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PHYSICOCHEMICAL STUDIES OF SYSTEMS AND PROCESSES

Silver Nanoparticles on Fibers and Films of *Bombyx mori* Silk Fibroin

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Abstract—Possibility of obtaining silver nanoparticles on fibers and films of silk fibroin was studied. A comparative assessment of the effect of reducing agents on the morphology and size of silver particles on the fiber surface was made.

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Natural-silk fibers are rather promising for use in medicine. They possess advantages characteristic of most of natural fibers: good sorption properties, air-permeability, and low electrifiability. At the same time, silk is more stable against moist treatment than cellulose fibers [1].

Technological processing of fibers of cultivated silkworm Bombyx mori yields a large amount of waste, which is of great value and can be reused as polymeric films via dissolution of the silk fibroin and its subsequent regeneration. As solvents for silk fibroin serve concentrated salt solutions [1], hexafluoroisopropanol [2], *N*-methylmorpholine *N*-oxide [3], and ionic fluids based on imidazole [4]. Fibroin films are also promising for application in biotechnology and medicine.

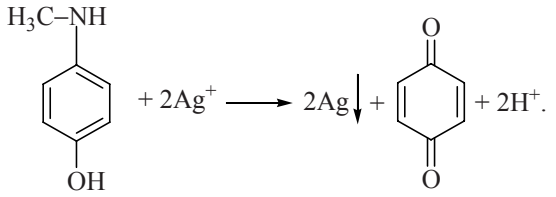
A topical task in this regard is to impart bactericide properties to silk fibroin fibers and films, e.g., by introduction of silver particles. Metal particles can be introduced into polymeric matrices in different ways, one of the best known of which is reduction of metal ions from salt solutions [5–8]. Particles of reduced silver can be formed within the fibrillar structure of a fibroin fiber or porous structure of a fibroin film, with the size of these particles in the sample bulk limited by pore sizes (up to 100 nm) [1, 3]. Larger agglomerates with sizes exceeding the nanometer range can be formed on the surface of fibers and films. Meanwhile, formation of nanosize silver particles would provide the maximum antibacterial effect of silver at its minimum amount on a fiber.

The aim of this study was to examine the possibility of obtaining silver nanoparticles on natural-silk fibers and fibroin films by reduction from a silver salt solution.

EXPERIMENTAL

Fibers of Bombyx mori silk, taken for the study, were washed to remove sericin and grease and wax substances in a 0.02 M Na_2CO_3 solution at 90°C, with the subsequent washing with distilled water. Silver ions were reduced on washed silk fibers under heterogeneous conditions in an aqueous solution of silver nitrate $AgNO_3$. Upon diffusion of Ag^+ ions into the fiber bulk during a certain time, a solution of a reducing agent with a calculated concentration was introduced under continuous agitation. The reducing agent was taken in an excess to quantitatively provide the most complete reduction of Ag^+ ions. As reducing agents were studied sodium borohydride and Metol, which reduce silver ions by the reactions

$$BH_4^- + 8Ag^+ + 8OH^- \rightarrow 8Ag\downarrow + B(OH)_4^- + 4H_2O,$$



After the reduction of silver, the fiber was separated from the solution on a glass frit and several times thoroughly washed, monitoring the content of the reducing agent and silver in the washings. The fiber was dried at room temperature to a constant weight.

To obtain films, silver fibers were dissolved in a 9 M aqueous solution of lithium bromide at 60°C. The resulting solution was subjected to dialysis for 24 h to completely remove the electrolyte. Films with a thickness of 0.04 mm were produced by casting onto fluoroplastic plates with subsequent drying. Silver ions on the films were reduced under heterogeneous conditions in an aqueous solution of silver nitrate $AgNO_3$. Upon diffusion of Ag^+ ions into the film bulk during a certain time, a solution of a reducing agent with a calculated concentration was introduced under continuous agitation. After the reduction, the film was several times thoroughly washed, monitoring the content of the reducing agent and silver in the washings, and dried at room temperature to a constant weight.

