# Copper Alginate-Cotton Cellulose (CACC) Fibers with Excellent Antibacterial Properties

Mary Grace, M.Sc.[1], Navin Chand, Ph.D.[2], Sunil Kumar Bajpai, Ph.D.[1]

[1]Government Medical Science College, Jabalpur, INDIA
[2]Advanced Materials and Process Research Institute, Bhopal, INDIA

Correspondence to:
Sunil Kumar Bajpai, Ph.D. email: sunil.mnlbpi@gmail.com

**ABSTRACT**

The present work describes synthesis of copper alginate-cotton cellulose (CACC) composite fibers and detailed investigation of antimicrobial action against the model bacteria *E.coli.* The CACC fibers were prepared by immersing cotton fibers in aqueous solution of sodium alginate, followed by ionic crosslinking of alginate chains within the cotton cellulose fibers with Cu(II) ions to yield CACC composite fibers. The resulting CACC fibers were investigated for their biocidal action against *E.coli*, by using zone inhibition and colonies counting method. Finally, CACC fibers were reduced with sodium borohydride to yield copper nanoparticles-loaded composite fibers and investigated for biocidal action. It was found that CACC fibers possessed both, the fair mechanical strength and antibacterial action. The extent of biocidal action was found to depend upon the amount of Cu(II) loaded and concentration of alginate into cotton- cellulose fibers. The fibers showed higher Cu(II) release in physiological fluid as compared to distilled water. Copper alginate-cotton cellulose (CACC) fibers show fair mechanical strength and release copper ions in the presence of physiological fluid and protein solution. These fibers have great potential to be used as dressing materials.

Key words: Mechanical strength, copper alginate, *E.Coli*, antibacterial activity.

## INTRODUCTION

Copper ions, either alone or in copper complexes have been used for centuries to disinfect liquids, solids and human tissues. Different forms of copper compounds were used by ancient civilization to treat people stricken with afflictions and to maintain hygiene [1].The ancient Egyptians sterilized drinking water and wounds using copper. The Romans catalogued numerous medicinal uses of copper for various diseases. The Aztecs treated sore throats with copper, while in Persia and India, copper was applied to treat boils, eye infections and venereal ulcers. Today, copper is used as a water purifier, algaecide, fungicide, nematocide, molluscicide and antibacterial and antifouling agent [2-3], copper has been frequently exploited to impart antimicrobial properties to various polymeric substances. Zhang et al [4], 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 of eukaryote and prokaryote target microorganisms [5]. Similarly, copper-fluoropolymer (Cu-CFX) nanocomposite films have shown strong inhibitory action against growth of Escherichia coli and Lysteria [6]. Apart from polymer-based antibacterial films, various synthetic and natural polymers, in the form of fibers, have also been developed with antibacterial properties.

Apart from showing strong antibacterial properties, Cu(II) is also reported to play a key role in collagen crosslinking, thus aiding in the normal formation of bone matrix [7]. Since burn injuries are associated with reduced bone formation and resorption in both adult and children [8], the copper ions may be expected to play a dual role in healing of burn injuries [9], namely preventing the wound from infection and helping in formation of bone matrix. Malakyan et al [10], 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. Recently, Qin et al [9], have reported release of copper ions from chitosan fibers and shown its strong antibacterial action against several species of bacteria commonly found in wound and skin. Similarly, Voruganti et al [11], evaluated status of Cu in burned children and assessed adequacy of supplementation.

Among the various fibrous products, alginate-based products are currently the most popular ones used in developing antimicrobial agents releasing systems or dressing materials. Edwards et al [12], have reported preparation of algino-cellulose conjugate through citric acid crosslinking. They also investigated the ability of algino-cellulose fibers to release neutrophil elastase inhibitor, oleic acid to neutralize destructively high level of neutrophil elastase found in burn wounds. Wang and co-worker [13], have recently demonstrated release of model drug salicylic acid (SA) from alginate/polyethylene blend fibers. The frequent use of alginate based fibers has been facilitated by its special properties such as low cost and easy availability, biocompatibility, ability to enhance healing of wounds, high moisture adsorption and strong ion-exchange capacity [14-15]. However, in spite of possessing excellent properties for being used as dressing material, alginate fibers can not be used alone due to their relatively poor mechanical strength. On the other hand, cotton cellulose fibers have been widely accepted as dressing materials due to their fair mechanical strength, biocompatibility, durability, ease of chemical modification etc [16-17]. However, lack of ion-exchange capability of cellulose fibers has also been recognized as a major drawback in this potential material.

Therefore, in order to develop a dressing material, which possesses excellent ion exchange capacity like alginate and also has fair mechanical strength like cotton cellulose, we have adopted a novel strategy to prepare cotton cellulose/ copper alginate composite fibers, loaded with Cu(II) ions as antimicrobial agent. These fibers show fair mechanical strength, excellent Cu(II)-releasing capacity due to ion-exchange property and a high degree of antibacterial activity against model bacterium *E.coli.* Moreover, the fibers have been treated with sodium borohydride to yield copper nanoparticles loaded alginate cotton cellulose fibers and their antibacterial action has also been investigated.

## EXPERIMENTAL

### Materials

Sodium – alginate (SA, average molecular weight 6000, M/G ratio 1.75±0.12, medium viscosity 200cP for 1% aqueous solution at 20$^{o}$C), anhydrous cuprous sulphate, potassium hexacyanoferrate, nutrient agar, agar-agar type-1, and nutrient broth were obtained from Hi Media Laboratories, 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 through the investigations.

