

## Bactericidal Effect of Cotton Fabric Treated with Polymer Solution Containing Silver Nanoparticles of Different Sizes and Shapes

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Received: 14 June 2019;

Accepted: 18 January 2020;

Published online: 30 May 2020;

AJC-19879

Stable silver nanoparticles in solutions of sodium-carboxymethylcellulose (Na-CMC) were synthesized and their structure and physico-chemical properties were evaluated. The form and sizes of silver nanoparticles formed in solutions of CMC and cotton fabrics were studied using UV-VIS spectroscopy, atomic force microscopy and transmission electron microscopy methods. It was found that silver nitrate concentration increase in sodium carboxymethylcellulose solutions, as well as photoirradiation of the hydrogel lead to the changes of the silver nanoparticles size and shape. Investigations have also shown that spherical silver nanoparticles with sizes of 5-35 nm and content of 0.0086 mass% in cotton fabrics possess high bactericidal activity. Stabilization of silver nanoparticles has preserved bactericidal and bacteriostatic activities during the washing of cotton fabrics and textiles on their base.

**Keywords:** Biomaterials, Silver nanoparticles, Sodium carboxymethylcellulose, Cellulose fibers, Cotton fabrics.

### INTRODUCTION

Huge interests are shown for the elaboration of cellulose fibers, textile based biomaterials and their products having a high bactericidal action against pathogenic growth and microorganisms [1]. Bactericidal and bacteriostatic action in cellulose based biomaterials and their products may happen by incorporation of silver particles in their structure and following their reduction into nanoparticles, their interaction with the polymer matrix is carried out through the coordination bonds formation [2]. Silver and its compounds have received the most attention as antimicrobial agents for their use in textile materials [3-8]. Silver is a leaching antimicrobial mediator [9,10], whose effectiveness depends upon the concentration of silver cations ( $\text{Ag}^+$ ) or nanoparticles discharged from the studied material fibers in which they exist. After being released into the studied environment, these species act as a toxin to a wide range of microorganisms, for example, Gram-negative and Gram-positive bacteria, growths, molds, infections, yeasts and algae. Notwithstanding its antimicrobial properties, silver is expected to offer the extra advantage of scope of not constituting a significant hazard to humans, particularly in low concentrations [11-13]. At present, it is resolved that the silver and its

compounds can suppress the development of and execute more than 650 kinds of bacteria, viruses and fungi, therefore continuing awareness of the microelement, which is an essential part of the fabric of all living organisms [14].

Silver nanoparticles have an incredibly enormous large surface area, which increases when in contact with bacteria, viruses and fungi. This fundamentally builds their bactericidal movement by diminishing the sizes of silver nanoparticles and by expanding their surface area to volume proportion [15]. As indicated in the literature, the mechanism of the antimicrobial activity of  $\text{Ag}^+$  and AgNPs are fundamentally the same as one another. Both  $\text{Ag}^+$  and AgNPs can take intermolecular interaction with the cell membrane of microbes. Besides, silver particles smaller than 10 nm have been reported to penetrate into the interior walls of microorganism cells, where they bind to the thiol groups of enzymes and nucleic acids [16,17]. In spite of the discoveries that the utilization of  $\text{Ag}^+$  and AgNPs at low concentration is relatively non-toxic to human cells and causes no genuine hazardness to humans [18], problems with respect to the safety of silver towards people and the earth are as yet present. It was studied that people can be exposed to silver particles through various routes, including ingestion, inhalation and assimilation through the skin [11]. The last course

is the most widely recognized in this context since sweat and other discharges facilitate the release of silver particles from fiber material to the skin surface. It has been shown that AgNPs smaller than 30 nm can be retained through the skin [19].

From the literature, it is obvious that silver altered clinical materials do not lead to cytotoxicity [20], irritation of the skin [21] and argyria [22]. Moreover, they have no unfavorable impacts on the biological balance of human skin microflora [23]. It was also found that the utilization of wound dressings with silver even accelerates the wound healing [24] and prevents the postoperative diseases [25]. The aim of this investigation is to elaborate on the presence of silver nanoparticles in bactericidal, antifungal fibers, textile biomaterials and its products, as well as to study their structural, physico-chemical and bactericidal properties.

## EXPERIMENTAL

Cellulosic fibers, cotton fabrics and purified sodium carboxymethylcellulose (Na-CMC) with degrees of substitution (DS) = 0.65-0.85 and degrees of polymerization (DP) = 200-400 were used as polymer matrices. An aqueous solution of silver nitrate was used to form silver nanoparticles in the solutions of Na-CMC. Strain pathogen microorganisms of human and animal diseases, *Staphylococcus epidermidis* and *Candida albicans* were used. For the formation of stabilized silver nanoparticles, 0.1-0.4 wt.% aqueous solution of purified samples of Na-CMC with various DS and DP were used.

**General procedure:** Calculated quantities of 0.1-0.001 mol/l aqueous solutions of AgNO<sub>3</sub> were added dropwise to Na-CMC solution until a homogeneous solution of Ag<sup>+</sup>CMC<sup>-</sup> was obtained. Photochemical reduction of silver ions was performed in the structure of Ag<sup>+</sup>CMC<sup>-</sup> at 25 °C through irradiation by a mercury lamp (DRSh-250) under high pressure until nanoparticles were obtained. To obtain dispersions of silver nanoparticles, the dispergator with the model number UZDN-1, Y-4.2 was used.