The total content of silver in the samples was determined by two methods: after burning and on a SOLAAR M6 Unicam atomic-absorption spectrometer upon dissolution of a 0.2-g portion of a sample.

The size of silver particles and their distribution on the surface of fibers and films was studied using electron micrographs obtained with a Jeol JSM-35 CF scanning electron microscope (SEM) at magnifications of 5000 to 30000. The results of a statistical processing of the electron micrographs were used to construct histograms of the silver particle size distribution on the fiber surface.

The structure and size of silver crystallites in the samples were examined by the wide-angle X-ray scattering method.

The bactericide properties of fibroin fibers containing reduced silver were tested on *Staphylococcus aureus* ATTC 6538 bacteria. The reproduction rate of the bacteria was determined by counting the number of colonies on a sample upon thermostating in a nutritional medium at a temperature of 36°C for 24 h. As a control served bacteria placed in a nutritional media on a laboratory glass.

Synthesis of silver nanoparticles on silk fibers. The total amount of silver reduced within the structure and on the surface of silk fibers from a silver nitrate solution depends on the type of the reducing agent and on the reduction duration. Kinetic curves describing the reduction of silver from 0.3 M silver nitrate solutions with the use of Metol and sodium borohydride and without a reducing agent are shown in Fig. 1.

It can be seen that the maximum amount of the metal in samples is reached after 30 min of reduction. The most quantitatively complete reduction at the same silver nitrate concentration in solution is provided by sodium borohydride. In the absence of a reducing agent, silver is also sorbed in the zero-valence form within the structure and on the surface of a fiber via interaction with active centers of fibroin.

Figure 2 shows how the total content of silver within the structure and on the surface of silk fibers depend on the silver nitrate concentration in solution, with different methods used for reduction during a time of 30 min.

These data suggest that the silver content of samples can be varied by changing the silver nitrate concentration in solution. The amount of silver in a sample (Fig. 2, curves *1–3*) and the size and shape of silver particles on the fiber surface also depend on the nature of a reducing agent. The sizes and shapes of

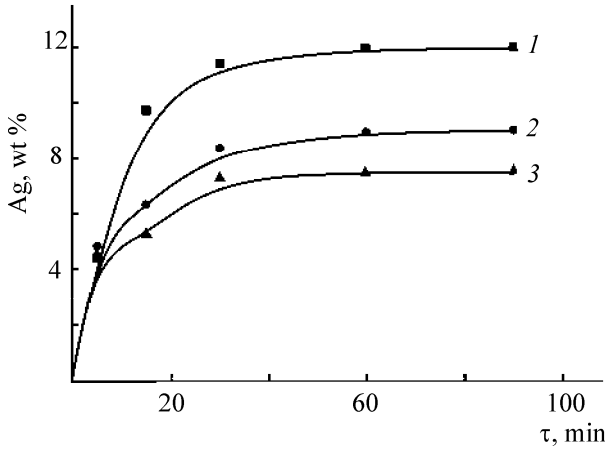


Fig. 1. Silver content Ag in a sample vs. time τ of reduction (25°C, silver nitrate concentration 0.03 M) with various reducing agents. (*1*) Sodium borohydride, (*2*) Metol, and (*3*) no reducing agent; the same for Figs. 2 and 8.

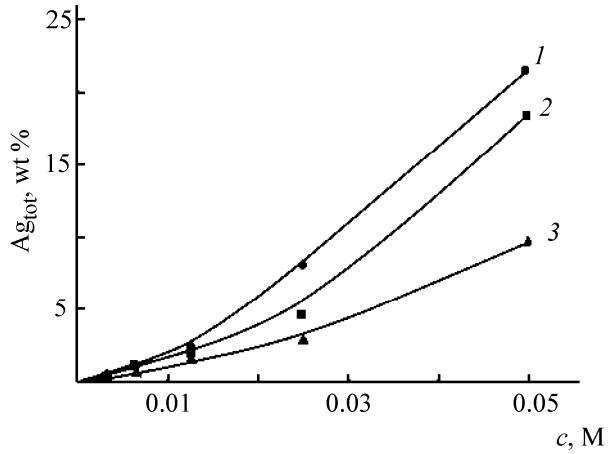


Fig. 2. Total silver content Ag_{tot} of a sample vs. silver nitrate concentration c in solution, with the use of various reducing agents.

silver particles formed on the fiber surface can be visually compared using the micrographs of the fiber surface in Fig. 3.