### Method

#### Preparation of CLCC and CACC composite fibers

Sodium – alginate was dissolved in distilled water at a concentration of 2% (w/v). Pre-weighed cotton fibers were put in sodium – alginate solution for 4 h at room temperature. The solution was stirred thoroughly to ensure complete absorption of sodium – alginate into cotton fibers. Thereafter, cotton fibers were immersed in 100ml of solution containing pre-determined quantity of crosslinker cuprous sulphate for 2h. Finally the resulting copper alginate cotton cellulose (CACC) composite fibers were washed with distilled water and then dried in a dust-free chamber at 40$^{0}$C. These fibers shall be designated as CACC (X) where the number X in parenthesis denotes the concentration of Cu(II) solution, in wt%, used to crosslink alginate chains. We also prepared copper loaded cotton cellulose (CLCC) fibers by just immersing cotton cellulose fibers in the 100ml of aqueous Cu(II) solution for 2h, followed by washing with distilled water and then drying at 40$^{o}$C. These fibers will also be designated as CLCC(X).

#### Preparation of copper nanoparticles loaded alginate cotton cellulose fibers

In order to prepare Cu nanoparticles loaded fibers, the CACC fibers were to react with sodium borohydride in aqueous medium. For this, a pre-weighed quantity of CACC fibers was immersed in 100 ml of 5.6mM sodium borohydride solution for a period of 24 h. The resulting copper nanoparticles loaded fibers were allowed to dry in vacuum chamber at 50$^{o}$C. *Figure 1* depicts cotton cellulose fibers, copper alginate-cotton cellulose composite fibers and nano copper loaded alginate-cotton cellulose composite fibers.

**FTIR spectral analysis**

FTIR spectra of plain cotton cellulose, sodium alginate entrapped cellulose and copper alginate/ cotton cellulose composite fibers were recorded with Shimadzu spectrometer (UV 1700) using KBr mixed disc/pallet.

**TEM analysis**

The size of the copper nanoparticles was determined using a Tecnai F 12 TEM instrument. TEM samples were prepared by dispersing 2-3 drops of solution, obtained by putting nano copper-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 plain cotton cellulose and copper-alginate - cotton cellulose (CACC) and nano copper loaded alginate-cotton cellulose fibers were obtained at room temperature on LR 100K machine. Diameter and length of fibers were 0.5 mm and 60 mm respectively and they were tested at a stretching speed of 300 mm/min. The measurements were made with five samples and average values have been reported in the data.

**Cu(II) Release studies**

To investigate the release of Cu(II) ions from the CACC composite 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 Pharmacopia [18]. Moreover, the Cu(II) ions release was also studied in the 2.9%(w/v) aqueous solution of water soluble protein soy bean as suggested by James and Taylor[19].The estimation of Cu(II) released was done spectrophotometrically [20].

**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.*

For qualitative measurement of antimicrobial activity, the CLCC composite 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 30ºC for 2 days. The presence of any clear zone around the 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 CLCC composite fibers, *E.coli* cells were grown in 100 ml of nutrient broth (NB) supplemented with a pre-weighed fibers at 37ºC with continuous stirring. The cylindrical sample container 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 ml [21].

