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Research Article

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Synthesis and coating of ZnO-BTCA composite on cotton for Antibacterial activity

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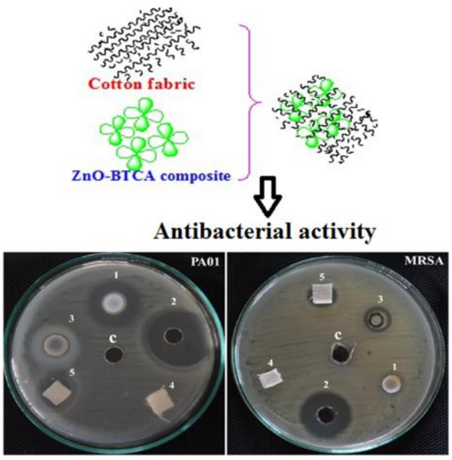
Graphical Abstract



Abstract

Zinc oxide-1,2,3,4-butanetetracarboxylic acid (ZnO-BTCA) composite was synthesized by chemical method. It was coated by pad-dry-cure method on cotton fabric and characterized by FT-IR and SEM with EDX techniques. ZnO, ZnO-BTCA composite powder and ZnO-BTCA composite coated cotton fabric were tested for antibacterial activity against Gram positive (*Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli)* by agar well diffusion method. Antibacterial activity result reveals that inhibitory effect of ZnO-BTCA composite coated on cotton fabric against *Escherichia coli* was more potent than *Staphylococcus aureus*.

Keywords: surfaces, sol-gel chemistry, infrared spectroscopy, surface properties.

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Introduction

Antibacterial agents are of capital importance in various industries like water disinfection, textiles, packaging, construction, medicine and food. Qilin et al., (2008). Growing resistance of microorganisms to potent antibiotics has renewed a great interest towards investigating antibacterial properties of nanoparticles and their nanocomposites. Cotton is one of the most important natural fibers, widely used as clothing materials due to its excellent characteristics, including regeneration, biodegradation, softness, comfort, cool and hygroscopic properties. Chi-Wai et al., (2011). Zinc oxide (ZnO) exhibits significant bacterial growth inhibition than other metal oxide nanoparticles. ZnO/Fe_2O_3 composite nanoparticles synthesized by basic hydrolysis of Fe^{2+} and Zn^{2+} ions in aqueous continuous phase containing gelatin showed improved antibacterial activity against *Staphylococcus aureus* than *Escherichia coli.* Tamar et al., (2011) . Nanostructured ZnO was deposited on cotton bandage in an aqueous solution under mild conditions at room temperature and neutral pH. The coated bandage showed better antibacterial activity against Gram-positive as well as Gram-negative bacteria. Manna et al., (2013). The suspensions of ZnO nanofluids showed better antibacterial activity against *E. coli.* Zhang et al., (2007). Ag_2O and ZnO were used as catalysts in the antimicrobial formulation on cotton fabric for improved antibacterial activity Lam et al., (2012). ZnO nanoparticles were synthesized in diethylene glycol (DEG) medium by forced hydrolysis of ionic Zn^{2+} salts. Particle size and shape were controlled by the addition of small molecules and macromolecules such as tri-n-octylphosphine oxide, sodium dodecyl sulphate, polyoxyethylene stearyl ether, and bovine serum albumin. The results confirmed that *E. coli* cells after contact with DEG and ZnO were damaged showing a Gram-negative triple membrane disorganization. Brayner et al., (2006). The antibacterial activity of ZnO nanoparticles synthesized by wet chemical method was examined against *E. coli* and *P. aueroginosa.* The maximum inhibition was observed at a concentration of 100 µl. Chitra et al., (2013). Nano-zinc oxide coated on cotton fabrics could impart functional properties such as better strength, air permeability and UV-absorption. Yadav et al., (2006). Crosslinking agents are used for the stabilization of nanoparticles on cotton fiber as there is no attraction between inorganic particles and cotton. Non-formaldehyde based crosslinking agents containing carboxylic acids such as 1,2,3,4-butane tetracarboxylic acid (BTCA), citric acid, succinic acid and maleic acid are used for this purpose. El-Tahlawy et al., (2005). Finishing of cellulosic fabrics with chitosan-polyethylene or glycol-siloxane improves antibacterial properties. Abdel-Rahman et al., (2013). Application of BTCA-Nano TiO_2 on cotton fabric was used for crosslinking cellulose, which yielded higher Nano TiO_2 absorption and enhanced self-cleaning properties. The treated fabrics indicated an antibacterial activity against both *S. aureus* and *E. coli* bacteria. Nazari et al., (2013). Polycarboxylic acid as crosslinker, sodium hypophosphite as catalyst, nano ZnO were used to prepare composite by step by step process and coated on cotton to enhance the flame retardant property. Abd El-Hady et al., (2013).

