TEXTILE TECHNOLOGY

Investigation of Antibacterial Properties of Silver Nanoparticle-loaded Poly (acrylamide-co-itaconic acid)-Grafted Cotton Fabric

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ABSTRACT

Cotton fabric was grafted with a poly (acrylamide-co-itaconic acid) co-polymer via ceric ammonium nitrate (CAN) induced graft co-polymerization in an aqueous medium. The grafted cotton fabric was loaded with Ag nanoparticles by entrapment of Ag⁺ ions into a grafted polymer network, followed by borohydride reduction. The resulting Ag nanoparticle-loaded grafted fabric was characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) analysis. The Ag nanoparticles were almost monodisperse in nature and their average diameter was approximately 12.5 nm. The fabric shows antibacterial activity against *Escherichia coli* depending upon the extent of grafting of the polymer network onto the fabric and the amount of nano Ag loaded into the grafted fabric. Finally, the Ag-loaded grafted fabric is found to suppress the bacterial growth approximately 3 h after the immersion of the fabric into a nutrient broth at 37 ºC.

Recently, an awareness of general sanitation, contact disease transmission, and personal protection has led to the development of antibacterial fibers to protect wearers against the spread of bacteria and diseases rather than to protect the quality and durability of the textiles (Chun and Gamble, 2007). Most of the processes to create antibacterial fibers entail the attachment of biocidal or bacteriostatic agents to the fabric surface, for example, N-halamine, enzyme, quaternary ammonium salt, chitosan, or zinc oxide.. The various mechanisms used to attach these agents to the fabric include the graft polymerization of N-halamine monomers onto textile substrate (Liu and Sun, 2006), the addition of N-halamine additives into the electrospinning dope of

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fibers (Obendorf and Sun, 2007), the immobilization of enzymes onto ester-cross-linked cotton fabrics (Ibrahim et al., 2007), the placement of quaternary ammonium salt onto cotton fabrics using a covalently bound adduct (Son et al., 2006), the attachment of chitosan to chitosan fabrics via cross-linking agents (El-Tahlawy et al., 2005), and the impregnation of zinc oxide–soluble starch nanocomposites onto cotton fabrics (Vigneshwaran, et al. 2006). Because of the developing resistance of bacteria against bactericides and antibodies and the irritant and toxic nature of some antimicrobial agents, biomaterial scientists have focused their research on nano-sized metal particles such as Ag, titanium dioxide, and copper. Silver, in its many oxidation states (Ag^o, Ag^+, Ag^{2+}, and Ag^{3+}) has been recognized as an element with strong biocidal action against many bacterial strains and microorganisms (Lansdown, 2002). It is believed that the high affinity of Ag towards sulfur or phosphorous is the key element of its antibacterial property. as sulpher and phosphorous are found in abundance throughout the cell membrane, Ag nanoparticles react with sulfur-containing proteins inside or outside the cell membrane, which in turn affects cell viability (Morones et al. 2005). Mastsumure et al. (2003) proposed that Ag^+ ions, released from Ag nanoparticles (Ag^o) can interact with phosphorous moieties in DNA resulting in inactivation of DNA replication, or can react with sulfur-containing proteins to inhibit enzyme functions. These properties allow the incorporation of Ag nanoparticles into various matrices such as polymer networks, textiles, and wound dressing materials (Bajpai et al., 2007; Caruso et al. 2004; Hilton et al. 2004; Ip et. al., 2006; Lee and Huang, 2007; Lee, et al. 2007). However, most of the processes require multiple steps and complex reagents to prepare Ag-coated matrices.

