Preparation and Characterization of Silver Containing Chitosan Fibers

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ABSTRACT: Silver containing chitosan fibers were prepared by blending fine particles of a silver sodium hydrogen zirconium phosphate compound into the spinning solution. It was possible to distribute the silver containing particles in the chitosan fiber because of the high viscosity of the spinning solution and the small diameter of the particles. Because the silver ions are imbedded inside the sodium hydrogen zirconium phosphate complex, the chitosan fibers remain white in color without being oxidized by the silver ions. The release of silver ions from the silver containing chitosan fibers were studied by placing the fibers in contact with distilled water, solution A, and aqueous protein solutions. Results showed that the release of silver ions was low in water, while in solution A and protein solutions, the silver ions are activated through ion exchange and chelation. The silver ions can significantly enhance the antimicrobial properties of the chitosan fibers. Experimental results showed that when placed in contact with the silver containing chitosan fibers, the reduction in bacteria count can be more than 98%. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 3622–3627, 2007

Key words: coextrusion; fibers; drug delivery systems; polysaccharides

INTRODUCTION

Chitosan is a biocompatible, biodegradable, and nontoxic polymer with abundant supply in nature. In recent years, chitosan has found widespread uses as a novel biomaterial.[1,2] Fibers made from chitosan have been known for a long time[3–5] and in recent years, woven, nonwoven, and knitted structures of chitosan fibers have been made and used in wound care and other biomedical fields. As a fibrous material, chitosan fibers possess both the novel bioactivities of chitosan and the good processibilities of a fiber.

As a polymeric amine, chitosan is positively charged when wet. As bacteria cell walls are negatively charged, bacteria can be adhered to the chitosan fibers, resulting in the containment of bacteria growth. Many studies have confirmed the natural antimicrobial properties of the chitosan fibers.[6,7] Since these fibers are relatively expensive, in the traditional textiles and apparel industry, chitosan fibers are normally blended with other conventional fibers, utilizing their antimicrobial properties and high moisture content.

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Silver has a long history as an antimicrobial agent,[8–12] especially in the treatment of burns. While metallic silver is relatively inactive, silver ions are effective against a wide range of bacteria. When low concentrations of silver ions accumulate inside cells, they can bind to negatively charged components in proteins and nucleic acids, thereby effecting structural changes in bacterial cell walls, membranes, and nucleic acids that affect viability.[13–15] Interestingly, although silver is a highly effective antimicrobial agent, it has a limited toxicity to mammalian cells.[16] It was found that the use of silver containing wound dressings can increase the rate of epithelialization by 28%, indicating a beneficial effect of silver ions to skin regeneration, in addition to its antimicrobial activity.

In recent years, silver has been gaining importance as an effective antimicrobial agent that does not result in bacteria resistance. Silver containing antimicrobial products have been developed so that a low concentration of silver ions can be released over time. A number of laboratory studies have shown the excellent antimicrobial performances of the silver containing antimicrobial products.[9,11]

This study aims to combine the natural antimicrobial properties of the chitosan fibers with the excellent antimicrobial effect of silver ion to produce a fiber that can both contain and kill bacteria growth.

EXPERIMENTAL

A spinning dope was prepared by dissolving 3 kg of chitosan powder in 97 kg of 1% aqueous acetic acid solution. Thirty gram of AlphaSan RC5000 (a silver sodium hydrogen zirconium phosphate containing 3.8% by weight silver) was added into the solution and thoroughly mixed with the chitosan solution. After storage at room temperature for 2 days to remove the bubbles, fibers were produced by extruding the dope through a spinneret with 4000 holes (hole diameter 80 μm) at 12 m/min into an aqueous coagulation bath containing 4% NaOH. The as-spun fibers were taken up at 7.2 m/min and then stretched at 80°C to the maximum extent. The fibers were then washed with water and acetone before being dried by hanging in air. Finally, the dry tow was cut to produce 50-mm length staple fibers.

