Photocatalytic Inactivation of Hospital-Associated Bacteria using Titania Nanoparticle Coated Textiles

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Summary: Modification in hospital textiles to include disinfection properties may help in the reduction of nosocomial infections. In this study, antibacterial properties were imparted to cotton fabric by modifying it with pure and (1%) silver doped titania nanoparticles. The nanoparticles were prepared by liquid impregnation process and characterized using X-ray Diffraction (XRD) spectroscopy, Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS). These nanoparticles were attached to cotton fabric using a cross linking agent succinic acid. Samples were washed at three different temperatures (30, 60 and 90°C), with and without detergent and for different number of cycles to test the durability of nanoparticles to the fabric. Scanning Electron Microscopy (SEM) was used for studying surface topography of fabric. Energy Dispersive X-ray fluorescence (ED-XRF) spectrometer was used to detect the titanium present on the fabric. Catalytic spectrophotometry using UV/visible spectrophotometer was used to determine titania concentration in washing effluent. The antibacterial activity of the modified fabric was examined against Methicillin Resistant Staphylococcus aureus (MRSA) under UV and fluorescent light. The maximum durability of titania nanoparticles to the fabric was retained after washing without detergent at 30°C. The overall results of durability testing showed that coating of nanoparticles on fabric was durable against washing at various conditions, hence suitable from an environmental perspective. Antibacterial testing showed 100% photocatalytic inactivation of MRSA after 4 and 24 h of UV and fluorescent light exposure respectively. The potential of using such textiles in hospital environment was validated through the use of modified bed linen in a local hospital for a period of three days consecutively. The viable count indicated the reduced bacterial contamination on nano-coated fabric as compared to uncoated fabric. Bed linen, curtains, staff uniforms, lab coats and medical garments developed from titania nanoparticle coated fabric may improve hospital environment against antibiotic resistant bacteria.

Keywords: Titania nanoparticles, Photocatalytic inactivation, Nosocomial infections, Hospital environment, Bed linen, Durability.

Introduction

The risk of nosocomial infections is increasing alarmingly over the world. This is due to the growing antibiotic resistance of pathogenic micro-organisms [1]. The most significant bacterial pathogens that cause nosocomial infections include Escherichia coli (E. coli), Klebsiella pneumonia (K. pneumonia), Pseudomonas aeruginosa (P. aeruginosa) and Staphylococcus aureus (S. aureus) that are difficult to be treated by common antibiotics [2-4].

These bacterial pathogens, that cause nosocomial infections, have various modes of transmission. The most important and recognized transmission is via direct-contact between an infected person and a susceptible host. Airborne transmission may also occur in which bacteria become airborne, usually through coughing or sneezing [5]. Another risk factor is endogenous transmission of the host's own microbiota from one part of the body to another [6]. Additionally, infections may be transmitted indirectly through contaminated objects such as instruments, floor, over-bed tables, phones, computer keyboards, needles, gloves and textiles [7-9].

Textiles used in hospitals such as bed linen, curtains, staff uniforms, lab coats and medical garments can be both, the mode of transmission and reservoir, for pathogenic bacteria [5, 10]. Therefore, with an increasing level of antibiotics resistance, textiles contamination is another significant environmental factor in the spread of nosocomial infections [3, 11].

In order to reduce the spread of these nosocomial infections to some extent, antibacterial textiles are receiving a lot of attention [12]. For this purpose antibacterial nanomaterials are applied to impart such properties in textiles due to their unique physical and chemical properties and great surface-to-volume ratio. There are various kinds of nano-sized antibacterial materials such as chitosan, calamine, copper (Cu), silver (Ag), zinc oxide (ZnO), magnesium oxide (MgO), and titanium dioxide (TiO_2) [4, 7].

