Absorption and Release of Zinc and Copper Ions by Chitosan Fibers

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ABSTRACT: Chitosan fibers were treated with aqueous solutions of $ZnCl_2$ and $CuSO_4{\cdot}5H_2O$ to prepare zinc and copper containing fibers, respectively. Significant weight gains were obtained as the zinc and copper ions were absorbed onto the fibers through chelation with the primary amine groups. The fibers were then placed in contact with aqueous solutions containing NaCl and water soluble proteins, respectively, to assess the release of zinc and copper ions. Results showed that the release of zinc and copper ions were affected by the treatment temperature, time, and the composition of the contacting media. More metal ions were released when the fibers were in contact with aqueous protein solutions than in NaCl solution, indicating the binding abilities of the protein molecules for zinc and copper ions. The zinc and copper containing fibers were tested for their antimicrobial effects against several species of bacteria commonly found in wound and skin. Results showed that these metal containing chitosan fibers had much stronger antimicrobial properties than the original chitosan fiber. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 527–532, 2007

Key words: adsorption; biofibers; fibers; ion exchangers; polysaccharides

INTRODUCTION

It is well known that chitosan is a natural chelating polymer with excellent absorption capacities for copper, zinc, silver, and many other heavy metal ions.[1,2] As they have a large specific surface area, chitosan fibers are particularly effective in absorbing metal ions through the primary amine groups in its structure. A previous study has shown that it is possible to attach silver ions onto the chitosan fibers by a treatment of the fibers with an aqueous silver nitrate solution.[3] In another study, it was found that when they were fully treated with $ZnCl_2$ and $CuSO_4$, the chitosan fibers contained up to 6.2% and 9.0% zinc and copper ions, respectively.[4]

Many metal ions are useful for biomedical applications. Silver is known for its excellent antimicrobial effect, while zinc and copper ions are useful in wound and skin care. After evaluating the role of zinc in the overall human physiology, Schwart et al.[5] found that zinc plays an important role in skin health and function, with zinc deficiency causing moderate to severe dermatitis. Zinc-based enzymes and proteins that direct the process of skin generation is especially evident during wound healing and inflammation reduction, where the high needs for zinc can be supplemented externally through topical zinc delivery, for example, by the use of zinc paste bandage.[6,7]

In addition to zinc ions, Voruganti et al.[8] found that major burn wounds are associated with impaired Zn(II) as well as Cu(II) ions. Both metal ions are essential for bone matrix formation, linear growth, and wound healing. During the healing of burn wound, Zn(II) and Cu(II) concentrations exceeded plasma concentrations, suggesting that inflammatory wound exudate was a primary route for Zn(II) and Cu(II) loss, and at the same time, indicating the need to supplement Zn(II) and Cu(II) ions during the healing process for burn wounds. Malakyan et al.[9] determined the efficacy of a Cu(II) complex in facilitating recovery from burn injury. They found that treatment with Cu(II) complex produced effects consistent with a facilitation of Cu-dependent immune-mediated physiological inflammatory response to burn injury. It was concluded that treatment of burn injury with Cu(II) complex supports Cu-dependent physiological responses involved in overcoming burn injury. Canapp et al.[10] evaluated the effects of topical glycyl-L-histidyl-L-lysine tripeptide-copper complex (TCC) on healing in ischemic open wounds. A significant decrease in wound area was seen in the TCC group. On day 13, initial wound area had decreased by 64.5% in the TCC group, as compared with 28.2% in the control group.

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TABLE I
Preparation Conditions for Zinc Containing Chitosan Fibers

Sample no.	Dry weight of the original fiber (g)	Weight of $ZnCl_2$ (g)	Weight gain during the treatment (g)	Weight increase (%)
1	6.922	0.692	0.295	4.26
2	7.647	1.912	0.761	9.95
3	9.164	4.582	1.299	14.17
4	10.853	21.706	2.026	18.67

This article studied the absorption and release of zinc and copper ions by chitosan fibers. In view of the already well known wound healing properties of chitosan fibers, their combination with zinc and copper ions can generate further improvement in their performances, and at the same time, enhance their application as a novel biomaterial, especially in the area of wound and skin care.

EXPERIMENTAL

The chitosan fibers used in this work were of a commercial grade fiber from Haixiao (Qingdao, China). Elemental analysis results showed that this fiber had a carbon content of 38.72% and a nitrogen content of 6.962%. The ratio between nitrogen and carbon contents is 0.1798 and the degree of acetylation for the chitosan is 24.3%.

