Copper (II) Ions and Copper Nanoparticles-Loaded Chemically Modified Cotton Cellulose Fibers with Fair Antibacterial Properties

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ABSTRACT: This work describes the release of copper(II) ions from cellulose fibers, which have been chemically modified by periodate-induced oxidation of cellulose, followed by covalent attachment of biopolymer chitosan. The release of copper(II) ions has been investigated in physiological fluid (PF) and protein solution (PS) both at 37°C. Fibers have demonstrated excellent antibacterial activity against *E. coli*. Finally, their borohydride-induced reduction has yielded copper nanoparticle-loaded fibers, with average diameter of particles, nearly 28.94 nm. The formation of copper nanoparticles has been established by surface plasmon resonance and FTIR spectroscopy. These fibers also show fair biocidal action against *E. coli*. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 757–766, 2009

Key words: fibers; mechanical property; TEM; FTIR; *E. coli*; antibacterial activity

INTRODUCTION

Recently, there has been a growing interest to develop metal nanoparticle-loaded fibers that could be used in a number of applications such as in medical devices, wound dressings, healthcare (including disposables), personal care products, veterinary, military and biodefence, protective suits, clothings. etc.[1–8] Fibers, made from natural sources, especially polysaccharides, have been considered as the most promising materials because of their excellent biocompatibility, nontoxicity, and potential bioactivity. These include alginates,[9] chitosan,[10] cellulose,[11] etc. However, these biopolymers have their own advantages and disadvantages, which must be taken into account while using them for the preparation of metal nanoparticle-loaded antibacterial materials. For example, products made from pure chitosan fibers have not been commercially viable because of the high processing cost involved (deproteination, demineralization, and deacetylation processes are required to produce chitosan materials of adequate purity), and the availability of such purified material is still insufficient for the production on large scale. Poor textile processing properties of resulting fibers has also been a major problem.[12] However, the antibacterial nature of chitosan and its capability to immobilize metal ions at its amine functionality are attractive features. Similarly, cotton cellulose fibers have been considered as a biocompatible, nontoxic, and easily available material with fair mechanical strength, durability, and cost effectiveness.[13] However, lack of ion-exchange capability of cellulose fibers has been recognized as a major drawback of this potential biopolymer.[14]

It is well known that metal nanoparticles of silver, zinc oxide, and titanium oxide have been exploited frequently as antibacterial agents in fibers/fabrics. Recently, Chen and Chiang[15] have grafted chelating monomer, glycidyl methacrylate-iminodiacetic acid (GMA-IDA) onto cotton fabric, and incorporated silver nanoparticles into grafted network. The size of the silver nanoparticles was found to be around 75 nm, as confirmed by TEM analysis. The nanosilver-loaded fabrics demonstrated excellent antibacterial property against *E. coli*. Similarly, Gupta et al.[16] have prepared poly(acrylamide-*co*-itaconic acid)-grafted cotton fabric and introduced silver nanoparticles by equilibration in silver nitrate solution, followed by borohydride reduction. The fabric showed fair antibacterial action against bacteria such as *E. coli* and also fungi like *Aspergillus* and *Fugerium*. In fact, silver has been recognized as an antibacterial agent since ancient time. Recently, Singh et al.[17] have reviewed wide spectral applications of silver nanoparticles. In another work,

Yadav et al.[18] have reported a simple method to prepare nano ZnO and coated the same on cotton fabric to impart UV-radiation blocking properties. The nano ZnO (2%)-coated cotton fabric absorbed nearly 75% of the incident UV radiation. The air permeability of ZnO-coated fabric was found to be higher than plain fabric. Apart from UV-blocking properties, ZnO nanoparticles also show fair biocidal action. Recently, Vigneshwaran et al.[19] have impregnated cotton fabric with zinc oxide-soluble starch nanocomposites and investigated biocidal action of resulting fabrics against two representative bacteria, *Staphylococcus aureus* and *Klebsiella*. The fabric showed excellent antibacterial action.

Copper, in its different forms, has also been used by ancient civilization to treat people stricken with afflictions and to maintain hygiene.[20] Recently, Zhang et al.[21] have introduced copper into medical polymer, polyethylene (PE), by means of copper plasma immersion ion implantation (CPIII) technique and investigated its antimicrobial properties. Copper nanoparticles-embedded poly(vinyl methylketone) films have also been investigated for their biocidal action against growth eukaryote and prokaryote target microorganisms.[22] In a work by the same group of workers, copper-fluoropolymer (Cu-CFX) nanocomposite films have shown strong inhibitory action against growth of *E. coli* and *Lysteria*.[23] Apart from polymer-based antibacterial films, various synthetic and natural polymers, in the form of fibers, have also been developed with antibacterial properties.

Similarly, Gabbay et al.[24] have reported that impregnation or coating of cotton and polyster fibers with cationic copper endows them with potent broad-spectrum antibacterial, antiviral, antifungal, and antimite properties. The biocidal properties of fabrics containing 3–10% copper-impregnated fibers are permanent and not affected by extreme washing conditions and do not interfere with the manipulation of the final products (e.g., color, press).