Most recently, we reported a detailed investigation of graft co-polymerization of the monomers acrylamide (AAm) and itaconic acid (IA) onto cotton fabric using ceric ammonium nitrate (CAN) as the initiator in an aqueous medium at room temperature (Gupta et al., 2008). In this paper we report a process that incorporates Ag nanoparticles into a polymer-grafted cotton fabric that yields an almost uniform

distribution of monodispersed Ag nanoparticles within the grafted fabric (Figure 1). This fabric was investigated for its antibacterial activity against the model bacteria *Escherichia coli*.
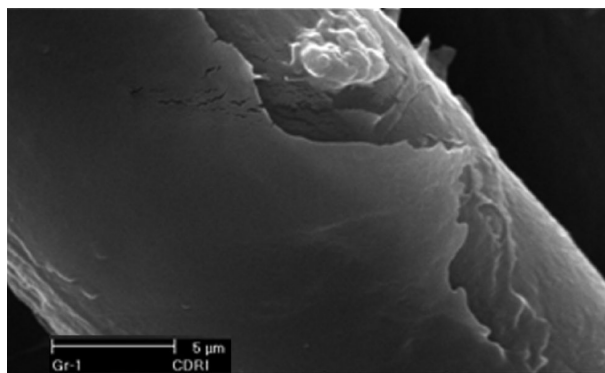

Figure 1. SEM photograph of co-polymer grafted fabric.

MATERIALS AND METHODS

Materials. Acrylamide, IA, N, N'-methylene bisacrylamide (MB), and CAN were obtained from Hi Media laboratories, Mumbai, India. Acrylamide was recrystallized in methanol to remove the inhibitor. Silver nitrate and sodium borhydride salts were obtained from Qualigens Fine Chemicals, Mumbai, India. Nutrient broth (NB) and nutrient agar (NA) were purchased from Hi Media laboratories. Cotton fabric was obtained from a local textile mill.

Preparation of poly (AAm-co-IA)-grafted fabric. All procedures, from solution preparation to the graft co-polymerization, were performed at room temperature. Both the initiator (CAN) and monomer/cross-linker were dissolved in 0.1M HNO_3 and bubbled with N_2. Unless otherwise stated, a pre-weighed cotton fabric piece was put in 10 ml of 20 mM CAN for 15 min, blotted with tissue paper to remove extra CAN, and then immersed in 20 ml of a solution containing predetermined quantities of the monomers AAm, IA, and the cross-linker MB. After the graft polymerization reaction, each substrate was equilibrated in distilled water to remove unreacted salts. Finally, the grafted fabric was put in acetone to remove any homopolymer formed (Taghizadeh and Darvishi, 2001) and to remove water from grafted fabric, and placed at 40 °C in a dust-free chamber until the fabric was completely dry.

The percent grafting (*G*) was calculated using the expression: = $(Wg\text{-}Wo) / Wo$ X 100

where w_o and w_g are the sample weights before and after graft co-polymerization respectively.

To check the reproducibility of the grafting yield, the graft-co-polymerization process was performed in triplicate and the average value of percent grafting is given in the data.

Preparation of nano Ag-loaded grafted fabric. A dry preweighed piece of grafted fabric was equilibrated in distilled water for 24 h. Thereafter, the swollen fabric was put in an aqueous solution of Ag nitrate prepared by dissolving 15 mg $AgNO_3$ in 30 ml of double-distilled water for 24 h. Next the Ag ions present in the fabric were reduced to Ag nanoparticles by putting the fabric in 0.66 mM sodium borohydride solution at 30 °C for 24 h. The resulting dark brown color of the grafted fabric indicated the formation of Ag nanoparticles within the polymer network part of the grafted fabric. Finally the fabric was rinsed with distilled water for 15 s and put in a dust free chamber at 40 °C until it gained constant weight. The dark brown color of the fabric might be a limiting factor for using this fabric as decorative clothing material; however this preparation of Ag-coated fabric can be used as a universal antiseptic substrate for clinical applications. In addition, this process could be used for the fabrication of antibacterial surgical gloves and pads, face-masks, uniform of defense people, particularly for those who are deputed in cold regions like glaciers and have severe infection.

Characterization of nano Ag-loaded grafted fabric. The Fourier transform infrared (FTIR) spectra of grafted and Ag-loaded grafted fabric were recorded with an FTIR-Spectrophotometer (Shimadzu, 8400 S Columbia, USA) using KBr. For this, fabric was cut into a number of small pieces and mixed with KBr. The scans recorded were the average of 100 scans and the spectral range was 400 to 4000 cm^{-1}. The contribution of the background was also accounted for. Transmission electron microscopy (TEM) measurements were performed with a JEOL JEM-2000 Washington,USA, operating at 200 kV. The sample was prepared by equilibrating Ag-loaded fabric in distilled water for 7 d, followed by sonication to get a solution of Ag nanoparticles. Approximately 2 to 3 drops of solution were dispersed on a 3 mm copper grid and allowed to dry for 24 h. Scanning electron microscope (SEM) images were taken with a Hitachi S-4700 (New Jersey USA) operating at an acceleration voltage of 15 kV.

