Novel Strategy for Synthesis of ZnO Microparticles Loaded Cotton Fabrics and Investigation of their Antibacterial Properties

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ABSTRACT

In this work, ZnO microparticles were in situ in the chitosan – attached cotton fabric following 'equilibration – cum - hydrothermal' approach to yield zinc oxide microparticles loaded cotton (ZOMLC) fabrics. The ZnO microparticles were characterized by surface plasmon resonance (SPR), X-ray diffraction (XRD) and scanning electron microscopy (SEM) techniques. The ZOMLC fabric showed fair antibacterial action against model bacteria *E.coli.*

INTRODUCTION

Today, there is an increasing demand for health safeguarding products in market. The requirement for hygienic living conditions has led to a great need of antimicrobial materials that do not allow microbes to attach, survive or at least proliferate on materials surfaces. Textiles, being the chiefly used product by mankind, efforts are being made to use them to safeguard our health. For this purpose, antimicrobial properties are being imparted to textile materials by chemically or physically incorporating various functional antibacterial agents onto fibers or fabrics by different techniques [1-2]. For years, organic compounds such as halogenated hydantoins have been employed in textile finishing processes as antibacterial agents [3-4]. However, N-helamine compounds have adequate solubility in water to allow a residual halogen concentration in excess of that required for disinfection [5]. Also a number of microbial trends have demonstrated an increasing resistance towards antibiotics and thus there has been extensive search for newer and better modes, to render antimicrobial functions to the fabrics surfaces [6]. With the emergence of nanotechnology, material scientists have focused their attention on immobilizing nobel metals based nano-particles like silver, gold, copper, etc on fibers because these metal nanoparticles show excellent biocidal action [7-12]. A number of methods are used for the surface modification of fibers and fabrics with these nanoparticles, including blending of the nanoparticles in the polymer matrix before spinning or chemical grafting of the desired functional groups onto the fibers.

Recently, Gupta et al. [13] 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 against fungi. Similarly, Chen et al [14] 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*.

Although silver and gold nanoparticles have proved to be highly efficient against microbes, but, the high cost of these metals has limited their use as antibacterial agents on industrial basis. Therefore, currently nanoparticle metal oxides, such as ZnO, have emerged out as a new class of important materials that are increasingly being developed for use in research and health related applications, due to their low cost, easy availability and unique chemical and physical properties. Recently, there have been several reports regarding the antimicrobial activity of ZnO nanoparticles [15]. It has been reported, on the basis of preliminary growth analysis, that ZnO nanoparticles have higher antibacterial affects on microorganism like *S. Aureus* than other metal oxide nanoparticles [16]. Similarly, Tam et al [17] have reported antibacterial activity of ZnO nanorods prepared by hydrothermal method exhibited fair activity against *E. Coli* and *B. Atrophaeus*, but it was considerably more effective in the later case (at 15 mM versus 5mM concentration respectively, showing zero viable cell count). For both organisms, damage of cell wall was observed. Recently, Padmavathy et al [18] have investigated biocidal action of ZnO nanoparticles of different sizes. The nanoparticles were characterized by SEM, TEM and XRD analysis. It was observed that ZnO nanoparticles showed enhanced activity as compared to ZnO microparticles. They suggested that both the abrasiveness and the surface oxygen species of ZnO nanoparticles promote the biocidal properties.

In another work, Yadav et al. [19] have reported a simple method to prepare nano ZnO and coat 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 property, ZnO nanoparticles also show fair biocidal action. Recently, Vigneshwaran et al. [20] 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.

Although ZnO nanoparticles are most suitable for antimicrobial finishes in textile sectors, but in most of the research works carried out in recent past, the nanoparticles were coated on to the cotton fabrics. However, the attachment of microparticles to fabric is almost physical in nature in this work; we have proposed a unique approach which involves attachment of chitosan molecules onto cotton cellulose fabric via coupling reaction induced covalent attachment followed by incorporation of ZnO microparticles into chitosan layer by 'equilibration-cum-hydrothermal' approach. We used chitosan in this work due to its excellent biocompatibility and its strong binding tendency with metal ions. The most important property of chitosan is its chelating power with excellent adsorption capacities for a number of metal ions due to its parent amino (–NH2) and hydroxyl (–OH) groups in its structure [21]. The fabric has been investigated for its biocidal action against *E.coli*.

