Copper Nanoparticles Loaded Alginate-impregnated Cotton Fabric with Antibacterial Properties

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ABSTRACT: In the present study, calcium alginate-impregnated cotton fabric has been loaded with copper nanoparticles to impart antimicrobial properties. The fabric, so prepared, has been characterized by TEM and FTIR analysis. There has been no adverse effect found on the mechanical properties of fabric due to alginate impregnation. The release of Cu(II) ions has been studied in the physiological fluid at 37°C under different experimental conditions, such as varying concentrations of sodium alginate and the crosslinker calcium chloride. The fabrics showed an appreciable release of Cu(II) ions, extended over a period of 50 h. The amount of Cu(II) ions released showed a negative dependence on the amount of alginate present within the fabric network and the concentration of crosslinker calcium chloride used. The release data was fitted on the Higuchi diffusion-controlled release model successfully. Finally, the antibacterial activity of fabric was tested by zone inhibition method against *E. coli* as model bacteria. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: FTIR; nanoparticles; diffusion TEM; chain; kinetics

INTRODUCTION

Synthesis of noble metal-based nanoparticles for applications in the field of biomedical and other-related areas has been a focus of research. Recently, an awareness of general sanitation, contact disease transmission, and personal protection has led to the development of antimicrobial textiles. With the development of nanotechnology, there have been continuous efforts to explore possibilities of using noble metals like Ag, Cu, and gold etc. to impart antimicrobial properties to fabrics.[1,2] Because of high cost of metals like silver and gold, material chemist have focused their attention on exploring possibilities of using copper nanoparticles as ultimate antimicrobial agent. Today, copper is used as a water purifier, algaecide, fungicide, nematocide, molluscicide, and antibacterial and antifouling agent.[3,4]

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.[5] Since burn injuries are associated with reduced bone formation and resorption in both adult and children,[6] the copper ions may be expected to play a dual role in healing of burn injuries,[7] namely, preventing the wound from infection and helping in formation of bone matrix. Malakyan et al.,[8] 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.,[9] 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.[10,11] The frequent use of alginate-based fibers has been facilitated by its special properties such as low cost and easy availability, biocompatibility, and ability to enhance healing of wounds, high moisture adsorption and strong ion-exchange capacity.[12,13] 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.[14,15] However, lack of ion-exchange capability of cellulose fibers has also been recognized as a major drawback in this potential material.

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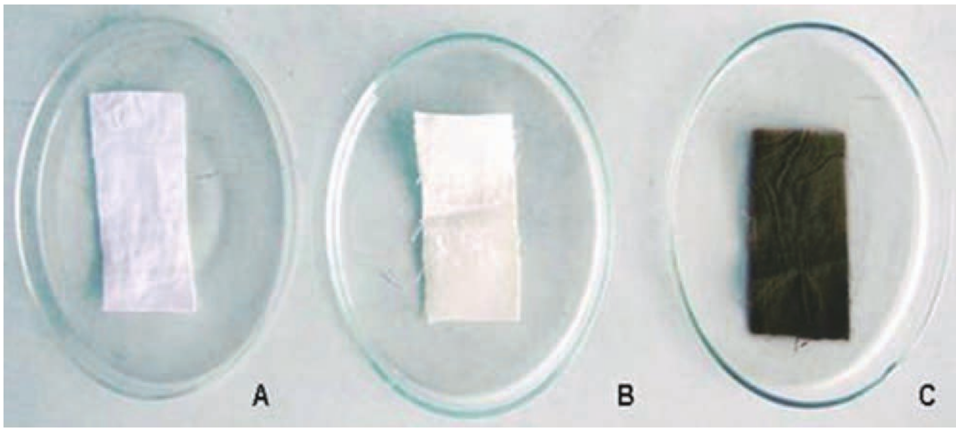

Figure 1 Photograph showing (A) plain cotton cellulose fabric (B) calcium alginate-impregnated cotton fabric and (C) copper nanoparticles loaded calcium alginate-impregnated cotton (CNLCAIC) fabric. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The present study describes a new strategy to entrap copper nanoparticles into calcium alginate-impregnated cotton fabric. The fabric, so obtained, demonstrates excellent Cu(II)-releasing capacity due to ion-exchange property and also possesses a high degree of antibacterial activity against model bacterium *E. coli.*

EXPERIMENTAL

Materials

Sodium-alginate (SA, average molecular weight 8000, M/G ratio 1.82 ± 0.12, and medium viscosity 220cP for 1% aqueous solution at 25°C), anhydrous cuprous sulfate, potassium hexacyanoferrate, nutrient agar, agar-agar type-1, and nutrient broth were obtained from Hi Media Laboratories, Mumbai, India. Sodium borohydride and calcium chloride were obtained from HiMedia Chemicals, Mumbai, India. Cotton fabric was gifted by a local textile mill. Double distilled water was used through the investigations.