When analyzing the silver ion contents in the chitosan fibers, 0.5 g fibers were treated with 7 mL concentrated sulphuric acid until the fibers were fully digested. The mixture was then diluted to 100 mL with distilled water and filtered. The silver ion concentration was determined by using atomic absorption spectrometer.

When testing the release of silver ions from the fibers, the fiber sample was placed in contact with 40 times its own weight of either distilled water, solution A, or aqueous solutions containing different amount of protein. The British Pharmacopeia specified solution A as an aqueous solution containing 142 mmol of sodium chloride and 2.5 mmol of calcium chloride, representing the typical ion concentrations of body fluid. The protein used was water soluble soya bean protein. After conditioning at specified temperatures for different periods of time, 5 mL solution was taken out and tested for silver ion concentration by using atomic absorption spectrometer.

To assess the effect of silver on the antimicrobial properties of the chitosan fibers, five test tubes were each added 10 mL 0.5% peptone water and 0.1 mL bacteria suspension with a concentration of 1×10^8 cfu/mL *Escherichia coli*. Then, to four of these tubes were added 0.1 g sterilized chitosan fiber, silver containing chitosan fiber, copper containing chitosan fiber, and zinc containing chitosan fiber, respectively, the later two samples being made according to a previously reported method.[17] The test tubes were then placed in a 36°C water bath and shaken at a speed of 120 r/min for 12–15 h. The antimicrobial efficacy can be judged by the clarity of the solution around the fibers.

Quantitatively, the antimicrobial activity of the fibers was tested against three common strains of bacteria, i.e., *Candida albicans, Staphylococcus aureus, and Pseudomonas pyocyanea*. The bacteria were suspended in 0.5% peptone water with the bacteria concentration at about 1.5×10^4–1.5×10^5 cfu/mL. Thirty-five milliliter of 0.5% peptone water were measured into 100 mL conical flasks and to each of them were added 2.5 mL of the bacteria suspension, with the bacteria concentration in the conical flask controlled at between 1×10^3 and 1×10^4 cfu/mL. After that, 0.375 $\pm$ 0.002 g of sterilized silver containing chitosan fibers were added into the conical flasks respectively. After the fibers were placed in contact with the bacteria suspension, the conical flasks were placed in a 36°C water bath and were shaken at a speed of 180 r/min for 8 h. Bacteria containing solution (0.1 mL) was then taken out to measure the colony forming units.

The reduction in the number of bacteria is calculated in the following equation:

$$\text{Reduction in bacteria} = [A - B]/A \times 100\%$$

where, A is the average bacteria concentration in the control sample after shaking, cfu/mL; B is the average bacteria concentration in the test sample after shaking, cfu/mL.

RESULTS AND DISCUSSION

Since chitosan fibers have in their structure primary amine groups, it can be easy to attach silver ions onto the chitosan fibers by treating them with aqueous silver nitrate solution. A previous study has shown that it is possible to apply different amount of silver onto the chitosan fibers by controlling either the weight ratio between chitosan and silver nitrate or by controlling the treatment time.[18] However, in this method, the silver ions can oxidize the chitosan fiber and on exposure to light, the fiber quickly turns black, which affects its application in such fields as medical and apparel textiles.

To preserve the white appearance of the fiber, which is critical in many applications, the present study used AlphaSan RC5000, which is a silver sodium hydrogen zirconium phosphate compound containing 3.8% silver. This microbiologically active ingredient is a synthetic inorganic polymer. Under scanning electron microscope, it resembles cube shaped crystals, with an average particle size of about 1 μm (about the size of an average bacterium). It consists of a three-dimensional, repeating framework of sodium hydrogen zirconium phosphate, with many equally spaced cavities containing silver. Silver provides the main antimicrobial properties, while the framework matrix acts to distribute silver evenly without clumping or pooling throughout the individual fibers.

