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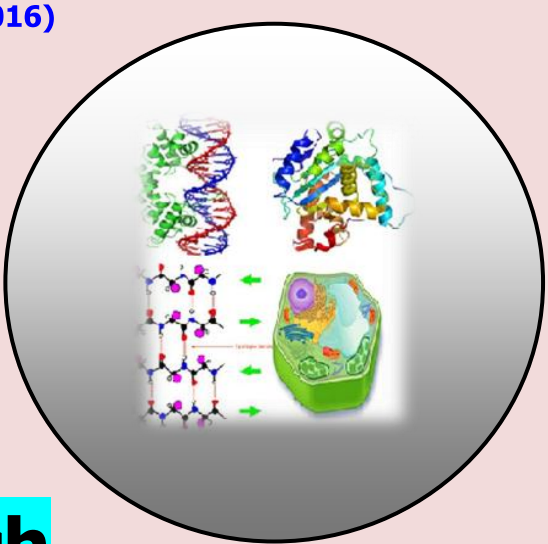

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RESEARCH PAPER

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Inclusion of Antimicrobial titanium dioxide on β-cyclodextrin Grafted Cotton

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ABSTRACT

There is a vast resource of natural antimicrobial agents which can be used for imparting useful antimicrobial property to textile substrates. The present work deals with the inclusion of metal oxide on β-CD grafted cotton for enhanced antimicrobial activity and durable finishing. Optimization of higher percent of β-CD grafting were carried on cotton using citric acid as crosslinking agent and sodium hypophosphite as catalyst. Inclusions of prepared TiO_2 particles were processed on β-CD grafted cotton and β-CD ungrafted cotton for the comparative analysis. FT-IR analysis confirmed the grafting of β-CD and loading of TiO_2 particles on β-CD grafted cotton. TiO_2 particles loaded on β-CD grafted cotton exhibit the significant antibacterial and antifungal action against the tested pathogens. While antimicrobial textiles provide the benefits in hygiene, odor control and protection of the fabric from microbial attack, bacterial resistance to the biocides used and their toxic breakdown products in the household and environment have been concerns. Most biocides used on commercial textiles can induce bacterial resistance to these substances, which can lead to increased resistance to certain antibiotics in clinical use. Bacterial resistance may be a particular concern because large quantities of biocides are needed on the textiles to achieve adequate activity and durability. Such concerns can be satisfied by the inclusion of antimicrobial agents by the inclusion phenomena.
***Keywords:** β-Cyclodextrin (β-CD), TiO_2 particles and Cotton.*

Antibacterial and Antifungal Studies of TiO_2 Loaded on β-CD Grafted Organic Cotton

INTRODUCTION

Cyclodextrin (CD), also called cycloamyloses are a family of compounds made up of sugar molecules bound together in a ring which are commonly termed as cyclic oligosaccharides. Cyclodextrins are produced from starch by means of enzymatic process. They are used in food, pharmaceutical, and chemical industry, as well as agriculture and environmental engineering [Loftsson et al, 2005].

Cyclodextrins are composed of five (or) more α-D glucopyranoside units linked by 1, 4 linkage which is as in amylose a fragment of starch. Cyclic oligosaccharides, also known typical cyclodextrins contain a number of glucose monomers ranging from six to eight units in a ring, creating a cone shape [Williams, 2005].

β-CD can be incorporated onto textile by means of spraying, printing, padding, grafting, surface coating, impregnation and ink jet printing various techniques like. Various feasible interactions occur between β-CD and some textile fibers [Buschmann, 2001]. Cotton is a natural vegetable fiber produced in the cotton plant in many countries of the world. Cotton fiber is one of the oldest natural fibers which is familiar to human beings and widely used for numerous purposes. It is predominantly composed of cellulose, along with hemicelluloses; there are certain non-cellulosic matters also attached and present in the cotton fiber such as sugars, starch protein and some inorganic matters.