METHOD

Preparation of calcium alginate-impregnated cotton fabric

The preweighed piece of cotton fabric was immersed in 4% (w/v) aqueous solution of sodium alginate for 1 h and then taken out, hung vertically for 4 min to remove extra loosely bound alginate, and then transferred into 3% (w/v) solution of $CaCl_2$ to crosslink the entrapped alginate chains through ionotropic gelation[16] for a period of 30 min at room temperature. The crosslinked CAIC fabric was taken out and allowed to dry at 50°C till it attained a constant weight.

Preparation of copper nanoparticles loaded CAIC fabric

To prepare copper nanoparticles loaded fabric, the CAIC fabric, obtained above, was placed in Cu(II) aqueous solution of desired concentrations for a period of 30 min, the fabric, loaded with Cu(II) was now transferred into 2% aqueous solution of sodium borohydride at 37°C for a period of 4h. This resulted in reduction of copper ions into copper nanoparticles within the CAIC fabric. The fabric shall be designated as copper nanoparticles loaded calcium alginate-impregnated cotton (CNLCAIC) fabric. Plain, alginate-impregnated and Cu nanoparticles loaded alginate-impregnated fabrics are shown in Figure 1.

FTIR spectral analysis

FTIR spectra of CAIC fabric and CNLCAIC fabrics were recorded with Shimadzu Spectrophotometer (UV 1700) using KBr mixed disc/pallet.

TEM analysis

The size and distribution of copper nanoparticles within the fabric was determined using a JEOL 1010 Transmission Electron Micrograph were taken as a 100-nm thick microtommed section, cut parallel to the surface of the fabric

XRD analysis

The X-ray diffraction method was used to identify copper nanoparticles loaded in the fabric. These measurements were carried out on a Rikagu diffractometer (Cu radiation = 0.1546 nm) running at 40 kV and 40 mA.

Mechanical properties

Tensile strength and elongation at break of the fabrics were realized at YG026B.

Electronic fabric strength tester (Ningbo Textile Instrument Factory, China) according to ISO13934-1. Test length was 30 cm and speed was 200 mm/min. The samples were conditioned in a room (20°C, a relative humidity of 65%) for 48 h before measurement.

Cu(II) release studies

To investigate the release of Cu(II) ions from the CNLCAIC fabric, samples of known weight were placed in contact with 40 times their own weight of physiological fluid (PF), composed of 142 m*M* of sodium chloride and 2.5 m*M* of calcium chloride, thus representing the typical ion concentration of body fluid as specified by the British Pharmacopoeia.[17] The estimation of Cu(II) released was done spectrophotometrically.[18]

Antibacterial study of fabric

The antibacterial activities of the fabric were tested qualitatively by an inhibition zone method with *E. coli* as model bacteria. For qualitative measurement of antimicrobial activity, the CNLCAIC composite fabrics 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 the fabric on the plates was recorded as an inhibition against the microbial species.

RESULTS AND DISCUSSION

Formation of CNLCAIC composite fabrics

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 residue.[19] Guluronic acid blocks are known to form rigid buckled structures. Two such sequences 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 $-COO^-$ groups 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.[20] Now, the overall formation of CNLCAIC fabrics may be explained as follows: when cotton fabric is put in sodium alginate aqueous solution, alginate chains are sorbed into the space available within the fibrous networks of fabric. They also get into the ultra-sized pores within the fibers. When this alginate-impregnated fabric is put in $CaCl_2$ solution, the alginate molecules are crosslinked ionically by incoming calcium ions, thus developing crosslinked egg-box like structures within the fabrics as shown in (Fig. 2). In this article, it is worth mentioning that $-COO^-$ groups present in alginate chains bind electrostatically to calcium ions. Now the calcium alginate entrapped cotton fabric is put in aqueous Cu(II) solutions to load copper ions into the alginate-entrapped fabric matrix. When Cu(II)-loaded fabric is put in aqueous solution of sodium borohydride, the Cu(II) ions present within the polyguluronate residue in "egg-box" cavities and also attached to polymannuronate chains are reduced to yield copper nanoparticles as shown below: in addition, the copper ions bound to –OH functionalities of cellulose also undergo reduction to yield copper nanoparticles.