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Among these various nanomaterials, TiO_2 nanoparticles are found to be one of the suitable disinfectants. As a photocatalyst, titania after absorbing radiation from UV or visible range produce reactive oxygen species (ROS) such as peroxide and superoxide radicals. These ROS are responsible for antibacterial activity by destruction of DNA and disruption of cell membrane morphology along with electron transport chain [13, 14]. Various studies have indicated that titania nanoparticles are very effective in photocatalytic inactivation of E. coli, S. aureus and P. aeruginosa [15-17]. In addition, metal doped titania nanoparticles are even more effective for the inactivation of bacteria [4, 18]. Hence titania nanoparticles may be used to impart antibacterial properties to textiles. Other than antibacterial applications, there are multifunctional properties of titania nanoparticle coated fabrics such as UV protection, self-cleaning and flame resistance [14, 19-21].

Different methods have been reported for the application of nanomaterials to textiles such as sonochemical [22-24], dip-pad-dry-cure [19, 25], dip-coating [26], sol process at low temperature [27, 28], pre-treatment by UVC-light and RF-plasma in vacuum and under atmospheric pressure [29, 30].

In addition to these various methods, cross-link process can also be used to coat cotton fabrics with titania nanoparticles. This method implies the use of cross-linking agents for fixation of nanoparticles on the cotton fabric [20, 21, 31]. Succinic acid can be used as a cross-linking agent having two free carboxylic groups (Fig. 1). The cellulose in cotton fabric is a polysaccharide consisting of several free hydroxyl groups on its surface. Firstly, succinic acid will be attached with cellulose through esterification of one carboxylic group of succinic acid by a hydroxyl group of cellulose. TiO_2, having a strong electrostatic interaction with carboxylic groups, will be anchored on cellulose through the other carboxylic group of attached succinic acid [32]. Attachment of titania nanoparticles on the cotton by cross linking is a simple and durable method that presents effective antibacterial properties [21].

Durability is an important characteristic to be considered in the use of titania nanoparticle coated textile. It is relevant to consumer safety from probable hazards associated with nanoparticles exposure along with environmental contamination on possible release of nanoparticles in effluents [7, 23].

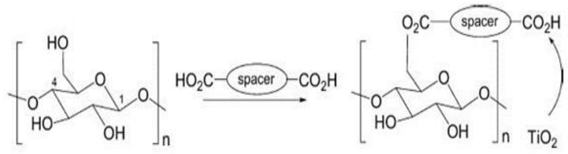

Fig. 1: Binding scheme of succinic acid with cotton cellulose and titania. Source: [32]

In the work represented here, we modified the cotton fabric with pure and 1% silver doped titania nanoparticles using succinic acid as a cross linking agent and investigated the biocidal activity and durability of this fabric. The disinfection efficacy of modified textile was investigated by studying its ability to inactivate methicillin resistant S. aureus (MRSA) under visible and ultraviolet light. Washings of modified fabric and analysis of this fabric along with washing effluent is a comprehensive method for assessment of durability. The effective antibacterial properties also signify the durability of the modified fabric. Potential of using such modified fabric in hospitals is also investigated through an experiment conducted in a local hospital where titania nanoparticle coated fabric in the form of bed linen were used by patients and monitored for the level of bacterial contamination as compared to uncoated fabric used in the same manner.

Experimental

Reagents and Materials

For this study, white cotton textile was obtained from the local market. Titanium dioxide (Sigma-Aldrich Labor chemikalien) was used for the synthesis of pure titania nanoparticles. Hydrogen peroxide, sodium hydroxide pellets (Fischer Chemical Limited) and ammonia (Merck) were used for the pretreatment of cotton textile. Coating of titania nanoparticle on cotton textile was done through succinic acid (Panreac Sintesis) and sodium dihydrogen phosphate (Honeywell). Methylene blue, ascorbic acid and sulphuric acid were used during the durability testing of modified cotton fabric. Nutrient agar and Luria-Bertani broth were obtained from Oxoid Ltd. Bacterial cultures of MRSA were grown in the laboratory by means of a standard protocol. Throughout the entire experimentation, distilled water was used. A fluorescent lamp with a wavelength range of 500–600 nm and a UV-A lamp with peak intensity at 365 nm were used to test the photocatalytic activity of modified cotton fabric.