By means of work up treating cellulosic fibers, cotton fabrics and textile products with solutions contained the stabilized silver nanoparticles and after their subsequent heat treatment obtained crosslinked water insoluble compositions of cellulosic fibers and cotton fabrics with antibacterial and antifungal properties.

**Detection method:** The size and shape of silver nanoparticles in the Na-CMC solution was controlled by spectrophotometric method in UV spectroscopy (model Specord M210) in the wavelength range from 200 to 900 nm. The size and shape of the silver nanoparticles in the cotton fabrics were determined through electron microscopy, using a transmission electron microscope (type TEM-100, Ukraine) and atomic-force microscope (AFM-5500, Austria). The variation coefficient was determined by treating the corresponding micrographs with the Math Cad program.

The content of silver nanoparticles in cotton fabric was determined through atomic absorption spectroscopy (model Perkin-Elmer 3030 B, USA) with a flame analyzer (acetylene-air). Bactericidal activity of cotton fabrics containing silver ions and nanoparticles was investigated using the microbiological method [26] at the pathogenic bacterium *Staphylococcus epidermidis* and fungus *Candida albicans*.

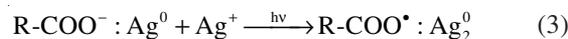
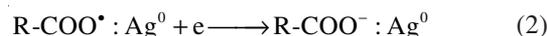
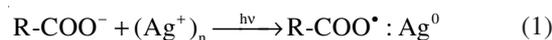
## RESULTS AND DISCUSSION

At the first stage of investigation, the formation of silver nanoparticles in the 0.2-0.4 wt.% concentrate solutions of Na-CMC with different DS = 0.85 and DP = 400 was carried out by photochemical reduction of silver ions [27]. It has been established that addition of silver ions in Na-CMC solutions causes an increase in the viscosity of the system due to the coordination bonding between molecules with the formation of Ag+CMC<sup>-</sup> polycomplexes [28].

Formation of nanoparticles from silver ions in the structure of dilute solutions of Na-CMC with different DS = 0.65-0.85 and DP = 200-600 were carried out by photochemical reduction of silver ions [29,30]. It has been established that added silver ions in Na-CMC solutions increase the viscosity of system due to a decrease in the solubility of Ag<sup>+</sup>CMC<sup>-</sup> and appearance of coordination bonds between molecules with formation of polycomplexes. Increasing the relative viscosity of Na-CMC contained silver cations dependence on decreasing the solubility due to formation of intermolecular coordination bonds between the silver ions and carboxylate anions in macromolecules of Na-CMC [31,32].

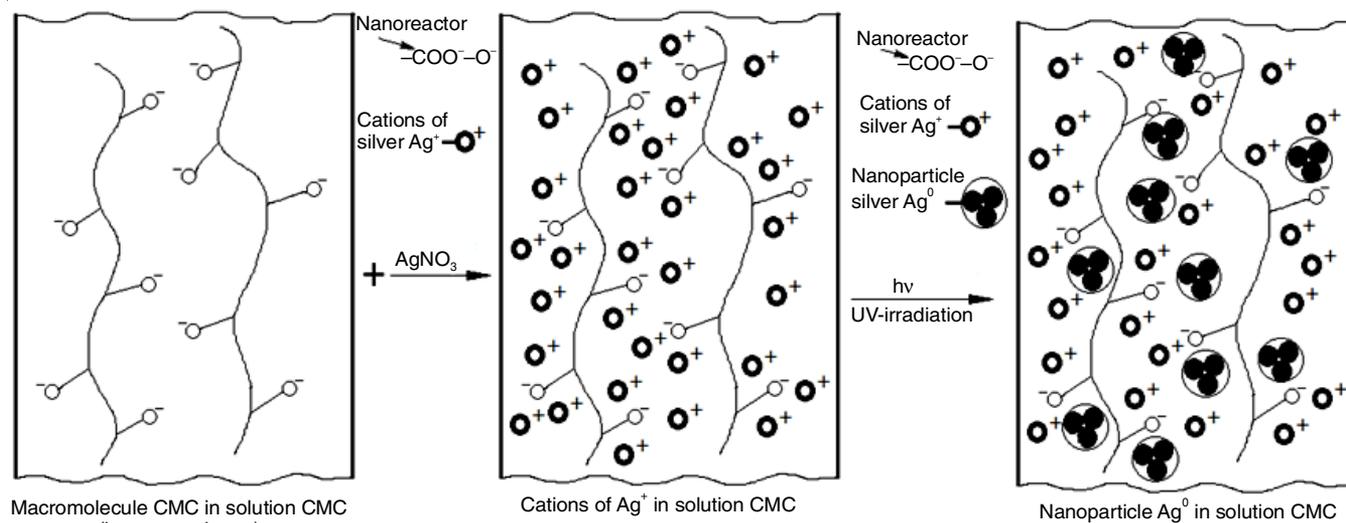
It is well known that colloidal silver particles formed at the base of the photographic process can be explained through the Mott-Gurney theory [29,33]. During photochemical reduction, the optically generated electron migrates and catches with the electron catcher at the bedding interface and near the surface. The negative charge attracts one of interstitial Ag<sup>+</sup> ions presented in thermodynamic equilibrium, which move to the trapped electron. This is the first step in the sequence of the electrons and interstitial atoms trapping and formation of silver clusters and nanoparticles as a basis of latent image.