In this study, it was aimed to use BTCA as crosslinker, sodium hypophosphite as catalyst, nanoZnO as antibacterial agent to prepare the composite by a single in situ step method and to coat on cotton to get the final product as antibacterial and non-formaldehyde based finish.

Materials and methods

Woven cotton fabric of 80 count was obtained from South Indian Textile Research Association (SITRA), Coimbatore. Pretreatment was carried out for the grey fabric by one pot method. The recipe used was 0.4 ml HCl, 3.9 g Na_2CO_3, 1.9 g NaOH and 1.9 ml H_2O_2 in 200 ml water. Grey cotton fabric (8cm x 4cm) was immersed in the bath and the temperature was kept at 80°C for 90 minutes.
Nano ZnO (Sigma Aldrich, 97%) with an average particle size of 50 nm, 1,2,3,4-butane tetracarboxylic acid (Alfa Aesar, 98%), sodium hypophosphite (S.D fine, 98%) were purchased and used as such.

Synthesis of ZnO-BTCA composite
3 g BTCA, 2 g sodium hypophosphite (SHP) and 0.2 g nano ZnO were taken in a beaker containing 100 ml water. The pH of the mixture was noted as 4.5. Stirring was carried out for 2 h. White colloidal solution was obtained. It was assumed that ZnO might be converted to zinc hydroxide in the presence of acidic medium. Thus, the colloidal solution obtained might be containing zinc hydroxide.

Coating on cotton
Bleached cotton fabric was padded in the prepared colloidal solution in two dip and two nip, and squeezed to a wet pick-up for 40 minutes. The coated fabric was dried at air and the crosslinking of the ingredients was effected by curing at 180°C for 4 min in a curing chamber. It is assumed that during curing, the colloidal zinc hydroxide present in the coated fabric might be converted back to ZnO. The cured fabric was then rinsed with de-ionized water three times to remove residual BTCA and SHP.

Characterization
The formation of ester linkage, the possible Zn-O and Zn-O-H groups in coated fabric were examined by FT-IR spectra using RXI [Perkin Elmer] in the range of 400-4000 cm^{-1}. The surface morphology of the fiber before and after coating was investigated using Scanning Electron Microscope (SEM) images obtained using FEG quanta 250.

Antibacterial analysis
ZnO, BTCA, ZnO-BTCA composite, ZnO-BTCA composite coated fabric, uncoated fabric were tested for antibacterial activity. *Pseudomonas aeruginosa* PAO1 and methicillin resistant *staphylococcus aureus* (MRSA) ATCC 33591 were used for this study. These strains were grown in Luria Bertani (LB) broth and tryptic soy broth (TSB) respectively and incubated at 37°C. For all the assays, overnight cultures of *P. aeruginosa* PAO1 and *S. aureus* MRSA were used in 1:100 dilutions. Antibacterial activity was assessed by disc diffusion susceptibility test [Clinical and Laboratory Standards Institute, 2006] in Muller–Hinton agar (MHA) (Himedia Laboratories) medium. Overnight cultures of MRSA and PAO1 were subcultured in the TSB and LB respectively, until a turbidity of 0.5 McFarland (1 X 10^8 CFU ml^{-1}) was reached. Using a sterile cotton swab (Himedia Laboratories), the cultures were uniformly spread over the surface of the agar plate. Wells with a diameter of 4 mm were then punched out of the swabbed plates, and loaded with 100 µl of the test compounds and treated fabrics (1cm X 1cm). Wells with dimethyl sulphoxide (DMSO) was used as control. The MHA plates were incubated at 37°C for 24 h and observed for the zone of inhibition.

Results and discussion

XRD analysis

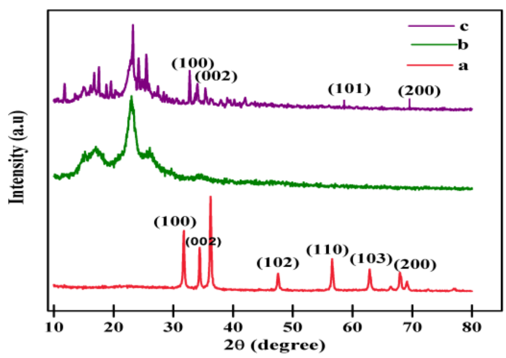

Figure.1.XRD pattern of (a) pristine ZnO (b) Uncoated cotton (c) BTCA-ZnO composite coated cotton

