Antibacterial Properties of Raw and Degummed Silk with Nanosilver in Various Conditions

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Received 17 November 2009; accepted 11 February 2010
DOI 10.1002/app.32289
Published online 19 May 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: This article is concerned with the effects of nanosized silver colloids on the antibacterial properties of silk fibers against two kinds of bacteria: *Staphylococcus aureus* and *Escherichia coli*. Different concentrations of silver nanoparticles (Ag NPs; 10, 25, 50, and 100 ppm) were applied to silk fibers by an exhaust method. The effect of medium pH on the Ag NP uptake on the fibers was studied. Also, sodium carbonate and sodium chloride were added to the liquor as auxiliaries. Scanning electron microscopy was used to observe the morphology of the silk fibers. The antibacterial activity was examined by a bacterial counting method. Energy-dispersive X-ray spectroscopy was also used to show the elements on the surface of the silk fibers. We observed that the antibacterial activity increased with silver treatment. It also increased with decreasing pH, especially for the raw silk. The use of NaCl improved the uniformity of the Ag NPs on the fiber surface and increased the antibacterial activities. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 253–258, 2010

Key words: fibers; nanotechnology; surfaces

INTRODUCTION

Nanoparticles are very important for the modification of textile products.[1–6] Nanosilver is a nanotechnological product with high utility that is particularly known for its antimicrobial properties.[7] It can remove more than 650 bacteria, virus, and fungi species by catalysis.[7] Silver nitrate is commonly used as an antimicrobial agent. Despite its excellent antimicrobial activity, silver nitrate is not appropriate for application to textile materials as it stains to black–brown when exposed to air and light because of an uncontrolled reduction processes.[8] However, a desirable level of antimicrobial activity without significant color change can be obtained with silver nanoparticles (Ag NPs) deposited on textile materials.[8] Nanosilver can be used for many specific applications, such as clothing designed for hygiene, sport, and the military.[7]

Antibacterial treatment of textile fabrics can be easily achieved by various compounds.[7,9,10]

Ag NPs have the properties of a high surface area, very small size (<10 nm), and high dispersion.[11] The reduction of silver ions (Ag^+) to silver metal (Ag°) is often achieved through the use of chemicals such as sodium borohydrate or hydrazine, which are rather hazardous chemicals. Safer chemicals, such as sugar or alcohol, can also be used to reduce Ag^+ to Ag° but then require the use of surfactants to stabilize the nanoparticles.[12] Recently, nanosilver was synthesized with liposome as an environmentally safe compound.[6] Several studies have been undertaken to explain the antibacterial properties of Ag^+ ions toward bacteria.[11] Polymeric or fibrous nanocomposites of polymers and Ag NPs have been reported to have special properties.[5,11] It is believed that the mechanism of the antibacterial effect of silver ions involves the shrinkage of the cytoplasm membrane or its detachment from the cell wall.[11] As a result, DNA molecules become condensed and lose their ability to replicate upon infiltration of Ag ions. Silver ions also interact with the thiol groups of proteins, which induces the inactivation of bacterial proteins.[11]

Ultrasound has been widely used in chemistry and the dyeing, finishing, and cleaning industries because of its obvious advantages in particle treatment, including dispersion and agglomeration effects.[13] During recent years, the treatment of nanomaterials with ultrasound has been a research hot topic, and many research findings have been achieved in this field.[13] Meanwhile, the applications of nanomaterials have received considerable attention in textile finishing, and some valuable functional textiles, such as antibacterial antiultraviolet products, have been brought to the market.[12] Fundamental studies have been carried out on the effects

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Journal of Applied Polymer Science, Vol. 118, 253–258 (2010)

of ultrasound on fine particles;[13] the results showed that the scattering effect on fine particles mainly came from the cavitation effect, which was closely related to the ultrasonic frequency, power, and viscidity of dispersions.[13] Also, sonochemical treatment can be combined with other techniques to increase the efficiency of destruction. These hybrid techniques can be cited as sonication followed by sonophotochemical destruction[14] and sonoelectrochemical methods.[15]

Several studies about the effect of salt addition to solutions have been reported. Okuwa[15] reported that the sound velocity increased with an increase in the concentration of NaCl in a dioxin–water mixture. Wang et al.[16] reported that the degradation rate of Reactive Brilliant Red K-BP in aqueous solution was substantially accelerated by Fe^{2+}, NaCl, and Fenton reagent addition.