Most recently, Tao et al.[25] have reported antibacterial properties of nanocopper/polyacrylonitrile composite fibers against *E. coli*, *Bacillus subtilis*, *S. aureus*. The average efficiency of antibacterial properties was more than 90% within 15 h. The mechanical properties of nanocopper/polyacrylonitrile composite fibers were found to be superior to copper/polyacrylonitrile fibers.

Cu(II) is reported to play a key role in collagen crosslinking, thus aiding in the normal formation of bone matrix.[26] Because burn injuries are associated with reduced bone formation and resorption in both adult and children,[27] the copper ions may be expected to play a dual role in the healing of burn injuries, i.e., preventing the wound from infection and helping in the formation of bone matrix. Copper functions as a cofactor of several cellular enzymes that are involved in free radical scavenging, electron transport system, pigmentation, and elastin and collagen crosslinking. Adequate status of this mineral may be particularly difficult to monitor in children with burns because of the rapid growth and hormonal changes. Malakyan et al.[28] determined the efficacy of 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. Vorunganti et al.[29] evaluated the status of Cu in burned children and assessed adequacy of supplementation.

Therefore, to develop an ideal fiber with fair ion binding and releasing properties, mechanical strength, easy processibility, excellent biocompatibilty, and excellent commercially production feasibility, we hereby propose a novel cellulose-based fiber, obtained by periodate-induced oxidation of cotton cellulose fibers to give dialdehyde cellulose, followed by covalent attachment of $-NH_2$ group of chitosan through coupling reaction. This novel chitosan-bound cellulose (CBC) fiber has been used for the immobilization of Cu(II) ions and copper nanoparticles to obtain fair antibacterial property against the model bacteria *E. coli*.

EXPERIMENTAL

Materials

Chitin was deacetylated in 50 wt % NaOH at 90°C in nitrogen atmosphere for 2 h.[30] The final chitosan flakes were washed three times with deionized water (Millipore Milli-Q) and dried at 50°C in a vacuum. The physical properties of chitosan flakes such as degree of deacetylation and molar mass were determined. The former was obtained to be 94 mol % following the method of Guibal et al.[31] The molar mass was found to be 1.42×10^6 using the well-known Mark–Houwink equation and viscosity data of solutions containing different amounts of chitosan in 0.1 mol/L acetic acid and 0.2 mol/L NaCl.[32] Anhydrous cuprous sulfate, potassium hexacyanoferrate, nutrient agar–agar type-1, and nutrient broth were obtained from Hi Media Labroratories (Mumbai, India). Sodium borohydride and soya protein were obtained from SRL (Mumbai, India). Cotton fibers were gifted by a local textile mill. Double distilled water was used for the investigations.

Preparation of oxidized cellulose fibers

The method given by Varavinit et al.[33] was followed with slight modifications. 2.0 g of prewashed and dried cotton fiber was soaked in 500 mL of 0.03M periodic acid for 2 h, and then the pH of the

solution was adjusted to 3.0, followed by heating in water bath at 70°C under constant stirring for 15 h. The fibers were taken out and washed three times with distilled water before drying.

Covalent attachment of chitosan

The sample (1.0 g) of cellulose dialdehyde fibers, prepared as earlier, was immersed in 30 mL of 1% chitosan solution prepared in 2% acetic acid (w/v) solution and allowed to react for a period of 1 h at 50°C. Then the fibers were removed and washed with 750 mL of distilled water, and then dried in oven at 40°C till they attained constant weight. These chitosan-attached cellulose fiber shall be designated as chitosan-attached cellulose (CAC) fibers.

Immobilization of Cu(II) onto CAC fibers

A known quantity of CAC fibers was placed in Cu(II) solution of known concentration for 4 h at 30°C. Now the fibers were washed with distilled water and then dried in a dust-free chamber. These fibers shall be named as "copper-bound chitosan-attached cellulose" and designated as CBCAC(X), where X in parenthesis denotes the percent concentration of Cu(II) solution used for the immobilization of copper ions. To establish the superiority of the proposed CBCAC fibers, we also prepared copper-loaded cellulose (CLC) fibers by immersing cellulose fibers into Cu(II) aqueous solutions.

Preparation of nanocopper-loaded CAC fibers

Copper nanoparticles were produced within the CAC fibers by borohydride-induced reduction of Cu(II).[34] Preweighed quantity of CLCAC fibers was immersed in 100 mL of 5.6 mM sodium borohydride solution for a period of 24 h. The resulting copper nanoparticles-loaded fibers were allowed to dry in vacuum chamber at 50°C. Figure 1 depicts plain cotton cellulose fibers, copper(II)-bound CAC fibers, and nanocopper-loaded CAC fibers.

FTIR spectral analysis

FTIR spectra of plain cotton cellulose, CAC, and CBCAC fibers were recorded with Shimadzu spectrophotometer (UV 1700) using KBr-mixed disc/pellet.

DSC thermal analysis

DSC analysis of fibers was performed with a Mettler DSC-30 thermal analyzer with plain and grafted fibers of known weight in sealed aluminum pans. The samples were heated from 40 to 260°C at the heating rate of 20°C/min under the constant flow of Argon gas.