Analyzing spectroscopic data of nanocomposites based on silver and Na-CMC suggested that the negative ions of carboxymethyl group is "catcher" for the positive charged of silver ions. The reaction mechanism sequence of Mott-Gurney are as follows:



Thus, the photostimulated formation of silver nanoparticles in the Ag<sup>+</sup>CMC<sup>-</sup> hydrogel can be considered as electron-stimulated nuclear process that may be based on Mott-Gurney theory [29] as the photography process. Moreover following the photochemical reduction, an electron and a proton are released in the process of regeneration of carboxylic anions of sodium-carboxymethylcellulose [34]. During the photochemical reduction, the optically generated electron migrates and cached in electron catcher at the interfaces and near the surface (**Scheme I**).

Silver cations attracts carboxymethyl anion of CMC to form an ionic bonds. Reduction reaction begins with connected silver cations at nanoreactors with the formation of clusters, which turn into nanoparticles, due to recovery of cations near nanoreactors. This is the first step in the sequence where the



**Scheme-I:** Proposed scheme of formation nanoparticles of silver in structure Na-CMC

electrons and interstitial atoms catching, forming clusters and silver nanoparticles, which make on basis latent image.

Synthesized silver nanoparticles, by photochemical method, in Na-CMC solution provided a high stability and didn't form agglomeration at long times as compared with the formation of nanoparticles reduced from silver ions in aqueous solutions by chemical agents.

After photolysis of Na-CMC solutions containing silver ions formed had enough stable colloidal systems of nanosilver pale-yellow colour having optical spectra at  $\lambda_{\text{max}} = 416$  nm, which characterizes silver nanoparticles with sizes 5-25 nm [35] (Fig. 1, curve -3). It can be seen that no changes were observed in the initial solutions of Na-CMC and  $\text{Ag}^+\text{CMC}^-$  at the 250-900 nm (Fig. 1, curves 1 and 2).

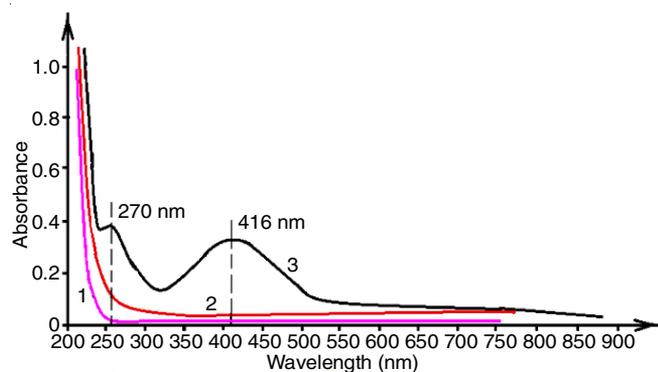


Fig. 1. UV-visible absorption spectra of the samples: 1) Na-CMC, 2)  $\text{Ag}^+\text{CMC}^-$ , 3)  $\text{Ag}^0\text{CMC}^-$ . Time of UV - irradiation for samples was 25 min;  $[\text{Na-CMC}] = 0.008$  mol (2%);  $[\text{AgNO}_3] = 3 \times 10^{-5}$  mol (0.25 wt.%)

With increasing photolysis time, the colour of solutions was changed from pale yellow to brown, which might be probably due to increase in the number and size of formed silver nanoparticles. For confirmation, the absorption were taken at conduction at different irradiation times of systems  $\text{Ag}^+\text{CMC}^-$  at concentration of Na-CMC 0.2% and  $1 \times 10^{-2}$  mol/L silver nitrate.

As seen from Fig. 2, after 5 min of photo-irradiation, a shoulder band at  $\lambda_{\text{max}} = 270$  nm was observed, which could be

due to stable polyanions charged silver clusters, approximately  $\text{Ag}_8^{2+}$  [36,37] (Fig. 2, curve 2). After 15 and 20 min of photolysis, an increase in the absorption intensity band was observed at  $\lambda_{\text{max}} = 270$  nm, which depends on with the formation of large stabilized clusters of silver with sizes 2-8 nm [37] (Fig. 2, curve 3 and 4). Further irradiations at  $\lambda_{\text{max}} = 290$  and 420 nm resulted in the appearance of new absorption peaks, which were due to large clusters and nanoparticles of silver with sizes 5-35 nm [37] (Fig. 2, curve 5). Homogeneity in nanoparticles with different sizes can be obtained since Na-CMC macromolecules shielded the nanoparticles' charged shell around them to prevent the nanoparticles' aggregation. Commonly, when polymer concentration is high, it tends to stabilize the nanoparticles.

