56, pp. 276-281.

31. Stepanov A.L. Optical properties metallic nanoparticle, synthesized in polymer by method ion implantation (review). Journal technical physics, 2004, no. 74 (2), pp. 1-12.

32. Lopatina LI, Sergeev VG. Influence of molecular weight and structure polyacrilic acids on formation «dark blue silver». Vestn. Mosk. Univ. – Bulletin of the Moscow University, Ser. 2: Khim., 2010, no. 51, pp. 398-401.

33. Sergeev B.M., Kiryuhin M.V., Prusov A.N., Sergeyev V.G. Preparation of silver nanoparticles in aqueous solution of polyacrylic acid. Bulletin of Moscow State University, series of Chemistry- I, 1999, no. 40 (2), pp. 129-133.

34. Zhao Y., Wang Y., Ran F., Cui Y., Liu C., Zhao Q., Wang S. A comparison between sphere and rod nanoparticles regarding their in vivo biological behavior and pharmacokinetics. Scientific Reports, 2017, no. 7, p. 11.

35. Kiryukhin M.V., Sergeev B.M., Prusov A.N., Sergeyev V.G. Photochemical reduction of silver cations in a polyelectrolyte matrix. Polym. Sci., Ser. B, 2000, no. 6 (42), pp. 1069-1073.

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