When preparing zinc containing chitosan fibers, the original fibers were first dried at 105°C for 4 h to obtain the dry weight before being treated with aqueous solutions containing different amounts of zinc chloride. The chitosan fiber and $ZnCl_2$ were placed in contact with 100 mL deionized water together with 1 g NaCl and conditioned at 40°C for 24 h. The fibers were then washed with deionized water and dried at 105°C until constant weight. Table I shows the preparation conditions for zinc containing chitosan fibers and the weight gain during the treatment.

When preparing copper containing chitosan fibers, the original fibers were first dried at 105°C for 4 h to obtain the dry weight before being treated with aqueous solutions containing different amount of $CuSO_4{\cdot}5H_2O$. The chitosan fiber and $CuSO_4{\cdot}5H_2O$ were placed in contact with 100 mL deionized water together with 1 g NaCl and conditioned at 40°C for 24 h. The fibers were then washed with deionized water and dried at 105°C until constant weight. Table II shows the preparation conditions for copper containing chitosan fibers and the weight gain during the treatment.

When testing the release of zinc and copper ions from the chitosan fibers, the fiber samples (sample 4 and sample D for zinc and copper, respectively) were first placed in contact with 40 times their own weight of either 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.[11] The protein used was water soluble soyabean protein. After conditioning at specified temperatures for different periods of time, 5 mL solution was taken out and diluted to 50 mL before the zinc and copper ion concentrations were tested by using atomic absorption spectrometer.

To assess the effect of zinc and copper ions on the antimicrobial properties of the chitosan fibers, to five test tubes each were added 10 mL of 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, copper containing chitosan fiber, zinc containing chitosan fiber, and silver containing chitosan fiber, respectively. The silver containing chitosan fiber was prepared under conditions reported in a previous study.[12] The test tubes were then placed in a 36°C water bath and shaken at a speed of 120 r/min for 12 to 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 five common strains of bacteria, i.e., *Candida albicans, Staphylococcus aureus,*

TABLE II
Preparation Conditions for Copper Containing Chitosan Fibers

Sample no.	Dry weight of the original fiber (g)	Weight of $CuSO_4{\cdot}5H_2O$ (g)	Weight gain during the treatment (g)	Weight increase (%)
A	9.522	1.904	0.942	9.89
B	7.423	3.712	2.226	29.99
C	8.931	8.931	3.699	41.42
D	11.112	33.336	5.203	46.82

Bacillus sabtilisin, Pseudomonas pyocyanea, and Escherichia coli. The bacteria were suspended in 0.5% peptone water with the bacteria concentration at about $1.5 \times 10^4 - 1.5 \times 10^5$ cfu/mL. Thirty-five milliliters of 0.5% peptone water was taken in 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 ± 0.002 g of sterilized fiber samples were added to 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. Around 0.1 mL of the bacteria containing solution 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, in cfu/mL and B is the average bacteria concentration in the test sample after shaking in cfu/mL.

RESULTS AND DISCUSSION

Because of the primary amine groups in the chitosan fibers, zinc and copper ions can be easily attached onto the fibers by a treatment of the fibers with aqueous solutions containing the respective ions. As can be seen in Tables I and II, when the chitosan fibers were in contact with aqueous solutions containing $ZnCl_2$ and $CuSO_4{\cdot}5H_2O$, significant weight gains were produced. This provides an easy method to produce zinc and copper containing fibers. By adjusting the ratio between the weight of chitosan fiber and those of $ZnCl_2$ and $CuSO_4{\cdot}5H_2O$, it is also easy to apply different amounts of zinc and copper ions to the fibers. A previous study has indicated that at saturated point of absorption, the fibers treated with $ZnCl_2$ and $CuSO_4$ contained 6.2% and 9.0% by weight of zinc and copper ions, respectively.[4]

To study the release of zinc and copper ions from the fibers, they were placed in contact with an aqueous solution at different test temperatures and over different periods of time. When testing the release of metal ions, it is important to point out that body fluid has a complex composition and the various components have different binding abilities to zinc and copper ions, and hence the choice of contacting media is important. In a study of the composition of serous fluid formed after axillary dissection, Bonnema et al.[13] found that on the first postoperative day, the drainage fluid contained blood contents and a high concentration of creatine phosphokinase. After day 1 it changed to a peripheral lymph-like fluid that contained different cells and more protein. Frohm et al.[14] analyzed the fluid from a postoperative wound, six leg ulcers, and a large blister. They found that wound fluid contains fragments of peptides. Trengrove et al.[15] found that wound fluid collected from leg ulcers contained 0.6–5.9 mmol/L glucose and 26–51 g/L protein. James and Taylor[16] showed that a typical wound fluid contained 2.9% protein.