Silk is a natural polymer and possesses special properties, such as thermoinsulation and adaptation to skin. To achieve these desirable properties, it is necessary to separate the sericin of silk, such as luminosity, from the fibroin.[17] Recently, natural polymers have attracted the attention of researchers because of their availability of resources, cost, easy handling, and minimum damage to the environment. In addition, they have unique properties, such as being nontoxic, having biological degradability, and their adaptive nature.[17] In recent years, attempts have been made to make use of natural polymers, such as chitin and alginate, by surface modification to improve their properties.[9,17] Chitosan grafting on silk has been successfully carried out by enzymatic reactions in solution.[17] By grafting chitosan, the number of hydrophilic groups ($-NH_2$) increase and cause the formation of hydrogen bonding by water molecules.[17]

In the formation of silk filament, the cocoon shell is composed of two proteins named *fibroin* held together by a gumlike protein called *sericin*.[18] The removal of the sericin from silk fibroin is accomplished by a process called *degumming*.[18] Most of the sericin must be removed during raw silk production at the reeling mill and the other stages of silk processing. Sericin is readily solubilized by boiling aqueous solutions containing soap, alkali, synthetic detergents, or organic acids.[19] Nowadays, the batch degumming of silk is mostly carried out in alkaline baths containing soap and alkali. Soap is replaced by synthetic detergents in continuous degumming systems because it cannot compensate for the acidity of sericin hydrolysis products accumulating in the bath and, thus, limits the use of the degumming bath for weekly degumming cycles.[19] The sericin protein is made of 18 amino acids, most of which have strongly polar side groups, such as hydroxyl, carboxyl, and amino groups.[10] This protein can be crosslinked, copolymerized, and blended with other macromolecular materials, especially synthetic polymers, to produce materials with improved properties.[18]

Silk possesses ionizable groups on the side chains of various amino acid residues, whose dissociation state depends on the surrounding pH conditions because silk is amphoteric in nature.[20] Moreover, it has been reported that the Ag NPs develop a negative charge around their surfaces.[20]

In this study, the effect of pH on Ag NP uptake on raw silk in an ultrasonic bath was examined. Also, the effect of the addition of Na_2CO_3 and NaCl on Ag NP uptake by an ultrasonic bath was investigated. However, to date, there has been no report on these effects in the ultrasound-related literature.

EXPERIMENTAL

Materials

Raw silk yarn (44 Den, 150 twists per meter, Ozbakestan) was used for the study. Ag NPs (Pars Nano Nasb Co., Tehran, Iran), Keliab (Kashan, Iran) extract from a plant named Oshnuo, Alcalase 2.5 L Type-DX (microbial-origin, serine-type protease with 2.5 AU/p activity from Novozyme (Denmark) EC 3.4.21.14). All other chemicals were laboratory grade (Merck, Germany).

Experiments

Method 1

Silk degumming was performed with raw silk yarn. Silk yarns were treated with 20 wt % Keliab (liquor ratio = 40 : 1) at 98°C for 35 min (pH = 11) were measured. Degummed silk was thoroughly rinsed and dried at room temperature.

Method 2

Enzymatic degumming processes were carried out as explained in Table I.

Scanning electron microscopy (SEM) was used to visualize the morphology of the raw and degummed silk fibers. Also, the raw silk fibers were scoured with 1 g/L nonionic detergent at 60°C for 20 min.

The concentration of colloidal silver solutions was varied (10, 25, 50, and 100 ppm) by the dilution of each nanosized silver colloid solution with distilled water.

Ag NPs were applied to the silk fibers (pH = 5 without the addition of acid and alkali) by an exhaust method and with an ultrasonic bath (Power Sonic, 35 KHz, Germany; with the application of suitable mechanical agitation).

The effects of the pH of treatments with weak acid and alkali agents (pH's = 2, 4, and 9) on the

TABLE I
Enzymatic Degumming Process

Material (g/L)	Process conditions
Alcalase 2.5 L	0.6
$NaHCO_3$	5.0
Nonionic surfactant	1.0

Liquor ratio = 30 : 1.

raw and degummed silk were studied. The treatment was carried out at 40°C for 30 min. The samples were air-dried in a dark room without rinsing.