β-CD is the most accessible, the lowest priced, and generally the most useful compound created from the enzymatic degradation of starch and it is also called Schardinger's dextrin. Cyclodextrins are torus-shaped cyclic oligosaccharides made of six, seven, and eight glycosidic units linked by α (1, 4) bonds into a ring in the most common forms called α-, β-, and γ- respectively. They can entrap a vast number of lipophilic compounds into their hydrophobic cavity depending on their size and molecular structure. In the last decades, cyclodextrins have been used extensively in cosmetics, food, pharmaceutical, and textile industry. In recent years, researchers such as Buschmann, Hara et al., Martel et al., and Yong have prepared fragment fabrics and gained desirable results [Yuan, 2008]. Polycarboxylic acids can crosslink cotton fabric in the presence of alkali metal salts of phosphorus-containing acids such as sodium dihydrogen phosphate and sodium hypophosphite. Cyclodextrin crosslinked wool fabric has also been investigated. In addition to cyclodextrin, its derivative monochlorotrizinyl β-cyclodextrin was used for the preparation of aroma and antimicrobial finished cotton fabrics [Abdel-Halim, 2011].

Titanium dioxide is one of the most prominent oxide materials for performing various kinds of industrial applications related to catalysis among which the selective reduction of compounds in stationary sources and photocatalysis for pollutant elimination or organic synthesis, appear as rather important. It can be used as a white pigment in paintings, as part of photovoltaic devices, or electrochromic devices, sensors, as a food additive, in cosmetics, as antimicrobial agent in textiles and as a potential tool in cancer treatment. TiO_2 occurs in nature in three different polymorphs which, in order of abundance, are rutile, anatase and brookite. Among the three forms, anatase has higher photocatalytic activity with a band gap of 3.2eV and is widely used in solar energy conversion. The increase in surface area of nanoscale TiO_2 compared to bulk has the potential to improve the efficiency of the material function. The nanostructure affects other important properties of the TiO_2 material, of importance in its technological applications [Forzatti, 2000, Tamas Zoltan Agocs, 2016, Fujishima et al., 2000, Haider et al., 2017, Hoffman et al., 1995].

Hence the present work deals with the inclusion of metal oxide on β-CD grafted cotton for enhanced antimicrobial activity and durable finishing.

EXPERIMENTAL DETAILS

MATERIALS

Plain bleached organic cotton was purchased from Erode textile industries, India. β-cyclodextrin was obtained from Chemico Glassware and Scientifics Limited from Erode, India. Citric acid was availed from Merck Specialties Limited, India. Sodium hypophosphite, ethanol and sodium hydroxide were purchased from Himedia, India. Titanium (IV) propoxide was availed from Acros Organics.

Optimization of Various Parameters for the Grafting of β-CD on Organic Cotton

Citric acid acts as cross linker and sodium hypophosphite acts as catalyst for the grafting of β-CD. Before grafting the fabric with β-CD, various parameters such as concentration of citric acid, concentration of sodium hypophosphite, concentration of β-CD, temperature and time should be optimized. Initially concentration of β-CD is varied from 0.2% -1.0% by rising with regular intervals of 0.2%, citric acid is 0.2%, SHP is 0.15%, Temperature is 150˚C and time is 10 min are kept constant. Percentage of grafting or grafting yield is noted for each variation of β-CD. Higher grafting yield obtained for particular concentration is taken for further parameters. Then concentration of citric acid is varied from 0.05% to 0.25% by rising with regular intervals of 0.05% by fixing the optimized β-CD concentration and other parameters are taken as such in pervious step. Hence optimized concentration or citric acid also arrived. Similarly, SHP, citric acid, temperature and time were optimized. Finally the fabric was grafted with the optimized values.

Grafting of β-CD on Organic Cotton

Cotton fabric was grafted with β-cyclodextrin by immersing a weighed quantity of fabric in a solution containing optimized β-CD, citric acid, sodium hypophosphite for 30 minutes. The fabric was squeezed and dried at 80˚C in a hot air oven. After grafting, weight percentage of β-CD grafting should be calculated. Hence, the weight percentage is calculated from the weight difference between the ungrafted and grafted fabric.

$$\text{Percentage of grafting} = \frac{(W_2 - W_1)}{W_1} \times 100$$

Where,

W_1 and W_2 are the weight of fabric before and after grafting of β-CD.

Preparation of TiO_2 Particles

Titanium isopropoxide (6 ml) was mixed with 2 ml of 10% acetic acid with continuous stirring using JSGW 13162 magnetic stirrers. After 5 min, 56ml of ethanol was added drop wise with continuous stirring. Then pH of the solution was adjusted to 1-2 by adding 2ml of con. HCl. The mixture was magnetically stirred well for 45 min. The obtained sol-gel TiO_2 was used for treating of cotton fabric.