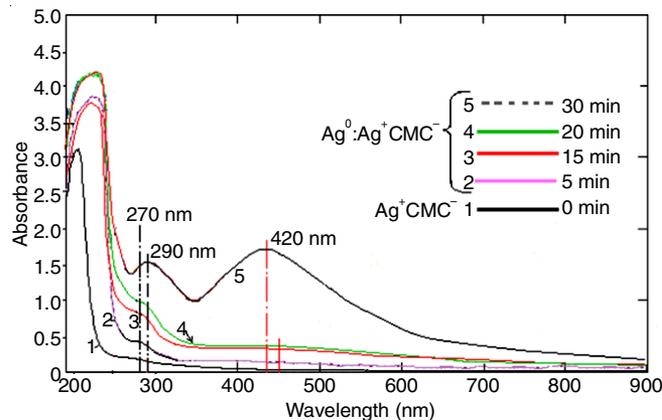


Fig. 2. Absorption spectra of photochemical reduced silver ions samples  $\text{Ag}^0\text{CMC}^-$ . Concentration of  $[\text{Na-CMC}] = 0.2$  %; and  $[\text{AgNO}_3] = 1 \times 10^{-2}$  mol/L. Time of UV-irradiation 0 (1) 5 (2) 15 (3) 20 (4) 30 (5)

Increasing the concentration of Na-CMC on the surfaces of silver nanoparticles created the electrostatic and steric stabilization, and on the other hand, it also creates an interaction of radicals generated by UV-photolysis of Na-CMC. In order to confirm the stability of formed silver nanoparticles in polymer matrix, the UV spectra of Na-CMC solutions containing stabilized silver nanoparticles at different time periods has been investigated. As seen from Fig. 3, the absorption peaks practi-

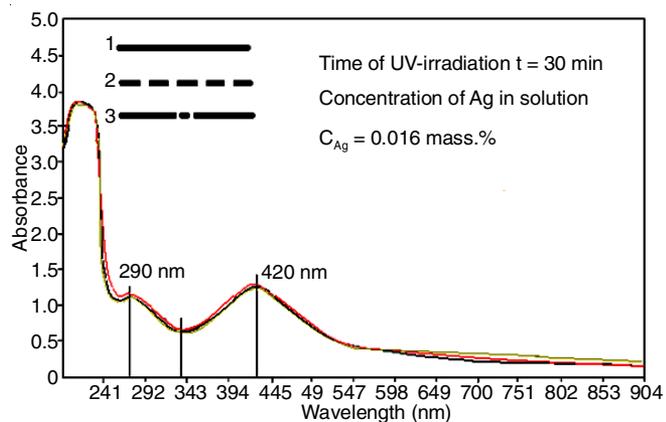


Fig. 3. UV-absorption spectra of solutions of Na-CMC, contained silver nanoparticles for keeping during some times. 1) 1 - month. 2) 2 - months. 3) 6 - months

cally unchanged for the silver nanoparticles in Na-CMC solutions at different time periods. Based on the experimental results, it could be concluded that depending on the molecular weight, degree of substitution and the ratio components of Na-CMC and  $AgNO_3$  able to control the size and shape of silver nanoparticles.

With the aim of determining the form and sizes of silver nanoparticles in the structure of Na-CMC, samples have been investigated through the atomic force microscope. It can be seen from the AFM photographs (Fig. 4) that at a low concentration of silver ions, spherical polydisperse nanoparticles of sizes 2-8 nm were formed (Fig. 4a). With an increase in the concentration of silver ions after photolysis, monodisperse silver nanoparticles with sizes 5-35 nm (Fig. 4b) were obtained. This can be explained by the fact that silver cations in CMC coordinated with carboxylic anions (nanoreactor) were reduced