Two exhaust treatment methods were performed with 25 ppm nanosilver in an ultrasonic bath at 40°C for 30 min.

In the first method, after 10 min, 5% sodium chloride was gradually added to the liquor, and in the second method, after 10 min, 5% sodium carbonate (pH = 8–9) was gradually added to the liquor.

Antibacterial test

The antibacterial activity of the nanosilver coatings was examined by a bacterial counting method. The coating specimens were sterilized. Different concentrations (10^8, 10^7, 10^6, and 10^5 cfu/mL) of bacterial suspension were prepared. The bacterial suspension (0.1 mL) was dripped onto the coating surfaces (for easier counting, the solution of bacteria was diluted). The coating specimens with the bacterial solution were covered with an aseptic polyethylene film and incubated at 37°C for 24 h. Afterward, the bacterial solution was collected and inoculated onto a standard agar culture medium. Finally, after incubation at 37°C for 24 h, the active bacteria were counted.

Characterization

SEM of the Ag NP treated silk was performed with a scanning electron microscope (XL30, Philips, Holland; S-4160, Hitachi, Japan). To verify and confirm the silver presence in fibers, energy-dispersive X-ray spectroscopy (EDX) measurement were performed (Cambridge, Cam Scan, MV 2300). The antibacterial activity against two different bacteria, *Escherichia coli* (ATCC no. 8739) and *Staphylococcus aureus* (ATCC no. 6538), was examined by the bacterial counting method. The antibacterial efficiency was obtained through eq. (1):

$$\text{Reduction of bacteria}(\%) = (A - B/A) \times 100 \qquad (1)$$

where A is the number of bacteria on the untreated fiber after 24 h and B is the number of bacteria on the treated fiber after 24 h.

RESULT AND DISCUSSION

Comparison of the two different degumming methods

The scanning electron micrographs of the degummed silk [Fig. 1(b)] did not show any fibrillation. However, Keliab acted as a strong alkaline reagent (pH = 11), which may have damaged the silk fibers.

The sericin coating was clearly evident in the control silk before degumming [Fig. 1(a)]. In the untreated silk yarn micrographs [Fig. 1(a)], sericin appeared as a nonuniform coating on the surface of the yarn. However, the micrographs of the treated samples and those degummed by alcalase and Keliab showed perfect degumming and no sign of destruction or damage to the surface of the yarn (Fig. 1).

The surface morphology of the silk fibers after Keliab and enzymatic degumming is shown in Figure 1(b,c). The dull appearance and stiff handle of the raw fabric disappeared, and the degummed fiber became shiny and soft. The closer SEM examination of the yarn showed that the individual silk filaments split off, and their surface was clean and free of sericin [Fig. 1(b,c)].

Antibacterial study

The silk fibers were treated with Ag NPs by an exhaust method to impart antibacterial activity. The treated samples were tested for antibacterial activity against the Gram-positive bacterium *S. aureus* and the Gram-negative bacterium *E. coli*. Figure 2 shows the SEM images of the silk fiber treated with 100 ppm Ag NPs. Table II presents the results of the

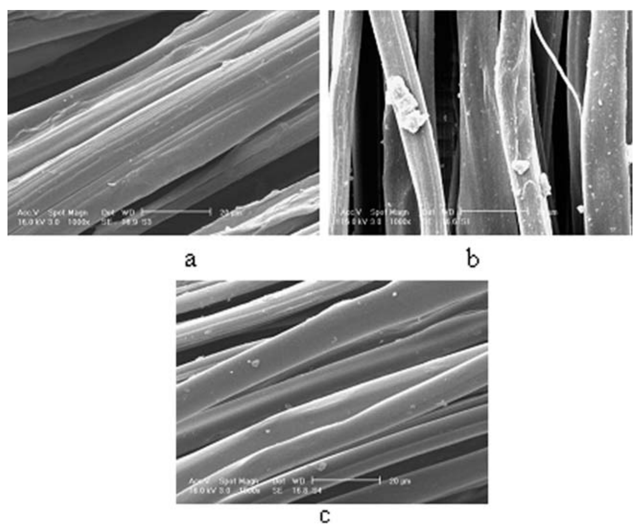


Figure 1 SEM micrographs of the (a) raw silk, (b) degummed silk with Keliab, and (c) degummed silk with enzyme.