Loading of TiO_2 Particles on β-CD Grafted Cotton Fabric

The prepared TiO_2 particles were loaded on β-CD grafted cotton fabric by direct application system of pad dry cure method. The cotton fabric specimen of dimension 2 cm × 2cm optimized β-CD grafted fabric was used for the loading of TiO_2 particles. Variable concentration of TiO_2 was undertaken from 0.2N – 1.0 N solution with regular intervals of 0.2N was used. Optimized β-CD grafted cotton fabric samples were immersed in TiO_2 particles solution and stirred for about 30 min. Then this fabric samples was padded continuously for about few minutes in a two bowl padding mangle. Finally the fabric was dried and cured at 110°C for 9 minutes. The same procedure was followed for the different concentration of TiO_2 particles to prepare TiO_2 particles loaded β-CD grafted organic cotton fabric. Percentage of loading of TiO_2 particles also is calculated for each and every concentration to evaluate the optimized concentration from the weight difference between before and after loaded TiO_2 particles β-CD grafted organic cotton fabric.

Characterization Techniques

Fourier Transform Infrared Spectroscopy

Grafting of β-CD on cotton fabric, loading of TiO_2 and rose extract on β-CD grafted cotton fabric were confirmed by Fourier transform infrared spectroscopic (FT-IR) technique. The vibrational frequency of bond stretching/bending can be approximated by relative contributions of bond strengths and atomic masses. The instrument software is opened and the required parameters like recording range (4000 – 400 cm^{-1}) are furnished before beginning the recording of spectra for the unknown sample. Now, the scanning of the instrument is started to record the characteristic absorbance/transmittance spectra for the sample under investigation. The plot of wave number Vs absorbance/transmittance is manipulated using the characteristic behavior of various functionalities present in the unknown chemical substance. FT-IR spectra of the cotton, β-CD substance, β-CD grafted cotton fabric and TiO_2 loaded β-CD cotton fabric were recorded in transmission mode using a Perkin Elmer RX1 spectrometer with 4 cm^{-1} resolution.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is commonly used to study surfaces, structures, morphologies and forms of materials. SEM analysis was conducted in vacuum environments. Surface morphology of treated cotton fabrics were examined by scanning electron microscopic images. SEM images of treated cotton fabrics were recorded on a JEOL JSM 6390 SEM instrument using an accelerating voltage of 15kV. EDX can be used to find the chemical composition of cotton fabrics. EDX capabilities provide fundamental compositional information for a wide variety of materials. EDX spectrum of treated cotton fabrics were recorded on a JEOL JSM 6390 SEM instrument with thermo EDX attachment using an accelerating voltage of 15 kV to determine the exact elemental composition.

Antibacterial and antifungal study by agar diffusion method

Antimicrobial activity such as antibacterial and antifungal study of the β-CD grafted organic cotton and TiO_2 loaded organic cotton fabrics were carried out by agar diffusion method. Agar diffusion method, also known as Kirby-Bauer method is a relatively quick and easily executed semiquantitative test for the testing of antimicrobial activity. Antibacterial activity of the samples was evaluated against *Staphylococcus aureus*ATCC-11229 (*S. aureus*), gram positive bacteria and *Escherichia coli* ATCC-25923 (*E. coli),* gram negative bacteria by agar diffusion method. Antifungal activity was determined against *Candida albicans* ATCC-10231 (*C. albicans*) fungal strain. The antimicrobial activity of the treated cotton fabrics was evaluated by measuring the zone of inhibition against the test organisms. Zone of inhibition is the area in which the bacterial or fungal growth is stopped due to bacteriostatic or fungistatic effect of the compound and it measures the inhibitory effect of compound towards a particular microorganism. Finally, diameters of zone of inhibition of the control strain and test can be measured with a ruler or calipers

RESULTS AND DISCUSSION

Evaluation of β-CD grafting yield

Cross linking of organic cotton and β-CD was done by the crosslinker citric acid in presence of catalyst sodium hypophosphite at high curing temperature. Fig. 1. shows schematic representation of the reaction mechanism. Citric acid undergoes dehydration on heating in presence of sodium hypophosphite to give five-member cyclic anhydride, which produces ester linkage with hydroxyl group of cellulose [Yuag, 2003]. Subsequently, the neighboring carboxylic acid groups on adjacent carbon atom undergo dehydration to form cyclic anhydride [Gawish, 2009]. Now, the second cyclic anhydride cleaves to form ester more preferentially with primary alcoholic group of β-CD. Fig. 2 (a)-(e) represent the grafting yield for variation of parameters such as β-CD, CA, SHP, temperature and time [Martel et al., 2002].