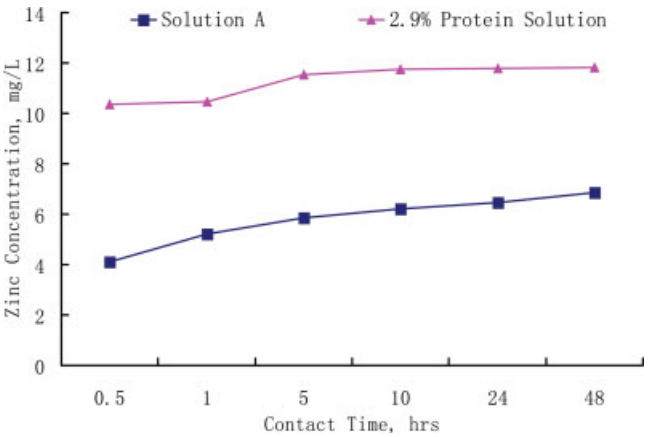

Figure 1 Effect of contact time on the release of zinc ions in solution A and 2.9% aqueous protein solution at 37.5°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

This study used solution A and aqueous solutions with different protein contents as the contact media. Solution A was used because the zinc and copper ions can be released from the fibers through ion exchange, while protein was used to assess the effect of chelation by the protein molecules. Figure 1 shows the effect of contact time on the release of zinc ions in solution A and 2.9% aqueous protein solution at 37.5°C. It can be seen that the amount released from the fibers is considerably higher in the protein solution than in solution A, indicating the importance of chelation by the protein molecules. It is also important to note that the release of zinc ions is a relatively slow process, with the zinc ion concentration rising from 4.1 mg/L at 30 min to 6.2 mg/L after 10 h. In the protein solution, the ion concentration stabilized much quickly than in solution A. The zinc concentration reached 10.4 mg/L after 30 min, as compared with 11.8 mg/L after 48 h.

Table III shows the effect of temperature on zinc release in 2.9% aqueous protein solution. After 30 min, the Zn(II) concentration was 7.4 mg/L at room temperature, while it increased to 10.4 mg/L at 37.5°C, representing an increase of 39.1%. After contacting for 10 h, the respective concentrations were 9.9 and 11.8 mg/L, with the concentration at 37.5°C showing an increase of 19.1% over that at room temperature. This shows that high temperature can speed

TABLE III
Effect of Temperature on Zinc Release in 2.9% Aqueous Protein Solution

	Zn(II) concentration in the contact solution (mg/L)	
Time (h)	25°C	37.5°C
0.5	7.4	10.4
1	8.1	10.4
5	9.0	11.6
10	9.9	11.8

up the release of zinc ions from the zinc containing chitosan fibers.

Table IV shows the release of zinc ions in two aqueous solutions at 37.5°C with different protein contents. After 30 min, the 1.0% aqueous protein solution contained 5.7 mg/L zinc ions, while in the 2.9% aqueous protein solution, the concentration was 10.4 mg/L. This significant difference is an indication that the metal ion binding sites on the protein molecules contributes significantly to the release of zinc ion from zinc containing chitosan fibers. The ratio between Zn(II) concentration in the 2.9% and 1.0% protein solution varied within a small range, between 1.49 and 1.83, in the different contact times, further indicating the relationship between ion binding sites on the protein molecules to the release of zinc ions from the fibers.

In clinical practice, zinc oxide is a common component of wound dressings.[17] Zinc oxide particles are virtually insoluble in water, but their solubility is significantly increased when in contact with proteinaceous wound fluid, when zinc ions are liberated continuously into the wound.[18] In this form, topical zinc oxide has been found to aid treatment of leg and pressure ulcers.[19,20] The zinc ions can generate immunomodulatory and antimicrobial effects, as well as activation of matrix metalloproteinases that facilitate autodebridement and keratinocyte migration.[21] Since the zinc containing chitosan fibers can liberate zinc ions when in contact with protein, they can offer an effective route for delivering zinc to wounds.