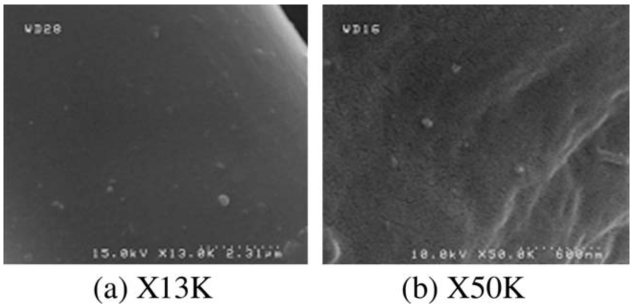


Figure 2 SEM micrographs of the silk fiber treated with 100 ppm Ag NPs.

antibacterial activity of silk fibers treated with various concentrations of Ag NPs. Fibers treated with Ag NPs showed approximately 100% antibacterial activity above 50 ppm. Also, Table II showed that, for antibacterial activity, there were no significant differences between degumming with alcalase or Keliab. Figures 3 and 4 show the number of bacterial colonies grown on the nanosilver coatings (in a solution of bacteria with a concentration of 10^7 cfu/mL). The percentage reduction of *S. aureus* seeded on 50 and 100 ppm nanosilver coatings was 100% after 24 h of incubation.

Effect of pH

The results (Table III) show that the antibacterial activity increased in acidic pH and decreased in alkali pH. Degummed silk is amphoteric in nature because of the ionizable groups present as end groups and on the side chains of various amino acid residues. Their dissociation state depends on the pH of the surrounding medium.[20] This characteristic of silk facilitates the attraction and binding of charged metal ions.[20] When immersed in an aqueous solution of metal salts, silk exhibits the tendency to absorb metal cations, and the rate and extent of uptake depend on various factors, such as the kind of metal and its valence state and pH.[20] The low uptake of the Ag NPs by the fibers in alkaline pH could be attributed to the negative–negative repulsion effect as the silk fibers developed a more negative charge because of their amphoteric nature. However, because of the same amphoteric nature, the silk developed more positive charges in acidic pH and, hence, attracted the negatively charged Ag NPs; this resulted in a higher uptake.[20] On the basis of further study, we observed (Table III) that a 100% antibacterial activity for the raw silk fibers treated with 25 ppm in pH = 2 was obtained, whereas the treated degummed silk with identical conditions (pH = 2) did not show 100% antibacterial activity. This may have been related to the effect of sericin. The hydrophilic amino acids of sericin to 70% could account for the good water absorption and solubility. It was found that the aromatic amino acids in sericin were very low (accounting for 6.6% of the total of amino acids)[21] compared with fibroin. In addition, as discussed in the previous section, the silk fiber developed more positive charges in acidic pH and, thus, attracted the negatively charged Ag NPs; also, these

TABLE II
Antimicrobial Activity of the Degummed Silk Treated with Ag NPs

Concentration of Ag NPs (ppm)	Degumming method	Antibacterial activity against *S. aureus* (%)	Antibacterial activity against *E. coli* (%)
0	Raw silk	4	7
0	Alcalase	0	2
	Keliab	2	2
10	Alcalase	10	12
	Keliab	8	14
25	Alcalase	58	38
	Keliab	59	76
50	Alcalase	100	94
	Keliab	100	100
100	Alcalase	100	100
	Keliab	100	100

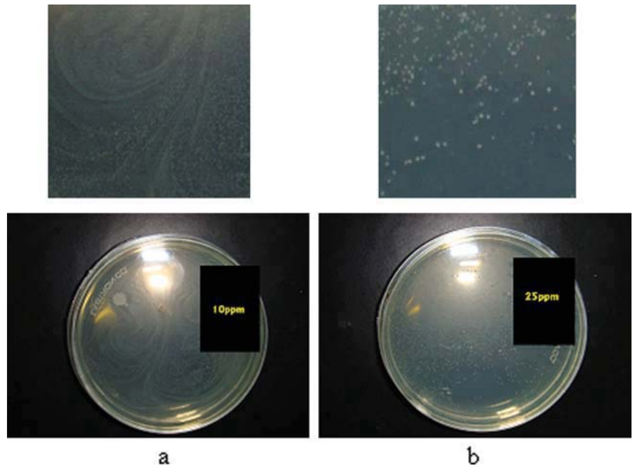


Figure 3 Antibacterial results on *S. aureus* after 24 h of incubation with (a) 10 and (b) 25 ppm Ag NPs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