At the same content of silver on a fiber (1.5–3 wt %), reduction with Metol yields a small amount of coarse irregularly shaped particles (Fig. 3b), whereas in reduction with sodium borohydride or in the absence of a reducing agent, a large number of small metal particles is formed (Figs. 3a and 3c, respectively). The silver particle size distribution can be judged from results of statistical processing of the electron micrographs, shown in the form of histograms in Figs. 4–6.

It can be seen that, in reduction of Ag^+ ions by sodium borohydride, silver particles 5–100 nm in diameter are formed on the fiber surface. Comparison of the histograms in Figs. 4a–4c shows that the relative content of smaller particles 5.0–50 nm in diameter tends to increase and, accordingly, the fraction of coarser particles tends to decrease as the content of silver on the fiber grows within the range under study. Possibly, this occurs because of the specific effect of sodium borohydride as a reducing agent.

In reduction by Metol, silver forms on the fiber surface large agglomerates with diameters mostly larger than 100 nm, with their size increasing with the content of the metal.

In the absence of a reducing agent, silver agglomerates are also formed on the fiber surface, with the sizes of the overwhelming majority of these aggregates exceeding 100 nm.

The results obtained suggest that the mechanism by which silver particles are formed in reduction of silver

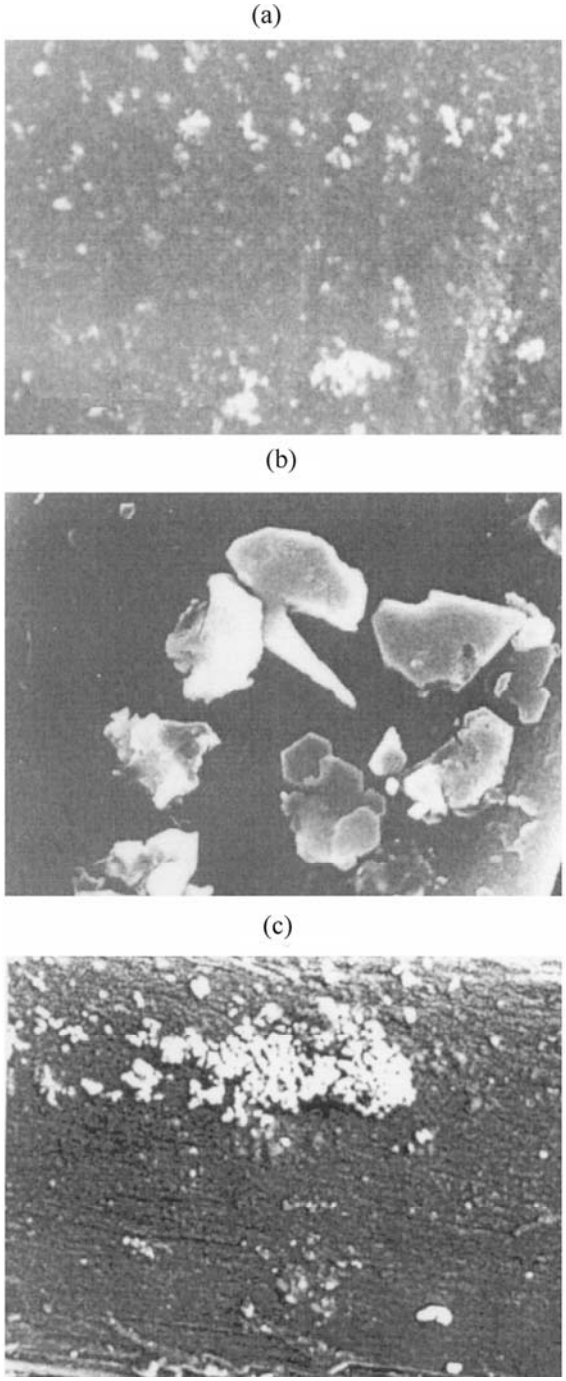


Fig. 3. SEM micrographs of silver particles on the fiber surface at a silver content of 1.5–3 wt %. (a) Sodium borohydride, (b) metol, and (c) no reducing agent.