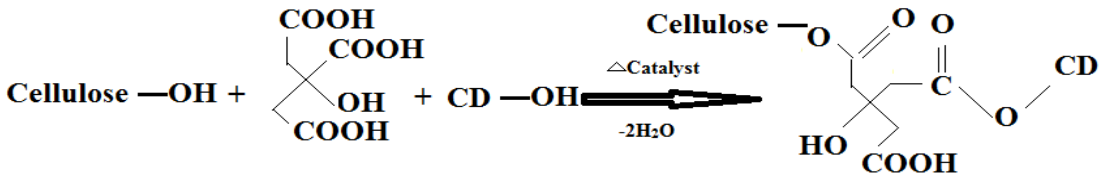


Figure 1. Schematic representation of the reaction involved in β-CD grafting on cotton.

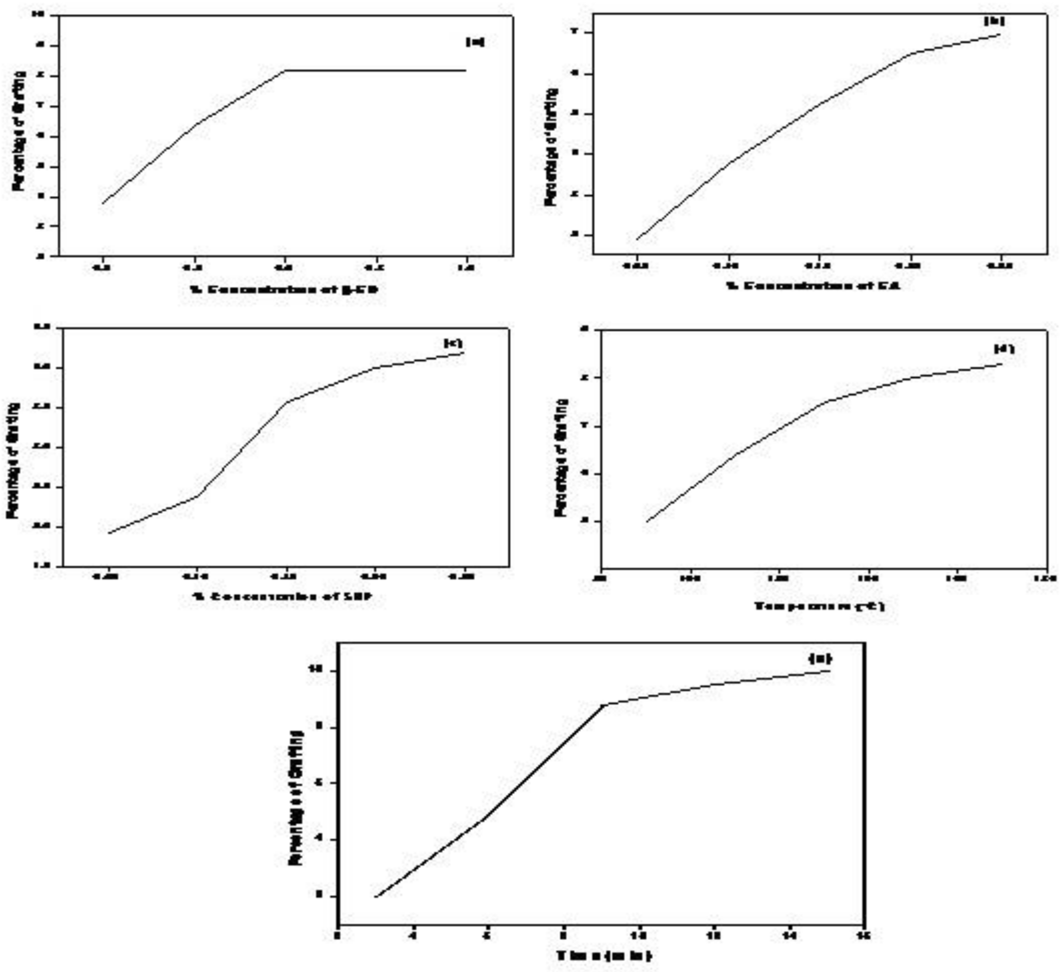


Figure 2. Effect of parameters (a) Concentration of β-CD, (b) Concentration of CA, (c) Concentration of SHP, (d) Temp and (e) Time on grafting.