EXPERIMENTAL

Material

Chitin was purchased from Hi Media (Mumbai, India) and its deacetylation was carried out with 50% NaOH (w/v) at 90°C in N_2 atmosphere for 2 h [22]. The chitosan flakes thus obtained were washed till neutrality and then dried at 50°C in an electric oven (Tempstar, India). Glacial acetic acid, potassium periodate, zinc chloride and sodium hydroxide were obtained from E. Merck (Mumbai, India). Nutrient broth, nutrient agar and were obtained from Hi Media Chemicals (Mumabi, India) and used as received. The Department of Biotechnology (Govt. Model Science College, Jabalpur, M.P., and India) provided standard cultures of the organisms. Mill-scoured cotton fabric of 125 g / m^2.were obtained from a local textile mill (Cotton Textile Mill, Indore, India). Double distilled water was used through the investigations.

Preparation of Chemically Modified Cellulose Fabrics

Chemically modified cellulose fabric was prepared by oxidizing the fabric by potassium periodate and then chitosan was covalently attached to the modified fabric before loading with ZnO microparticles. In brief, cotton fabrics were washed thoroughly and dried in a dust free chamber. Oxidised fabrics were prepared by following the method given by Varavinit et al [23] with slight modifications. 1 gm of cotton fabric was soaked in 250 ml of 0.01M of potassium periodate at 50˚C for 24hrs. This lead to the oxidation of fabrics, thus resulting in formation of cellulose dialdehyde fabrics. Now, 1g of cellulose dialdehyde fabric, prepared as mentioned above, was immersed in 30ml of 1 and 2 percent chitosan solutions prepared in 2 percent acetic acid (v/v) solution, and allowed to react for a period of 2 h at 50°C. The fabric changed to pale yellow from white due to the attachment of chitosan on the surface. The chitosan attached fabrics were then further used to load ZnO microparticles.

Preparation of ZnO Microparticle Loaded Fabrics

500 mg of chitosan attached fabrics were immersed in 2% (w/v) aqueous solution of Zinc chloride for 12h at 30^0C. The loaded fabric was transferred into 0.02M aqueous solution of sodium hydroxide. After 4h, the fabric was taken out and kept in electric oven (Tempstar India) at 70^0C for 2h for complete conversion of $Zn(OH)_2$ into ZnO by thermal curing. Finally, the fabric was washed with de-ionized water and then put in vacuum chamber till further use. The sample thus prepared were designated as ZOMLCA

(X) fabric where the number X in parenthesis denotes the concentration of solution used for equilibration. For example, the fabric prepared, as above, will be designated as ZOMLCA (2). *Figure 1* shows optical images of plain, chitosan-attached and ZnO microparticles loaded fabrics.

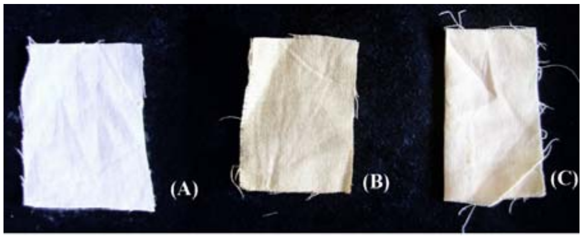

FIGURE 1. Photograph of (A) plain cotton fabric (B) chitosan attached cotton fabric (C) ZnO microparticles loaded chitosan attached fabric.