The optimised BTCA was crosslinked with ZnO to impart the focussed applications. The synthesized BTCA-ZnO composite coated cotton can be ascertained by the XRD analysis. The synthesized composite coated cotton was examined for XRD analysis and the results obtained were mentioned in the Fig 1.
Fig.1 a-c shows the XRD pattern of the pristine ZnO, blank cotton fabric and BTCA-ZnO composite coated cotton fabric. The XRD spectra in Fig.1a shows that all of the ZnO powder

target are polycrystalline with hexagonal structure, but (002) orientation is still dominant. There are three peaks corresponding to the (100), (002) and (101) orientation of hexagonal ZnO. Fig.1b shows that there is no identical peaks were observed in the untreated cotton fabric. Fig.1c shows the sharp diffraction peak of the ZnO implies that the ZnO is crystalline in nature. It indicated by corresponding peaks to the planes of (100), (002),(101). Brayner et al., (2006). The crystallite size of BTCA-ZnO composite coated fabric was calculated by using Scherrer's formula was found to be 17 nm. All the peaks match very well with the standard ZnO of the hexagonal structure (JCPDS card 06-7848).

FT-IR analysis

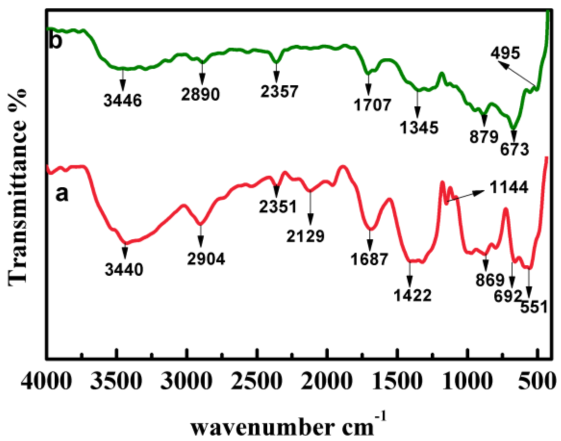

Figure.2.FTIR spectra: (a) Uncoated cotton and (b) BTCA-ZnO composite coated cotton.

The vibrations observed at 3440 and 3426 cm^{-1} are attributed to O-H stretching vibration of primary alcoholic groups present in the glucose units of uncoated and coated fabric respectively. The peaks observed at 2904 cm^{-1} and 2351 cm^{-1} are attributed to C-H bending vibration and O-H bending vibration of uncoated fabric respectively. The peak at 2890 cm^{-1} is attributed to C-H anti-symmetric stretching vibration of secondary CHOH groups present in the glucose units of uncoated cotton. Peak at 2129 cm-^{1} present in the uncoated fabric disappears in the coated fabric, which indicates the modification of the fabric. Castro et al.,(2004). Peak at 1687 cm^{-1} is attributed to the bending vibration of C-O bonds in the uncoated cotton. This peak is shifted to 1707 cm^{-1} in the coated cotton corresponding to the formation of ester and carboxylate in the crosslinked cellulose. Yazhini et al., (2015). Peak at 1144 cm^{-1} attributes to the C-O-C stretching vibration of the cyclic anhydride of uncoated fabric. The peak at 1422 cm^{-1} is attributed to the C-H asymmetric deforming vibration of coated fabric. Peak at 1345 cm^{-1} is attributed to C-H symmetrical deforming vibration of coated fabric (Fig.1a and b). Peak at 496 cm^{-1} is attributed to the Zn-O vibration of the coated cotton fabric. Kavkler et al., (2011).

SEM analysis

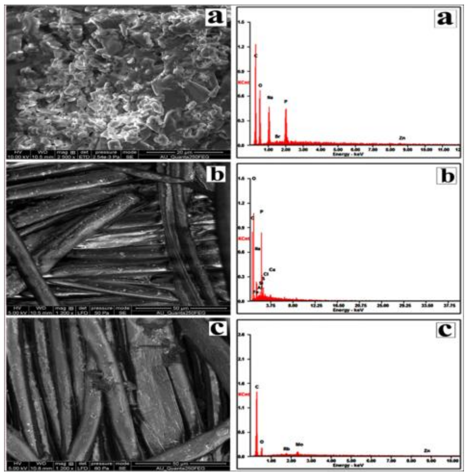

Figure.3.SEM with EDAX images: (a) ZnO-BTCA powder (b) Uncoated Cotton and (c) coated Cotton.

ZnO-BTCA composite material shows flower like pattern in the SEM images. The EDAX spectrum shows the presence of Zn, O, P, Na in the composite (Fig.2a and c). Noticeable change in the topography between coated fabric and uncoated fabric is observed from the images (Fig.2b and c). It is clearly seen that the surface of the uncoated fabric is comparatively rougher than the treated fabrics as the coatings obviously led to the flattening of the fiber surface.