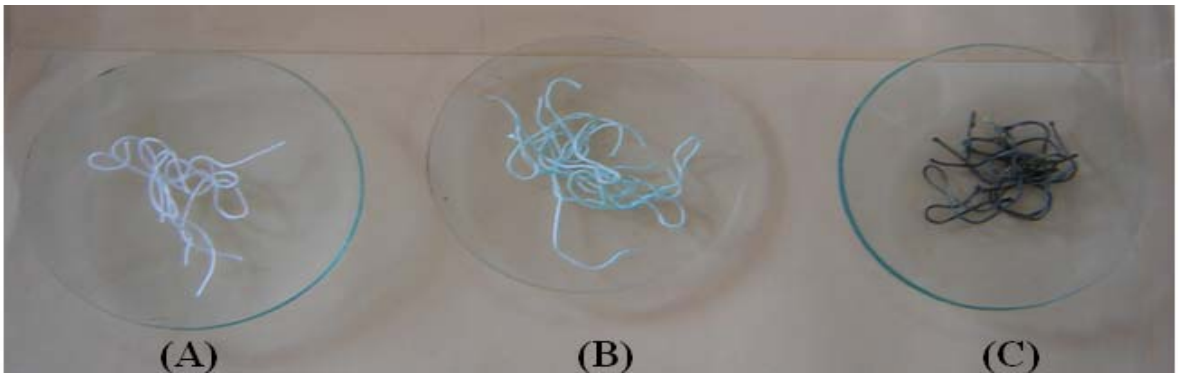
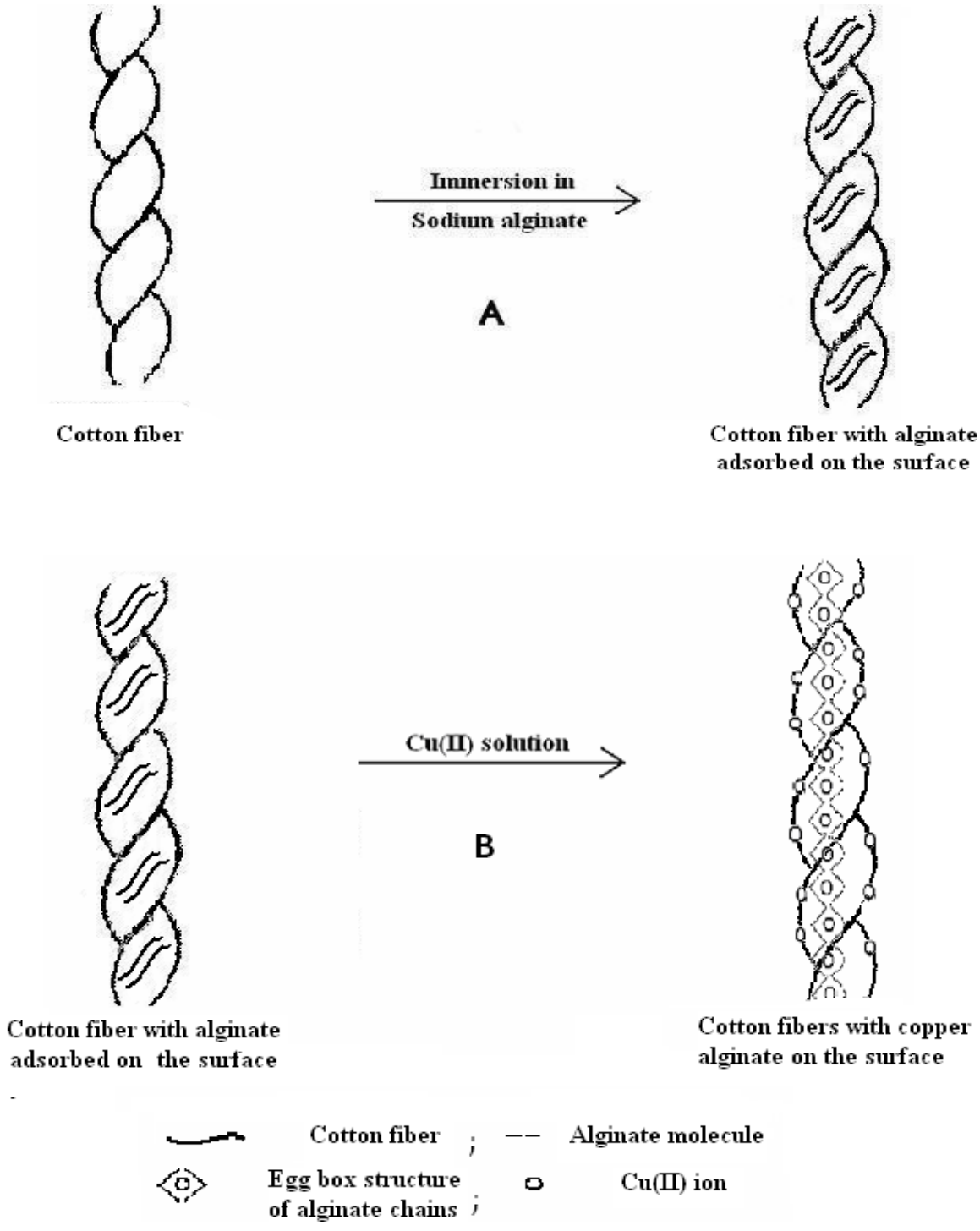



**FIGURE.1. Photograph showing (A) plain cotton cellulose fibers, (B) copper alginate-cotton cellulose (CACC) fibers, and (C) copper nanoparticles loaded alginate-cotton cellulose fiber.**




**SCHEME I: (A) Sorption of alginate chains into cotton cellulose fibers. (B) Crosslinking of alginate chains by Cu(II) ions thus forming 'egg-box' cavities.**

## RESULTS AND DISCUSSION

### Formation of CACC composite fibers

Alginate is an anionic linear polysaccharide with 1,4'-linked D-mannuronic acid and L-guluronic acid residues either as block of the same unit or as random sequence of these two sugar residues **[22].** Guluronic acid blocks are known to form rigid buckled structures. Two such sequence make an 'egg-box' array upon contacting divalent ions like $Ca^{++}$, $Cu^{++}$, $Zn^{++}$, thus forming an ionically crosslinked structure in aqueous environment.

The crosslinking of the polymer is due to binding of divalent cations to the $–COO^-$ group of α-L-gluronic acid block in a highly co-operative manner and the size of the co-operative units is more than 20 monomers **[23].** Each alginate chain can dimerize to form junction with many other chains and as a result gel networks are formed rather than insoluble precipitate **[24].** Now, the overall formation of CACC fibers may be explained as follows: When cotton cellulose fibers are placed in dilute solution of sodium alginate, macromolecular chains of alginate get adsorbed onto the surface of the ultrafine cellulose fibers as shown in Scheme-I (A). Later on, when these alginate containing cotton cellulose fibers are placed in Cu(II) ions solution, copper ions enter into the polyguluronate and polymannuronate residue, thus resulting in formation of Cu(II)-crosslinked alginate network with Cu(II) ions occupying the space within the 'egg-box' cavities as shown in Scheme I(B).Moreover, Cu(II) ions also bind to the –OH group of cellulose network in cotton fibers. In this way, CACC composite fibers with higher copper content are produced. On the other hand, the CLCC fibers were simply prepared by immersing cotton fibers in Cu(II) ions solution, and therefore loading of copper ions did not occur to such a great extent as in the case of CACC composite fibers. **Here we would like to mention that although plain cotton cellulose fibers also possess also sufficient strength, their inability to undergo ion-exchange was the major reason for discussing copper alginate chains in the introduction.**

### Formation of nano Cu- loaded fibers

When CACC fibers are put in aqueous solution of sodium borohydride, the Cu(II) ions present within the polyguluronate residue in 'egg-box' cavities are reduced to yield copper nanoparticles. In addition, the copper ions bound to –OH functionalities of cellulose also undergo reduction to yield copper nanoparticles. The dark appearance of fibers, as depicted in *Figure 1* also indicates formation of nanostructures.