Figure 2 shows the effect of time on copper ion release in solution A and 2.9% aqueous protein at 37.5°C. With the copper containing chitosan fibers, the release of copper ions was low in solution A. However, the addition of protein into the contacting solution had a significant effect. In the 2.9% aqueous protein solution, the Cu(II) ion concentration was 6.4 mg/L after 30 min; it rose to 15.2 mg/L after 24 h, which compares to 5.4 mg/L in solution A at the same time. When compared with similar results shown in Figure 1, it can be seen that the protein component in the contacting solution had a greater effect on Cu(II) release than on Zn(II), indicating the effect of the strong binding between Cu(II) ions and the amino acid groups on the protein molecules.

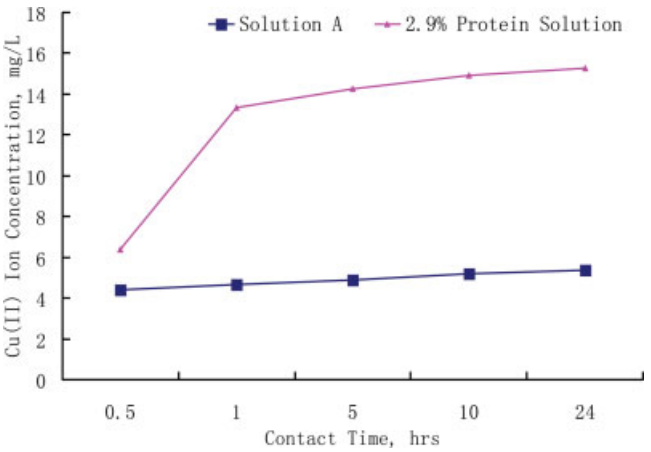

Figure 2 Effect of time on copper ion release in solution A and aqueous protein at 37.5°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table V shows the effect of temperature on Cu(II) ion release in 2.9 and 10% aqueous protein solutions. It can be seen that both the contact temperature and time had a significant effect on the release of Cu(II) ions. At 25°C and when protein concentration was 2.9%, the Cu(II) concentration slowly rose from 3.3 mg/L at 30 min to 15 mg/L at 24 h. When the contact temperature was 37.5°C and protein concentration was 10%, the Cu(II) concentration reached 15.5 mg/L after only 30 min.

TABLE IV
Release of Zinc Ions in Two Different Protein Solutions at 37.5°C

Time (h)	Zn(II) concentration in the contact solution (mg/L)		Ratio between Zn(II) concentration in 2.9% and 1.0% protein solution
	1.0% protein	2.9% protein	
0.5	5.7	10.4	1.83
1	6.5	10.4	1.60
5	7.1	11.6	1.62
10	7.9	11.8	1.49

TABLE V
Effect of Temperature on Cu(II) Ion Release in 2.9% and 10% Aqueous Protein Solutions

	Cu(II) ion concentration (mg/L)			
	25°C		37.5°C	
Time (h)	2.9% protein solution	10% protein solution	2.9% protein solution	10% protein solution
0.5	3.3	6.2	6.4	15.5
1	8.2	8.2	13.4	19.0
5	9.5	12.2	14.3	25.8
10	10.4	16.1	14.9	34.8
24	15.0	20.4	15.2	40.0