Characterization

The FTIR spectra of fabrics were recorded with FTIR spectrophotometer (Shimadzu 8400 S, (Shimadzu, Japan) using KBr. The surface plasmon resonance measurement of the fabrics was performed by using Systronics 2201 UV spectrophotometer (Systronics 2201, India). XRD analysis for ZnO microparticles loaded chitosan attached fabrics were also performed. Transmission electron microscopy (TEM) studies of the chitosan attached fabric and Zn microparticles loaded chitosan attached fabric were also performed.

Antimicrobial Activity

The antibacterial activity of plain, chitosan attached and ZnO microparticles loaded chitosan attached fabric was tested qualitatively and quantitatively by zone inhibition methods [24]. In all these methods, *E. coli* were taken as model bacteria. For qualitative measurement of antimicrobial activity, the fabrics (plain, chitosan attached and ZnO microparticles loaded chitosan attached were cut into discs of known diameter and antimicrobial activity was tested using modified agar diffusion assay (disc test). The plates were examined for possible clear zones after incubation at 37°C for 24h. The presence of clear zone was recorded as inhibition against the microbial species.

For percent reduction test, the test fabric was shaken in a known concentration of bacterial suspension and the reduction in bacterial activity was measured. The percent reduction was calculated as:

$$R = (A-B) \times 100/A \qquad (1)$$

where R is percent reduction, A is the number of bacteria in the broth inoculated with the fabric sample immediately after inoculation i.e. at zero contact time and B is the number of bacteria recovered from the broth inoculated with the fabric after the desired period (18 h).

RESULTS AND DISCUSSION

Fabrication Of ZnO Microparticle Loaded Fabrics

In the present study, we attached chitosan macromolecular chains to the cotton cellulose fabric and then used these polymeric chains as templates for introducing ZnO microparticles. In this way, there occurs almost uniform distribution of ZnO microparticles throughout the fabric and the incorporated ZnO microparticles are well protected within the attached chitosan chains. The overall scheme for formation of ZOMLCA fabric may be explained as shown in *Figure 2*.

When the fabrics are treated with potassium periodate solution, the C_2-C_3 linkage in cellulosic chains is cleaved and cellulose dialdehyde is formed (*Figure 2 (a)*). Treatment of chitosan solution with the fabric at this stage leads to coupling of the amino ($-NH_2$) groups of chitosan, with the aldehydic groups of cellulosic fabrics (*Figure 2 (b)*). The chitosan attached fabrics are then immersed in aqueous solution of salt solution where Zn binds to the ($-NH_2$) and hydroxyl (–OH) groups of chitosan and cellulosic networks (*Figure 2 (c)*). *Figure 2 (d)* shows that on treatment with NH_4OH, $Zn(OH)_2$ are formed which then yields ZnO microparticles on thermal curing.

Characterization Of ZnO Microparticle Loaded Fabrics

The FTIR spectra of plain cotton fabric (A), chitosan attached cotton fabric (B) and silver nanoparticle loaded chitosan attached cotton fabric (C) are shown in the *Figure 3*. The plain cotton fabric shows a broad band of stretching vibrations of –OH group from 3540 cm^{-1} - 3120 cm^{-1} [25]. The C-O-C stretching vibrations are found 1445 cm^{-1}. In the FTIR of chitosan attached fabric a slightly broader peak is seen around 3540 cm^{-1} - 3120 cm^{-1} due to the broad peaks of OH as well as NH stretching vibrations. A sharp peak at 1730 cm^{-1} is characteristic peak of C=O of aldehydes formed during oxidation of fabric to cellulose dialdehyde as explained in the scheme in *Figure 2(b)* and a very intense peak at 1658 cm^{-1} due to N-H bending of NH_2 and at 1587 cm^{-1} due to C=N stretching. These are clear evidences of attachment of chitosan chains on cotton fabric. The FTIR of the ZnO nanoparticles loaded fabric show shortening of the peaks around 3540 cm^{-1} - 3120 cm^{-1} and a slight shifting of NH bending vibrations to 1680 cm^{-1} which is an evidence of attachment of Zn to the NH_2 groups of chitosan chains. The asymmetric and symmetric stretching vibrations in all the three cases is at 2925 cm^{1} and 2853 cm^{1} respectively.