Antibacterial test for Ag-loaded grafted fabric. The antibacterial activity of the fabric was tested qualitatively and quantitatively by the inhibition zone method (Qin et al., 2006) and viable cell count

method (Pal et al., 2007) respectively. In both the methods, the model bacteria was *E. coli.*

For qualitative measurement of antimicrobial activity, the Ag-loaded fabric was cut into 6-mm diam. discs and tested using the modified agar diffusion assay (disc test). The plates were examined for possible clear zones after incubation at 30 ºC for 2 d. The presence of a clear zone around the circular disc on the plate medium was recorded as inhibition against the microbial species.

To examine the bacterial growth or killing kinetics in the presence of Ag nanoparticle-loaded grafted fabric, *E. coli* cells were grown in continuously stirred 100 ml NB at 37 ºC supplemented with a preweighed piece of fabric . The cylindrical sample containers were placed horizontally on an orbital shaker platform and agitated at 200 rpm. Growth or killing rates and bacterial concentrations were determined by measuring the optical density (OD) at 600 nm. The OD values were converted into concentrations of *E. coli* colony forming units (CFU) per ml) using the approximation that an OD value of 0.1 corresponded to a concentration of 10^8 cells per ml (Pal et al., 2007).

RESULTS AND DISCUSSION

Preparation of poly (AAm-co-IA)-grafted fabric. On the basis of the mechanism suggested for Ce(IV)-induced graft-co-polymerization of acrylamide and ethylacrylate onto cellulose (Gupta and Khandekar, 2006), we propose the following mechanism for grafting the monomer AAm and IA onto cotton cellulose.

In the presence of HNO₃ (HA), primary radical species formation occurs as a result of the action of acid on Ce(IV):

$$Ce^{4+} + HA \rightarrow Ce^{3+} + H^+ + A^\bullet$$
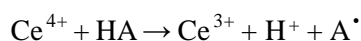
Once the free-radical species (A•) is formed, cellulose macroradicals are produced via direct H abstraction of cellulose molecules:

$$Cell\text{-}OH + A^\bullet \rightarrow Cell\text{-}O^\bullet + HA$$
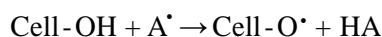
where Cell–OH represents the cellulose molecule. Cellulose macroradicals can also be formed by direct attack of Ce⁴⁺ ions on the cellulose molecule via H abstraction.

$$Cell\text{-}OH + Ce^{4+} \rightarrow Cell\text{-}O^\bullet + Ce^{3+} + H^+$$
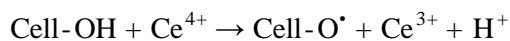
These cellulose macroradicals act upon the double bonds of vinyl monomers— AAm and IA— forming covalent bonds with the creation of a free radical on the monomer which initiates a chain formation.

graft propagation
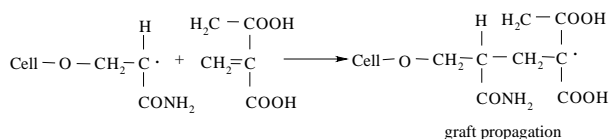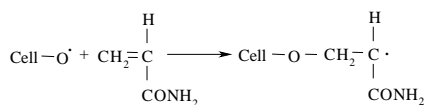
Termination of the growing grafted chain can occur via reaction with the initiator, coupling, or combination and disproportion (Mostafa, 2005).