TEM analysis

The size of the copper nanoparticles was determined using a Technai F12 TEM instrument. TEM samples were prepared by dispersing 2–3 drops of solution obtained by adding nanocopper-loaded fibers in distilled water for 24 h, on a copper grid and drying at room temperature.

Mechanical strength analysis

The stress–strain curve of CBC and copper-loaded CAC and nanocopper-loaded CAC fibers were obtained at room temperature on LR 100K machine. Diameter and length of fibers were 0.5 and 60 mm,
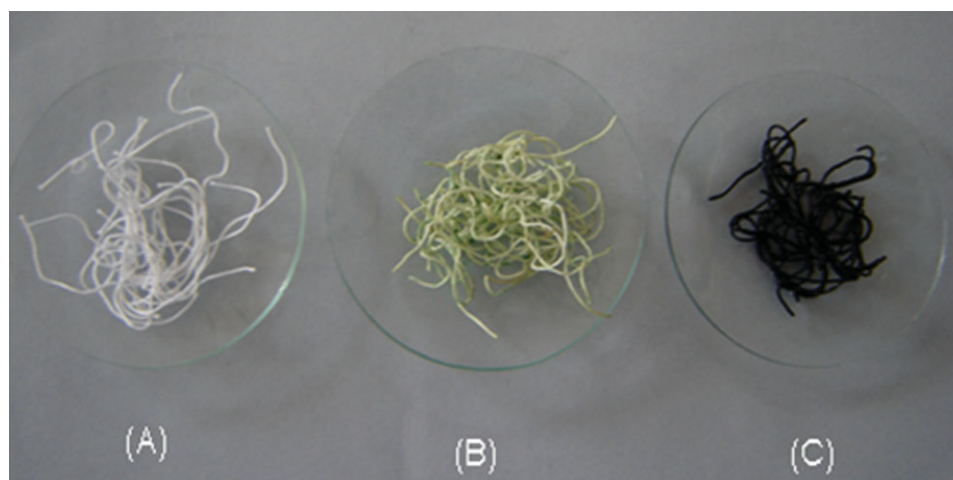
Figure 1 Photograph showing (A) plain cotton cellulose fibers, (B) copper-bound chitosan-attached cellulose (CBCAC) fibers, and (C) nanocopper-loaded chitosan-attached cotton (NCLCAC) fibers. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
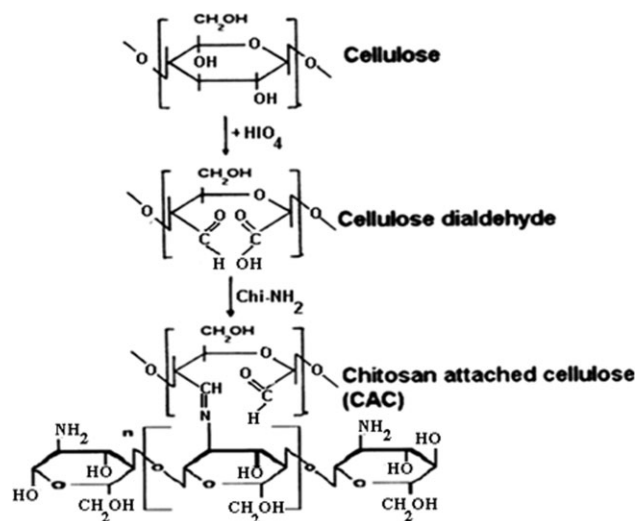
Figure 2 Scheme showing the formation of chitosan-attached cellulose (CAC) fibers.

respectively, and they were tested at a stretching speed of 300 mm/min.

Cu(II) release studies

To investigate the release of Cu(II)ions from the CBCAC fibers, samples of known weight were placed in contact with 40 times their own weight of physiological fluid (PF), composed of 142 mM of sodium chloride and 2.5 mM of calcium chloride, thus representing the typical ion concentration of body fluid as specified by the British Pharmacopoeia.[35] Moreover, the Cu(II)ions release was also studied in the 2.9% (w/v) aqueous solution of water-soluble protein soyabean as suggested by James et al.[36] The estimation of Cu(II) released was done spectrophotometrically.[37]

Antibacterial study of fibers

The antibacterial activities of the fibers were tested qualitatively and quantitatively by an inhibition zone method and a viable cell count method, respectively. In both the methods, the model bacteria were *E. coli*.[38] For qualitative measurement of the antibacterial activity, the CBCAC fibers were cut in small pieces, put together to form a circular zone, and the antimicrobial activity was tested using modified agar diffusion assay (disc test). The plates were examined for possible clear zone after incubation at 30°C for 2 days. The presence of any clear zone around fibers on the plates was recorded as an inhibition against the microbial species. To examine the bacterial growth or killing kinetics in the presence of CBCAC fibers, *E. coli* cells were grown in 100 mL of nutrient broth (NB) supplemented with preweighed fibers at 37°C with continuous stirring. The cylindrical sample containers were placed horizontally on

an orbital shaker platform and agitated at 200 rpm. Growth or killing rates and bacterial concentration were determined by measuring the OD at 610 nm. The OD values were converted into concentrations of *E. coli* (CFU per milliliters) using the approximation of 10^8 cells per milliliter.[39]