Figure 4 shows X-ray diffraction pattern of micro ZnO-loaded fabrics. The peaks obtained at different crystal planes of micro zinc oxide were very close to reported data (JCPDS76-0704) [26]. Our values were also resembling with those obtained by Li et al [27], who reported 2θ values of 31.7, 34.4, 36.2, 47.5, 56.6, 62.8, and 69.1 for reflections at (100), (002), (101), (110), (103), and (112) planes. The values of 2θ as displaced in *Figure 4* are 31.6, 34.4, 36.0, 47.5, 56.6, 63.0 and 69.2 respectively which are almost identical with the reported values. In addition to this, the presence of chitosan is also confirmed by the diffraction peaks observed at 2θ = 8.5, 15.8 and 23.0 which also match with the values reported by Zhong et al [28]. Here it is also worth mentioning that some insignificant additional small peaks have also been observed.

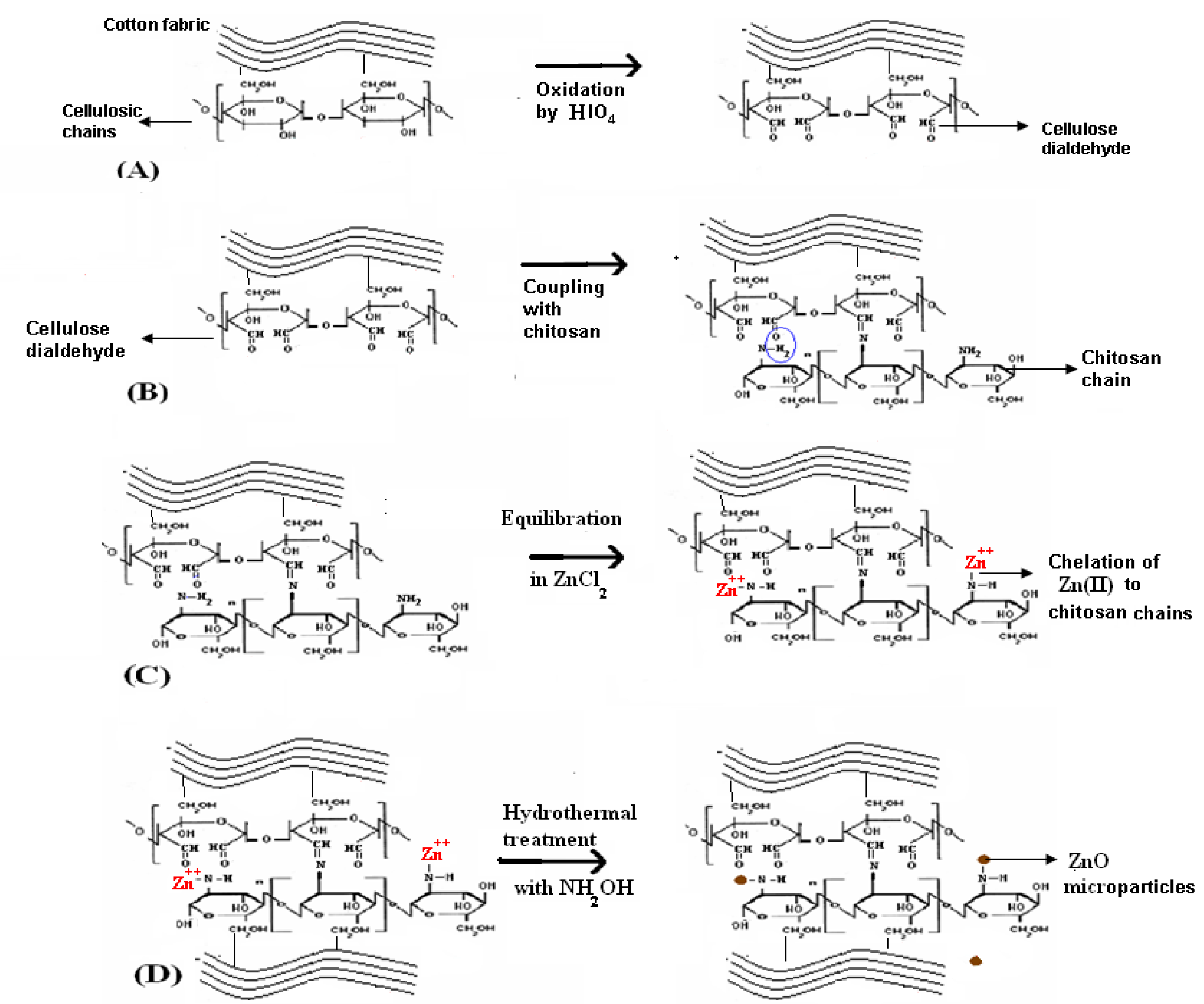

FIGURE 2. Scheme showing the (A) oxidation of plain cotton fabric; (B) coupling of chitosan to fabric; (C) equilibration of fabric in solution and (D) formation of ZnO microparticles loaded chitosan attached fabric.

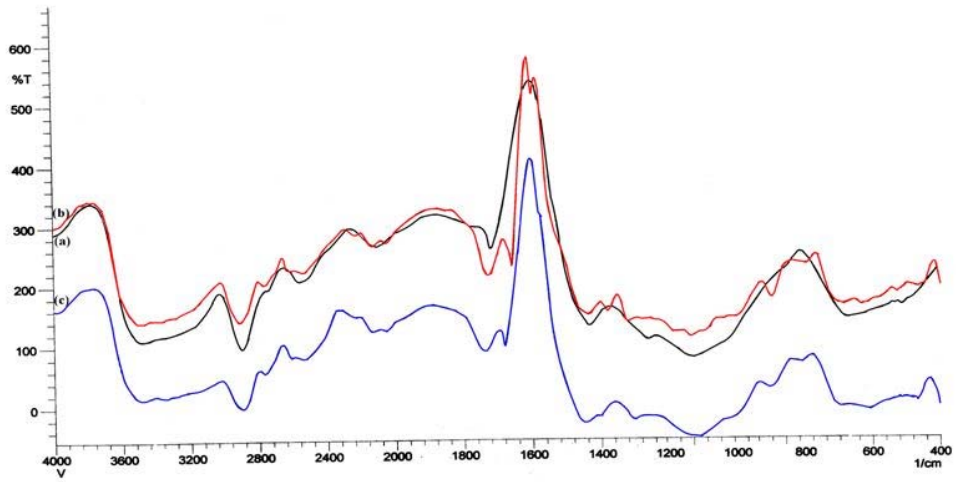

FIGURE 3. FTIR spectra of (A) Plain cotton fabric, (B) chitosan attached cotton fabric and (C) ZnO microparticles loaded chitosan attached fabric.