Formation of Ag-loaded grafted fabric. Because of the applications of Ag-loaded antibacterial fabrics in various biomedical fields, we were interested in developing a facile method to prepare Ag-loaded grafted fabric. The major advantage of our method is that it provides the uniform distribution of Ag nanoparticles within the grafted polymer network onto the fabric. The overall formation of Ag nanoparticles within the grafted network is shown in Fig. 2. When poly (AAm-co-IA)-grafted fabric is put in distilled water for 24 h, the grafted polymer network undergoes appreciable swelling due to the hydrophilic nature of the monomers and the presence of charged –COO⁻ groups along the macromolecular chains that result from the ionization of IA moieties in the network. These three forces—hydrophilicity, chain-relaxation, and osmotic swelling pressure (Bajpai and Dubey, 2004)—cause the network to swell (Fig. 2A). When the swollen fabric is placed in an aqueous solution of AgNO₃, Ag ions enter into the swollen polymer network due to following factors: (a) the ion-exchange process between H⁺ ions present in the swollen network and Ag⁺ ions present in the external solution, and (b) the affinity of Ag⁺ ions to bind with the electron-rich N and O atoms of amide and carboxylate functionalities (Mohan et al., 2007).

In this way, it may be realized that Ag⁺ ions are strongly localized within the three-dimensional cross-linked network (Fig. 2B). Finally, when swollen fabric containing Ag⁺ ions is put in an aqueous sodium borohydride solution, the Ag⁺ ions are reduced to Ag , converted into Ag nanoparticles, and distributed almost uniformly throughout the network (Fig. 2C). The proposed scheme is a slight modification of our previously

reported work (Bajpai et al., 2007), in which we explained the formation of Ag nanoparticles within the poly (acrylamide-co-acrylic acid) hydrogel network.
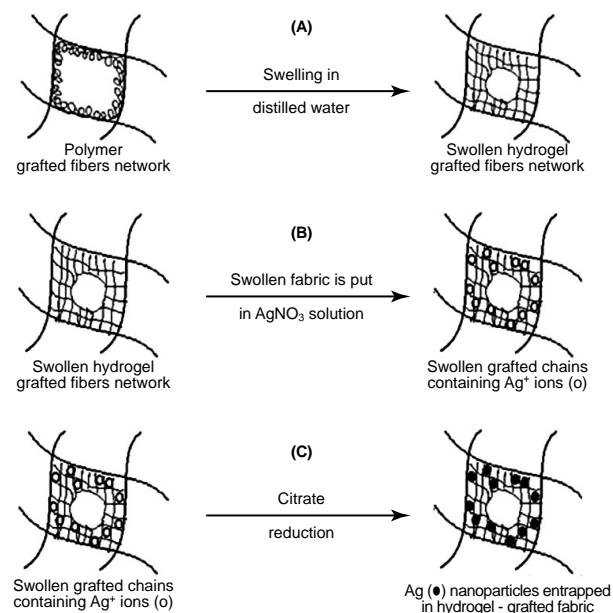

Figure 2. Diagram showing the formation of Ag nanoparticles within the grafted polymer network onto cotton fabric.

Characterization of Ag-loaded fabric. The SEM photograph of Ag-loaded grafted fabric at 500 times magnification is shown in Fig. 3. The dark texture of the ultra fine fibers is caused by the presence of Ag nanoparticles with high electron density. In addition, the almost uniformity of the dark texture suggests that Ag nanoparticles have been distributed uniformly within the grafted-polymer network on the fabric. To confirm the formation of Ag nanoparticles within the polymeric graft chains along the cellulosic fiber network, we carried out TEM analysis. The results shown in Fig. 4 clearly indicate the formation of Ag nanoparticles with an average diameter of approximately12.5 nm. In addition, nanoparticles do not appear to form aggregates, which may be attributed to the stabilization of Ag nanoparticles within the three-dimensional cross-linked polymer network. The uniformly distributed amide and carboxylate functionalities provide electron-rich N and O atoms as binding sites for Ag ions that are later reduced to give uniformly distributed Ag nanoparticles. The size distribution was obtained by measuring the diameter of 88 particles present in an arbitrarily chosen area of TEM image (see inset). Approximately 64% of particles have an average diameter of 12.5 nm and

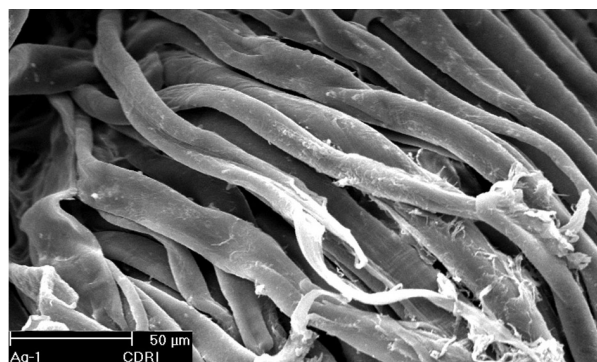the overall distribution falls within the narrow range of 2 to 30 nm.