Synthesis of Titania Nanoparticles

Titania nanoparticles were prepared through liquid impregnation method [4]. For preparation, general purpose titania reagent, after adding to 300 ml distilled water, was stirred on a magnetic plate for 24 h. The resulting slurry was allowed to settle for another 24 h and oven-dried at 105°C for 12 h. Using mortar and pestle, the dried solids were crushed. The powder resulting from this step was calcined at 400°C for 6 h in a muffle furnace. 1% silver doped titania nanoparticles were prepared through same method using $AgNO_3$ with general purpose TiO_2 reagent.

Characterization of Nanoparticles

Crystalline phase and size of synthesized pure and silver doped titania nanoparticles were analyzed through X-ray diffraction (XRD) spectroscopy. X-ray diffraction patterns were recorded on JEOL JDX-II with Cu-Kα radiation (λ=1.54060 nm; voltage=20 kV; current=5 mA) and range of diffraction angles (2θ) from 20° to 80°. The morphology of these nanoparticles was examined through scanning electron microscopy (SEM) by using JEOL JSM-6460 at an acceleration voltage of 20kV. For the elemental analysis of nanoparticles, energy dispersive spectroscope (EDS) Oxford INCA X-sight 200 attached with SEM was used.

Synthesis of Titania Nanoparticle Coated Cotton Textile

Cotton fabric was modified with titania nanoparticles through the cross-linking method [32]. To remove stains from the cellulose fibres, cotton fabric was initially pre-treated. For this purpose, fabric sample was bleached with 5% hydrogen peroxide (H_2O_2), treated with 5% sodium hydroxide (NaOH) aqueous solution and washed with water. An ammonia (5%) treatment was then applied that was removed from the sample fabric through drying in an oven at 100°C.

Sample was then dipped in an aqueous solution of succinic acid (6%) with sodium dihydrogen phosphate (NaH_2PO_2) as catalyst (4%) for 1 hour. The fabric sample was then oven dried for 3 min at 85°C and cured for 2 min at 180°C.

Lastly, in order to load titania nanoparticles on sample fabric, a 5 g/l TiO_2 nanoparticles suspension was prepared and sonicated for 30 min. Immersed into this aqueous suspension of titania nanoparticles, fabric sample was heated at 75°C for 30 min with continuous stirring on magnetic plate and again heated at 75°C for 30 min in an oven. After drying at 100°C for 1 hour, the weakly bonded titania nanoparticles were washed out from the modified cotton fabric in distilled water through sonication for 5 min.

Characterization of Titania Nanoparticle Coated Cotton Textile

The topography of modified textile was studied through SEM analysis. Energy Dispersive X-ray fluorescence (ED-XRF) spectrometer (JOEL JSX 3202 M) was used to determine the titania content on modified textile.

Durability Testing

The durability of titania coated textile was tested through a modified version of standard method of AATCC Test Method 61-2013 [33]. In this method, 45 min of washing in 2g/L detergent at a temperature of 50°C and speed of 42 rpm is considered equivalent to five home launderings at 38 ± 3°C. In our approach, this washing process was done at varying temperatures and cycles [7, 23]. Effect of detergent was also analyzed by washing the fabric with and without the addition of detergent. Coated samples with size 10 cm×10 cm were used in the washing process. Each sample was immersed in 100 ml distilled water at a set temperature: 30, 60 and 90°C at 100rpm. Wash water was stirred and the required temperature was maintained using a magnetic hot plate. Washing was done for 45 min (equal to five home launderings) and 135 min (equal to fifteen home launderings). After washing, each fabric was dried in an oven at 70°C for 15 min. The same method was repeated for samples immersed in 100 ml distilled water containing 0.2g detergent. These washed fabric samples were also analyzed through SEM analysis and ED-XRF spectrometer to determine their titania content after washing process.