Moreover, in order to investigate whether the formation of copper nanoparticles occurred on the surface of the fibers or in the bulk, the fibers were cut and the cross-section was viewed through microscope. It was found that there was dark black appearance only on the surface of the fibers thus supporting our argument, given in the previous section and also in scheme I, that copper alginate formation takes place only on the surface of the fibers due to inability of high molecular weight alginate chains to penetrate the fibers.

The result of the TEM analysis, as shown in the *Fig. 2*, clearly indicates that particles are almost monodisperse in nature. We also carried out particle size distribution analysis, selecting copper nanoparticles from different arbitrarily chosen area of TEM image (see inset). On the basis of distribution curve the average diameter of the copper nanoparticles was found to be 37.5 nm. Here it is worth mentioning that the inset shown in the TEM image, describes size of copper nanoparticles present on the surface of the cotton fibers. To record TEM image, when copper nanoparticles loaded alginate-cotton fibers are put in the distilled water, copper nanoparticles are released in the medium. The drops of this solution were put on copper grid for TEM image.

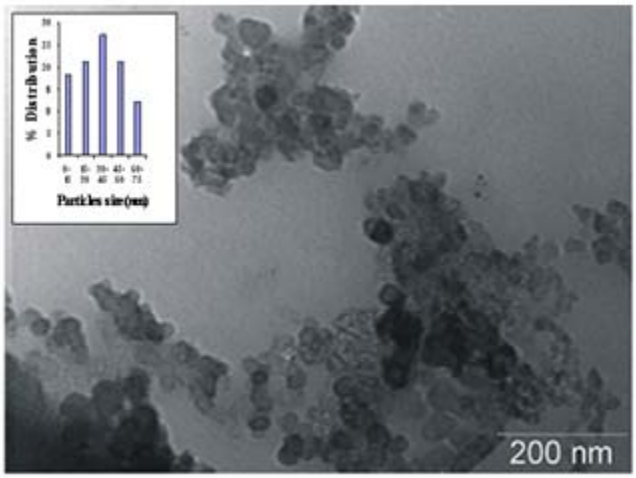



**FIGURE 2. TEM analysis of copper nanoparticles.**

### FTIR spectral analysis

The FTIR spectra of plain cotton cellulose fibers, sodium alginate-sorbed cotton fibers and copper-alginate-cotton cellulose (CACC) composite fibers

have been shown in *Figure 3*. In *Fig. 3(A)* a broad peak corresponding to –OH group of cellulosic cotton fibers is observed in the range 3600-3200 $cm^{-1}$ and peak of –CO carbonyl group is also observed at 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 $cm^{-1}$.

*Fig. 3(B)* shows intermolecular hydrogen bond between –OH group of cellulosic cotton fibers and carboxylate moiety of sodium-alginate at 3600$cm^{-1}$**.** In *Fig 3(C)* a broad peak is observed in the range 3400-3200 $cm^{-1}$ shows the formation of co-ordinate bond between the carboxylate moiety of sodium – alginate and Cu(II) ions. Copper crosslinking caused lesser shift at 3400 $cm^{-1}$ which is probably due to the strong ionic (and weak co-ordinate) nature of the carboxylate copper interaction, which allow more

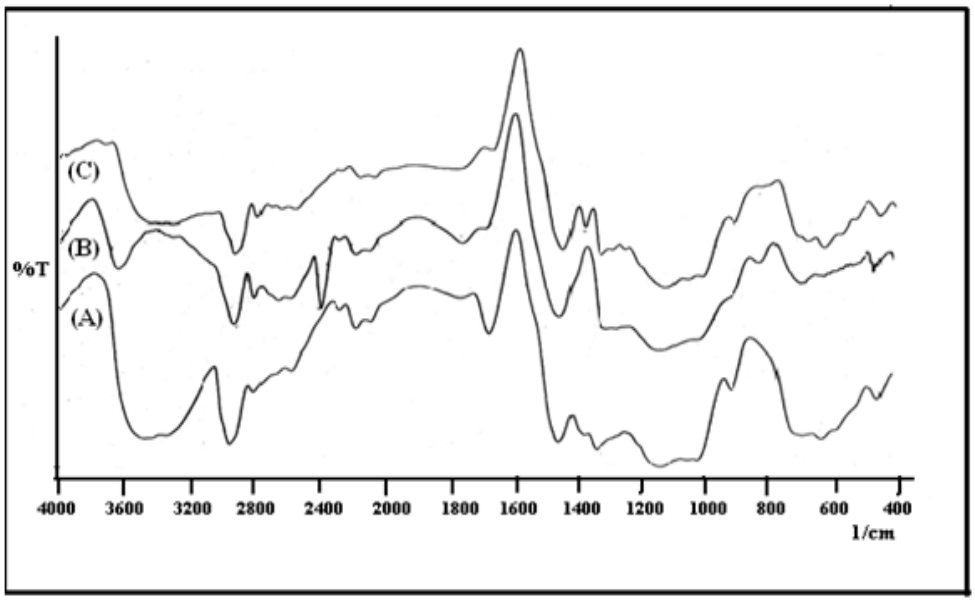



FIGURE 3 FTIR spectra of (A) cotton cellulose fibers, (B) sodium alginate-absorbed cotton cellulose fibers, and (C) copper alginate-cotton cellulose fibers.

free delocalization of the carboxylate electrons and reduce the double – bond character of the carboxylic – carbonyls

## Mechanical Strength of fibers

*Table I* describes the mechanical analysis data for the cotton fibers, copper alginate and copper nanoparticles loaded alginate-cotton fibers. A close look at the various value displayed clearly indicates that there is not any significant improvement in the mechanical properties of plain cotton fibers on incorporation of copper alginate. This may simply be due to the fact that alginate chains are only adsorbed on the surface of cotton fibers. Therefore formation of copper alginate coating does not make any significant contribution towards mechanical strength. So it may be concluded that coating of cotton fibers with copper alginate does not alter mechanical properties significantly.