EXPERIMENTAL RESULTS

### Preparation of Cu(II)-bound chitosan-attached cellulose fibers

Cellulose, with a large amount of hydroxyl groups, has been widely employed as a substrate for graft copolymerization of vinyl monomers[40,41] and for the immobilization of macromolecules such as enzymes.[33] In this study, the covalent attachment of chitosan to the cellulose has been carried out by periodic acid-induced oxidation of cellulose to dialdehyde followed by coupling reaction between amino group of chitosan and aldehyde group of oxidized cellulose as shown in the Figure 2. The CAC fibers, so produced, were used to immobilize Cu(II) at nitrogen atom present in amine groups of chitosan chains. However, the binding of Cu(II) at oxygen atom of —OH groups of cellulose chains is also to be taken into consideration. In a novel work, Lee et al.[39] suggested that —OH groups present in cellulose network in cotton fabric act as binding sites for immobilization of silver ions. Therefore, their observation may equally hold good in this study also.

FTIR spectral analysis

The FTIR spectra of plain cotton cellulose fibers, chitosan-attached cellulose fibers, and CBCAC fibers are shown in Figure 3. In Figure 3(A), a broad peak corresponding to —OH group of cellulosic cotton fiber is observed in the range 3600–3200 cm^{-1} and peak of —CO carbonyl group is also observed at
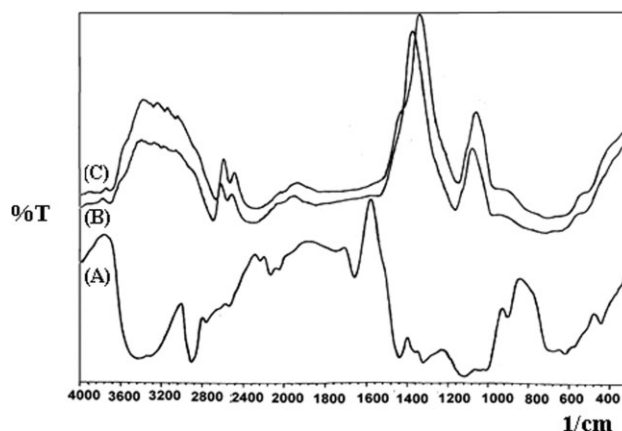
Figure 3 FTIR spectra of (A) plain cotton cellulose fibers, (B) chitosan-attached cellulose (CAC) fibers, (C) copper-bound chitosan-attached cellulose (CBCAC) fibers.
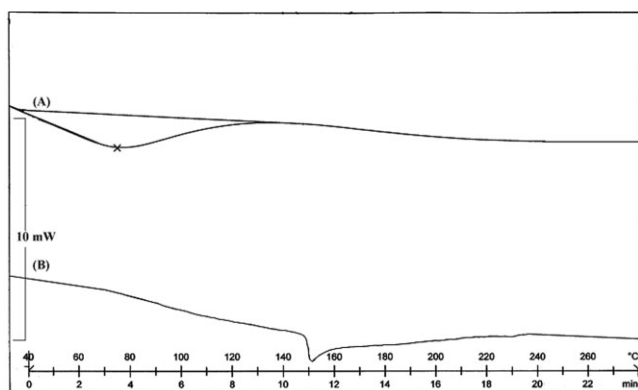
**Figure 4** DSC thermal analysis of (A) plain and (B) nano-copper-loaded fibers.

1666 cm^{-1}. The asymmetric C—H stretching of cellulose is obtained in the range 3000–2800 cm^{-1}, whereas the symmetric C—H stretching is obtained in the range 2500–2000 cm^{-1}.

Figure 3(B) shows the formation of intermolecular hydrogen bond between —NH$_2$ group of chitosan and —OH group of cellulosic cotton fibers in the range 3000–3500 cm^{-1}.

Figure 3(C) shows the crosslinking process of Cu(II) ions with —NH$_2$ group of chitosan at 3300 cm^{-1} because of the formation of C=N, and this is because of the reaction of imines between amino group from chitosan and copper(II) ions.

DSC analysis

Figure 4 displays DSC thermogram for plain (A) and copper nanoparticles-loaded fibers (B). It is clear that glass transition temperature T_g for these samples are nearly 70 and 150°C, respectively, thus confirming the thermal stability of fibers because of the coating of copper–nanoparticles-containing alginate.
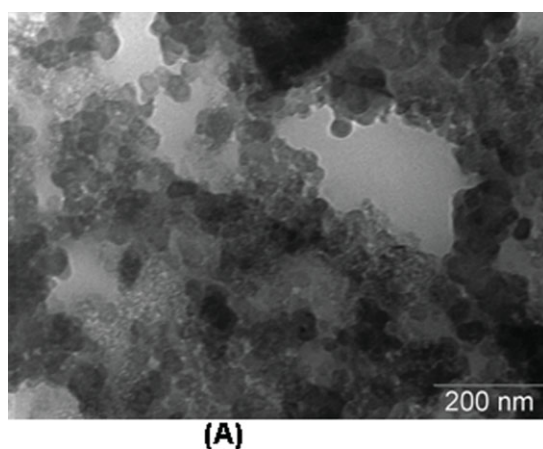
TEM analysis of NCLCAC fibers

The result of the TEM analysis, as shown in Figure 5(A), clearly indicates that particles are almost monodisperse in nature. The almost uniform sizes of copper nanoparticles may probably be attributed to the fact that nitrogen atoms of —NH$_2$ groups of chitosan and O atoms of hydroxyl groups of cellulose act as binding sites for immobilization of Cu(II) ions. Hence, on the reduction of copper(II)-loaded CAC fibers, these functionalities act as active sites for the formation of copper nanaoparticles. We have also carried out particle-size distribution analysis selecting copper nanoparticles from different arbitrarily chosen areas of TEM image [see Fig. 5(B)]. Based on the distribution curve, the average diameter of the copper nanoparticles was found to be 28.9 nm.