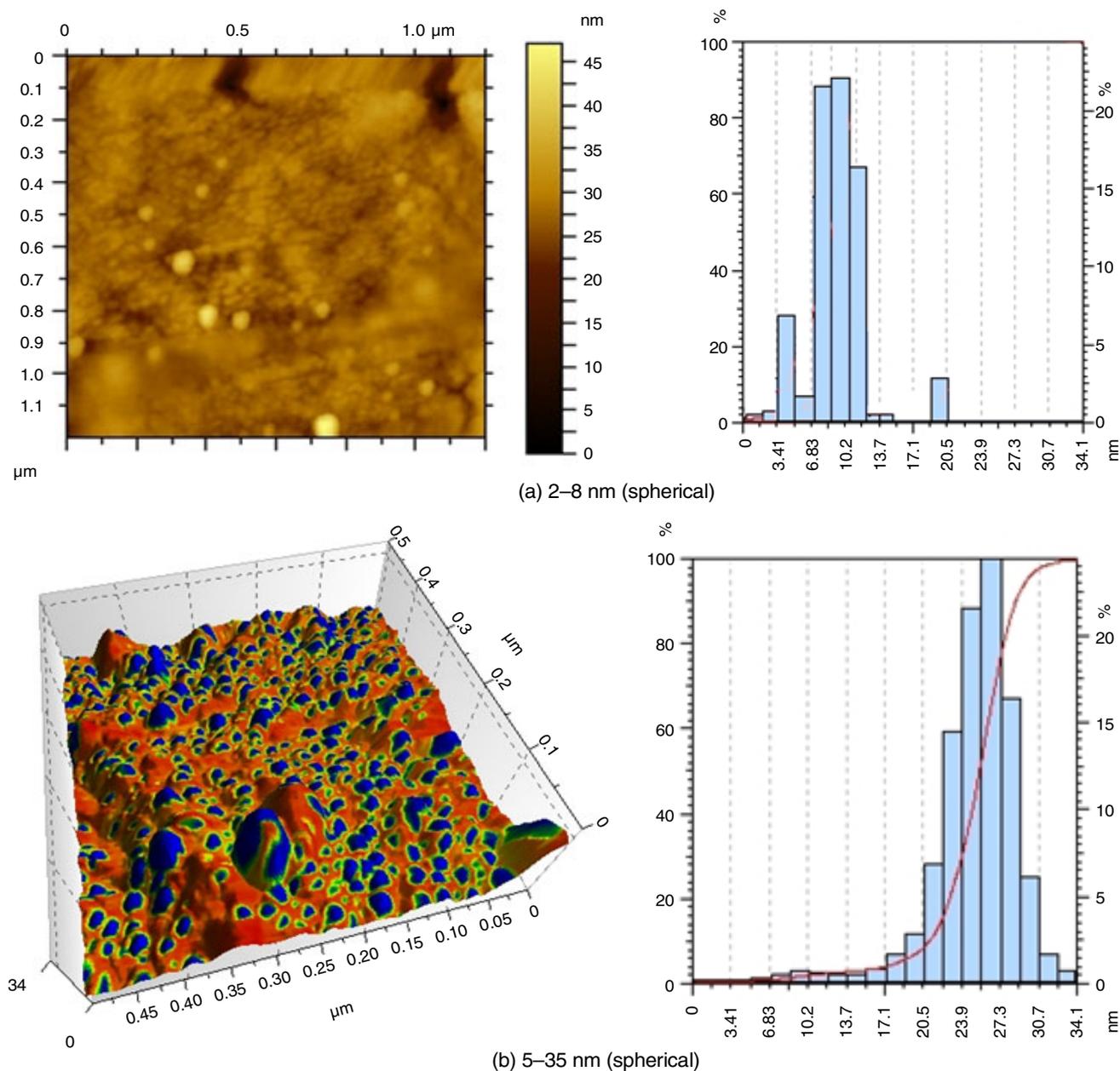


Fig. 4. AFM microphotographs of Na-CMC solutions containing silver nanoparticles (a), and their distribution by size (b). Concentration of  $[Na-CMC] = 0.008$  mol (0.2 wt.%);  $[AgNO_3] = 3 \times 10^{-6}$  (a) and  $3 \times 10^{-5}$  mol (b). Time of UV-irradiation = 30 min

by increasing the photolysis time [34]. These processes occurred with different speed, which probably increases the polydispersity of formed silver nanoparticles.

Thus, the size and shape of silver nanoparticles formed by the photochemical reduction of silver cations in  $\text{Ag}^+\text{CMC}^-$  systems depend on degree of substitution and the concentration of solution of Na-CMC, concentration of  $\text{Ag}^+$  and photochemical irradiation times. It was also found that by keeping the concentration of carboxylate anions constant in Na-CMC solution with increasing content of silver cations resulted in the increase the size of silver nanoparticles and their contents.

To obtain the bactericidal cotton fibers, biomaterials and fabrics were treated with Ag-CMC solutions. The low concentration of Na-CMC solution and the sizes of silver nanoparticles and ions promoted their penetration, *i.e.* inter-fiber and intermolecular free spaces of the materials and fabrics obtained from cellulose [38]. The wet materials and cotton fabrics and products obtained were subjected to additional UV-irradiation, where restoration of unreacted silver ions in Na-CMC matrix structures was carried out [39]. The samples have been investigated through the transmission electron microscope. From Fig. 5b, it can be seen that the sizes of spherical silver nanoparticles were in the range of 2-30 nm at cotton fabrics of 0.0086 mass%.

Fig. 6 shows the supposed formation of silver nanoparticles in the structure of cotton fabrics, complex fibers and elemental cellulose fibers. When cotton fabrics, complex fibers and the elemental fibers of cellulose (Fig. 6) grafted with the mixture solutions of Ag-CMC and  $\text{Ag}^+\text{CMC}^-$ , they squeezed in-between the free spaces of fibers (Fig. 6a) and those of the complexes of fibers (Fig. 6b). It was found that the Na-CMC macromolecules containing silver nanoparticles were able to squeeze in

between free spaces of fibrils elemental of fiber cellulose (Fig. 6b), which indicates that the above-mentioned particles and ions of silver were trapped.

After repeated photoirradiation of wet fibers and the silver ions situated in between the strings, fibers and fibrils, the space in the structure of Na-CMC was reduced to nanostructure, which, after drying, changed into an insoluble state through the formation of intermolecular hydrogen and covalent bonds between the carboxyl and hydroxyl groups of the Na-CMC macromolecules and cellulose. This can be explained by the stability of the silver nanoparticles present in the structure of fibers and fabrics after repeated washing.