ions depends on the chemical nature of the reducing agent. Metal particles are formed in reduction of ions from solutions of their salts in several stages. In the first stage, there occurs chemical reduction of silver nitrate; in the second, nuclei of the solid phase of zero-valence silver are formed and undergo condensation;

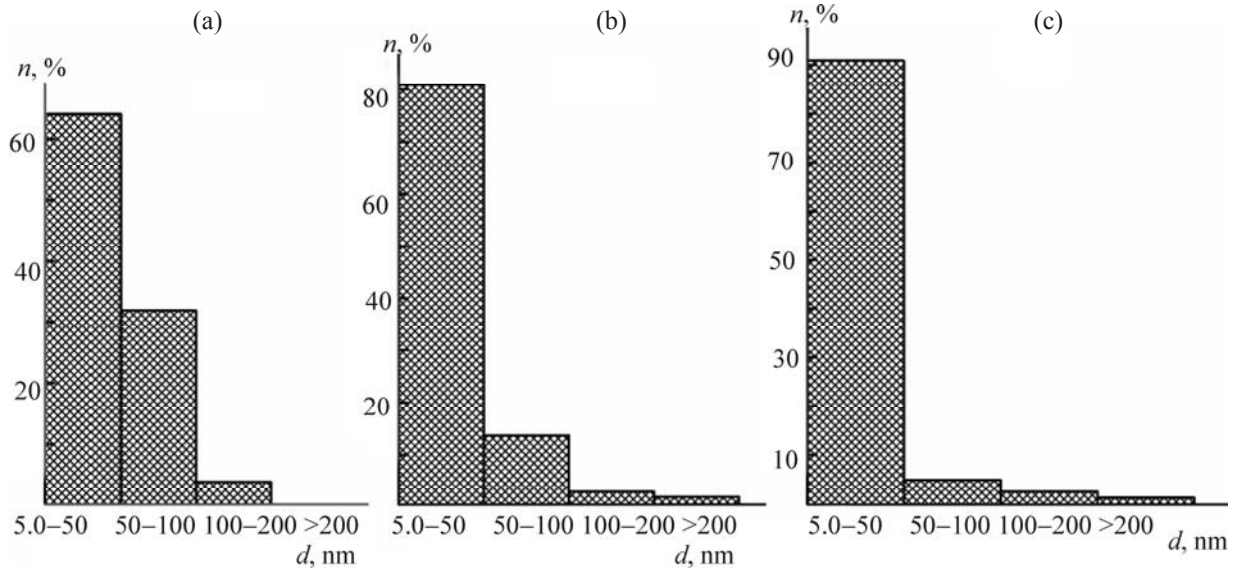


Fig. 4. Histograms of the distribution of the number n of silver particles over their size d on the fiber surface at a silver content of a sample of (a) 0.895, (b) 1.22, and (c) 2.31 wt %. Reducing agent: sodium borohydride.