UV–visible spectral analysis

Furthermore, to confirm the formation of copper nanoparticles–nanocopper-loaded chitosan-attached cellulose (NCLCAC) fibers, we carried out UV–visible absorption studies. In Figure 6, a strong characteristic absorption peak around 567 nm is noted for the copper nanoparticles in NCLCAC fibers because of the surface plasmon resonance effect. However, Cu(II)-bound CAC fiber did not show any such peak.

Mechanical strength of fibers

The results of the mechanical strength (i.e., tensile strength) analysis has been well depicted in Figure 7. The tensile strength of CAC, CBCAC, and NCLCAC fibers was found to be approximately 30.58, 59.77, 38.88 MPa, respectively. It is quite clear that CBCAC fibers possess more tensile strength than plain CAC fibers, which is obviously due to the presence of Cu(II) ions that bind firmly with the N atoms of amino group of chitosan chains in CBCAC fibers.
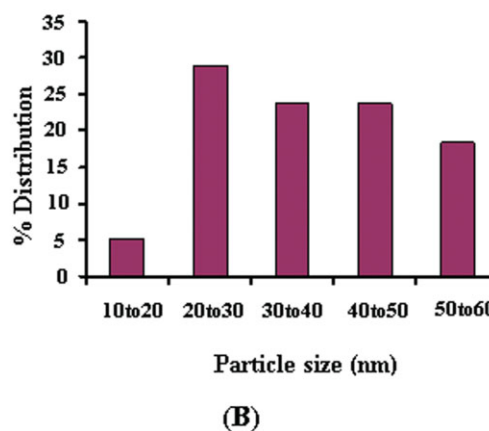
Figure 5 TEM image of copper nanoparticles. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
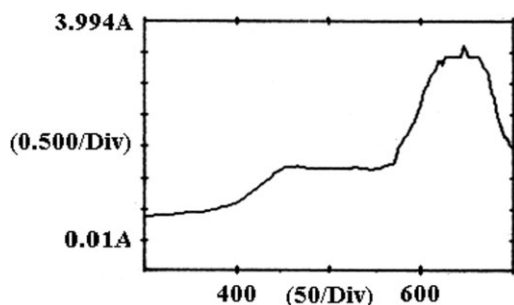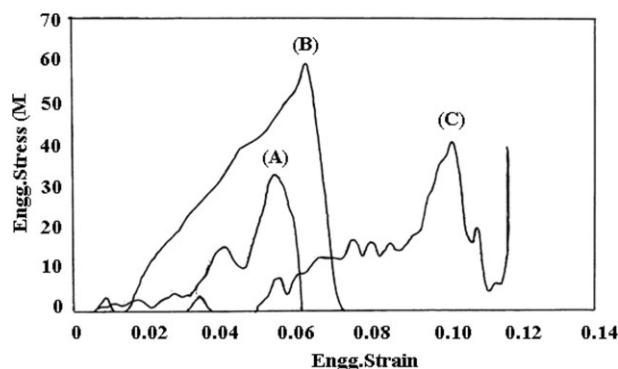
Figure 6 Surface plasmon resonance (SPR) for copper nanoparticles.

Figure 8 Dynamic release of Cu(II) ions from CBCAC (4) and CLC (4) fibers in the physiological fluid at 37°C.
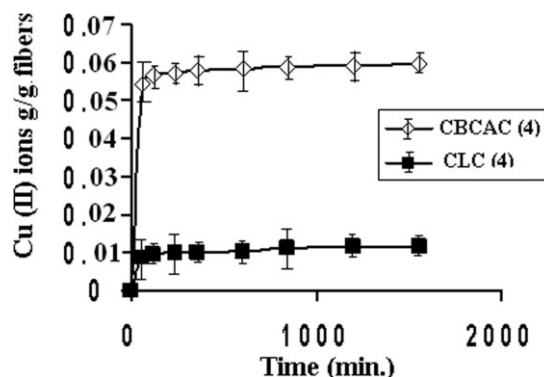
Thus, there is almost twofold increase in tensile strength because of the binding of Cu(II) ions. However, after carrying out borohydride-induced reduction of Cu(II) ions, the tensile strength of resulting NCLCAC fibers decrease to 38.88 MPa, which may be attributed to the fact that the reduction of Cu(II) to Cu^0, their binding with chitosan chains and with —OH group of cellulose networks decrease, thus resulting in less mechanical strength.