Bactericidal activity of the obtained samples of cotton fabrics grafted with  $\text{Ag}^+\text{CMC}^-$  solution in test cultures of *Staphylococcus epidermidis* and *Candida albicans* was studied. To determine the antimicrobial activity of the samples, test-tubes containing thioglycolic environments (for *Staphylococcus epidermidis*) and saburo (for *Candida albicans*) were added to the following systems: (i) control cotton fabrics physiological solution; (ii) cotton fabric +  $\text{Ag}^+$ ,  $C_{\text{Ag}^+} = 0.0086$  mass%; (c) cotton fabric +  $\text{Ag}^0$ ,  $C_{\text{Ag}^0} = 0.0086$  mass%; (d) cotton fabric +  $\text{Ag}^0$ ,  $C_{\text{Ag}^0} = 0.086$  mass%.

As a control in the same medium was added physiological solution of 10% NaCl compared to the volume of medium. Within six hours, test culture was added in the final concentration of 150 CFU/mL in each test-tube. Samples were incubated at 34 °C for 48 h (*Staphylococcus epidermidis*) and 72 h (*Candida albicans*). The result of microbiological investigations showed that all samples have a degree of antimicrobial activity against pathogenic microorganisms of human. Results of investigations samples of fabric are shown in Table-1.

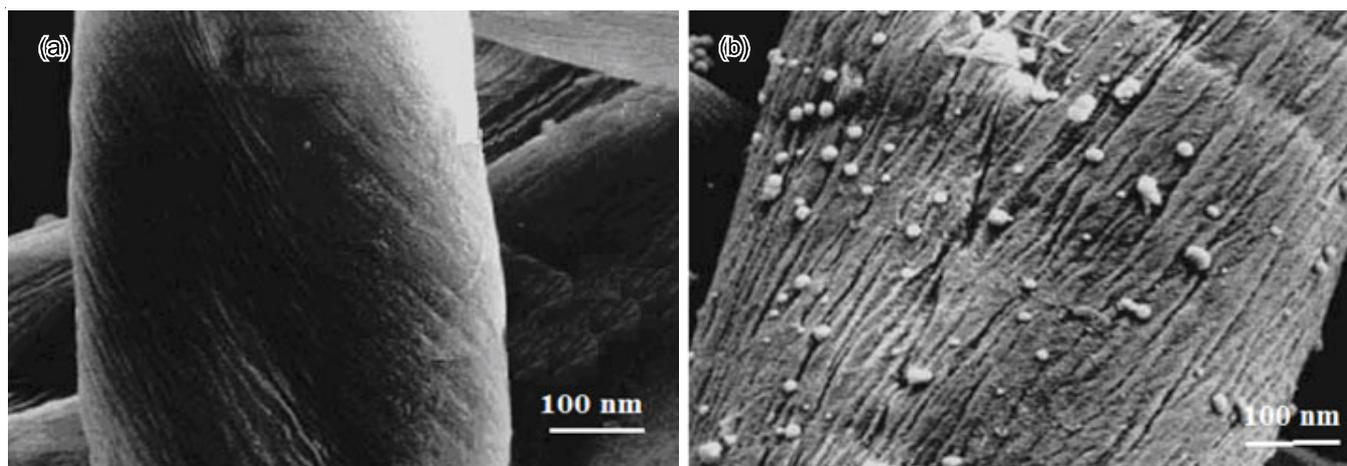
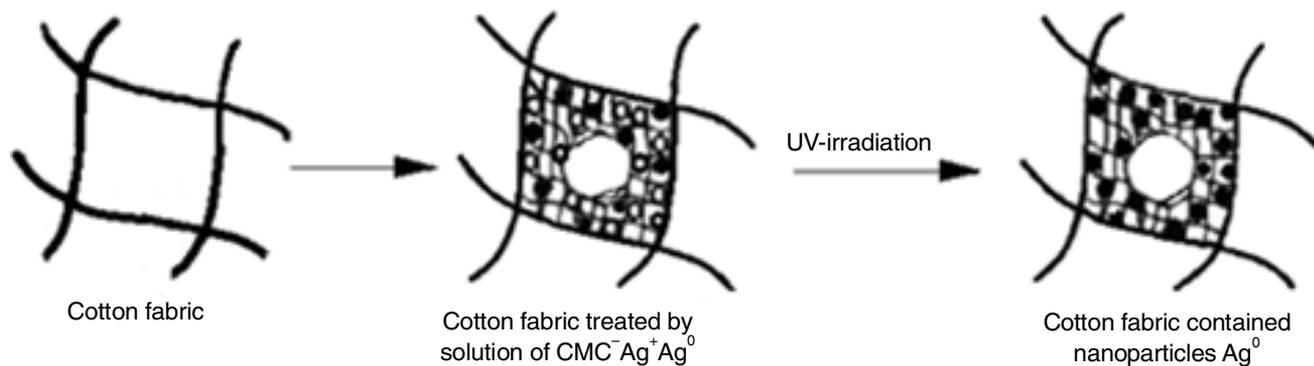


