



SYNTHESIS OF SILVER NANOPARTICLES IN A BIOPOLYMER MATRIX BASED ON DIALDEHYDE CARBOXYMETHYLCELLULOSE/SERICIN

Sherzod A. Yuldoshov, Khaydar E. Yunusov Kh.E., Imomjon I. Sharibov, Ilkhom Sh. Goyibnazarov, Jamila S. Norboyeva, Abdushkur A. Sarymsakov

Tashkent Scientific Research Institute of Chemistry and Technology, Tashkent, Uzbekistan

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Corresponding author

sherzodbek_y@mail.ru

ABSTRACT

In this study, the formation of silver nanoparticles (AgNPs) within a graft copolymer matrix based on dialdehyde carboxymethyl cellulose (DCMC) and silk sericin was investigated. The formation of Schiff bases between the aldehyde groups of DCMC and the amino groups of sericin resulted in the development of a stable biopolymer network, which enabled the simultaneous reduction and stabilization of nanoparticles. The mechanism of the photochemical reduction of silver ions into their metallic form and their interaction with the polymer matrix was comprehensively analyzed.

The structure and morphology of the obtained nanocomposites were characterized using Fourier-transform infrared (FTIR) and UV-Vis spectroscopy methods. According to the results, stable silver nanoparticles with spherical and needle-like morphologies, ranging in size from 20 to 80 nm, were uniformly distributed within the polymer matrix. The findings demonstrate that DCMC/sericin-based biopolymer systems possess significant potential for application as functional nanocomposites, particularly in the development of antimicrobial materials.

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Introduction

In recent years, there has been a sharp increase in the demand for sustainable, biodegradable, and bio-based materials, significantly enhancing the interest in developing functional materials based on natural polymers, particularly polysaccharides and proteins [1-4]. Cellulose and its derivatives, including sodium carboxymethyl cellulose (Na-CMC), are widely used in various scientific and practical fields due to their high hydrophilicity, biocompatibility, and susceptibility to chemical modification [5-8]. Furthermore, functionalizing cellulose derivatives to enhance their reactivity and create new composite systems is becoming increasingly viable [9-11].

Oxidizing Na-CMC to obtain DCMC introduces highly reactive aldehyde groups into the cellulose chain [12-14]. This makes DCMC an efficient matrix for forming chemical bonds with proteins and biopolymers containing amino groups via Schiff bases [15-17]. In particular, hydrogels and composites based on DCMC have shown high mechanical stability, biocompatibility, and functional properties in numerous studies [18-20].

In recent years, silk sericin has gained significant attention as a natural protein. Its biological activity, including antioxidant, antimicrobial, and regenerative properties, opens up opportunities for its use in medicine and biomaterials [21-24]. However, its low mechanical stability, high

hydrophilicity, and polydispersity limit its use as a standalone material [25-27]. Therefore, chemically grafting sericin onto polysaccharides to create stable and functional biopolymer systems is an important scientific challenge [28-30].

The formation of metal nanoparticles in biopolymer matrices has become one of the rapidly developing areas in nanotechnology and biomedicine in recent years [31-33]. In particular, silver nanoparticles are widely used in medicine, pharmaceuticals, and the food industry due to their high antimicrobial, bactericidal, and fungicidal properties [34-37]. Conventional chemical synthesis methods often require toxic reagents and stabilizers, which pose environmental and biological safety concerns [38-39].

Therefore, "green synthesis" approaches, i.e., the simultaneous reduction and stabilization of metal ions in biopolymer matrices, have gained significant importance [40-42]. The functional groups (-OH, -COO⁻, -NH₂) in polysaccharides and proteins play a crucial role in forming complexes with metal ions, reducing them, and stabilizing the nanoparticles [43-45].

Nevertheless, the formation of silver nanoparticles in dialdehyde carboxymethyl cellulose and sericin-based graft copolymer matrices, as well as the factors affecting their formation mechanism, morphology, and stability, have not been adequately studied. In particular, there is limited data on the effect of the network structure formed through aldehyde-amine interactions on the size and distribution of nanoparticles.