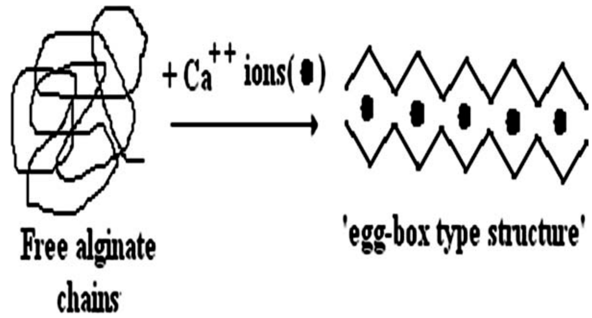

Figure 2 Scheme showing the egg box structure of CAIC fabric.

$$CuCl_2 + 2NaBH_4 \rightarrow Cu + 2NaCl + B_2H_6 + H_2$$

To investigate whether the formation of copper nanoparticles occurred on the surface of the fabric or in the bulk also, the fabric was cut and the cross section was viewed through microscope. It was found that there was dark black appearance only on the surface of the fabric thus supporting our argument.

Characterization of fabric

The results of the TEM analysis are shown in the Figure 3. The image clearly reveals an almost uniform distribution of copper nanoparticles throughout the calcium alginate-impregnated cotton fabric sample. The particle size distribution curve (see inset) was obtained by choose measuring size of individual particles arbitrarily selected from different areas of image. On the basis of the distribution curve obtained, it was found that nearly 45% particles had an average diameter of 60–80 nm. The X-ray

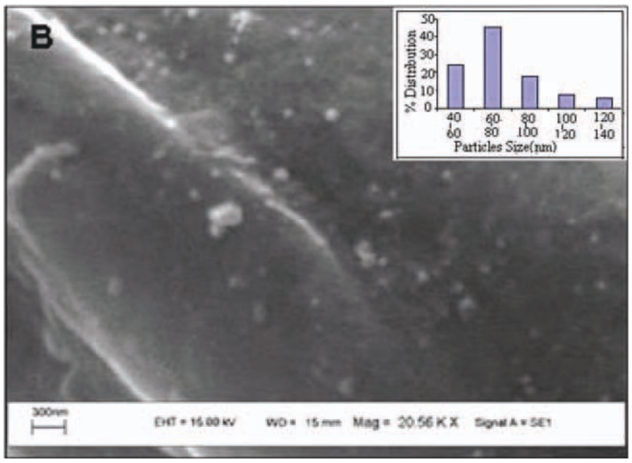

Figure 3 TEM analysis of CNLCAIC fabric. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

diffraction pattern of nano copper-loaded fabric is shown in the Figure 4. It can be observed that three characteristic peaks of copper nanoparticles appear at 2θ value of 43.6, 50.7, and 74.5 degree, which correspond to reflections at (111), (200), and (220) planes respectively. The values obtained are in close agreement with those reported by Thelvasanthi et al.,[21] who reported reflections at of 43.6°, 50.8°, and 74.4°, respectively. Apart from these peaks, the XRD pattern shows amorphous nature of calcium alginate present in the fabric. The crystallinity index (CI) of the fabric was calculated using ratio of peak height corresponding to (111) plane to the sum of heights of amorphous and crystalline peaks. It was found to be nearly 0.92, the indicating fair crystalline nature of the fabric

FTIR spectral analysis

The FTIR spectra of calcium alginate-impregnated cotton (CAIC) fabric [Fig. 5(a)] and copper nanoparticles loaded calcium alginate-impregnated cotton (CNLCAIC) fabric [Fig. 5(b)] are shown. In both the spectra, there is a broad peak corresponding to O–H stretching vibration coupled with intermolecular H-bonding between OH groups of cotton cellulose and alginate chains. In addition, a sharp peak is also visible at 2902 cm^{-1} due to CH stretching vibrations in both the spectra.