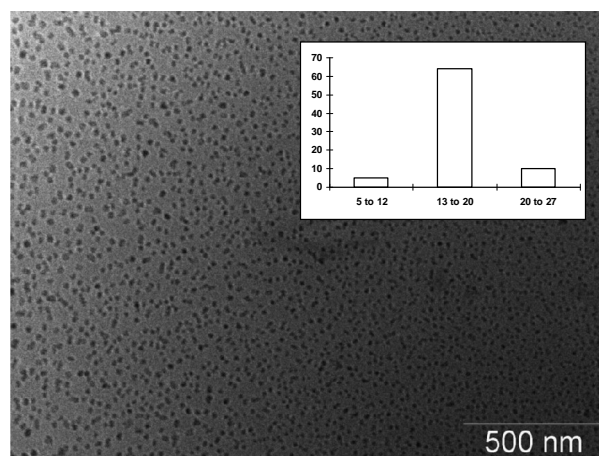**Figure 3. SEM photograph of nano Ag-loaded grafted fabric.**

Figure 4. TEM image of Ag nanoparticles formed in situ in the grafted network.

FTIR spectral analysis. A comparative depiction of FTIR spectra of plain, poly (AAm-co-IA)-grafted, and Ag-loaded grafted fabric is shown in Fig. 5A, B, and C, respectively.

In Fig. 5A a broad peak corresponding to the –OH hydroxyl group of cotton fabric is observed in the 3600-3200 cm^{-1} range and a peak of the –CO carbonyl group is observed at 1651cm^{-1}. Asymmetric C-H stretching is obtained in the 2500–2000 cm^{-1} range, whereas the symmetric peak is obtained in the 2800–2600 cm^{-1} range.

Figure 5B shows a broad peak at 3400–3600 cm^{-1} in the spectrum of the grafted sample due to both the presence of carboxylic groups of IA as well as –NH stretching of amide groups present in AAm and MB. However, in Fig. 5C this broad peak almost disappears in the spectrum of Ag-loaded fabric, thus indicating the binding of Ag with N and O moieties of these functional groups. In addition, the peak at 1672 cm^{-1}, corresponding