Thus, the aim of this work is to synthesize a graft copolymer based on dialdehyde carboxymethyl cellulose and sericin and to study the process of silver nanoparticle formation in this biopolymer matrix. The mechanism of nanoparticle formation, their morphology, size, and stability were analyzed using spectroscopic and microscopic methods.

Experimental

Materials. In the study, the following materials were used: DCMC obtained from Na-CMC of Asdacell HV brand, produced by "Promxim Impex" LLC (Uzbekistan) with an oxidation degree of 82% and molecular weight of 141 kDa, sericin derived from silk industry fibrous waste, and silver nitrate (AgNO₃) salt (Sigma Aldrich).

2.1. Preparation of Dialdehyde Carboxymethyl Cellulose (DCMC)

1 g of Na-CMC is dissolved in 30 ml of water with the help of a mechanical stirrer. To the resulting solution, 10% NaIO₄ solution is added. The solution's pH is adjusted to 3.5 by adding 1 M HCl dropwise. The oxidation reaction is carried out under the effect of high-intensity (10%) ultraviolet (UV) light at 70 W for 10 minutes, with cooling every 2 minutes. The temperature of the oxidation reaction is maintained below 35⁰C. The obtained mass is precipitated in 300 ml of 94% ethanol. The mass is filtered and washed with 70% ethanol until neutral. The obtained mass is dried in a lyophilizer for 6-8 hours.

2.2. Extraction of Sericin from Natural Silk Fibers

Sericin is extracted from natural silk fibers by thermal hydrolysis. Ground natural silk fibers are soaked in water at a ratio of 1:10 and boiled in an autoclave at 110⁰C for 24 hours. The resulting mass is filtered, and the filtrate is dried in a lyophilizer for 8-10 hours.

2.3. Preparation of DCMC/Sericin Graft Copolymer

A 2% solution of DCMC is prepared and mixed with a 3% solution of sericin in a 1:1 mass ratio. The Schiff base formation reaction is carried out at 60⁰C for 1 hour. The resulting solution is dried in Petri dishes at 37⁰C.

2.4. Formation of Silver Nanoparticles in the DCMC/Sericin Graft Copolymer Matrix

For the formation of silver nanoparticles in the DCMC/sericin graft copolymer matrix, a 0.1 M aqueous solution of pure AgNO₃ is used according to GOST 277-75 for analysis. A 2% aqueous solution of the copolymer is prepared, and 3-10 ml of a 0.1 M AgNO₃ solution (pH = 5.14) is added at 25⁰C. The solution is mechanically stirred at 1400 rpm for 30 minutes and treated in an ultrasonic disperser (UZDN-2, U-4.2, Russia) for 20 minutes. The resulting hydrogel and films based on them were analyzed using FTIR and UV-Vis spectroscopic methods.

2.5. FTIR Spectroscopy

FTIR spectroscopy was carried out using an Inventio-S IR Fourier (Bruker, Germany) device. Spectra were studied in the range of 500-4000 cm^{-1} with a resolution of 0.085 cm^{-1}

2.6. X-ray Spectroscopy

The crystalline structure of the samples was determined using wide-angle X-ray diffraction (XRD) with a "MiniFlex 600" diffractometer (Rigaku, Japan), with monochromated Cu-K α radiation as the source. Scanning was performed at a speed of 2°/min in the range of 5-40°, with a generator power of 22 kV and 12 mA. The samples were tested in powder form. Experimental diffraction peaks were processed using the "SmartLab Studio II" software and the PDF-2 database (2020 Powder diffraction file, ICDD). The crystallite size (t) was calculated using the Scherrer equation:

$$\tau = \frac{K \cdot \lambda}{\beta \cdot \cos\theta}$$

Where K is the shape factor (0.9), λ is the X-ray wavelength (1.5418 Å), β is the full width at half maximum of the diffraction peak in radians, and θ is the diffraction angle. The crystallinity index (CrI) (%) was calculated using the following equation:

$$\text{CrI} = \frac{I_t - I_a}{I_t} \cdot 100$$

2.7. UV-Vis Spectroscopy

The UV-Vis spectra of the samples were recorded using a "Specord M210" spectrophotometer in the range of 200-900 nm.

2.8. SEM Analysis

The surface morphology of the samples was analyzed using a scanning electron microscope (SEM) with 25 kV acceleration voltage.