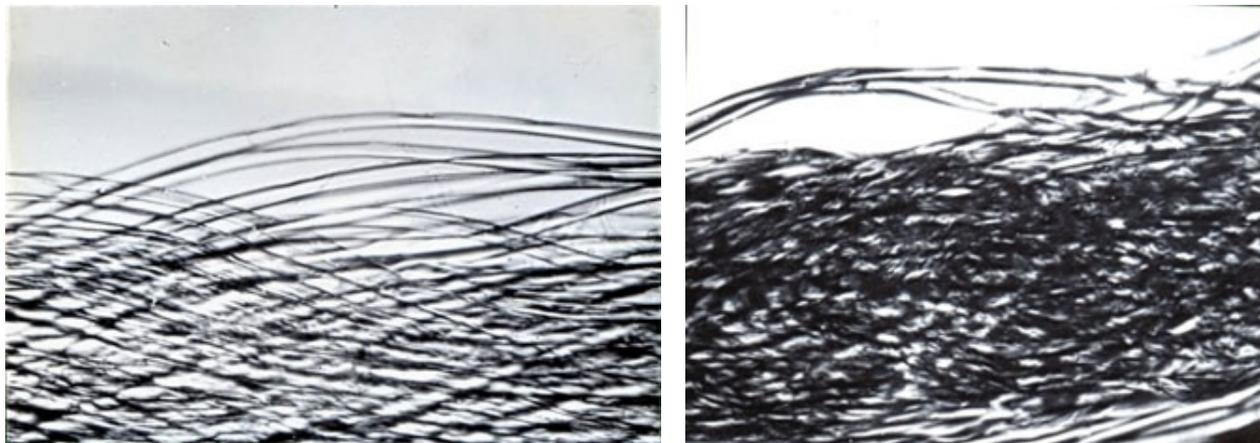
Fig. 5. TEM photographs of initial cotton fiber (a) and silver nanoparticles on the surface of cotton fiber (b)

TABLE-1  
ANTIMICROBIAL ACTIVITY OF COTTON FABRICS, CONTAINED SILVER IONS AND NANOPARTICLES AGAINST STRAINS OF *Staphylococcus epidermidis* AND *Candida albicans*

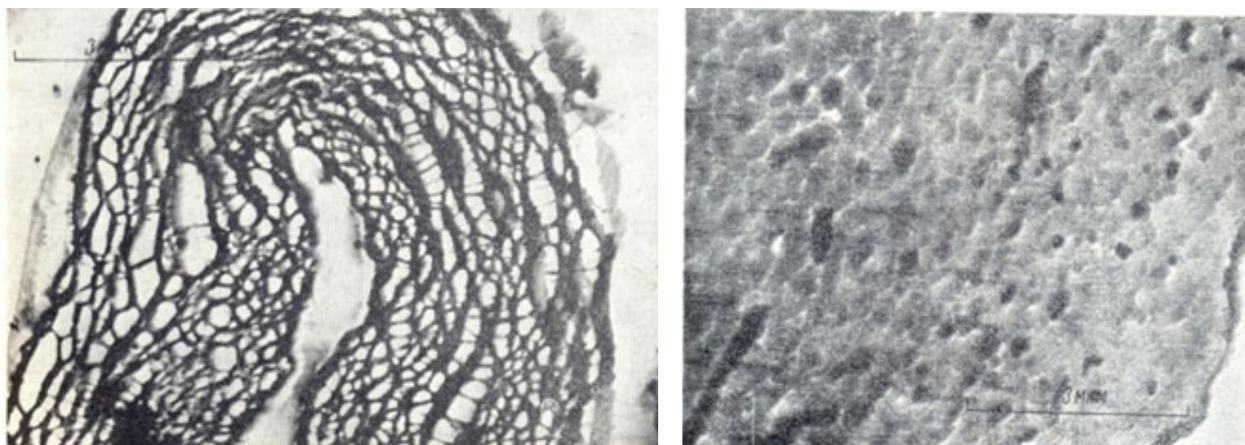
S. No.	Samples	Content of silver nanoparticles in the cotton fabric, mass%	Strains	
			<i>Staphylococcus epidermidis</i>	<i>Candida albicans</i>
1	Control	–	150 CFU/mL	150 CFU/mL
2	Cotton fabric + $\text{Ag}^+$	0.0086	24 CFU/mL	15 CFU/mL
3	Cotton fabric + $\text{Ag}^0$	0.0086	6 CFU/mL	Absent
4	Cotton fabric + $\text{Ag}^0$	0.0860	9 CFU/mL	Absent



(a) Cotton fibers



(b) Complex fibers



(c) Elemental of cellulose fiber

Fig. 6. Supposed scheme of formation and fixing of silver nanoparticles in the structure of (a) cotton fibers, (b) complex fibers, and (c) elemental of cellulose fibers

As observed, all the samples except the control and the sample number 2 (cotton fabric +  $\text{Ag}^+$ ,  $C_{\text{Ag}^+} = 0.0086$  mass %); completely inhibited the growth of *Candida albicans*. Low activity of cotton fabric can be explained as the contact of silver ions bounded to the surface of these strains to make bondings with functional groups. This results in the fast inactivation of the silver ions [40,41]. Samples 2, 3 and 4 inhibited the growth of *S. epidermidis* by 72%, 94.6% and 88%, respectively.