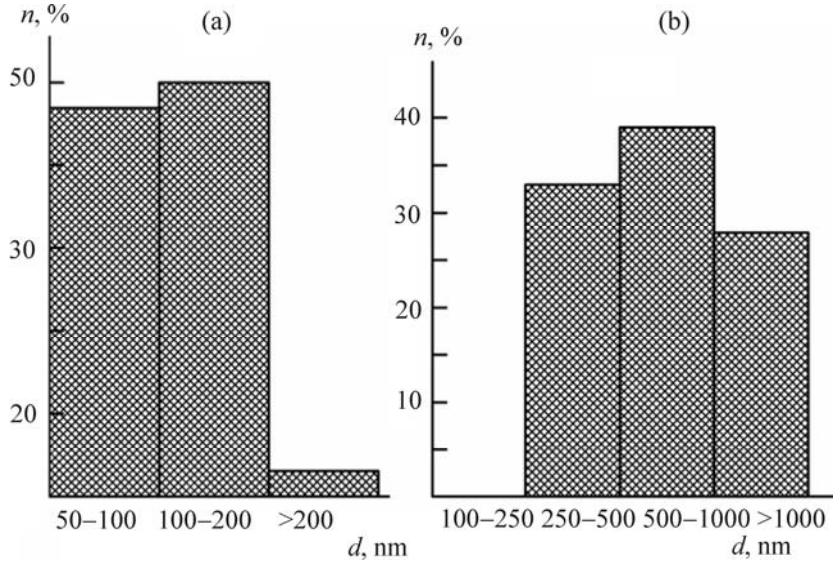


Fig. 5. Histograms of the distribution of the number n of silver particles over their size d on the fiber surface at a silver content of a sample of (a) 1.39 and (b) ≥1.66 wt %. Reducing agent: Metol.

and the third stage yields metallic particles. The solid-phase nuclei being formed are stable, which is due to the cohesion between metal atoms. It is commonly believed that the process predominantly occurs at the interface between the solid phase and a solution of the corresponding electrolyte [8, 9]. In reduction by sodium borohydride, the metal being reduced is predominantly expended for nucleation, and only its minor part goes to growth, with the result that fine metal particles are formed. In reduction by Metol, there occurs active growth of the solid-phase nuclei and, therefore, larger particles are formed.

Within the structure and on the surface of fibers, the reduced silver is in the crystalline form with cubic unit cell parameters $a = b = c = 0.408$ nm and $\alpha = \beta = \gamma = 90°$, as determined by X-ray diffraction analysis (Fig. 7). No reflections of silver oxide were found in the X-ray diffraction patterns.

The presence of silver within the structure and on the surface of silk fibers imparts bactericide properties to these fibers, as indicated by the data in Table 1.

It can be seen that bacteria on sample no. 2, which contains 2.31 wt % silver reduced by sodium boro-

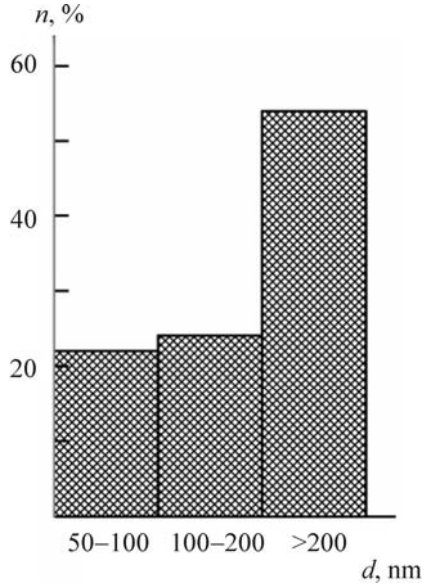


Fig. 6. Histograms of the distribution of the number n of silver particles over their size d on the fiber surface at a silver content of a sample of 3.23 wt % without a reducing agent.

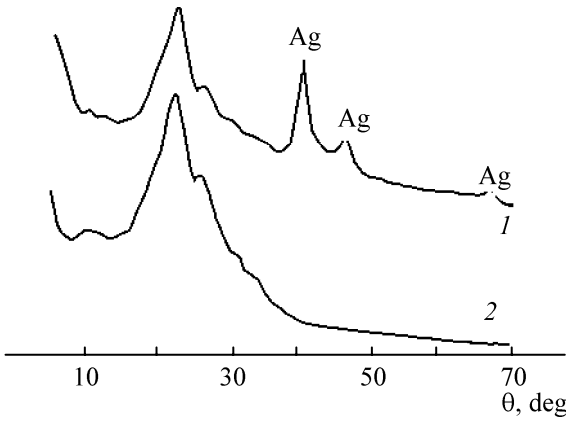


Fig. 7. X-ray diffraction patterns of silk fibroin fibers (*2*) before and (*1*) after reduction of Ag^+. (θ) Bragg angle; the same for Fig. 10.