When AlphaSan RC5000 is mixed with the chitosan solution, the fine particles can be evenly distributed in the spinning solution under a high rate of shearing. Because the particles are very fine and the viscosity of the chitosan solution is very high, they can be sus-

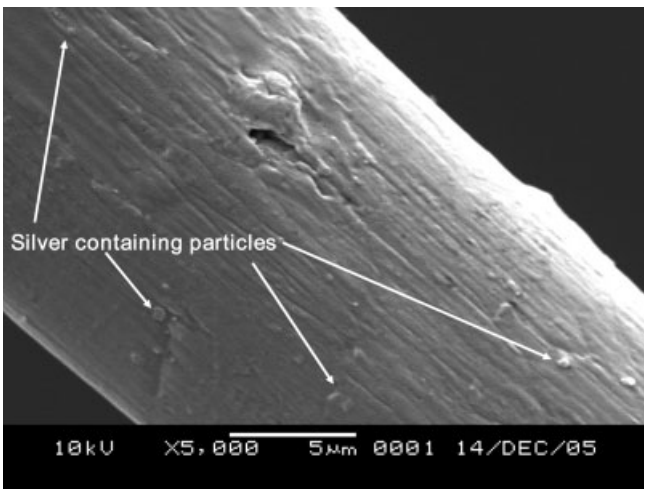

Figure 1 SEM Photomicrograph of the silver containing chitosan fiber.

pended uniformly while the solution is extruded to form fibers. Since the sodium hydrogen zirconium phosphate framework prevents the silver ions from oxidizing the chitosan, this type of silver containing chitosan fiber remains white even after sterilization with γ-irradiation.

Figures 1 and 2 show the SEM photomicrographs of the silver containing chitosan fibers. It can be seen that although the silver containing chitosan fiber generally has a smooth surface structure, the AlphaSan RC5000 particles are visible and can be seen embedded into the chitosan structure. When the fiber is wet with 0.1% aqueous acetic acid solution, it can be seen under optical microscope that the silver containing AlphaSan RC5000 particles are fairly uniformly distributed inside the fiber structure, acting as the reservoir for releasing the antimicrobial silver ions.

It should be pointed out that the diameter of the AlphaSan RC5000 particles are about 1 μm, while the diameter of a dry fiber is about 20 μm, making it possible to disperse these particles in the fiber without serious effect on the tensile properties of the fibers. Test results showed that the silver containing chitosan fiber had a tensile strength of 1.9 g/day, with an extension at break of 8.5%.

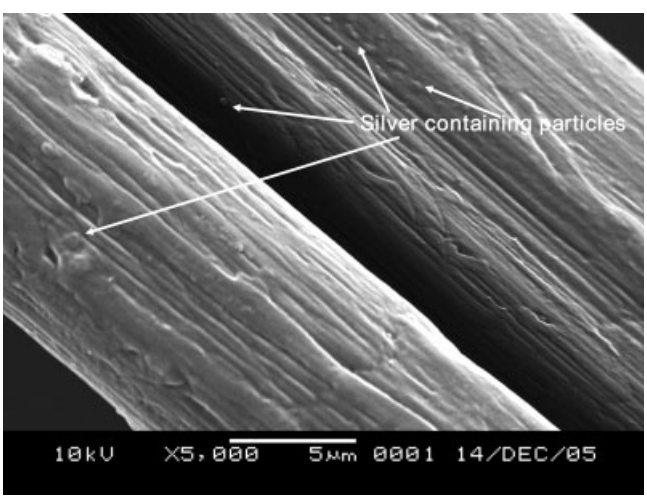

Figure 2 SEM Photomicrograph of the silver containing chitosan fiber.

Figure 3 shows the release of silver ions when the silver containing chitosan fibers are placed in contact with three different solutions at 37°C. With distilled water, the silver concentration was low and remained at about 0.137 μg/mL over extended period of time. In both solution A and 2.9% aqueous protein solution, the silver concentrations in the contacting solutions were much higher than in distilled water. With solution A, the silver concentration slowly rose from 0.402 μg/mL at 30 min to about 0.654 μg/mL after 24 h. The silver concentration in the 2.9% protein solution was much higher than in solution A. At 4 h, the silver concentration in the protein solution was 1.31 μg/mL, about twice those measured in solution A after same period of contact with the silver containing fibers.