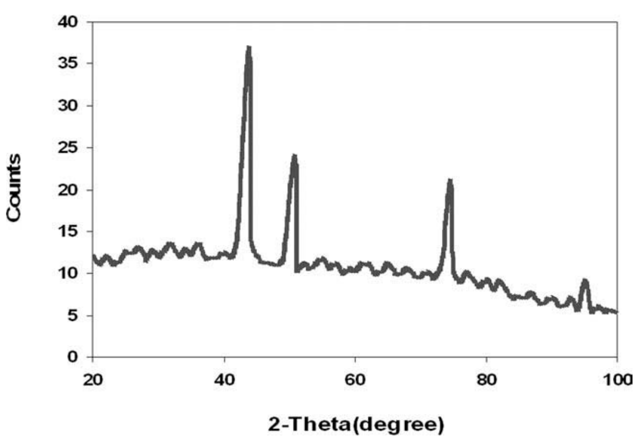

Figure 4 XRD pattern of copper nanoparticles loaded cotton fabric.

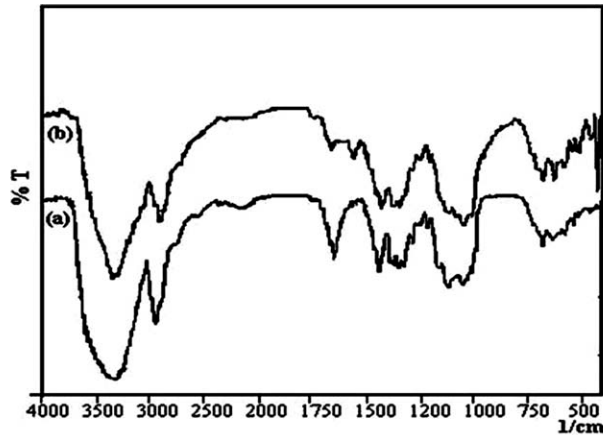

Figure 5 FTIR spectra of (a) CAIC fabric and (b) CNLCAIC fabric.

The strong absorption band, for carbonyl group appearing in spectrum (a) at 1647 cm^{-1} shifts to 1664 cm^{-1} with decrease in sharpness. In addition, carboxylate anion stretching vibrations at 1431 cm^{-1} (symmetric) and 1371 cm^{-1} (symmetric) for alginate-impregnated fabric [Fig. 5(a)] shifts to 1427 cm^{-1} and 1363 cm^{-1}, respectively, in spectra of copper nanoparticles loaded alginate-impregnated fabric. This accounts for binding of Cu with carboxylic groups and carboxylate anions.

Selection of physiological fluid

There have been various reports describing the composition of wound fluid. In a study by Bonnema et al.,[22] it was found that the composition of serum fluid formed after auxiliary dissection, 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. Similarly, Trengrove et al.,[23] reported that wound fluid, collected from leg ulcers, and contained 0.6–5.9 m*M*/L glucose and 26–51 g/L protein. Similarly, Frohm et al.,[24] 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, the authors decided to carry out our *in vitro* study in the PF, as suggested by British

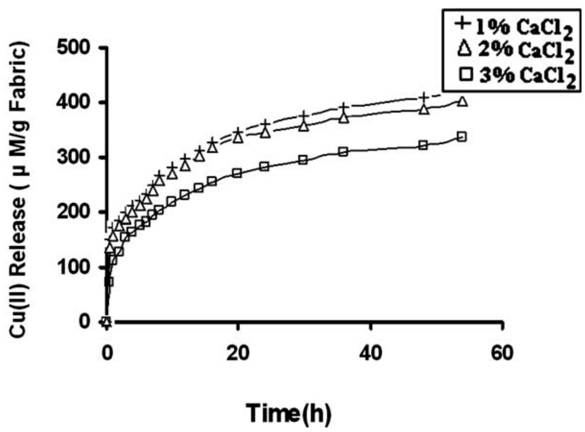

Figure 6 Dynamic release of Cu(II) ions from fabric samples with varying degree of crosslinking of impregnated alginate in physiological fluid at 37°C.

pharmacopoeia, which contained 142 m*M* of NaCl and 2.5 m*M* of $CaCl_2$.