Dynamic release of Cu(II) from CBCAC composite fibers

While investigating the release of metal ions, it must be noted that body fluid has a complex composition and the various components have different binding abilities to copper ions, and therefore the choice of release media is important. In a study of the composition of serum fluid formed after auxiliary dissection, Bonnema et al.[42] found that on the first postoperation day, the drainage fluid contained blood contents and a high concentration of creatine phosphokinase. After Day 1, it changed to peripheral lymph-like fluid that contained different cells and more proteins. Trengrove et al.[43] found that wound fluid, collected

from leg ulcers, contained 0.6–5.9 mM/L glucose and 26–51 g/L protein. Similarly, Froahm et al.[44] analyzed the fluid from a postoperative wound, leg ulcers, and leg blisters. They found that the fluid contained fragments of peptide. Looking to the variation in various wound fluids composition, we decided to carry out our *in vitro* study in the PF, as suggested by British Pharmacopoeia, which contained 142 mM of NaCl and 2.5 mM of $CaCl_2$.

The results of the release experiments carried out with copper-loaded cellulose fibers, i.e., CLC (4) and CBCAC (4) fibers in the PF, are shown in Figure 8. It is quite clear that CBCAC (4) fibers demonstrate higher release when compared with CLC (4) fibers. This may be explained on the basis of the fact that CBCAC (4) fibers have higher copper (II) loading because of the binding of Cu(II) ions with amino group of chitosan chains and also with —OH group of cellulose network. Therefore, these fibers, when placed in the PF, exhibit higher release owing to ion-
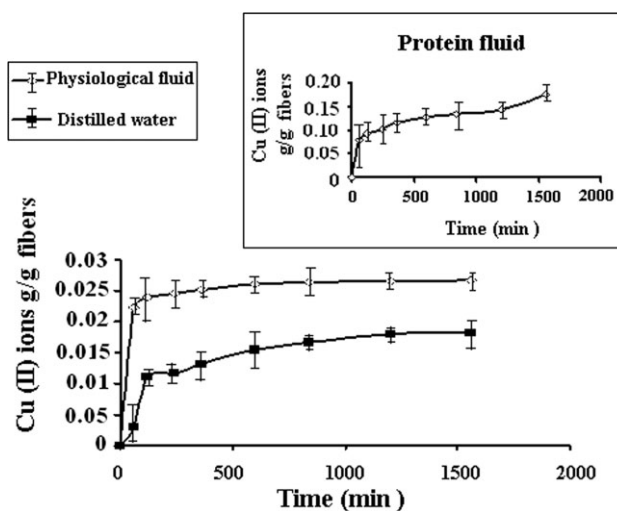

Figure 7 A comparative depiction of tensile strength of (A) chitosan-attached cellulose (CAC) fibers, (B) copper-bound chitosan-attached cellulose (CBCAC) fibers, (C) nanocopper-loaded chitosan-attached cellulose (NCLCAC) fibers.
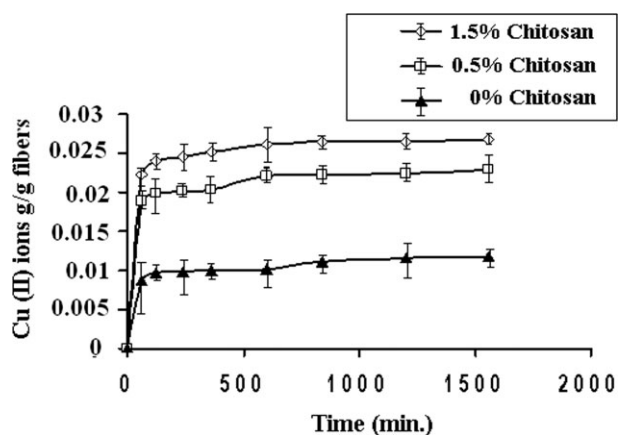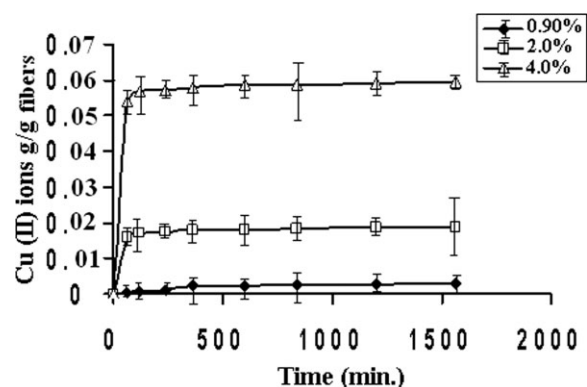

Figure 9 A comparative depiction of Cu(II) release profiles obtained for CBCAC (4) fibers in distilled water and physiological fluid at 37°C.

Figure 10 Dynamic release of Cu(II) from CBCAC (4) fibers containing distilled amounts of bound chitosan.