## Dynamic release of Cu(II) from CACC 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 [25], found that on the first post operation 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 [26], found that wound fluid, collected from leg ulcers, and contained 0.6-5.9 mM/l glucose and 26-51 g /l protein. Similarly, Froahm et al. [27], analyzed the fluid from a postoperative wound, leg ulcers and leg blisters. They found that 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 physiological fluid (PF), as suggested by British pharmacopia which contained 142mM of NaCl and 2.5 mM of $CaCl_2$.

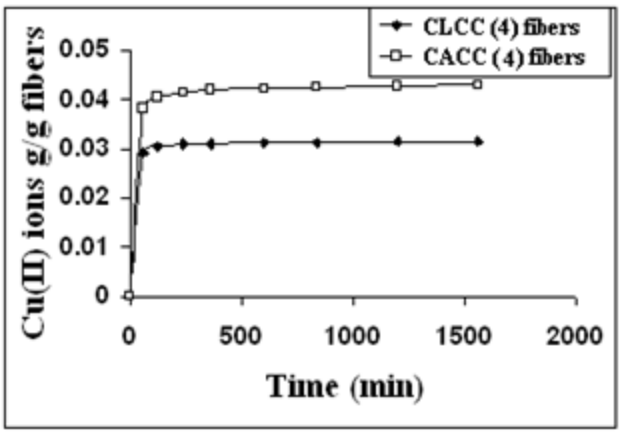



**FIGURE 4. Dynamic release of Cu(II) ions from CACC (4) and CLCC (4) fibers in physiological fluid at 37°C.**

**TABLE I**

**Data showing mechanical properties of sodium alginate-cotton cellulose (CACC) fibers and copper alginate-cotton cellulose (CACC) fibers (n=5).**

| Mechanical Properties | Plain cotton cellulose fibers | Copper-nano-alginate cotton cellulose fibers | Copper alginate-cotton cellulose (CACC) fibers |
|---|---|---|---|
| Maximum load(N) | 26.12 | 26.56 | 27.19 |
| Maximum stress($N/mm^2$) | 133.0 | 135.3 | 138.5 |
| Load break(N) | 26.12 | 26.56 | 27.19 |
| Stress break($N/mm^2$) | 133.0 | 135.3 | 138.5 |
| Extension break(mm) | 7.777 | 5.746 | 4.531 |
| Strain break (%) | 15.55 | 11.50 | 9.062 |

The results of release experiments, carried out with CACC (4) composite fibers and CLCC (4) fibers are well depicted in the *Figure 4*. It is quite clear that CACC (4) composite fibers demonstrate higher release as compared to CLCC (4) fibers. This may simply be attributed to the fact that CACC (4) composite fibers have higher copper loading due to binding of copper ions with – OH groups of cellulose network as well as with the carboxylate groups of alginate chains. So when CACC (4) composite fibers are put in physiological fluid, Cu(II) ions, present in the 'egg-box' cavities of crosslinked polyguluronate residues of alginate chains, undergo exchange with external $Ca^{2+}$ ions and hence are released into the medium. At the same time Cu(II) ions are also released from cellulose network of cotton fibers. Therefore overall release is faster. On the other hand, CLCC (4) fibers demonstrate slower release due to the poor copper loading and absence of any driving force like 'ion-exchange process'. In this way it may be concluded that CACC (4) composite fibers, not only possess higher copper loading, but they also release Cu(II) ions at a faster rate. Here it is also noteworthy to mention that the release was extended over a duration of nearly 24 h.

Is stated above, the major driving force for the observed release from CACC (4) composite fibers is the ion-exchange between Cu(II) ions present in the 'egg-box cavities' of alginate networks and Ca(II) ions present in the release medium. In order to confirm this, we compared the release profiles, obtained for CACC (4) composite fibers in water and physiological fluid at 37°C (See *Figure 5*).

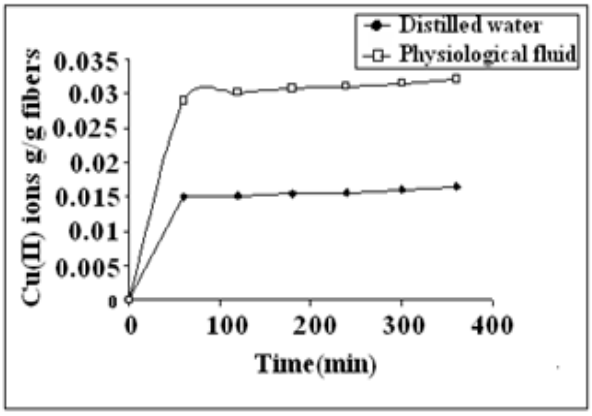



**FIGURE 5. A comparative depiction of dynamic release profile of Cu(II) from CACC (4) fibers in distilled water and physiological fluid at 37°C.**

It can be clearly seen that CACC (4) composite fibers demonstrate slower release in distilled water which may be attributed to the fact that since the release medium is distilled water, it does not contain any cations that can be exchanged with Cu(II) ions presents within the CACC (4) fibers. As a result, the 'ion-exchange mechanism' is not operative here and hence less amount of Cu(II) ions released. It should also be noted that the release observed may probably be due to the Cu(II) ions bound to the cellulose network, which come out due to fair solubility of copper ions in water. We also studied the Cu(II) ions release from CACC (4) composite fibers in 2.9% protein solution and compared the data with that obtained in distilled water (See *Figure 6*).