Effect of $CaCl_2$ concentration on Cu(II) release from CNLCAI Fabric

In the present study, alginate chains, entrapped within the cotton fabric, have been crosslinked ionically using calcium chloride solution and therefore extent of crosslinking appears to affect the Cu(II) release. To investigate this, the authors prepared three fabric samples, namely CNLCAIC(1), CNLCAIC(2), and CNLCAIC(3), where the number in parenthesis denotes the percent composition (w/v) of calcium chloride solution used to crosslink the alginate chains within the fabrics. The results of release experiments, carried out with above samples, are well depicted in the Figure 6. It is quite clear that amount of Cu(II) released at different time-intervals shows a negative dependence on the concentration of $CaCl_2$ solutions, i.e., the quantity of copper release followed the order: CNLCAIC(1) > CNLCAIC(2) > CNLCAIC(3). Although the release profiles obtained with fabrics crosslinked with 1 and 2% solutions of $CaCl_2$, are very close to each other, but the total amount of Cu(II) released differ appreciably. The total amount of Cu(II) released in 50 h was 422.6, 402.1, and 336.2 μ*M*/g of fabrics, respectively. The observed findings may simply be attributed to the fact that as the concentration of calcium chloride solution increases, the alginate chains present within the cotton fabrics are crosslinked more strongly, thus producing more tight network of calcium alginate chains within the fabric network. Hence, minimum quantity of Cu(II) is released from the fabric sample CNLCAIC(3), which was crosslinked with 3% $CaCl_2$ solution. On the other hand, the fabric sample CNLCAIC(1), which was crosslinked with 1% $CaCl_2$ solution possessed loosely crosslinked structure of alginate chains and hence demonstrated a faster release of copper ions.

The release data was analyzed using the following equation.[25]

$$M_t/M_\infty = k\, t^n \ldots \tag{1}$$

where M_t and M_∞ are the amounts of Cu(II) released at time t and at equilibrium, respectively, n and k are the release exponent and gel characteristic constant, respectively. The slopes and intercepts, obtained from ln M_t/M_∞ versus ln t linear plots (data not shown) were used to calculate values of n and k (see Table I). The values of release exponents, obtained in all the cases were below 0.45, thus indicating a diffusion-controlled mechanism or Fickian release mechanism.

Effect of alginate content on Cu (II) release

In the present study, sodium alginate, entrapped within the fabric network, has been used to capture the incoming Cu (II) ions via ion-exchange mechanism and therefore, amount of alginate in the soaking solution is expected to affect the release behavior of resulting fabrics. To investigate this, three fabric samples were prepared by immersing plain cotton fabrics in sodium alginate solutions of varying concentrations, i.e., 1, 2, and 3% (w/v), keeping other factors the same. These fabric samples may be designated as CNLCAIC(1%), CNLCAIC(2%), and CNLCAIC(3%), where the percent shown in parenthesis denotes the alginate concentration in the immersion solution. Figure 7 depicts the results of dynamic release of Cu(II) ions from these fabric samples under physiological conditions. It is clear that the amount of copper ions released at different time intervals decreases with the increase in sodium alginate concentration. The total quantity of Cu(II) released from the samples CNLCAIC(1%), CNLCAIC(2%), and CNLCAIC(3%) was found to be 359.7, 281.9, and 225.0 μ*M*/g fabrics, respectively. The observed negative dependence of Cu(II) release on alginate content in the dope and hence in the fabrics may be explained as follows: as the concentration of sodium alginate in the soaking

TABLE I
Diffusion Parameters for Calcium-Alginate Loaded Fabric Treated with Different Amount of Crosslinker $CaCl_2$

S. no.	Sample code[a]	n	k
1	CNLCAIC(1)	0.2298	14.69×10^{-2}
2	CNLCAIC(2)	0.2785	11.82×10^{-2}
3	CNLCAIC(3)	0.3341	6.97×10^{-2}

[a] The number in paranthesis denotes percent concentration (w/v) of $CaCl_2$.

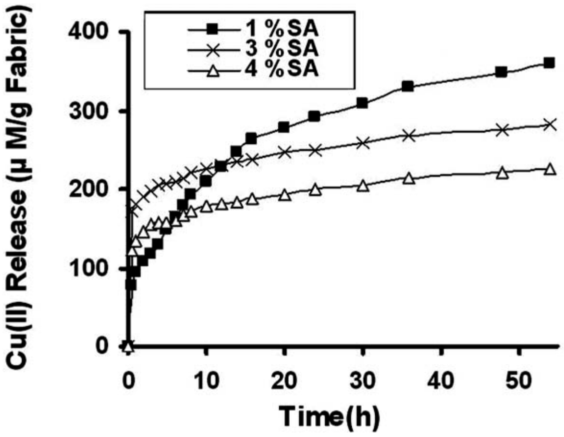

Figure 7 A comparative depiction of dynamic release profiles of Cu(II) from fabric samples with different alginate contents in physiological fluid at 37°C.