hydride, not only fail to grow in abundance (after 24 h of thermostating in a nutritional medium, the number of bacteria is 106 times smaller than that on a control sample under the same conditions), but even perish (the number of bacteria is 103.1 times smaller than that originally deposited). This effect is achieved even at a comparatively low content of silver because nanosize particles have a large active surface area. Indeed, the bactericide properties of sample no. 3, which contains 11.3 wt % silver in the form of large agglomerates, are almost the same as those for a sample with low content of the metal.

Synthesis of silver nanoparticles on silk fibroin films. Figure 8 shows how the total content of silver in fibroin films depends on the silver nitrate concentration in solution, with Metol or sodium borohydrate used as a reducing agent or without a reducing agent. The maximum amount of silver in the samples is obtained for silver nitrate solutions with a concentration of about 0.015 M, irrespective of the type of a reducing agent. Use of more concentrated silver nitrate solutions does not lead to an increase in the content of silver in the films. It can be assumed that this is due to morphological features of fibroin films and to a specific interaction of silver ions with active centers of the polymer on the film surface. A quantitatively more complete reduction of the metal is observed with sodium borohydride as a reducing agent. The total amount of the reduced metal in the films does

Table 1. Bactericide properties of fibroin fibers

Sample	Thermostating duration, h	Number of bacteria	Growth factor	Bacteriostatic activity	Antimicrobial activity
Control	0	2.43×10^4	–	–	–
Control	24	2.09×10^7	2.9	–	–
(1) Untreated fibroin fibers	24	1.34×10^7	–	0.2	–2.7
(2) Fibroin fibers containing 2.31 wt % silver	24	<20	–	6.0	3.1
(3) Fibroin fibers containing 11.3 wt % silver	24	<20	–	6.0	3.1

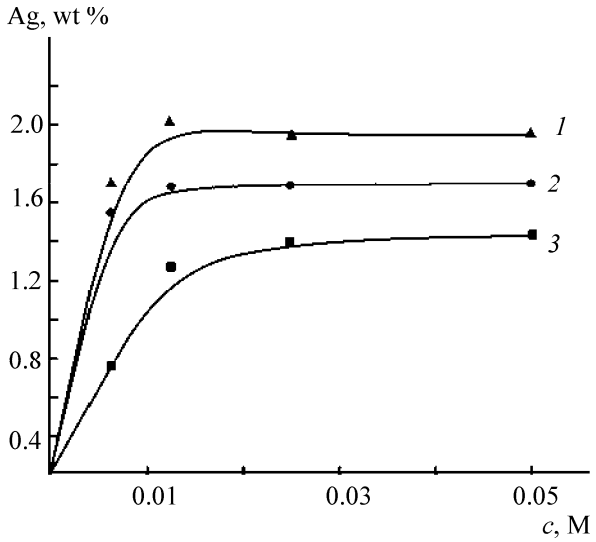


Fig. 8. Silver content Ag of films at a silver nitrate concentration c in solution.

not exceed 2 wt %. The particle sizes within the film structure are limited by the pore size and do not exceed 100 nm. Metal particles are distributed over the film surface in the form of particles with various shapes and sizes, which can judged from film surface micrographs (Fig. 9) and results of a statistical analysis of the particle size distribution (Table 2).

It can be noted that, in silver reduction with sodium borohydride, nuclei of the solid phase of the metal are formed on silk fibroin films and fibers without any pronounced subsequent increase in the size of these nuclei, so that fine metal particles are produced. For example, it can be seen in Table 2 that, upon reduction by sodium borohydride, more than a half of all particles on the film surface have sizes of 5.0–50 nm, and about a quarter are 50–100 nm in size.