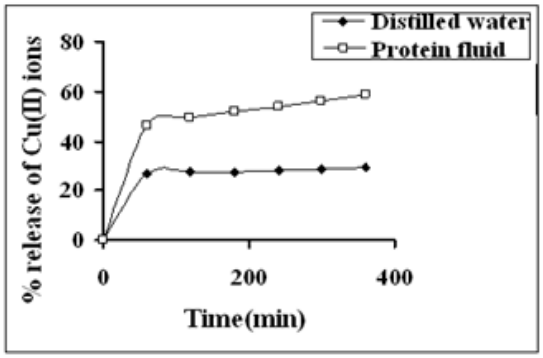



FIGURE 6. A comparative depiction of release profiles obtained for CACC (4) fibers in 2.9% protein solution and distilled water at 37°C.

It is quite clear that a faster release is obtained in protein solution as compared to distilled water. The observed faster release in protein solution may simply be attributed to the strong tendency of Cu(II) ions to bind with the soya protein molecules, thus resulting in faster release. We also compared the Cu(II) release from CACC (4) fibers in protein solution (2.9%) and physiological fluid at 37°C . The results, as depicted in the *Fig. 7*, reveal that amount of Cu(II) released from CACC(4) fibers in protein fluid at different time-intervals is almost the same as released in physiological fluid. It is well known that Cu(II) ions has a strong to bind with amino acid groups of molecules [7].

This may be treated as a key factor responsible for observed release in protein fluid. On the other hand, the release of Cu(II) in physiological fluid is simply due to the ion-exchange process as discussed earlier. Therefore, it may be concluded that CACC fibers exhibit almost the same release in protein fluid and physiological fluid.

**Study of biocidal activity of CACC fibers**

As stated in the section 'introduction', Cu(II) ions have shown strong biocidal activities, either alone or

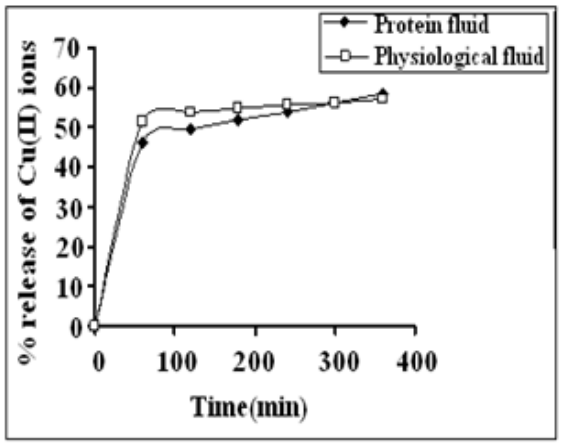



**FIGURE 7. A comparative depiction of release profiles obtained for CACC (4) fibers in 2.9% protein solution and physiological fluid at 37°C.**

in the form of complex. In order to investigate biocidal activity of CACC fibers in the present study, we prepared two samples, by immersing alginate-absorbed cotton cellulose fibers in 2 and 4 percent Cu(II) ions solutions. We designated these fibers as CACC (2) and CACC (4) respectively where number in parenthesis denotes percent composition (w/v) of crosslinker Cu(II) ions solutions. *Fig.8* shows the biocidal activity of these fiber samples against the plain cotton fibers taken as control. *Fig.8* (A), (B) and (C) shows the bacterial growth round the bunch of plain, cotton fibers, CACC(2) and CACC(4) fibers in petri dishes respectively.

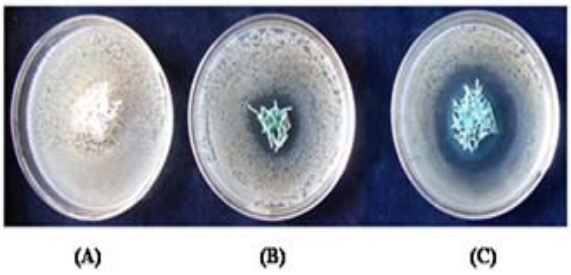



**FIGURE 8. Biocidal action of (A) plain fibers, (B) CACC (2), and (C) CACC (4) fibers against E.Coli as studied by Zone inhibition method.**

It is quite clear that petri dish, containing plain fibers, have dense population of bacterial colonies throughout. On the other hand, petri dishes, supplemented with fiber samples CACC (2) and CACC (4), show a clear 'zone of inhibition' around the bunch of fibers. It can also be observed that the area of inhibition zone increases with copper content within the fibers. The observed findings can be clearly attributed to the antibacterial action of Cu(II) ions which are released from the CACC fiber samples and kill the bacteria in effective manner.