The cotton fabrics grafted with a solution of Na-CMC, containing silver nanoparticles with sizes 2.8 nm exhibited the bactericidal activity against *Staphylococcus epidermidis*

more than 80%, while *Candida albicans* exhibited bactericidal activity to 98%. Moreover, the most active against *S. epidermidis* and *C. albicans* was the cotton fabrics grafted with solution of Na-CMC having spherical nanoparticles of silver sizes 5-35 nm. The reason could be explained by the content of silver nanoparticles and their surface area [42,43].

Cotton fabrics grafted with Na-CMC containing silver nanoparticles having sizes in length 130-420 nm and in width 15-40 nm was less bactericidal active compared with cotton fabric grafted with solution containing silver nanoparticles of spherical structures having size 5-35 nm.

TABLE-2  
COMPARATIVE RESULTS OF THE BACTERICIDAL ACTIVITY OF COTTON FABRICS GRAFTED WITH SOLUTIONS OF Na-CMC CONTAINED SILVER NANOPARTICLES WITH DIFFERENT SHAPES AND SIZES

S. No.	Samples cotton fabric treated with a solution of Ag <sup>0</sup> CMC at different concentrations of silver ions	Size of the silver nanoparticles in the solutions Na-CMC, nm	Strains	
			<i>Staphylococcus epidermidis</i>	<i>Candida albicans</i>
1	Control	–	5 × 10 <sup>12</sup> CFU/mL	1 × 10 <sup>7</sup> CFU/mL
2	C <sub>Ag</sub> = 0.000003 mol	2-8 nm	1 × 10 <sup>12</sup> CFU/mL	1 × 10 <sup>5</sup> CFU/mL
3	C <sub>Ag</sub> = 0.00003 mol	5-35 nm (spherical)	Absent	Absent
4	C <sub>Ag</sub> = 0.0003 mol	L1 = 130-420 nm, L2 = 15-40 nm (whiskers)	1 × 10 <sup>10</sup> CFU/mL	Absent

TABLE-3  
COMPARATIVE RESULTS OF THE BACTERICIDAL ACTIVITY OF COTTON FABRIC, AFTER WASHING SEVERAL TIMES

S. No.	Samples	Number of washings	Content of silver nanoparticles in cotton fabric, mass%	Strains	
				<i>Staphylococcus epidermidis</i>	<i>Candida albicans</i>
1	Control	–	–	5 × 10 <sup>12</sup> CFU/mL	1 × 10 <sup>7</sup> CFU/mL
2	Cotton fabric + Ag <sup>0</sup>	Without washing	0.0086	Absent	Absent
3	Cotton fabric + Ag <sup>0</sup>	1 time	0.0079	Absent	Absent
4	Cotton fabric + Ag <sup>0</sup>	2 times	0.0068	2 × 10 <sup>8</sup> CFU/mL	2 × 10 <sup>8</sup> CFU/mL
5	Cotton fabric + Ag <sup>0</sup>	3 times	0.0054	2 × 10 <sup>8</sup> CFU/mL	Absent
6	Cotton fabric + Ag <sup>0</sup>	4 times	0.0033	3 × 10 <sup>6</sup> CFU/mL	Absent
7	Cotton fabric + Ag <sup>0</sup>	5 times	0.0023	5 × 10 <sup>7</sup> CFU/mL	1 × 10 <sup>6</sup> CFU/mL

The comparatively high bactericidal activity of silver nanoparticles (Table-2) with the silver ions (Table-1) probably due to the fact that (a) silver nanoparticles cannot make chemical bonding with the functional groups [43] and at the surface studied strains and probably seems able to penetrate through wall of cell and into the cells nucleus and inhibited their growth and activity; and (b) lowering the size of silver nanoparticles incre-ased the total area of the surface and accelerating their ability to penetrate the cell nucleus of the studied strains.

The bactericidal activity of cotton fabrics after repeated five washings is presented in Table-3.

## Conclusion

In this work, optimal conditions of silver nanoparticles with different shapes and sizes formation in the structure of CMC solutions of different degrees of substitution (DS) and degrees of polymerization (DP) were determined by the photo-irradiation of solution. It was established that the replaced silver ions in Na-CMC macromolecules mainly subject to restoration and play the role of "nanoreactors" in which carboxylate ions trapped the positively charged silver ions and promote the photo-stimulated formation of silver nanoparticles. UV-spectroscopic method for control of the form and sizes of silver nanoparticles at process of their restoration was also investigated. It was established that depending on concentration of polymeric substrate, silver ions and UV irradiation, spherical and rod-like stabilized silver nanoparticles of different sizes in the structure of Na-CMC. Moreover, it was found that grafted cotton fabrics and products, which were stabilized through the contribution of silver nanoparticles, possessed bactericidal and bacteriostatic properties. The obtained bactericidal biomaterials based on the cellulose containing stabilized silver nanoparticles, possessed the good antifungal effect, prevented offensive odour, decreased the level of pathogenic germs and preserved the fungal disease.

## ACKNOWLEDGEMENTS

This work was supported by applied project of A-FA-2019-34 "Development of a new generation of nanopolymers for the treatment of various types of burns" for 2019-2021 years of Ministry of Innovative development of the Republic of Uzbekistan.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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