When silver containing chitosan fibers are in contact with liquid, silver ions can be released from the fibers in three different mechanisms. First, there is an ion exchange between the silver ions in the fiber and the sodium and calcium ions in the contacting solution. Second, silver ions can be chelated by protein molecules in the solution. Third, the silver containing AlphaSan RC5000 particles attached on the surface of the fibers can also be detached from the fibers and get into the solution. In the present study, it is clear from the results shown in Figure 3 that chelation with protein molecules plays the most important role in the release of silver ions from chitosan fibers containing AlphaSan RC5000 particles.

Silver ions released from the silver containing chitosan fibers can act as an effective antimicrobial agent. In the literature, it has long been known that a silver concentration of 0.0000001% killed the freshwater

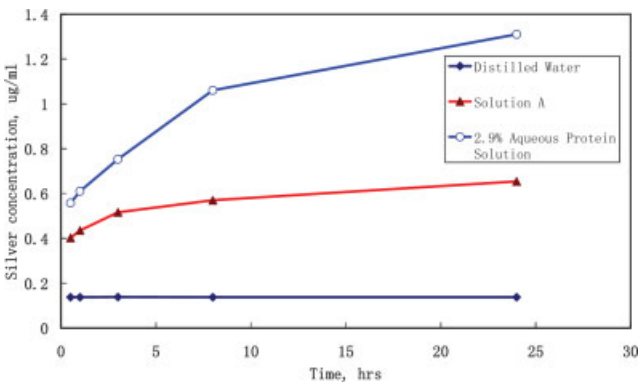

Figure 3 Silver ion concentrations after the silver containing chitosan fibers were in contact with three different solutions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

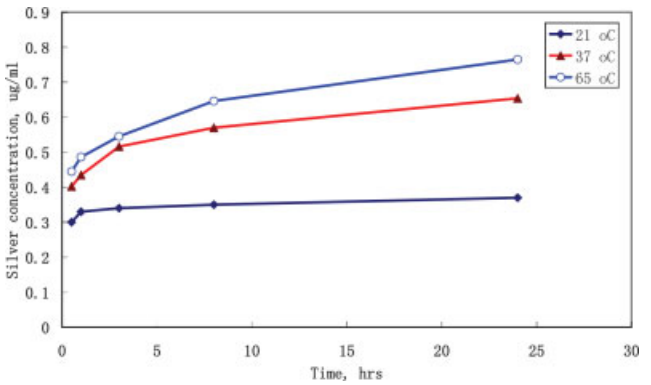

Figure 4 Effect of temperature on the silver release when the silver containing chitosan fibers were in contact with solution A. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

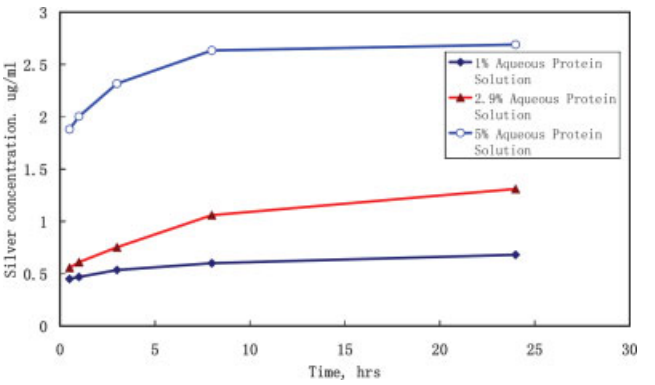

Figure 6 Silver ion concentrations when the silver containing chitosan fibers were in contact with aqueous solutions containing different amount of protein at 37°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