In the case of reduction by Metol, solid-phase nuclei actively grow and, therefore, coarse particles (50–100, 100–200, and >200 nm) are formed in approximately equal amounts.

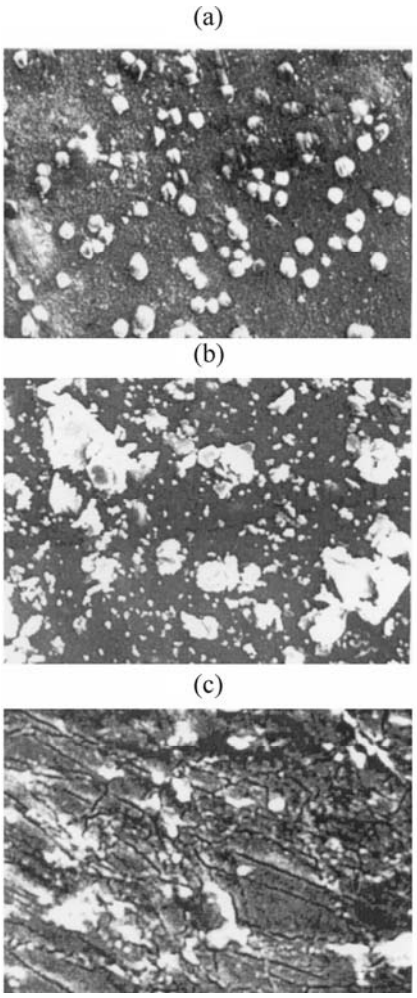


Fig. 9. SEM micrographs of the film surface with silver particles. (a) Sodium borohydride as reducing agent, silver content 1.75 wt %; (b) Metol as reducing agent, silver content 1.34 wt %; and (c) no reducing agent, silver content 1.64 wt %.

In the absence of a reducing agent, approximately a half of all particles of silver reduced on the film surface have sizes of 50–100 nm, and the remaining two quarters are 100–200 and 200–500 nm in size.

Table 2. Silver particle size distribution on the surface of fibroin films

Reducing agent	Ag content of a film, wt %	Relative content of particles with indicated diameter, nm					
		5.0–50	50–100	100–200	200–500	500–1000	>1000
Sodium borohydride	1.73	62	23	12	3	–	–
Metol	1.34	–	27	33	20	11	9
No reducing agent	1.64	–	57	21	22	–	–