to carboxyl groups of amide functionalities in a grafted sample, is shifted to 1693 cm⁻¹ in the spectrum of Ag-loaded fabric. This also indicates binding of an Ag with O in amide groups of AAm and MB.
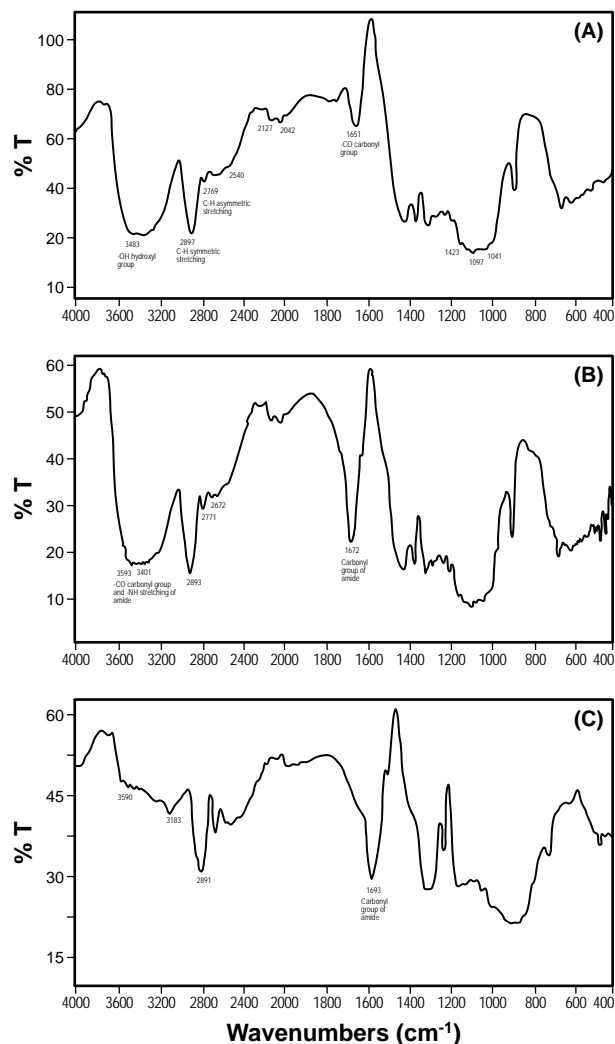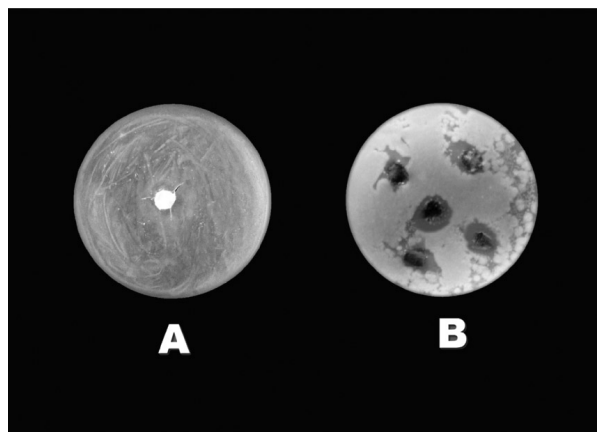

Figure 6. Antibacterial effect of Ag-loaded fabric on *E. coli*.plain fabric (A), Ag loaded fabric (B)

The bactericidal action of Ag-loaded fabric was expected to depend upon the extent of grafting of co-polymer onto fabric. To investigate this, two samples of grafted fabric (60% and 100% grafting) were put in a 20 mg/30 ml Ag nitrate solution and reduced with the same concentration of aqueous sodium borohydride solution. The antibacterial action, as compared with the plain fabric, the control, is shown in Fig. 7. Fabric with 100% grafting exhibited greater inhibiting power against growth of bacterial colonies as compared to the fabric with 60% grafting. The control, plain fabric, showed a dense population of bacterial colonies. The observed finding can be explained by Ag nanoparticles entrapped to a greater extent in the fabric with the relatively higher percentage of grafting (i.e.,100%) due to the availability of a greater number of binding sites for Ag⁺ ions. Therefore, the Ag-loaded fabric with 100% grafting showed more inhibiting power against bacteria. It may be concluded that the greater the amount of grafting on the fabric, the greater is its power to inhibit bacterial growth. We also prepared two Ag-loaded samples with different Ag ion content by immersing the grafted fabrics in AgNO₃ solutions of different concentrations (20 mg/30 ml and 50 mg/30 ml), followed by reduction with sodium borohydride. The results of these antibacterial tests (Fig. 8), clearly indicated that the fabric prepared by immersion in AgNO₃ solution with a concentration of 50 mg /30 ml demonstrated greater biocidal activity as indicated by larger area of inhibition zone. Therefore, the Ag content of the fabric is a key factor in controlling its antibacterial activity.
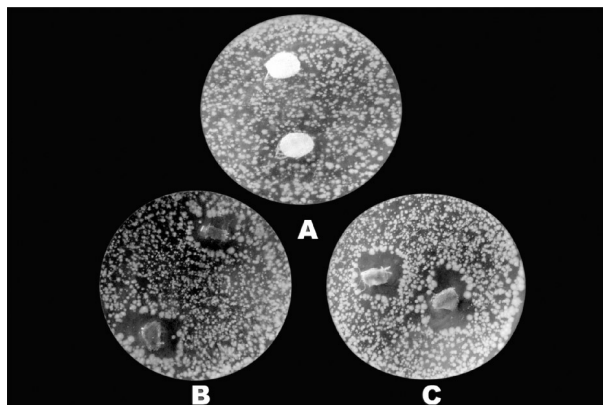

Figure 5. FTIR spectra of plain fabric(A), grafted fabric (B), Ag-loaded grafted fabric (C).