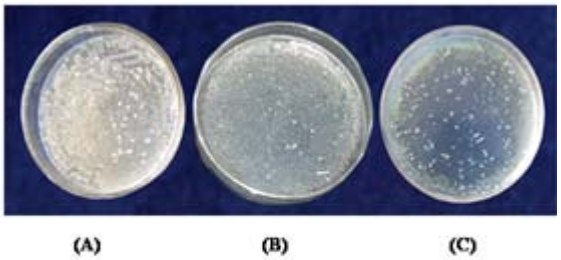



**FIGURE 9. Quantitative interpretation of biocidal action of (A) plain fibers, (B) CACC (2), and (C) CACC (4) fibers against *E.Coli* as studied by Zone inhibition method.**

In order to investigate the biocidal action in quantitative manner, fiber samples were cut into a large number of extremely small pieces, thoroughly mixed with culture media and then bacterial cells were grown as described in the experimental section. The results, as depicted in the *Fig.9*, clearly show that in the control set there are nearly 105CFU/cm$^2$ while no. of CFU in the petri dishes, supplemented with fiber samples CACC (2) and CACC (4), are nearly 11 and 5 respectively, thus indicating that with the increase in copper content within the fibers, the growth of bacterial colonies is suppressed in more effective manner.

Finally, *Fig.10* shows the kinetics of growth of bacterial colonies in the absences and presence of

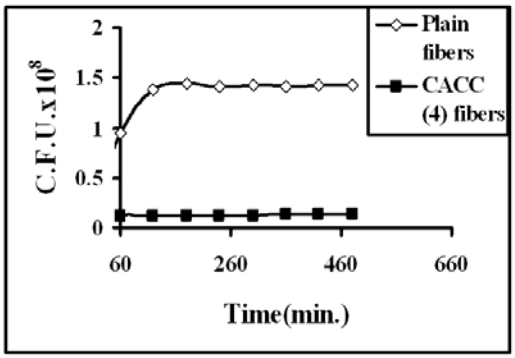



**FIGURE 10. Kinetic of growth rate of bacterial cells as a function of time for CACC(4) fibers, taken plain fibers as control set.**

CACC (4) fibers. It can be seen that there is continuous growth in bacterial cells in the presence of plain cotton cellulose fibers while due to strong killing kinetic action of released Cu(II)ions from the CACC (4) fibers, the bacterial growth is suppressed

to a great extent thus indicating that CACC (4) fibers are very effective in killing bacterial cells.

**Antibacterial activity of copper nanoparticles loaded fibers**

In order to investigate the biocidal action of nano copper loaded fibers on *E.Coli*, we carried out borohydride-induced reduction of CACC (4) fibers and studied antibacterial action of resulting fibers. *Fig.11* (A), (B), and (C) show a comparative depiction of plain fibers, CACC (4) fibers and nano copper loaded fibers respectively. It can be clearly seen that nanoparticles loaded fibers also show their efficiency to inhibit bacterial growth. The mechanism of biocidal action of copper nanoparticles may probably be explained on the basis of the fact that Cu nanoparicles release Cu(II) ions on contact with moisture. These copper ions bind with the –SH and –COOH groups of protein molecules of bacterial cell wall. Our argument is further supported by fact that copper nanoparticles loaded fibers, after showing biocidal action, has lost their black color (please see *Fig. 1(C)* and *Fig. 11(C)*) thus indicating that these particles must have released Cu(II) ions.

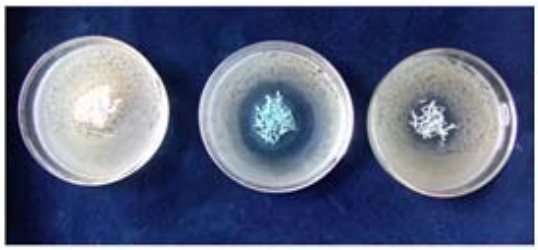



**FIGURE 11. Antibacterial activity of copper nanoparticles loaded alginate cotton cellulose fibers.**

Here it is to be noted that a close look at the *Fig. 11* (B) and (C) clearly reveal that Cu nanoparticles loaded fibers show less biocidal action as compared to CACC (4) fibers. This may probably due to the fact that CACC fibers contain Cu(II) ions that are directly released from the fibers and act upon the bacterial cell, while in the case of copper nanoparticle-loaded fibers the conversion of Cu into Cu(II) seem to be the governing factor to control their biocidal action.

## CONCLUSION

From the above study it may be concluded that copper alginate-cotton cellulose (CACC) fibers show fair mechanical strength, and release copper ions in the presence of physiological fluid and protein solution. The fibers show excellent antibacterial action against *E.Coli* and the extent of their biocidal action is more than that of copper nanoparticles loaded fibers, they have great potential to be used as dressing materials.

## REFERENCES


[1] Faundez, G., Troncoso, M., Navarrete P., Figueroa, G., "Antimicrobial activity of copper surfaces against suspensions of salmonella enterica and campylobacter", *BMC Microbial*, 4, 2004**,** 19.

[2] Borkow, G., Gabbay, J.,"Putting copper into action: copper-impregnated product with potent biocidal activities", *FASEB J.*, 18(14), 2004**,** 1728.

[3] Ibrahim, N.A., Abo-shosha, M.H., Gaffar, M.A., Elshafei A.M., Abdel-Fatah, O.M. , "Antibacterial properties of ester-crosslinked cellulose-containing fabrics post-treated with metal salts", *Polymer-Plastics Technol. Eng.,* 45(6), 2004, 719.

[4] Zhang, W., Zhang, Y. Yan, J-Ji, Q., Huang, A., and Chu, P.K., "Antimicrobial polyethylene with controlled copper release", *J. Biomed. Mater. Res. A,* 83(3), 2007,838.

[5] Cioffi N., Ditaranto, N., Torsi, L., Picca, R.A., Giglio, E. De, Sabbatini, L., Novello, L., Tantillo, G., Bleve-Zacheo, T., Zambonin, P. G., "Synthesis, analytical characterization and bioactivity of Ag and Cu nanoparticles embedded in poly (vinyl methylketone) films"**,***Anal. Bioanal. Chem* ., 382(8)**,** 2005,1912.