The concentration of titania in the washing effluent was analysed through catalytic spectroscopy using UV–Vis spectrophotometer (T-60U) [34]. In this method, concentration of the titanium (Ti) was determined through its catalytic effect on methylene blue-ascorbic acid redox reaction. For this purpose, firstly, a stock solution (1000 ppm) of Ti was prepared through the dissolution of 0.83 g of TiO_2 in 150 ml of hot sulphuric acid and diluting this solution in a volumetric flask (500 ml). Using this stock solution, further dilutions were prepared. In a series of 100 ml flasks, 20 ml of 2.02×10^{-5} mol/litre methylene blue solution, 10 ml of pH = 4 acetate buffer solution, 10 ml of standard Ti solution were added so that a concentration range of 20-120 ppm were obtained in the final solutions. Distilled water was added approximately upto a volume of 90 ml in 100 ml flasks. 10 ml of 2×10^{-3} mol/litre ascorbic acid was then added and diluted upto 100 ml mark with distilled water. The solution was mixed well. The time was taken as zero at which the last drop of ascorbic acid solution was added. After 5 min, solution was transferred into cuvette and analyzed in UV-Vis Spectrophotometer. Firstly the absorption spectrum of the methylene blue reduction product was obtained in the wavelength ranging from 400-800 nm using the solution with Ti concentration of 120 ppm. The calibration curve was then obtained by standard solutions (20, 40, 60, 80, 100, 120 ppm) of Ti. Concentration of Ti in washing effluents was obtained simply using the calibration curve.

Antibacterial Testing

Antibacterial test was performed on modified cotton fabric using a culture of methicillin resistant S. aureus (MRSA) prepared in Luria-Bertani (LB) broth. MRSA strain was selected as it was common in nosocomial infection studies. After sterilizing, fabric samples were transferred to test tubes containing 10 ml of freshly prepared culture. These test tubes were subjected to UV light for 4 h. Samples were collected at 0, 2 and 4 h interval. Bacterial load was analyzed in each sample through viable count on nutrient agar media after serial dilutions of the sample (upto dilution factor of 10^{-7}) in 0.85% saline solution. After incubation for 24–48 h at 37°C, colony forming units (CFU) were counted to verify the bacteria inactivation of the fabric samples. The same procedure was followed where modified cotton fabric were subjected to fluorescent light as well as dark conditions for 24 h.

Field Testing

In addition to the laboratory analysis of antibacterial properties of titania nanoparticle coated fabric, field analysis was done to study their application in hospital environment. For this purpose, white bed linen was coated with titania nanoparticles through the method explained in 2.4. Each bed linen was divided into three sections including uncoated (control), pure titania nanoparticle coated and silver doped titania nanoparticle coated as shown in Fig. 2.

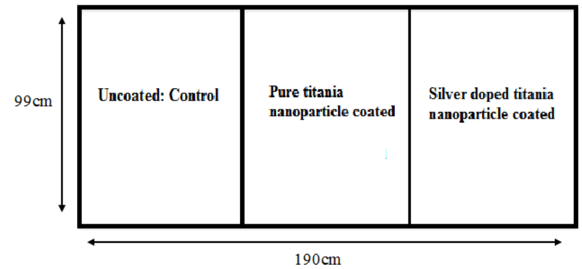

Fig. 2: Coating pattern and dimensions of bed linen.

The study was conducted in medical intensive care unit (MICU) of a local hospital where patients with severe and fatal illnesses and injuries require continuous monitoring from specialized equipment, long term care and medications in order to ensure normal bodily functions. Three patients were included in this study with details of their medical condition given in Table-1. These patients were in a state of low consciousness and completely reliant on medical personnel for all necessities. Modified autoclaved bed linen was placed on the beds of these patients consecutively for a period of 3 days (Fig. 3). After being used by the patients, each bed linen was placed in a clean plastic bag, sealed and transported to the microbiology laboratory within 30 min. From each bed linen, bacterial samples were collected from three different sites (each with an area of 10 cm^2) of every section. These samples were collected using sterile cotton-tipped swabs, pre-moistened with sterile 0.85% saline solution. The viable count was executed on nutrient agar plates after directly spreading the sample through rubbing the cotton swabs on agar plates and also after the serial dilutions (upto dilution factor of 10^{-2}) in 0.85% saline solution. The entire procedure was performed in a laminar flow hood. Colonies were counted after 24 h of incubation at 37°C to compare the level of bacterial contamination among different sections of bed linen.