Results and Discussion

In our previous studies, the synthesis of high-molecular-weight oxidized Na-CMC with an oxidation degree of 82% and a molecular weight of 141 kDa was investigated using microwave-assisted periodate oxidation [10]. In this case, the oxidation of Na-CMC samples in the presence of sodium periodate under microwave-assisted conditions proceeds according to the following equation:

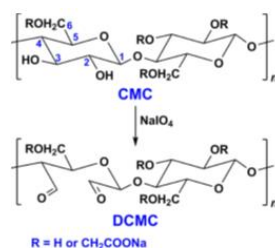


Figure 1. Oxidation reaction of Na-CMC in the presence of sodium periodate

Additionally, the conditions for the efficient extraction of sericin from silk industry fibrous waste using microwave-assisted treatment were determined in our previous study [11]. The process of synthesizing the graft copolymer based on the obtained DCMC and sericin is shown in the following figure [Figure 2].

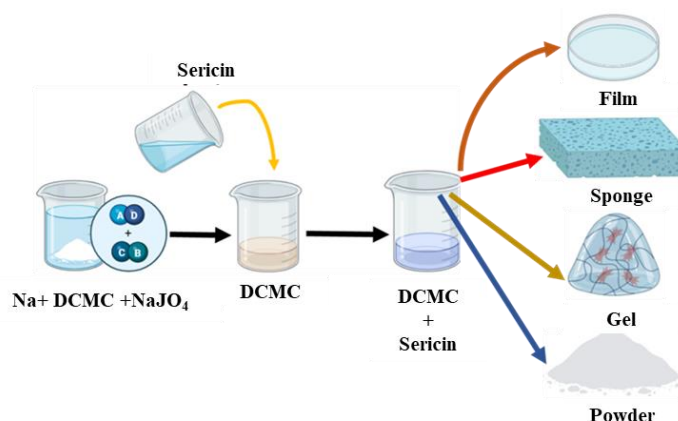


Figure 2. Synthesis of the DCMC/sericin graft copolymer

In this process, the aldehyde groups react with the amino groups in a nucleophilic substitution reaction [10], forming Schiff bases and creating a graft copolymer via imine (carbinolamine) bonding [12]. The new absorption regions in the DCMC-sericin graft copolymer were studied using the FTIR spectroscopy method.

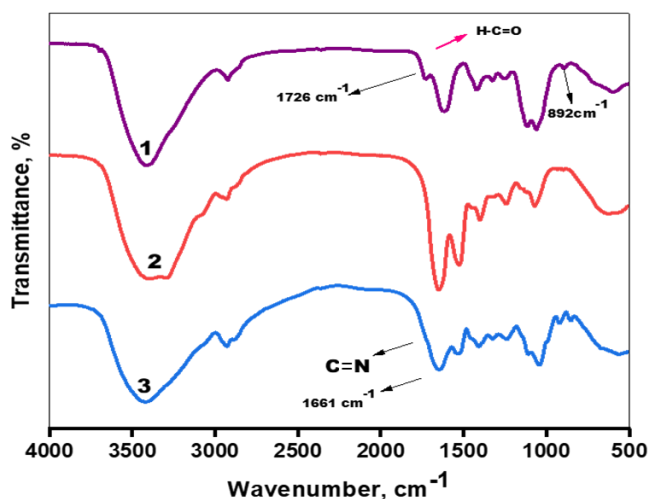


Figure 3. FTIR spectra of DCMC (1), sericin (2), and DCMC/sericin graft copolymer (3)

As shown in Figure 3, when the DCMC/sericin copolymer is formed, the absorption band characteristic of the carbonyl ($-C=O$) group of dialdehyde at 1726 cm^{-1} disappears. It was found that in the DCMC/sericin copolymer, absorption intensities characteristic of sericin's I, II, and III bands were observed at 1620 , 1516 , and 1240 cm^{-1} , respectively [13]. Furthermore, new absorption intensities at 1661 cm^{-1} , which are characteristic of the imine ($C=N$) linkage formed in the DCMC/sericin copolymer, were also observed.

The process of forming silver nanoparticles in the graft copolymer matrix was carried out according to the method described in [14]. The results of the UV-spectroscopic analysis of the silver nanoparticles formed in the graft copolymer matrix are shown in Figure 4.