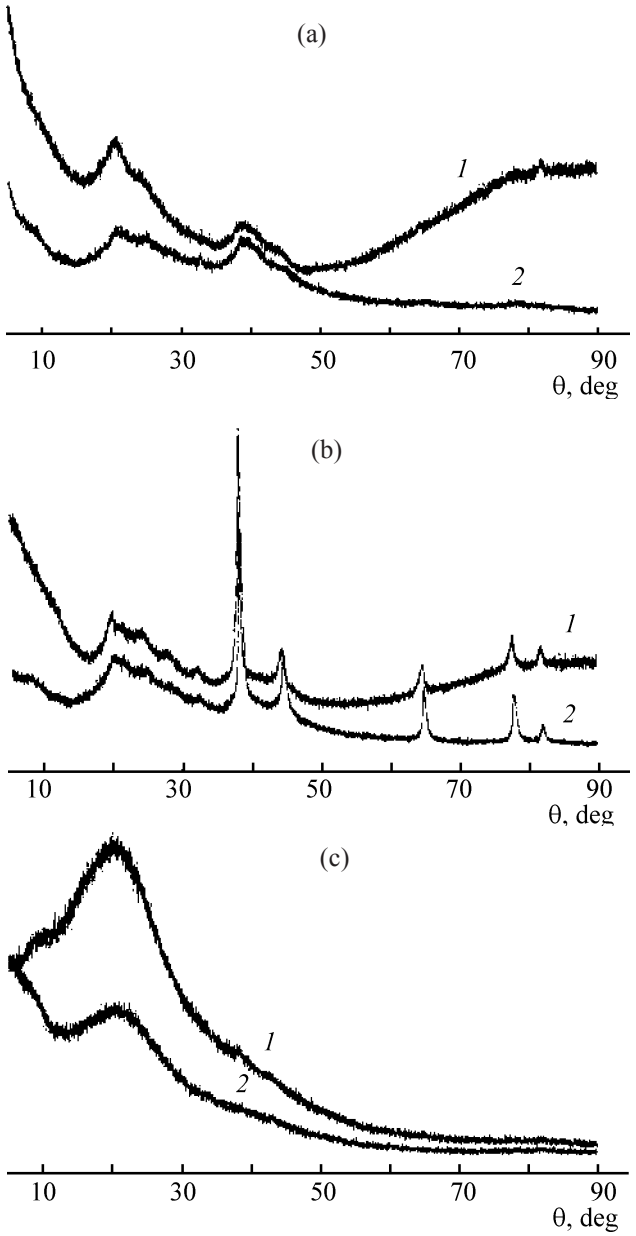


Fig. 10. X-ray diffraction patterns of silk fibroin films with particles of silver reduced by (a) sodium borohydride and (b) Metol and (c) deposited without using a reducing agent. Silver content of a sample (wt %): (a) (*1*) 1.73 and (*2*) 1.80; (b) (*1*) 1.49 and (*2*) 1.34; and (c) (*1*) 0.5 and (*2*) 1.64.

The morphologies of silver particles in samples obtained with different reducing agents are also different, which can be judged from a comparison of X-ray diffraction patterns of films in Figs. 10a–10c.

The zero-valence silver reduced by Metol has a crystalline form with cubic unit cell parameters $a = b = c = 0.408$ nm and $\alpha = \beta = \gamma = 90°$ (Fig. 10b) In the X-ray diffraction patterns of films with silver particles obtained with sodium borohydride and without a reducing agent (Figs. 10a and 10b, respectively), there are no reflections associated with a crystalline structure. It can be assumed that silver in these films is in the amorphous form.

CONCLUSIONS

(1) The amount and size of silver particles on a natural silk fiber and on a film produced from a silk fibroin solution depend on the chemical nature of a reducing agent and reduction conditions (time and silver nitrate concentration in solution). Silver particles with the minimum size are obtained on the surface of the fibers and films when sodium borohydride is used as the reducing agent.

(2) Zero-valence silver is reduced within the structure and on the surface of fibers as crystals with cubic unit cell parameters $a = b = c = 0.408$ nm and $\alpha = \beta = \gamma = 90°$. In films, crystalline silver is only formed with Metol as a reducing agent. With sodium borohydride and in the absence of a reducing agent, amorphous silver particles are formed in films.

(3) The presence of even a small amount of silver in the form of nanoparticles imparts antibacterial properties to the material.

REFERENCES

1. *Silk Polymers: Materials Science and Biotechnology*, Kaplan, D., Ed., Washington: Am. Chem. Soc., 1994.
2. WO Patent 9315244.
3. Sashina, E.S., Bochek, A.M., Novoselov, N.P., and Kirichenko, D.A., *Zh. Prikl. Khim*., 2006, vol. 79, no. 6, p. 881.
4. Sashina, E.S., Novoselov, N.P., Kuz'mina, O.G., and Troshenkova, S.V., *Khim. Volokna*, 2008, no. 3, p. 75.
5. Suzdalev, I.P. and Suzdalev, P.I., *Usp. Khim*., 2001, vol. 70, no. 3, p. 203.
6. Gubin, S.P., Koksharov, Yu.A., Khomutov, G.B., and Yurkov, G.Yu., *Usp. Khim*., 2005, vol. 74, no. 6, p. 539.
7. Tret'yakov, Yu.D., Lukashin, A.V., and Eliseev, A.A., *Usp. Khim*., 2004, vol. 73, no. 9, p. 974.
8. Kotel'nikova, N.E., Lysenko, E.L., Serimaa, R., et al., *Zh. Prikl. Khim*., 2006, vol. 79, no. 11, p. 1924.