algae Spirogyra and a concentration of 0.00006% prevented germination of *Aspergillus niger* spores.[12] The antimicrobial action of silver products has been directly related to the amount and rate of silver released and its ability to inactivate target bacterial and fungal cells. The microbicidal action of silver compounds at low concentrations reflects the ability of bacteria, trypanosomes, and yeasts to take up and concentrate silver from very dilute solutions.[13] It was shown that bacteria killed by silver may contain 10^5–10^7 Ag^+ per cell, the same order of magnitude as the estimated number of enzyme-protein molecules per cell. This showed that through chemical chelation, the enzyme-protein molecules in bacteria cell can absorb silver ions from the contacting solution. Hence when bacteria and the silver containing chitosan fiber are in contact with each other, silver ions can be transferred from the fiber to bacteria. In wound management, for example, when the silver containing chitosan fiber is in contact with wound fluid, because the fluid contains both inorganic ions and protein, silver ions can be released from the fiber through both ion exchange and chelation. The silver containing AlphaSan RC5000 particles can act as the silver reservoir with a sustained release of the antimicrobial silver ions over extended period.

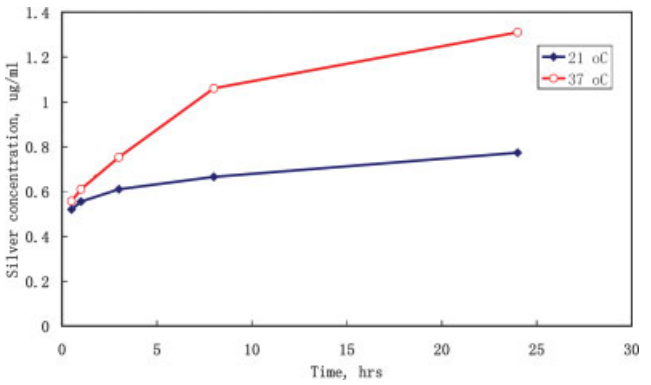

Figure 5 Silver ion concentrations at two different temperatures when the silver containing chitosan fibers were in contact with 2.9% aqueous protein solution. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 4 shows the effect of temperature on the silver release when the silver containing chitosan fibers were in contact with solution A. After 24 h of contact, the silver ion concentration in solution A were 0.370, 0.654, and 0.765 μg/mL at 21, 37, and 65°C, respectively, with the silver ion concentration at 65°C roughly double that at 21°C. This shows that the rate of silver ion release can be significantly elevated when temperature is increased. It is possible that at a higher temperature, the fibers can swell more as water penetrates inside the fiber. In addition, the ion exchange process can also be accelerated at an elevated temperature.

Figure 5 shows the silver ion concentrations at two different temperatures when the silver containing fibers were in contact with 2.9% aqueous protein solution. At the beginning of the process, the silver concentration was roughly same at 0.520 and 0.557 μg/mL respectively, at the two different contacting temperatures. As contacting periods extend, the silver concentration in the 21°C sample slowly rises but the rate of increase

TABLE I
The Antimicrobial Effect of Silver Containing Chitosan Fibers Against Three Common Bacteria

Type of bacteria	Bacteria concentration (cfu/mL)		Reduction in bacteria count (%)
	Control	Test sample	
Candida albicans	1.46×10^5	2005	98.63
Staphylococcus aureus	1.2×10^4	168	98.60
Pseudomonas pyocyanea	7.16×10^6	1	100

was very slow, with the silver concentration at 24 h at 0.773 μg/mL, representing an increase of about 48.6% over the concentration at 30 min. The silver concentration in the 37°C sample showed a more rapid rise over extended period of time, with the concentration at 24 h more than double the concentration at 30 min. This showed that when these silver containing chitosan fibers are used in wound dressings or underwear apparels, at body temperature, the silver ions in the chitosan fibers can be fairly easily released from the fiber, acting as an antimicrobial agent.