Antibacterial action of Ag-loaded fabric. Figure 6 shows the results of the antibacterial action of nano Ag-loaded grafted cotton fabric on the growth of *E. coli*. It is clear that the growth of the bacterial colonies around the Ag-loaded fabric is inhibited (Fig. 6B), whereas a dense population of bacterial colonies appear in the control set that contains pieces of plain fabric in the petri dish (Fig. 6A). The observed inhibiting action of nano Ag-loaded fabric is due to the release of Ag⁺ ions from the Ag nanoparticles present in the fabric. These Ag⁺ ions come in contact with bacterial cells and kill them.

Figure 7. Effect of degree of grafting on bactericidal action. (A) plain sample (B) sample with 60%, and (C) 100% grafting.
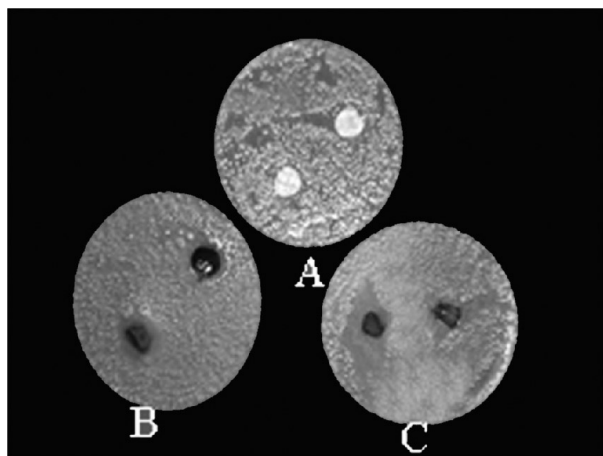

Figure 8. Effect of Ag content on bacteriacidal action. (A) plain sample, (B) sample prepared in 20 mg/30 ml AgNO$_3$ solution, and (C) sample prepared in 50 mg/30 ml AgNO$_3$ solution.

It is clear from Fig. 9 that in the initial phase bacterial growth is almost the same in the media containing plain and Ag-loaded fabrics. The killing action of Ag-loaded fabric began approximately 3 h after its incubation in NB medium. This may be attributed to the fact that when Ag-loaded fabric was put in the bacterial suspension, the fabric began to take up water slowly. As water entered the grafted polymer network, Ag nanoparticles diffused out as Ag$^+$ ions and killed the bacterial cells. The killing activity of Ag-loaded fabric began to diminish later, probably because nearly all the Ag nanoparticles were consumed in binding with bacterial cells.
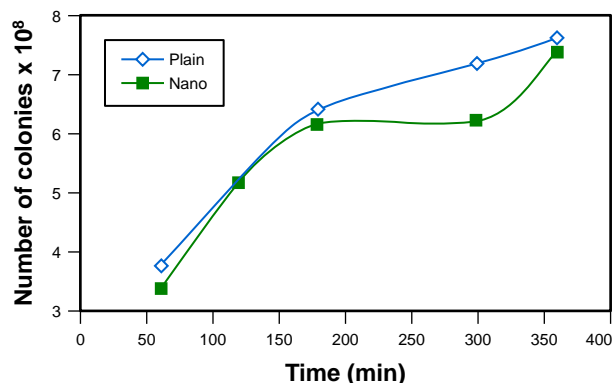

Figure 9. Kinetics of bacterial growth in the liquid NB medium in the presence of plain and Ag-loaded grafted fabric.

CONCLUSION

From this study we concluded that the in situ formation of Ag nanoparticles in a grafted polymer network of cotton fabric was an effective method for the preparation of antibacterial fabrics. The almost uniform distribution of narrow dispersed Ag nanoparticles is a major advantage of this method. These fabrics show biocidal action against the bacteria *E. coli*, thus showing great potential to be used as an antiseptic dressing or bandage, which are in high demand for biomedical applications.

ACKNOWLEDGMENTS

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