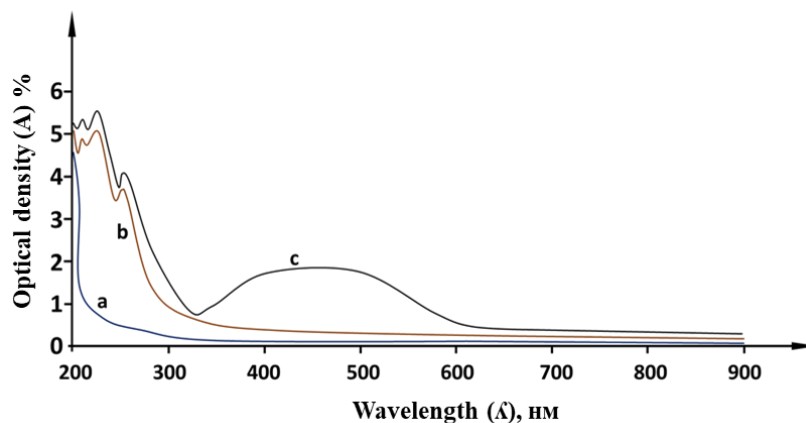


Figure 4. UV spectra of DCMC/sericin graft copolymer samples containing silver nanoparticles a) DCMC/sericin, b) Ag^+ /DCMC/sericin, c) Ag^0 /DCMC/sericin

The UV spectra of the DCMC/sericin graft copolymer and its modified samples containing silver ions and nanoparticles were analyzed in the 200-800 nm range (Figure 4). The obtained spectra revealed three distinct states: (a) the initial copolymer matrix, (b) the system complexed with Ag^+ ions, and (c) the system containing Ag^0 nanoparticles formed by photoreduction.

For the initial DCMC/sericin copolymer, a broad absorption band with low intensity was observed around 260-290 nm. This signal is related to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electronic transitions associated with aromatic amino acids (tyrosine, tryptophan) in the sericin, as well as carbonyl ($\text{C}=\text{O}$) and imine ($\text{C}=\text{N}$) groups [13,28]. This directly confirms the successful formation of Schiff bases in the copolymer network.

In the sample with added silver ions (Ag^+), an increase in absorption intensity and the widening of the spectral line in the 270-300 nm range was observed. This can be explained by the formation of coordination complexes between Ag^+ ions and the functional groups ($-\text{CHO}$, $-\text{COO}^-$, $-\text{NH}_2$) in the polymer matrix. Moreover, the redistribution of electron density results in the enhancement of $n \rightarrow \pi^*$ transitions. At this stage, metal nanoparticles have not yet formed, and ion-polymer interactions dominate in the system.

After photoreduction, the sample spectrum shows a distinct and intense absorption peak in the 400-450 nm range ($\lambda_{\text{max}} \approx 420\text{-}430$ nm). This peak is associated with the surface plasmon resonance (SPR) of silver nanoparticles, indicating the successful reduction of Ag^+ to Ag^0 [16,37]. The relatively broad and asymmetric shape of the SPR peak suggests that the nanoparticles are polydisperse and have different morphologies (spherical and partly needle-like).

Furthermore, the SPR peak located around 420 nm indicates that the nanoparticle size is approximately in the range of 20-80 nm. The shift of the peak toward longer wavelengths and the increase in intensity may be related to clustering in the polymer matrix and interparticle interactions. This confirms that the DCMC/sericin matrix not only acts as a reducing agent but also as an effective stabilizer.

An important aspect is that the hydroxyl, carboxylate, and amine groups in the polymer matrix adsorb on the surface of the silver nanoparticles, limiting their aggregation and ensuring colloidal stability. As a result, the nanocomposite system forms a stable structure that maintains optical stability over time.