Figure 11 A comparative depiction of release profiles obtained for CBCAC fibers prepared by the immersion in Cu(II) solutions of different concentrations.

exchange process between the Cu(II) ions bound with amino groups of chitosan and Ca(II) ions present in the release medium. This ion-exchange process seems to be mainly responsible for higher release observed. In addition, the Cu(II) ions bound to the —OH groups of cellulose chains are also released. On the other hand, copper-loaded cellulose (CLC) fibers do not contain chitosan and hence Cu(II) loading is poor and it is only due to the attachment of Cu(II) with —OH groups of cellulose network of fibers. Therefore, copper is released in small quantity. In this way, it may be concluded that CBCAC (4) fibers demonstrate higher release of Cu(II) ions when compared with the CLC (4) fibers. Therefore, covalent attachment of chitosan chains to cellulose results in composite fibers with higher loading and release of Cu(II) ions.

As mentioned earlier, the major driving force for the observed release from CBCAC (4) fibers in the PF is perhaps the "ion-exchange process" taking place between Cu(II) ions bound with amino residue of chi-

tosan chains and external Ca(II) ions present in the PF. To confirm this, we compared the release profiles obtained for CBCAC (4) fibers in the PF and distilled water at 37°C (see Fig. 9). It is clear that fibers demonstrate slower release in water when compared with PF, which may be explained by the fact that distilled water does not contain any exchangeable ions and therefore Cu(II) ions bound with chitosan chains in the fibers are not released. On the other hand, the ion-exchange process between Cu(II) and Ca(II) ions in PF enhances the release of Cu(II) ions and so a higher release is observed in the PF. Therefore, it can be claimed that CBCAC (4) fibers demonstrate faster release in PF, mainly due to ion-exchange process. Finally, we also studied the copper release from CBCAC (4) fibers in the protein solution (PS) consisting of 2.9% soyabean in aqueous medium (see inset). It was observed that maximum release took place in the PS, which may be attributed to the fact that copper possesses very strong tendency to bind with the protein. Therefore, the
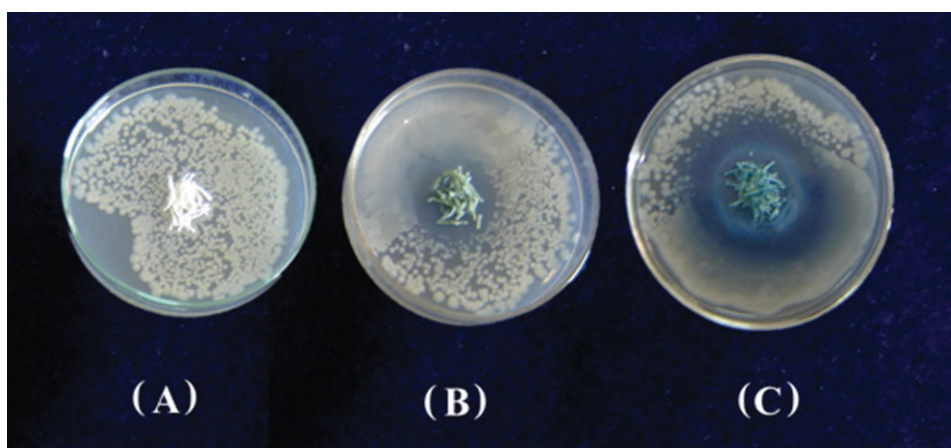
Figure 12 Biocidal action of (A) plain cellulose fibers, (B) CBCAC (2), and (C) CBCAC (4) fibers against *E. coli* as studied by zone inhibition method. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
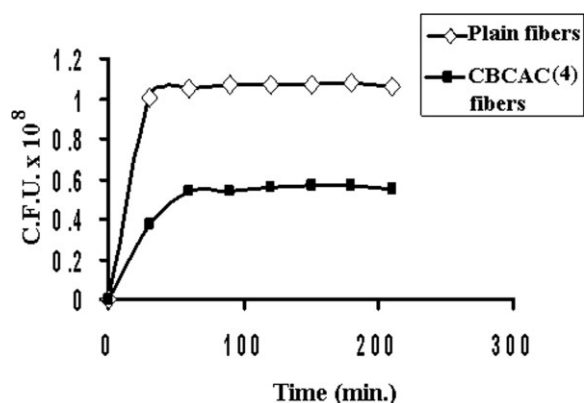
Figure 13 Kinetics of growth rate of bacterial cells as a function of time for CBCAC (4) fibers against plain fibers as control set.

extent of copper release in various media follows the order, protein fluid > PF > distilled water.

From the earlier discussion, it is now clear that the presence of chitosan in the composite fibers not only increases the copper loading but also enhances the release rate. Thus, the amount of chitosan bound to cellulosic chains in the composite fibers may exert great influence on the Cu(II) release from the fibers. To investigate this further, we prepared three fiber samples, with different amounts of bound chitosan, by immersing definite quantity of cellulose dialdehyde fibers in chitosan solutions of different concentrations, namely 0 (no chitosan), 0.5, and 1.5% (w/v) followed by their immersion in same Cu(II) solutions and studied copper release in PF at 37°C. The results, as depicted in Figure 10, clearly indicate that

as the amount of chitosan in the solution increases, the copper release from the resulting fibers also increases. This is simply due to the fact that with the increase in the concentration of chitosan solution, more and more chitosan is bound to cellulose dialdehyde fibers and hence copper loading in these fibers increases accordingly. When placed in PF, the amount of Cu(II) released from these fibers follow the same order, i.e., 0% < 0.5% < 1.5% chitosan (w/v). Therefore, by varying the amount of chitosan bound to the cellulose, copper release can be regulated.