Figure 6 shows the silver ion concentrations when the silver containing chitosan fibers were in contact with aqueous solutions containing different amount of protein at 37°C. It is clear that the silver ion concentration in the contacting solution is directly proportional to the protein concentration. The effect of a high protein concentration is fairly obvious; with the silver ion concentration in the 5% protein solution about four times that of the 1% protein solution after 30 min.

The silver ions released from the silver containing chitosan fibers can act as an effective antimicrobial agent. Although chitosan is already well known for its antimicrobial properties, this is mostly due to the positive charge on the amine groups which controls bacteria growth by its ability to combine the negatively charged bacteria cells with the positive charge on the amine groups. Though this action can help limit bacteria growth, its action is bacteriastatic rather than bactericidal.

Table I shows the antimicrobial effect of the silver containing chitosan fibers against three common strains of bacteria. It is clear that with all three types of bacteria, the silver containing chitosan fibers were effective in reducing the bacteria count by more than 98%. In Table II, the antimicrobial effect of chitosan fibers and silver containing chitosan fibers were compared against *Candida albicans*. Under the same test conditions, the reduction in bacteria count for the chitosan fiber was 78.62%, while for the silver containing chitosan fiber, the reduction was 97.22%. This clearly demonstrates that the silver containing chitosan fiber is more effective in controlling bacteria growth than the chitosan fiber.

Figure 7 shows the clarity of solutions in the control sample and in suspension of *Escherichia coli* containing silver containing chitosan fiber, chitosan fiber, copper containing chitosan fiber, and zinc containing chitosan fiber, respectively. With the control sample, as bacteria grows and as the bacteria count in the solution rises, the solution became turbid in appearance. With the chitosan fiber and chitosan fiber containing antimicrobial metal ions, since the fibers can control bacteria growth, the solutions in contact with the fiber appear clear, indicating a low concentration of bacteria in these solutions.

TABLE II
The Antimicrobial Effect of Chitosan Fibers and Silver Containing Chitosan Fibers Against *Candida albicans*

Sample	Control	Chitosan fiber	Ag chitosan fiber
Bacteria concentration (cfu/mL)	5.4×10^3	1155	150
Reduction in bacteria count (%)	–	78.62	97.22

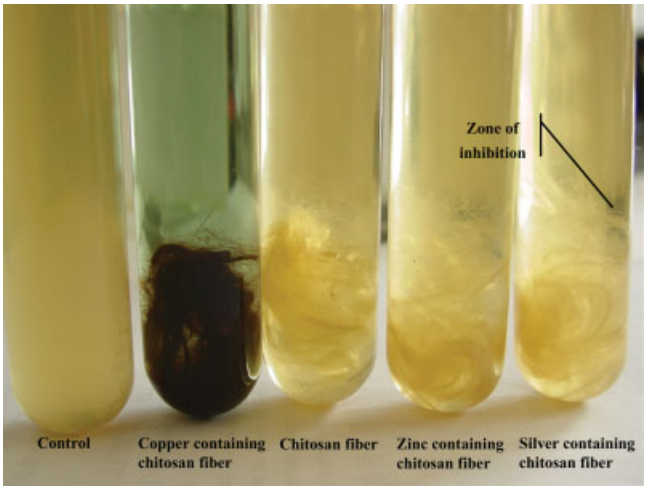

Figure 7 Clarity of solutions in the control sample and in suspension of *Escherichia coli* with copper containing chitosan fiber, chitosan fiber, zinc containing chitosan fiber, and silver containing chitosan fiber. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CONCLUSIONS

This study has shown that silver containing chitosan fibers can be made by blending silver containing AlphaSan RC5000 particles in the spinning dope. The AlphaSan RC5000 particles were found to be uniformly distributed inside the fiber, acting as the reservoir for the sustained release of silver ions. Experimental results showed that when the silver containing chitosan fibers were placed in contact with either solution A or aqueous protein solution, the silver ions can be released from the fiber through ion exchange and chelation, with chelation with protein molecules more effective in releasing the silver ions from the fiber. It was found that the silver containing chitosan fiber is more effective in controlling bacteria growth than the chitosan fiber.

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