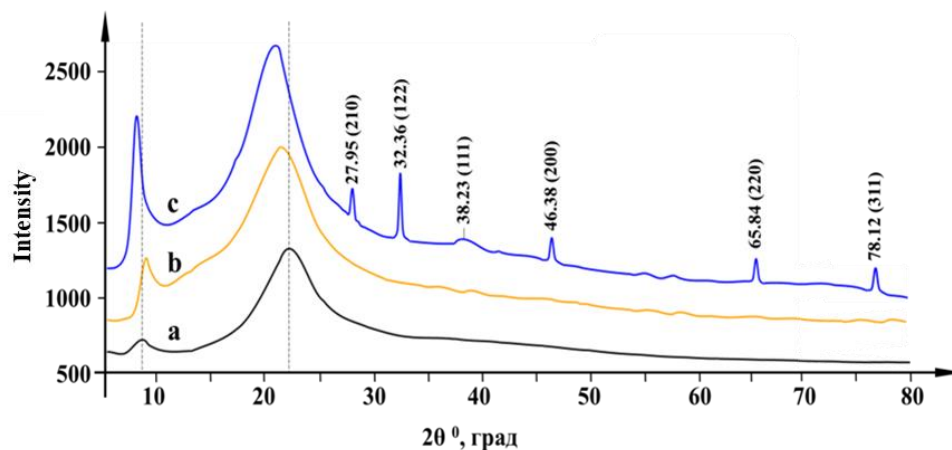


Figure 5. X-ray spectra of DCMC film samples containing Ag nanoparticles a) DCMC/sericin, b) Ag^+ /DCMC/sericin, c) Ag^0 /DCMC/sericin

The crystalline structure of the DCMC/sericin graft copolymer and its modified samples containing silver ions and nanoparticles was studied using wide-angle X-ray diffraction (XRD) method (Figure 5). The diffraction patterns revealed three main states: (a) pure DCMC/sericin copolymer, (b) copolymer modified with Ag^+ ions, and (c) composite with silver nanoparticles (Ag^0).

For the initial DCMC/sericin copolymer (a), a broad and low-intensity amorphous halo was observed around $2\theta \approx 20-22^\circ$. This indicates a low degree of crystallinity of the cellulose derivatives and the transition of polymer chains into an unordered (amorphous) state due to grafting with sericin. The amorphous nature of sericin and the interaction between functional groups (-CHO, -COO⁻) in the DCMC chains lead to the formation of a network structure, disrupting the crystalline domains.

In the sample with added Ag^+ ions (b), the amorphous halo is still present, but changes in its intensity and shape were observed. This is explained by the partial change in the macromolecular ordering due to the coordination bonding of silver ions with the functional groups of the polymer matrix. At this stage, no distinct crystalline reflections are observed, indicating that the Ag^+ ions have not yet transitioned into metallic silver nanoparticles.

After photoreduction, the diffraction pattern of the sample (c) shows new and distinct diffraction peaks. Specifically, the intense peaks observed at $2\theta \approx 38.2^\circ$, 44.9° , and 65.8° correspond to the (111), (200), and (220) crystal planes of silver's face-centered cubic crystal structure, respectively, confirming the successful reduction of Ag^+ ions into metallic Ag^0 nanoparticles.

Additionally, the extra peaks around $2\theta \approx 27.9^\circ$ and 32.3° corresponding to the (210) and (122) planes could be related to polymer-metal interactions or small-sized crystallites. This indicates the formation of a heterogeneous structure in the nanocomposite system.

The relatively broad width of the peaks related to silver nanoparticles suggests that the nanoparticle size is in the nanometer range. Based on the Scherrer equation, approximate calculations show that the crystallite size is in the range of 20-80 nm. The high intensity and sharpness of the peaks indicate that the nanoparticles are well-crystallized and evenly distributed in the polymer matrix.