Grafting yield (%) increases linearly with the increase of β-CD concentration, but beyond 0.6% concentration a limiting value is attained. It shows that a limiting concentration of β-CD (0.6%) is sufficient and further increase plays no significant role, because primary alcoholic groups of cellulose and β-CD are competitively involved in esterification with citric acid. When the concentration of citric acid increased, yield of grafting increases up to 0.8%, thereafter it slowly decreases. It has been reported by yang and collaborators that the stearic hindrance of polycarboxylic acid reduces accessibility of cellulosic hydroxyl groups and reducing the amount of crosslinked product but at the same time increasing the untreated anhydride intermediate on the cellulosic particles. In addition, yellowing of fabric takes place because oxidation occurs in cellulosic hydroxyl group at higher concentration of citric acid. This is the indication of adverse impact on the tensile strength of fabric. Sodium hypophosphite plays a vital role in crosslinking of cellulose, citric acid and β-CD. Esterification without SHP is impossible. It influences crosslinking up to 0.15% thereafter; there is no significant increase in the yield. Temperature plays an inevitable role in cellulose, CA and β-CD, since the primary step of cyclic anhydride formation is initiated by heat. According to yang, the ester formation increases as the temperature increases from 110°C to 190°C. The anhydride formation first increases and then starts to level off at 110°C once the product is formed. Hence, to prevent damage of fabric at higher temperature, an optimum temperature of 110°C is sufficient enough for better yield. Time of treatment influences the curing and fixation process. Hence, for every 3 min raise (3-15 min), there is notable improvement in grafting yield. Long duration of treatment at higher temperature produces damage to fabric. Therefore, an optimum duration of 9 min is enough for curing at 110°C. Percentage of grafting by the effect of various parameters is tabulated in Table 1.

Table 1. Effect of Various Parameters on Percentage of Grafting.

Optimization parameters	Variable Range	Percentage of Grafting (%)				
% Concentration of β-CD	0.2, 0.4, 0.6, 0.8 & 1.0	3.77	6.38	8.16	8.16	8.16
% Concentration of CA	0.05, 0.1, 0.15, 0.2 & 0.25	1.90	3.77	5.25	6.52	6.97
% Concentration of SHP	0.05, 0.1, 0.15, 0.2 & 0.25	1.92	2.38	3.57	4.01	4.20
Temperature (°C)	90, 110, 130, 150 & 170	5.03	6.38	7.50	8.00	8.30
Time (min)	3, 6, 9, 12 & 15	1.96	4.87	8.80	9.50	10.0

Evaluation of TiO_2 Particles Loading on β-CD Grafted Organic Cotton

Loading of TiO_2 particles was also optimized for the higher percentage of loading on β-CD Grafted Organic Cotton. Concentration of TiO_2 is increased; loading percentage of TiO_2 also increases. This can be clearly depicted from Table 2 and Fig. 3. But the concentration of TiO_2 above 0.8N shows no considerable increase in percentage of loading. Hence a limiting concentration of 0.8N is fixed. Hence further increase of concentration of TiO_2 plays no significant role in percentage of loading.

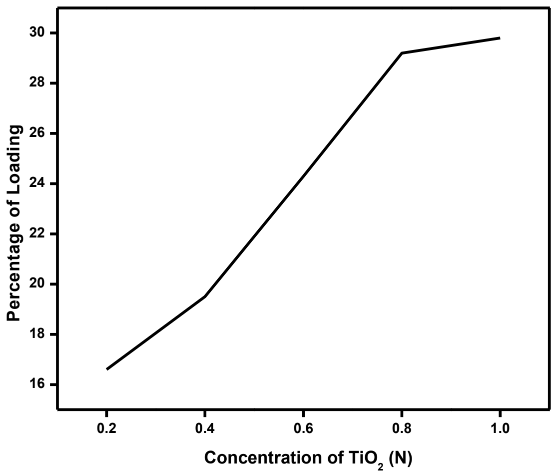


Figure 3. Effect of various concentration of TiO_2 loading on β-CD grafted cotton.