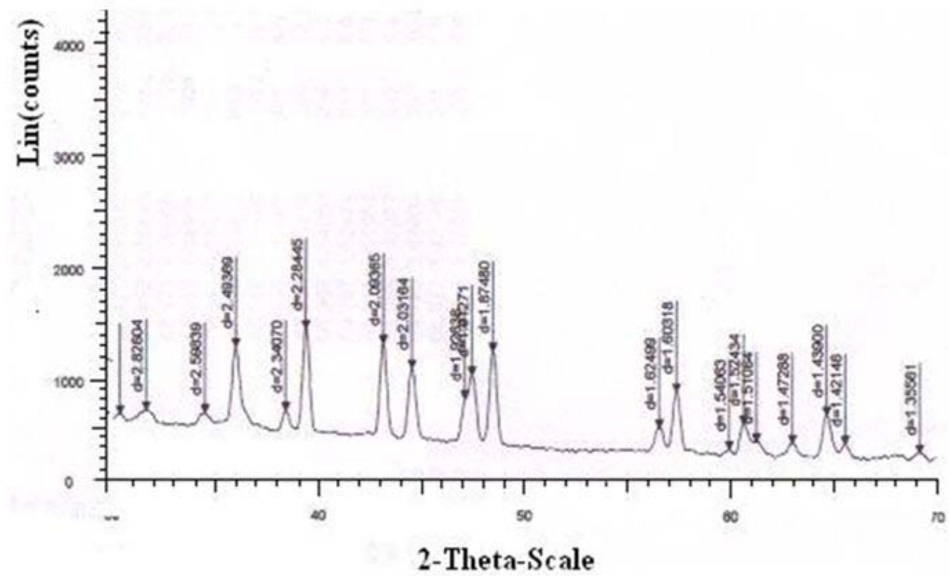

FIGURE 4. XRD pattern of the ZnO microparticles attached chitosan loaded fabric.

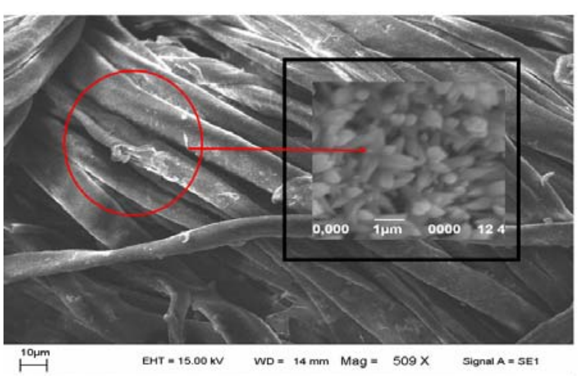

FIGURE 5. Scanning electron micrograph (SEM) of chitosan attached micro ZnO loaded fabrics. Inset shows the magnified portion of the fabric

Figure 5 shows the SEM images of micro ZnO loaded fabrics. It is clear that there is dense population of ZnO microparticles in the fabric prepared with 4 percent (w/v) $ZnCl_2$ solution. In addition, the almost uniform distribution of ZnO microstuctures establishes the superiority of present method involving in situ formation of zinc oxide microstructures within the chitosan attached ZnO microparticles loaded fabrics.

Antimicrobial Activities Of ZnO Microparticle Loaded Chitosan Film

A number of chemically modified fabrics were prepared for increasing the antibacterial efficiencies of the fabrics. Silver, gold nanoparticles demonstrate excellent antimicrobial activity, alone [29-30] or within the substrates [31-32]. But as mentioned in introduction, due to the high price of these metals cost effective nanoparticles like the ZnO are now preferred in fabrics [33]. To investigate the biocidal action of ZOMLCA fabrics synthesized in our work, we considered *E.coli* as model bacteria and observed their growth in the presence chitosan attached plain cotton fabric, ZnO microparticles– loaded chitosan attached fabrics by zone inhibition method, taking plain cotton fabric as a control as a control.

The results, as depicted in the *Figure 6*, clearly indicates the formation of inhibition zones of 2.5 cm around the circular ZnO microparticles-loaded chitosan attached fabrics (see *Figure 6(C)*) while there is a dense population of bacterial colonies in the petridishes with plain and chitosan attached fabric (see *Figure 6 (A)*and (B)).

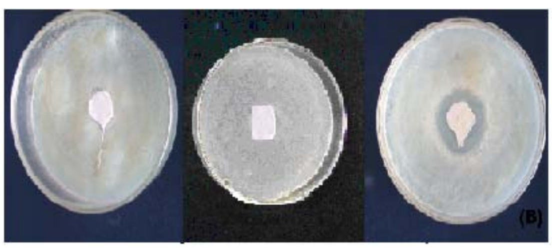

FIGURE 6. Photograph showing the inhibition zone of (A) plain cotton fabric disc (B) chitosan attached cotton fabric disc and (C) Zinc (II) loaded chitosan attached fabric.