[6] Cioffi, N., Ditaranto, N., "Analytical characterization of bioactive fluropolymer ultratrhin coatings modified by copper nanoparticles", *Analytical and Bioanalytical chemistry,* 381(3), 2005, 607.

[7] Tapeiro, H., Townsend, D. M., Tew, K. D.,"Trace elements in human physiology and pathology: Copper",*Biomed. Pharmacother*; 57, 2003, 386.

[8] Klein ,G. L., Herndon, D. N. , Goodman, W. G., Langman, C. B., Phillips, W. A.,

Dickson, I. R., "Histomorphometric and biochemical characterization of bone following acute severe burns in children", *Bone*, 17 (5), 1995,455.

[9] Qin, C., Zhu, J., Chen, D., Liang and G. Wo., "Absorption and release of Zinc and copper ions by chitosan fibers", *J. Appl. Polym. Sci*., 105, 2007**,** 527.

[10] Malakyan, M. H., Bajinyan, S. A., Abrahamyan, A. K., Petrosyan, Z. H. , Harutyunyan, N. K., Badiryan, V. A., Sorenson, J. R. *Inflammapharmacology*, 12, 2004**,** 321.

[11] Saroja Voruganti, V., Klein, G. L., Lu, H., Thomas, S., Freeland-Graves, J. H., Herndon, D. N., "Impaired zinc and copper status in children with burn injuries : Need o reassess nutritional requirements", *Burns*, 31, 2005, 711.

[12] Edward, J. V., Bopp, A. F., Batiste S. L., Goynes, W.R., "Human Neutrophil elastase inhibition with a novel cotton-alginate wound dressing formulation", *J. Biomed. Mater. Res. A*, 66(3**),** 2003, 433.

[13] Wang, Q., Zhang, N., Hu, X., Yang, J.,Du,Y., "Alginate/polyethylene glycol blend fibers and their properties for drug controlled release", *J. Biomed. Mater. Res A*, 82(1), 2007, 122.

[14] Knill, C.J., Kennedy, J.F., Mistry, J., Miraftab, M., Smart, G., Groocock , M.R., Williams, H.J., "Alginate fibers modified with unhydrolysed and hydrolysed chitosans for wound dressings",*Carbohydrate Polym*; 55, 2004,65.

[15] Bajpai, S.K., Tankhiwale, R., "Investigation of water uptake behavior and stability of calcium alginate/chitosan bipolyweric beads: Part-I",*Reactive and functional polymers*, 66, 2006, 645.

[16] Hipler, U.C., Elsner P., Flugr, J.W., "A new silver-loaded cellulosic fiber wiyh antifungal and antibacterial properties",*Curr. Probl. Dermatol*., 33, 2006, 165.

[17] Princi, E., Vicini, S., Proietti N., Capitani D., "Grafting polymerization on cellulose based textiles: A $^{13}C$ solid state NMR characterization", *Eur. Polym. J*., 41, 2005, 1196.

[18] *British pharmacopoeia Monograph for Alginate Dressings and packings*, 1994.

[19] James, T., Taylor, R., "In proceedings of a joint meeting between European Wound Management Assosition and European Tissue Repair Society; G.Cherry, K. Harding, Eds.", *Churchill Communication Europ*: London,1,1997.

[20] Bajpai,S.K., Rohit., V.K., "Cation exchanger saw dust CESD) as an effective sorbent for removel of Cu(II) from Aqueous solution". *Electronic Journal of Environmental, Agricultural and Food Chemistry* **,** 6(5) **,** 2007**,** 2053**.**

[21] Pal, S., Tak ,Y. K., Song, J. M., 'Does the antibacterial activity of silver nanoparticles depend on the shape of the nanopaticle? A study of the Gram-negative bacterium Escherichia coli", *Appl. Environ. Microbiol*., 73(6), 2007, 1712.

[22] Kikuchi, A., Kawabuchi, M., Sugihara, M., Sakuraiand, Y., Okano, T., 'Pulsed dextran release from calcium- alginate gel beads', J. Cont. Rel., 47, 1997, 21.

[23] Gombotz, W. R., Wee, S.F., 'Protein release from alginate matrices', Advanced Drug Delivery Reviews, 31, 1998, 267.

[24] Rees, D. A., Welsh, E. J., 'Secondary and tertiary structure 0f polysaccharides in solution and gels', Angew. Chem. Int. Ed. Engl., 16. 1977, 214.

[25] Bonnema, J.,Ligtenstein, D.A., Wiggers, T., Van Geel, A.N., *Eur. J. Surg.***,** 165, 1999**,** 9.

[26] Trengrove, N. J. ; Langton, S.R.; ; Stacey, M. C., *Wound Rep. Regen*., 1996**,** 4,234.

[27] Frohm, M. ; Gunne, H.; Bergman,A.C.; Agerberth, B.; Bergman, T.; Boman, A.; Liden, S.; Jornvall, H.; Boman, H. G., *Eur. J. Biochem*., 237, 1999, ,86.

**AUTHORS' ADDRESSES**

**M. Grace, M.Sc., , S. Kumar Bajpai, Ph.D.**
Government. Model Science College
Department of Chemistry
Pachpedi
South civil lines
Jabalpur, Madhya Pradesh 482001
INDIA

**N. Chand, Ph.D.**
Advanced Materials and Process Research Institute,
Bhopal
(Near Habibganj Naka, Hoshangabad Road)
Bhopal 462026
INDIA