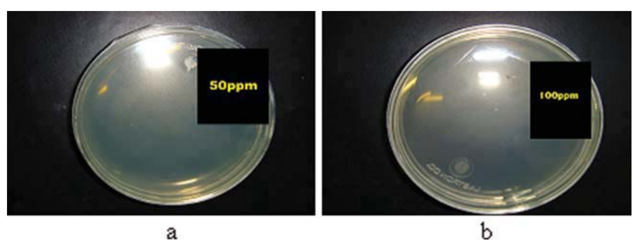


Figure 4 Antibacterial results on *S. aureus* after 24 h of incubation with (a) 50 and (b) 100 ppm Ag NPs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE III
Antimicrobial Activity of the Degummed and Raw Silk Treated with 25 ppm Ag NPs at Different pH's

pH of bath exhaustion	Silk	Antibacterial activity (%) against *S. aureus*	Antibacterial activity (%) against *E. coli*
2	Raw	100	100
	Degummed	46	76
4	Raw	32	27
	Degummed	84	62
9	Raw	10	22
	Degummed	25	18

TABLE IV
EDX Quantification of Various Samples

Silk treated with Ag NPs	pH	Ag (wt %)	Cl (wt %)
Degummed, 100 ppm	—	4.88	—
		5.23	—
Degummed, NaCl, 25 ppm	—	2.92	3.78
		3.71	3.30
Degummed, 25 ppm	2	1.26	—
		1.05	—
Raw, 25 ppm	2	3.14	—
		3.07	—
Degummed, 25 ppm	9	1.69	—
		0.59	—
Raw, 25 ppm	9	0.46	—
		0.43	—

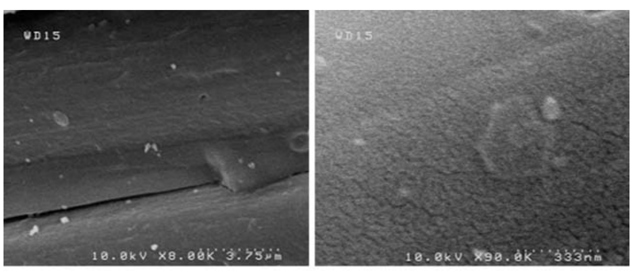
groups (NH_2 (side)) existed more in sericin as compared with fibroin.[22] To examine the morphology of the Ag NP treated silk [Fig. 5(a,b)], SEM was used, and the Ag NPs were observed. The presence of Ag NPs over the treated degummed and raw silk surfaces could be seen at pH = 2; in particular, in raw silk, Ag NP loaded on the gum of the silk.

In addition, the antibacterial activity of raw silk decreased at pH = 4. Like other proteins, sericin has an isoelectric point in which its solubility is lowest. From the results, it was clear that pH 4 was close to the isoelectric point, which was low in positive charge and limited the attraction of Ag NP. This led to a lower antibacterial activity.