Table 2. Percentage of loading for various concentrations of TiO_2

Concentration of TiO_2 (N)	Percentage of Loading (%)
0.2	16.6
0.4	19.5
0.6	24.3
0.8	29.2
1.0	29.8

FT-IR Analysis of β-CD grafted Organic Cotton

FT-IR analysis is a powerful technique for characterizing organic moieties in an unknown compound. FT-IR analysis was applied to confirm the grafting of β-CD on cotton. Fig. 4 shows the FT-IR spectrum for cotton (a), β-CD (b) and β-CD grafted organic cotton. Spectrum of cotton in Fig.5.4 (a) shows a broad peak at 3521 cm^{-1} may be attributed for –OH stretching vibration of cellulosic hydroxyl group and a band at 1600-1700 cm^{-1} may be assigned due to –OH in plane bending vibrations of cotton. Broadness nature of peak from 1500-3000 may be observed for the asymmetric strectching vibration of C-H. The peaks appeared below 1000 cm^{-1} can be depicted for the –OH in-plane and out-plane bending vibrations of cotton [Tas et al., 2000].

FT-IR spectrum of β-CD in Fig. 5.4(b) shows peaks characteristic of –OH stretching at 3364 cm^{-1} and strong complexed band at 1033, 1156, 1365 and 1419 cm^{-1} are characteristic of C-O stretching and –OH in-plane bending vibration of β-CD. β-CD grafted cotton in Fig. 5.4(c) depicts a broad peak between 3000-3100 cm^{-1} characteristic of –OH stretching of cellulose and carboxylic acid group of citric acid involved in crosslinking and –OH in plane bending vibration is shifted from 1600-1700 cm^{-1} to 1700-1800^{-1} due to the attachment of cyclodextrin with the –OH group of cellulose. Hence the grafting of β-CD on cotton is confirmed by the shifting of peak values [Huang et al., 2007].

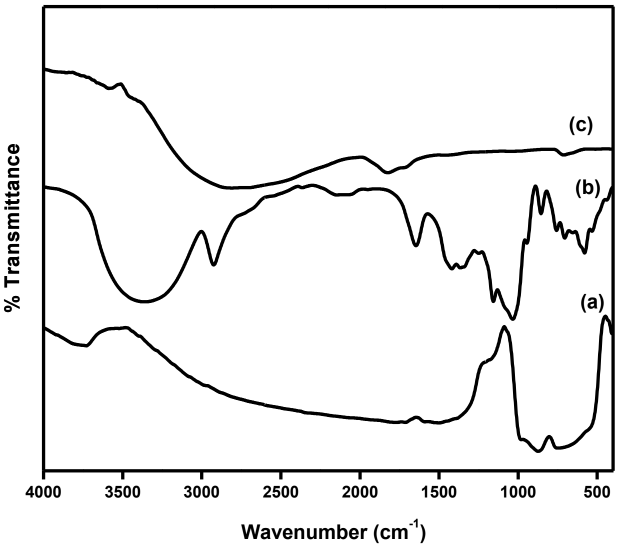


Figure 4. FT-IR spectrum for (a) Cotton (b) β-CD and (c) β-CD grafted organic cotton.

FT-IR Analysis of TiO_2 loading on β-CD grafted organic cotton

Loading of TiO_2 on β-CD grafted organic cotton is analyzed from its FT-IR spectrum which is shown in Fig. 5(a) & (b). The peak characteristic of Ti-O-Ti bonds of TiO_2 nanoparticles is greatly enhanced at 705cm^{-1} due to the condensation of surface hydroxyl groups of titanium hydroxide [Shivshankar et al., 2003]. A peak at 705 cm^{-1} in Fig. 5.4 (a) could be related to the Ti-O bonds in TiO_2. A broad band appeared at 3300-3500 cm^{-1} and a peak at about 1600 cm^{-1} could be attributed to –OH stretching vibrations of water molecule present in the moisture content. FT-IR spectrum of TiO_2 loaded cotton shows characteristic peaks at 662 cm^{-1} and 710 cm^{-1}. Hence TiO_2 was loaded successively into the cavity of β-CD by the inclusion phenomena [Mandal et al., 2006].

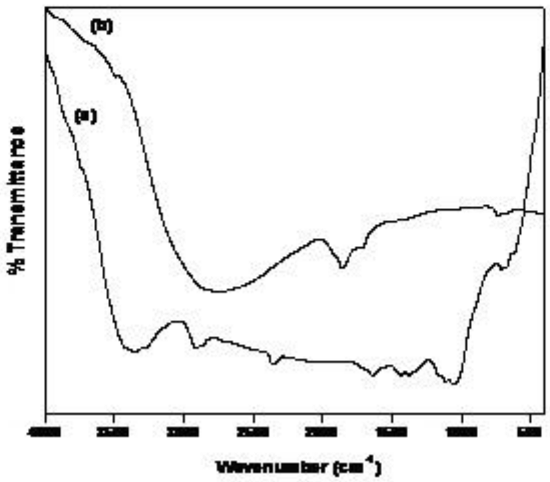


Figure 5. FT-IR spectrum for (a) TiO_2 particles and (b) TiO_2 loaded on β-CD grafted organic cotton.