Here it is worth mentioning that chitosan has also been reported to possess antimicrobial activity [34]. However, in the present study, the chitosan-attached fabric did not show any biocidal action. This could probably be due to the fact that chitosan used in this study was high molecular weight, which must have suppressed its biocidal action [35]. One more reason may be that since chitosan is attached to cellulose via coupling reaction between its –NH2 group and –CHO group of cellulose dialdehyde, thus reducing number of protonated amino groups in chitosan chains which are mainly responsible for antibacterial activity of chitosan. Finally, since chitosan chains are attached to the cotton fabric, their diffusion is restricted. This also suppresses its biocidal action.

In order to investigate the effect of amount of ZnO microparticles entrapped on the effectiveness of biocidal action, we prepared fabrics ZOMLCA (1), ZOMLCA (2), ZOMLCA (3) and ZOMLCA (4) and investigated their antibacterial efficiency.

The results, as depicted in *Figure 7*, clearly indicate that diameter of inhibition zone increases with concentration of Zn(II) solutions, being smallest for petridish supplemented with fabric sample ZMLCA (1) and maximum for sample ZOMLCA (4).

This may simply be attributed to the fact that as the concentration of Zn(II) solution increases, more and more Zn^{2+} ions get entrapped within chitosan chains, thus finally resulting in higher concentration of ZnO microparticles within the fabric. Hence it proves that higher the amount of Zn(II) salts used to treat the fabric, the greater will be the concentration of ZnO microparticles formed and more will be the antibacterial efficiency of the resulting fabric. In this way, the antibacterial tests were successful in exhibiting the biocidal properties of the synthesized fabrics loaded with ZnO microparticles.

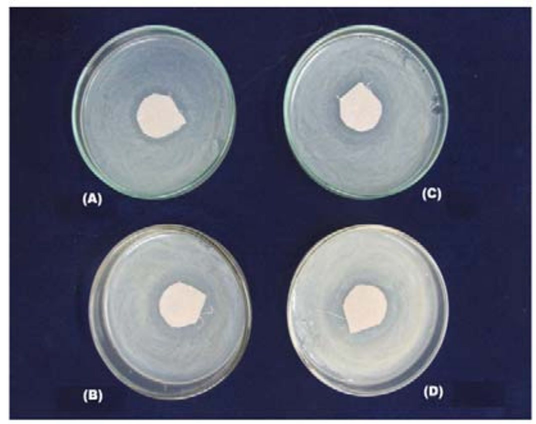

FIGURE 7. Photograph showing 'zone of inhibition' in petridishes supplemented with (A) ZOMLCA (1); (B) ZOMLCA (2); ZOMLCA (3) and (D) ZOMLCA (4) fabric samples.

Finally the wash durability testing of ZnO micro particles –loaded fabric i.e. ZOMLCA(2) was carried out using a neutral soap at 40 ^{o} C for 30 min, keeping the material to liquor ratio as 1:50, followed by rinsing, washing, and drying. After complete drying, the fabric was assessed for antibacterial activity using percent reduction method.

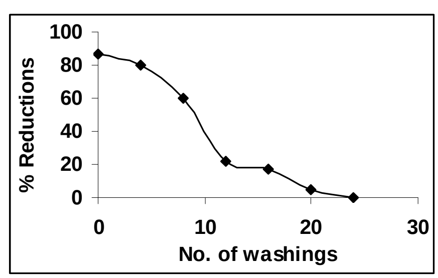

FIGURE 8. Percent bacterial reduction as a function of no. of washing cycles for ZnO microparticles loaded fabric.

The results, as shown in the *Figure 8* clearly indicate that the percent reduction continues to decrease with the increase in the number of washing cycles. It was observed that after 20 washing cycles the percent reduction was almost zero.

CONCLUSION

From the above study it may be concluded that covalent attachment of chitosan to oxidized cotton cellulose results in formation of a fair substrate into which ZnO microparticles can conveniently be entrapped to yield antibacterial fabric. The fabric shows excellent biocidal action against *E.coli*. The antibacterial action of fabric can simply be increased by increasing the amount of chitosan bound. The fabric shows great potential to be used in biomedical applications.

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