Effect of NaCl and Na_2CO_3

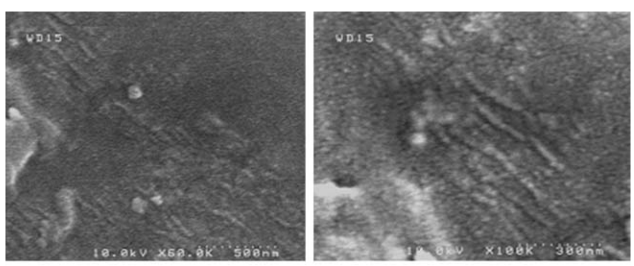
Table IV shows the effect of the NaCl on the Ag NP uptake by the degummed fiber. The uptake of Ag NPs by the fibers increased when NaCl was added to the aqueous solution of Ag NPs along with sonication (pH of Ag NP solution, without the addition of acid or alkali). This phenomenon could be attributed to the charging pattern of the silk substrate and the electrical potential of the Ag NPs. In addition to the positive charge of the fibers, the Na^+ ions were absorbed by negative charge, and then, the silk fibers were more attractive for the Ag NPs. Moreover, the addition of NaCl could increase the surface tension and ionic strength of the aqueous phase and decrease the vapor pressure.[17] All of these factors contributed to the more violent collapse of the bubbles. Furthermore, the sonication causing the collision of Na^+ ions with the Ag NPs led to a higher dispersion of nanoparticles in the solution and, consequently, the uniform absorption of nanoparticles on the fiber surfaces. Furthermore, despite the results of the antibacterial activity tests and the EDX of silk treated with Ag NP including NaCl, which showed a great amount of silver, the SEM images in Figure 6 did not show a great number of particles, as compared with Figures 2 and 4. This might have been due to size of the particles, which were small. Also, the antibacterial activity increased because NaCl addition not only increased the Ag NP uptake but also dispersed the nanoparticles. This helped to produce small-sized particles on the fiber surfaces with higher antibacterial efficiency. As shown in Table IV, the fibers treated with 25 ppm including the NaCl confirmed the 100% antibacterial activity.

Figure 7 shows the effect of Na_2CO_3 on the Ag NP uptake by the degummed fiber. As discussed in the previous section, the Ag NP uptake by the fiber decreased in alkali pH (pH = 8–9). The salt strongly interacted with water and added to the Ag NP/water solution. This led to a decrease in the amount of water around the Ag molecules, which resulted in the deposition of Ag NP in some areas of silk



(a) (b)

Figure 5 SEM micrographs of silk fibers treated with 25 ppm Ag NPs at pH 2: (a) raw silk and (b) degummed silk.



(a) X60K (b) X100K

Figure 6 SEM micrographs of silk treated with 25 ppm Ag NPs including NaCl.

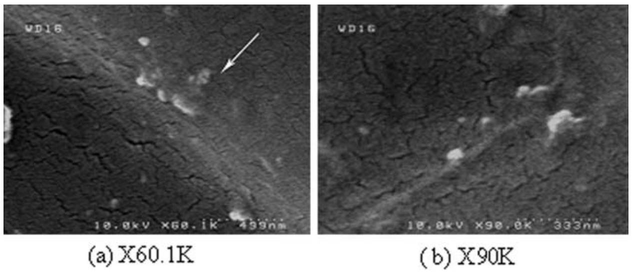


Figure 7 SEM micrographs of silk treated with 25 ppm Ag NPs including Na_2CO_3 (from different areas of the fiber).

surface (not the whole of silk fibers). The interaction of Na^+/water or CO_3^{2-} (HCO_3^-)/water were stronger than the interaction of Ag NP/water. The separation of an ethanol solution by the addition of Na_2CO_3 was already reported in the literature.[23]

The Ag NPs were generally well-dispersed on these surfaces. SEM micrographs of the samples showed the presence of variable amounts of Ag NPs on their surfaces. SEM images of the treated silk fibers (Fig. 6) did not show a considerable amount of Ag NPs on the surface, but the antibacterial activity and EDX quantification showed the presence of considerable amounts of nanosilver on the silk fiber, maybe because of the presence of the NaCl and sonication, the penetration of Ag NPs was enhanced or maybe the particles were small. Otherwise, Figure 7 shows the agglomeration of nanosilver deposited onto the silk fiber surface.

EDX analysis

Table IV lists the silver content and other elements on different silk fibers. The results confirmed the results of the antibacterial test as the antibacterial activity increased with increasing silver on the fiber surface.

CONCLUSIONS

Ag NPs were applied to silk by an exhaust method. Different concentrations of Ag NPs were treated on the silk fiber. The antibacterial activity increased with increasing silver treatment solution.

The effect of the pH medium on the Ag NP uptake by the fibers was studied. The antibacterial activity diminished at alkaline pH and increased at acidic pH; furthermore, Ag NP uptake by the fiber increased when Ag NPs were applied to raw silk at pH = 2. When sodium carbonate and NaCl were added to the liquor, not only did NaCl increase the Ag NP uptake but it also absorbed the Cl ions by the fiber, and Cl ions manifested antibacterial activity.

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