Antibacterial activity
Agar diffusion assay

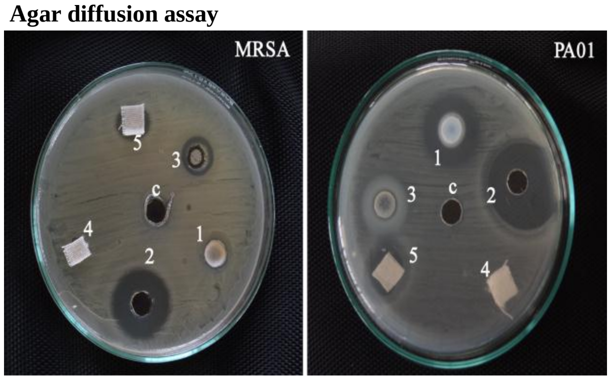

Figure.4.Samples and zone of inhibition; (1) ZnO, (2) BTCA, (3) ZnO-BTCA composite (4) uncoated fabric (5) coated fabric and (c) control sample.

The zone of inhibition was observed for all the test samples against PAO1 and MRSA bacteria. Among these, BTCA exhibited maximum zone of inhibition (Fig.3). Uncoated cotton fabric containing DMSO was served as control (c).
Agar diffusion method result reveals that BTCA exhibited the maximum zone of inhibition against test pathogens (Fig.3 and Table 1).

Table 1: zone of inhibition of test samples

Compound ID	Sample	Organism	Zone of inhibition (mm)			
			1	2	Mean	Standard deviation
1	ZnO-BTCA composite	*S. aureus*	12	13	12.5	0.71
		E. coli	21	23	22	1.41
2	ZnO-BTCA Treated fabric	*S. aureus*	11	14	12.5	2.12
		E. coli	19	20	19.5	0.70
3	ZnO-BTCA-CNT composite	*S. aureus*	19	21	20	1.41
		E. coli	23	22	22.5	0.70
4	ZnO-BTCA-CNT Treated fabric	*S. aureus*	17	18	17.5	0.70
		E. coli	-	-	-	-
5	Untreated fabric	*S. aureus*	-	-	-	-
		E. coli	-	-	-	-

Its efficacy was further confirmed by liquid assay against the same test pathogens in micro broth dilution method. IC_{50} value was assessed by liquid assay in 24 well polystyrene plates. The wells were loaded with 1:100 dilution of overnight test culture containing different concentrations of BTCA ranging from 7 to 350 µg ml^{-1} (Fig.4).

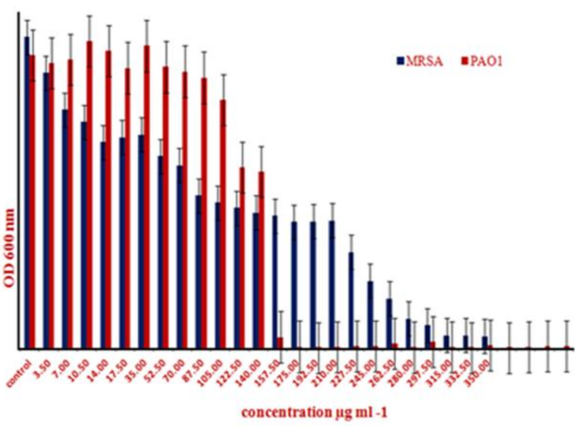

Figure.5. Effect of BTCA concentration on the cell density (absorbance measured at 600 nm) of MRSA and PAO1. Test pathogens without BTCA served as controls. Wells containing Luria Bertani broth and tryptic soy broth medium alone served as respective blanks. BTCA significantly reduced the cell density with increasing concentration against MRSA and inhibited 50 % at 525 µg ml^{-1} (IC_{50}), whereas at 700 µg ml^{-1} (IC_{50}) against PAO1 (Fig.4).

Conclusions

BTCA is one of the environment-friendly compounds used for the finishing of cellulosic fabrics as crosslinker. Nano ZnO particles were used in this study as an antibacterial agent. BTCA-ZnO composite was successfully prepared in a single step in situ process. The composite thus obtained was applied on the cotton fabric surface by pad-dry-cure method. Curing at 180 ° C for 3 min was found to be optimum for the effective crosslinking between BTCA and cellulose as well as effective regeneration of nano ZnO. The crosslinking of cotton fabric was confirmed from the shift in FT-IR peak at 1687 cm^{-1} to 1707 cm^{-1}. The modification of cotton fabric was also confirmed from the presence of peak at 2129 cm^{-1} in the uncoated fabric, which disappeared in the coated fabric. The BTCA-nano ZnO composite coated cotton fabric was tested for antibacterial activity. The BTCA-ZnO composite coated fabric showed improved antibacterial activity against *E. coli* and *S. aureus* than the pristine ZnO, pristine BTCA and pristine BTCA-ZnO composite powder. The coated fabric could be used for hospitality fabric.

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