SEM with EDX Analysis of TiO_2 Loaded on β-CD Grafted Organic Cotton

The surface morphology of the cotton fiber surface by the loaded TiO_2 particles can be analyzed by the SEM photographs in Fig. 6. SEM images of the TiO_2 loaded cotton clearly reveal that TiO_2 particles were homogeneously covered in larger proportions. EDX studies were carried out in order to obtain an indication of the treatment of cotton with TiO_2 particles. The treatment on the surface of the cotton fibers with TiO_2 particles were further confirmed by their EDX spectrum which is shown in Fig. 7. Carbon and oxygen peak which belongs to cellulose are also present in its EDX spectrum. The peaks featured for titanium at 5.8 and 6.3 keV, oxygen at 0.54keV and carbon at 0.25keV are present in EDX spectrum. SEM and EDX analysis support the hypothesis that successful loading of cotton with TiO_2 particles.

Figure 6. SEM Images of TiO_2 particles loaded on β-CD grafted organic cotton.

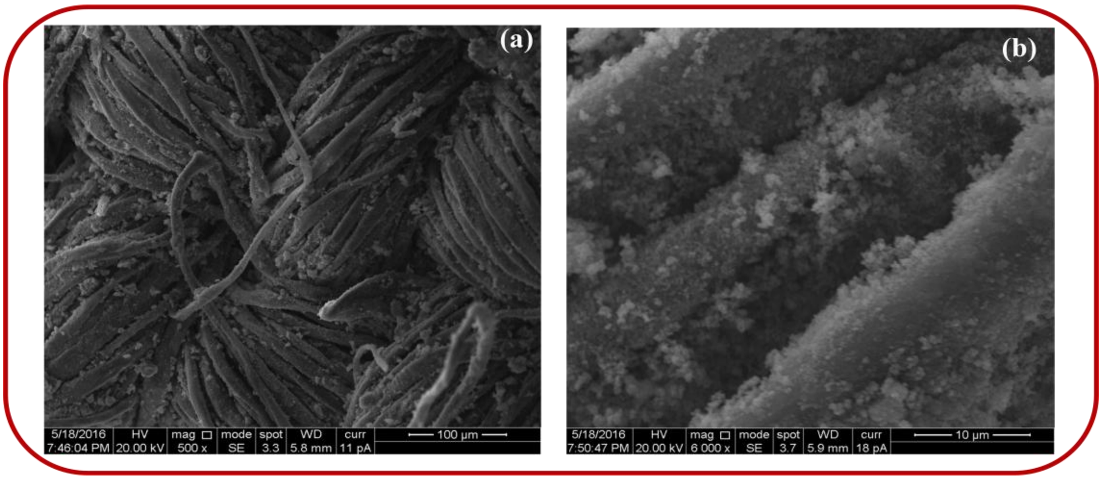
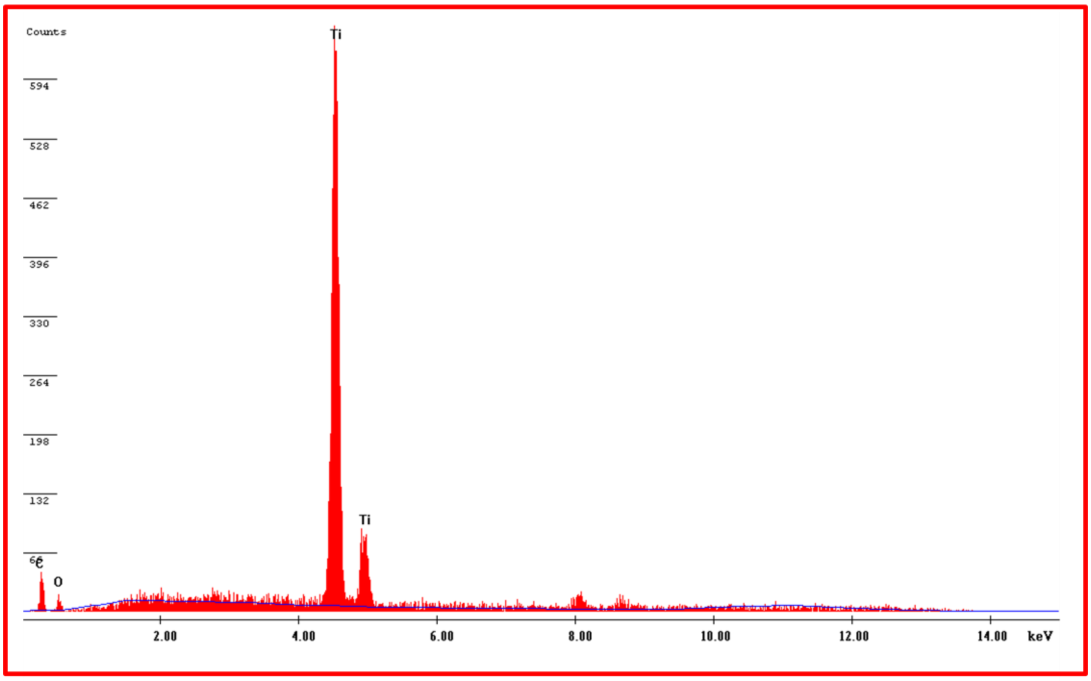