solution increases, more and more alginate chains are entrapped within the space available in the fabric networks, thus causing an increase in the amounts of alginate present within the fabric networks. This finally causes an increase in the polymer chains density, thus lowering the release rate. However, a close look at the Figure 6 reveals one interesting result for the initial duration of 6 h the Cu(II) release follows the order 3% sodium alginate >4% sodium alginate >1% sodium alginate. This can be explained as follows: the highest release from fabric may be due to the fact that the Cu(II) with 3% sodium alginate percent on the surface is release first, which is higher for higher SA content (i.e., 3%). However, as the fabric with 1% sodium alginate has fairly low content of calcium alginate, copper ions might be entrapped in lower concentration range, thus causing a slower release.

From the results described above, it may be inferred that the cotton fabric, prepared by immersing in 1% sodium alginate solution and then crosslinked with 1% $CaCl_2$ solution shows maximum release of Cu(II) ions. Therefore, the fabric sample CNLCAIC (1), seems to be suitable for antimicrobial studies.

Interpretation of kinetic release data

The authors selected the above sample to fit the kinetic data obtained onto the diffusion-controlled Higuchi model, proposed by Higuchi.[26] This model describes the release of drugs from nonswellable insoluble matrix as a square root of time-dependent process on the basis of Fickian diffusion [see eq. (2)]

$$Q_t = M_t/M_\infty = K_H\ t^{1/2}... \quad (2)$$

where Q_t is the fractional release or percent release of drug at time t and K_H is the Higuchi constant. The dynamic release data of sample CNLCAIC(1), was applied on eq. (2) and a curve was plotted between Q_t and $t^{1/2}$, as shown in Figure 8. The plot obtained was fairly linear with an excellent regression of 0.9719, thus confirming the suitability of the Higuchi diffusion-controlled release model for the fabric sample CNLCAIC(1). Therefore, it may be inferred that the diffusion of copper ions from the fabric occurs through the diffusion-controlled mechanism. This is further supported by our finding that release exponent "n" for these fabrics was less than 0.45, which is also indicative of diffusion-controlled release of Cu(II) ions.

Antibacterial activity of CNLCAIC fabric

To investigate the antimicrobial action of CNLCAIC fabric, three fabric samples were prepared by immerging cotton fabric in 1% sodium alginate solution, then crosslinking it with 1% calcium chloride solution and then dipping in varying concentrations of Cu(II) ions, i.e., 1, 2, and 3% followed by borohydride reduction these samples may be designated as Cu(1), Cu(2), and Cu(3), respectively, were the number in parenthesis denotes the percent composition of Cu(II) solutions.

The result of antibacterial action of these fabric samples against *E. coli* are shown in Figure 9. It can be seen that petriplates supplemented with fabric samples CNLCAIC(1), CNLCAIC(2), and CNLCAIC(3) show increasing trend of diameter of inhibition zone. This can be simply attributed to the fact that higher is the concentration of the solution, the greater is quantity of copper nanoparticles within the fabric and hence larger is the diameter of the inhibition zone. The diameters were measured as an average of longitudinal and transverse measurements and found to be 2, 2.5, and 3.5 cm, respectively. In this experiment, cotton fabric impregnated with calcium alginate was taken as control, therefore, it may be inferred that biocidal action of the

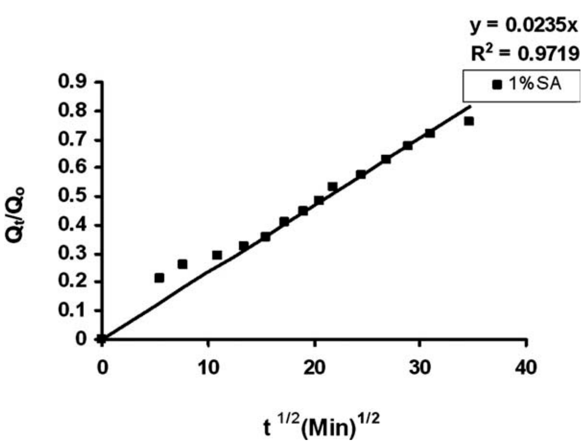

Figure 8 Higuchi plot for dynamic release of Cu(II) from the fabric sample CNLCAIC(1).