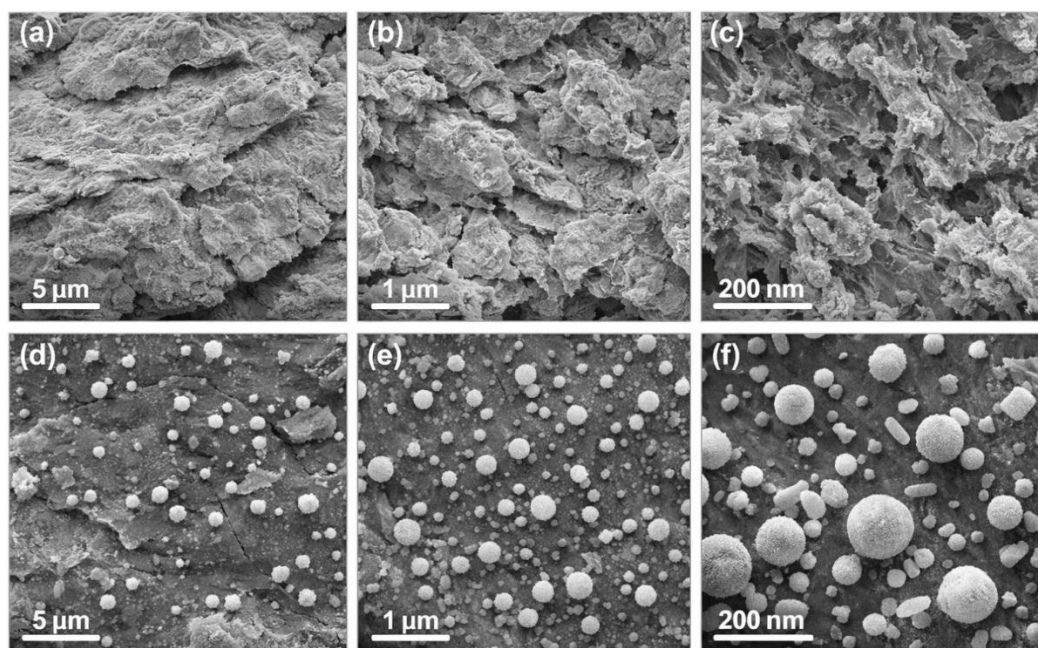


Figure 6. SEM images of DCMC/sericin graft copolymer films containing silver nanoparticles

The morphology of the DCMC/sericin graft copolymer and its modified samples containing silver nanoparticles was analyzed at different magnifications using scanning electron microscopy (SEM) (Figure 6).

For the initial DCMC/sericin copolymer (top row, a-c), the characteristic morphology was observed to be irregular, lamellar, and partially compact amorphous structure. This structure in the polymer matrix is explained by the network system formed between sericin and DCMC chains via Schiff bases. At the same time, surface micro-porosity and lamellar structure are formed as a result of the rearrangement of polymer chains and internal stresses during the drying process.

In the sample with silver nanoparticles (bottom row, d-f), significant changes in the morphology were observed. Specifically, nanoparticles evenly distributed on the polymer matrix surface were clearly visible. These particles predominantly have a spherical shape, although in some cases, elongated (rod-like) or needle-like morphology elements are also observed. This indicates the presence of anisotropic growth mechanisms in different crystal directions during nanoparticle growth.

The particle size is approximately in the range of 20-80 nm, as visually estimated, which correlates well with the XRD and UV-Vis results. While some nanoparticles have formed aggregates, they are generally uniformly distributed throughout the polymer matrix. This demonstrates the high stabilizing efficiency of the DCMC/sericin matrix.

The density and distribution of nanoparticles on the surface are associated with the coordination and electrostatic interactions between silver ions and the functional groups (-OH, -COO⁻, -NH₂, and -CHO) in the polymer matrix. These groups adsorb on the surface of the nanoparticles, limiting their aggregation and ensuring colloidal stability.

The clearer morphology of the nanoparticles in images (e-f) confirms that they are spherical and partially elongated. The presence of needle-like elements may be related to the rapid nucleation and subsequent growth stages in the photochemical reduction process.

Conclusion

This study investigated the possibility of forming silver nanoparticles in a DCMC/sericin graft copolymer matrix. SEM analysis results revealed that the polymer matrix had an amorphous, lamellar, and micro-porous structure, and significant morphological changes occurred after the formation of silver nanoparticles. Specifically, nanoparticles in the range of 20–80 nm, mainly

spherical and partially anisotropic, were observed. Their relatively even distribution within the matrix indicates the high stabilizing ability of the DCMC/sericin system. The physical-chemical analysis (SEM, XRD, UV-Vis) confirmed the crystalline nature and size of the nanoparticles. Furthermore, the presence of anisotropic morphology elements was linked to competitive kinetic mechanisms between nucleation and growth stages in the photochemical reduction process. Based on the conducted research, a stable silver nanoparticle-loaded DCMC/sericin copolymer was synthesized.

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