Figure 7. EDX spectrum of TiO_2 particles loaded on β-CD grafted organic cotton



Antibacterial and antifungal study of TiO_2 Loaded on β-CD Grafted Organic Cotton

Antimicrobial action of prepared cotton samples against E. coli, *S. aureus* and *C. albicans* were evaluated by agar diffusion method. Antibacterial and antifungal images are shown in Fig. 5.11. TiO_2 particles loaded on β-CD ungrafted and grafted cotton exhibit significant antimicrobial activity against test pathogens [Ling Lam et al., 2012]. TiO_2 particles and rose extract loaded on β-CD grafted cotton displays higher zone of inhibition when compared to ungrafted cotton especially against *S. aureus* bacterial pathogens and *C. albicans* fungal strain. Zone of inhibition values are tabulated in Table. 5.4. Grafted cotton affords more antimicrobial action due to the inclusion of more amount of antimicrobial compounds into its hydrophobic cavity. Moreover particles or extract loaded on β-CD grafted cotton have washing durability while comparing ungrafted cotton. Hence even after sever washings, particles or substances loaded on β-CD grafted cotton have remarkable biocidal action.

The proposed antibacterial mechanisms are first that the metal ions or natural extract can associate with the cell wall, cell membrane and cell envelope of microorganism. Mainly the positive charge of a antimicrobial agent is critical for antimicrobial activity, allowing electrostatic attraction between the negative charges of the bacterial cell membrane and positively charged metal particles causing cell membrane rupturing. Second, ions can react with nucleophilic amino acid residues in proteins thereby resulting in the metabolites efflux, interfering with DNA replication, inactivation and inhibition of bacterial growth. Third the antimicrobial action suggested to be related to the formation of free radicals and subsequent free radicals induced membrane damage [Jangra et al., 2012].

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Table 3. Zone of inhibition values of prepared cotton samples against tested pathogens.

S.No.	Cotton samples	Zone of inhibition (mm)		
		E.coli	***S.aureus***	***C.albicans***
1	TiO_2 particles loaded on β-CD ungrafted cotton	10	12	15
2	TiO_2 particles loaded on β-CD grafted cotton	13	15	18

CONCLUSION

There is a vast resource of natural antimicrobial agents which can be used for imparting useful antimicrobial property to textile substrates. Longer lasting and durable antimicrobial finishing can be afforded by the inclusion of antimicrobial agents into the cavity of β-CD using citric acid as crosslinking agent and sodium hypophoshite as catalyst. Higher percentage of grafting is obtained for the optimum concentration of β-CD – 0.6%, CA – 0.15%, SHP – 0.15%, temperature – 110°C and time – 9 min.

Higher percentage of loading on TiO_2 on β-CD grafted cotton observed is 0.8%. FT-IR analysis confirms the grafting of β-CD on cotton, loading of TiO_2 on β-CD grafted cotton. Structural morphology observed from SEM studies reveals that the TiO_2 particles were coated on β-CD grafted cotton. EDX spectrum confirmed the loading of TiO_2 particles t on β-CD grafted cotton. TiO_2 particles loaded on β-CD grafted cotton showed prominent antibacterial action against E. coli and S. aureus and antifungal activity against C. albicans. Antimicrobial action of antimicrobial agents is more effective against β-CD grafted cotton due to the more trapping of antimicrobial molecules into the β-CD cavity.

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