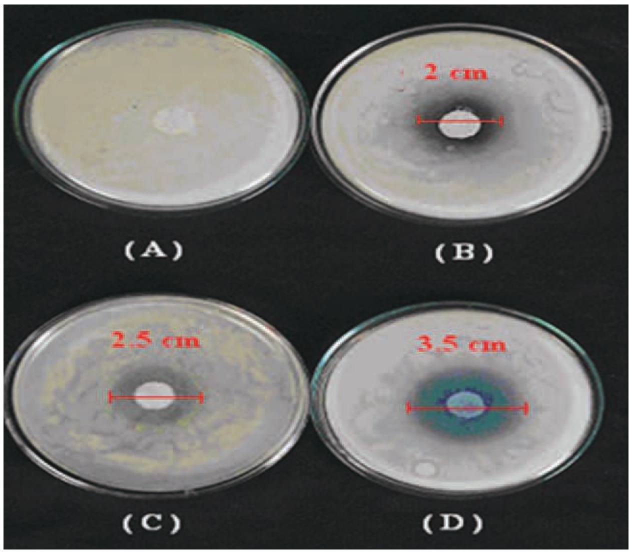

Figure 9 Zones of inhibition for Petriplates supplemented with (A) plain fabric, (B) CNLCAIC(1), (C) CNLCAIC(2) and (D) CNLCAIC(3) fabrics against *E. coli*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

proposed fabric depends upon quantity of copper nanoparticles within the fabrics.

Mechanical properties

The mechanical properties of breaking strength and breaking elongation of plain cotton fabric and CNLCAIC fabric (prepared by immersion in 3% sodium alginate followed by crosslinked with 2% $CaCl_2$ solution) are shown in Figures 10 and 11. On the whole, there is no significant in breaking strength and breaking elongation between plain cotton and CNLCAIC fabric. This indicates that incorporation of calcium alginate into ultrafine network of cotton fabric does not produce any adverse effect on the mechanical properties of fabric.

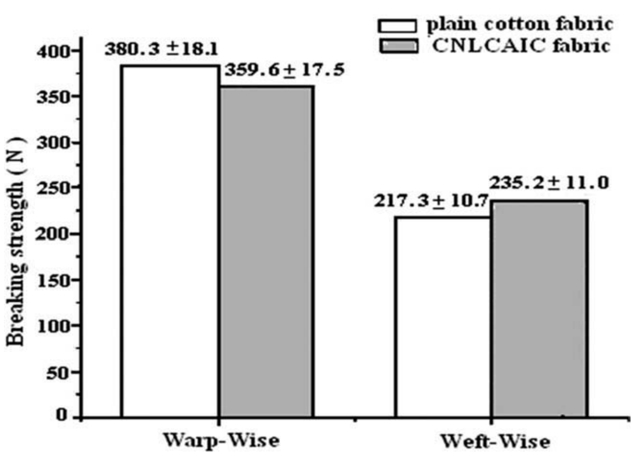

Figure 10 Breaking strength of plain fabric and CNLCAIC fabric.

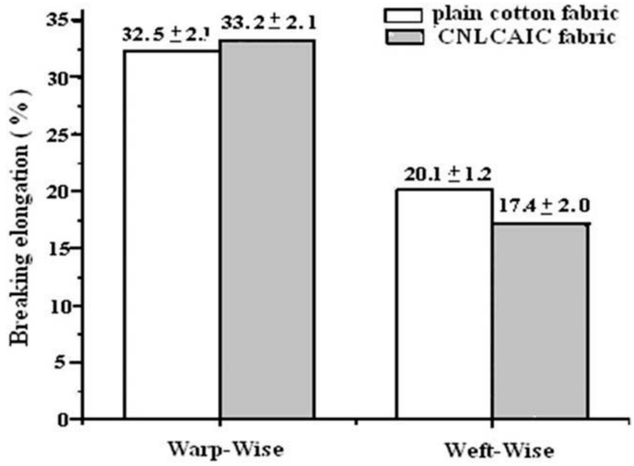

Figure 11 Breaking elongation of plain fabric and CNLCAIC fabric.

CONCLUSION

From the above study, it may be concluded that physical entrapment of sodium alginate into cotton fabric does not result in loss of mechanical strength of fabric. This fabric serves as a substrate for *in situ* formation of copper nanoparticles. The release of Cu(II) shows a negative dependence on amount of sodium alginate and crosslinker $CaCl_2$ used. The fabric shows fair mechanical strength and demonstrates strong biocidal action against *E. coli.*

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