For a given CBCAC fiber sample, the release of copper can also be regulated by varying the copper loadings into the solution. We prepared three composite fiber samples, namely, CBCAC (0.9), CBCAC (2), and CBCAC (4), where the number in parenthesis denotes the concentration of Cu(II) ions in the solution used for loading of copper into fiber. The results of release study, carried out in PF, reveal (see Fig. 11) that as the percent concentration of Cu(II) solutions increases, the amount of copper released from fibers also increases. This is simply due to the fact that with the increase in the copper content in the loading solution, the quantity of Cu(II) bound to the fibers also increases and hence causes faster release.

Antimicrobial studies

It is well known that Cu(II) ions demonstrate strong antibacterial action, either alone or in the form of complexes. We tested biocidal action of fibers by preparing two fibers, namely CBCAC (2) and CBCAC (4), where the number in parenthesis denotes the percent
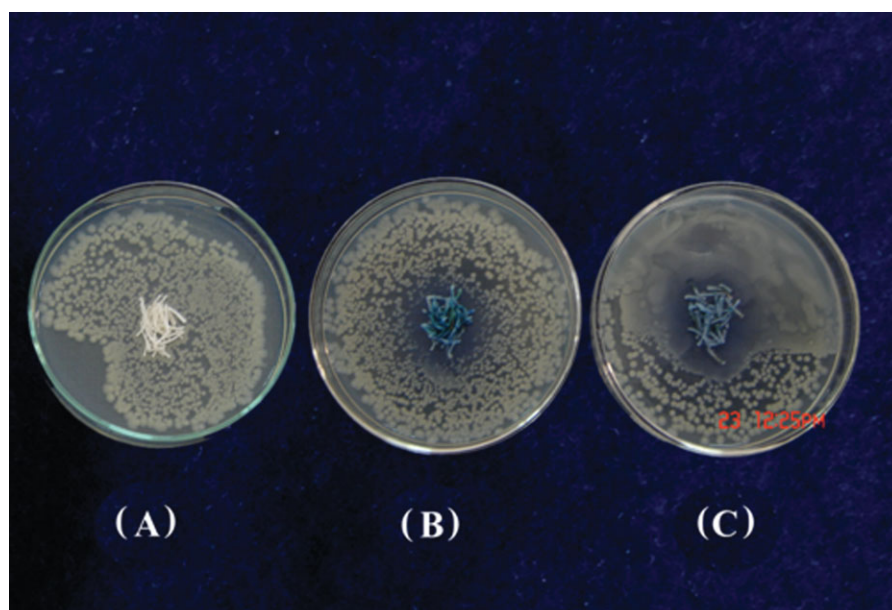
Figure 14 Bacterial growth in Petri dishes supplemented with (A) plain fibers, (B) CBCAC (2), and (C) CBCAC (4). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

concentration (w/v) of copper solutions used for loading copper ions into fibers. The results of the antibacterial test have been shown in Figure 12. It can be seen in Figure 12 that Petri dish containing bunch of plain fibers as control [see Fig. 12 (A)] shows dense population of bacterial colonies while Petri dishes, supplemented with fibers CBCAC (2) and CBCAC (4), as shown in the Figure 12 (B,C), respectively, show zones of inhibition around the bunch of the fibers. It is also be noted that radius of inhibition zone increases with the increase in the copper content of loading solutions. The observed findings may simply be attributed to the biocidal action of Cu(II) ions, which are released from the CBCAC fibers. In fact, as the copper content in the loading solutions increases, the amount bound to fibers also increases, and therefore, the antibacterial action of resulting fibers becomes more effective, thus resulting in the formation of "zone of inhibition" with greater area (or radius). Finally, we also studied the kinetics of growth of bacterial colonies in the presence and absence of CBCAC (4) fibers. It can be well seen in Figure 13 that the growth rate of bacterial colonies is suppressed to a great extent in the presence of CBCAC (4) fibers, which also support strong antibacterial action of Cu(II) ions being released from the fibers.

Finally, we also studied the antibacterial action of nanocopper-loaded fibers, namely, NCLCAC (2) and NCLCAC (4) against *E. coli*, taking plain fibers as control. The results, as depicted in the Figure 13, clearly indicate that Petri dish with plain fibers [see Fig. 14(A)] show dense population of bacterial colonies, whereas Petri dishes supplemented with nanocopper-loaded fibers, NCLCAC (2) and NCLCAC (4) [see Fig. 14(B,C), respectively], show less growth of colonies as indicated by a clear "zone of inhibition" around the bunch of amount of copper nanoparticles present in the fibers which, in turn, depends upon the concentration of Cu(II) solution used for copper loading.

CONCLUSION

From this study, it may be concluded that binding of copper(II) to chitosan-attached cellulose fibers results in the formation of novel antibacterial fibers with fair Cu(II) releasing capacity and biocidal action. In addition, the borohydride-induced reduction of these fibers also yields copper nanoparticles-loaded fibers, which also possess fair antibacterial property. These fibers have great potential to be used in burn/wound dressing and also in the fabrication of antibacterial dressing.

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