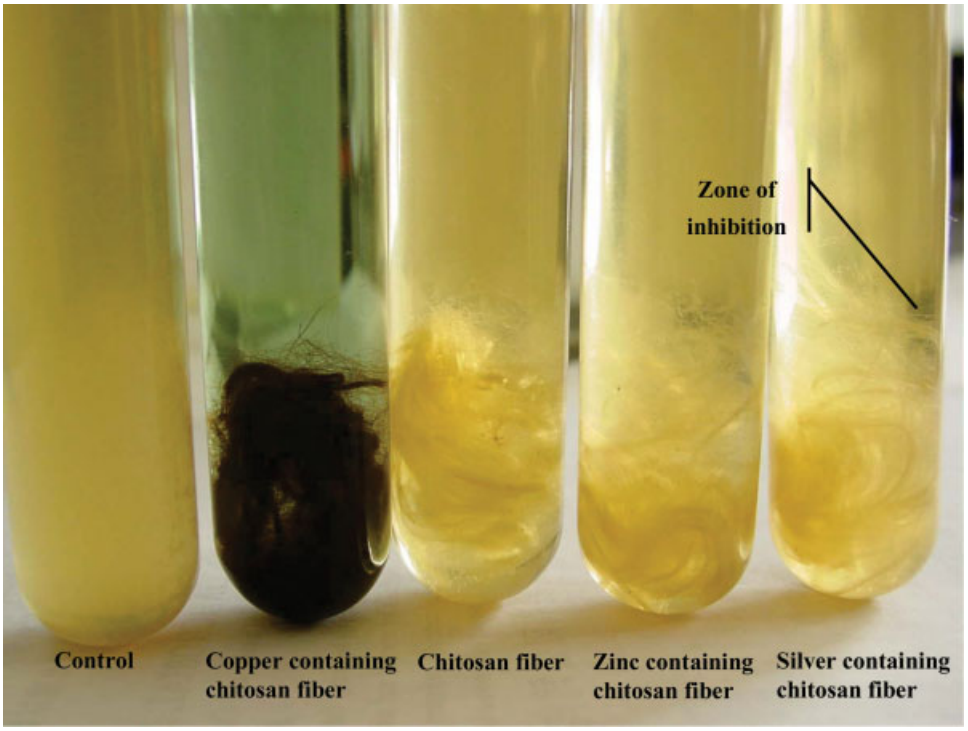

Figure 3 Clarity of solutions in the control sample and in suspension of *Escherichia coli* with copper containing chitosan fiber, chitosan fiber, zinc containing chitosan fiber, and silver containing chitosan fiber. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

As been mentioned before, both copper and zinc ions are useful in wound and skin care. Copper is an element essential to a number of physiologic processes in the human body. The presence of copper in living tissues has been known for more than 200 years and early medicinal applications of copper typically involved treatment of painful joints and muscles using copper bracelets, or copper-containing ointments.[22,23] As less than 1 mg of copper is available in the typical daily American diet, supplement of copper ions by novel materials, such as the present copper containing chitosan fibers could be useful.

Figure 3 shows the clarity of solutions in the control sample and in suspension of *Escherichia coli* with chitosan fiber, copper containing chitosan fiber, zinc containing chitosan fiber, and silver containing chitosan fiber. All the three metal containing chitosan fibers had visibly clear solution around the fiber, indicating their potent antimicrobial effect. The whole solution in contact with the copper containing fiber was clear, indicating that the large amount of copper ions released from the fiber had suppressed bacteria growth.

Table VI showed the antimicrobial effect against *Candida albicans* by different types of chitosan fibers. While the original chitosan produced a reduction in bacteria count of 78.6%, the respective figures for the copper and zinc containing fibers were 96.2% and 97.7%.

Table VII shows the effect of zinc containing chitosan fiber on the antimicrobial performances against five different species of bacteria. In all cases, the reduction in bacteria count was larger than 98%. This shows that in addition to being able to deliver zinc ions, the zinc containing chitosan fiber is also an excellent broad spectrum antimicrobial material.

TABLE VI
Antimicrobial Effect Against *Candida albicans* by Different Types of Chitosan Fibers

Samples	Bacteria count (cfu/mL)	Reduction (%)
Control	5.4×10^3	N/A
Chitosan fiber	1155	78.6
Cu(II) chitosan fiber	208	96.2
Zn(II) chitosan fiber	123	97.7

TABLE VII
Effect of Zinc Containing Chitosan Fiber on the Antimicrobial Performances Against Five Different Species of Bacteria

Type of bacteria	Bacteria count (cfu/mL)		Reduction (%)
	Control	Zinc chitosan	
Candida albicans	2.82×10^5	71	99.97
Staphylococcus aureus	1.84×10^5	0	100
Bacillus sabtilisin	1×10^4	40	99.60
Pseudomonas pyocyanea	9×10^3	150	98.33
Escherichia coli	5.0×10^5	19	99.96

CONCLUSIONS

This study has shown that chitosan fibers are an excellent carrier materials for zinc and copper ions. These two metal ions can be easily attached to the chitosan fibers by a treatment of the fiber with aqueous solutions containing the respective metal ions. The zinc and copper ions can be released from the fibers when they are in contact with aqueous solutions containing either NaCl and protein, indicating the fact that these metal ions can be released from the fibers when they are used in wound and skin care applications. The protein component in the contacting solution can greatly enhance the release of zinc and copper ions. It was shown that in addition to being able to deliver zinc and copper ions in clinical circumstances, the zinc and copper containing chitosan fiber have excellent antimicrobial properties.

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