Table-1: Clinical characteristic of patients.

Patients	Medical condition	Time period in hospital (till experiment) - days
1	Cerebral vascular accident (CVA), Diabetes mellitus (DM), Hypertension(HTN)	126
2	Cerebral vascular accident (CVA), Diabetes mellitus (DM), Sepsis	24
3	Pneumonia, Traumatic quadriparesis	55

Fig. 3: Placement of bed linen in MICU of a local hospital.

Results and Discussion

Characterization of Titania Nanoparticles

XRD Patterns: XRD spectroscopy was carried out using Cu-Kα radiations at an angle 2θ ranging from 20° to 80°. Peaks can be seen for both pure and silver doped titania nanoparticles at 25°, 37°, 38°, 48°, 54°, 55°, 63°, 68°, 71°, 76° and 77° in Fig. 4. These peaks characterized that titania nanoparticles were mostly in the anatase (crystalline) phase. This crystallinity was due to heat treatment of titania during calcination at 400°C for 6 h. In case of silver doped titania nanoparticles, there was some reduction in intensity of peaks corresponding to anatase phase and appearance of peaks at 27° and 36° corresponding to rutile phase. This transformation of anatase to rutile phase was because of the silver ions that increased oxygen vacancies at the surface of anatase lattice favouring the structural reorganization and ionic rearrangement for the formation of rutile phase [35]. These silver doped titania nanoparticles did not show any peak corresponding to silver species which can be attributed to very low proportion and complete dispersion of doped silver on titania surface [36]. The average particle size of pure titania nanoparticles was 43.1 nm and silver doped titania nanoparticles was 54.6 nm. The larger size of silver doped titania nanoparticles as compared to pure titania nanoparticles was due to anatase-to-rutile transformation. Adjacent anatase nanoparticles began to coalesce by diffusion of atoms to form larger anatase nanoparticles before converting to the rutile phase, leading to an increase in size of silver doped titania nanoparticles [37].

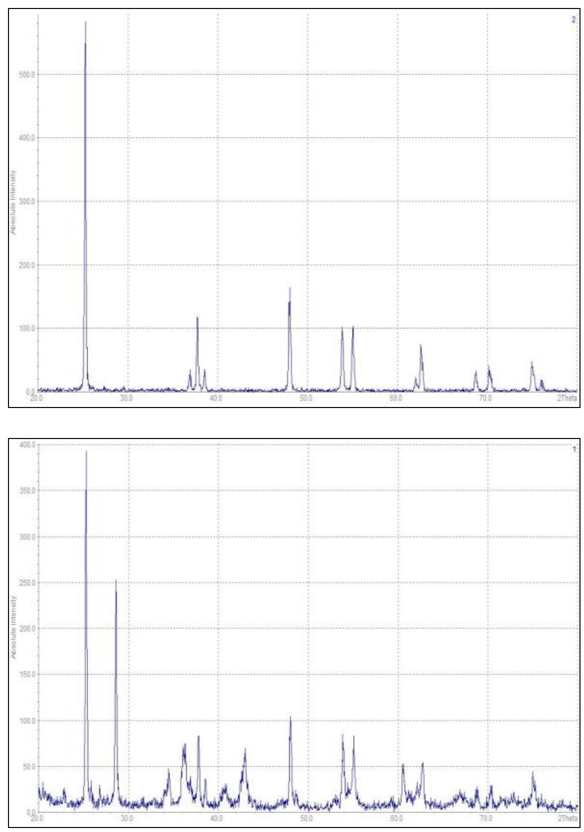

Fig. 4: XRD intensity plot for pure titania nanoparticles and 1% silver doped titania nanoparticles.

SEM observations: Fig. 5 shows the SEM micrographs of pure and silver doped titania nanoparticles at 5000 magnifications. Most of the nanoparticles were spherical in shape and occur in the form of micro-sized aggregates. This aggregation is advantageous for their removal from aqueous environment after treatment [4].

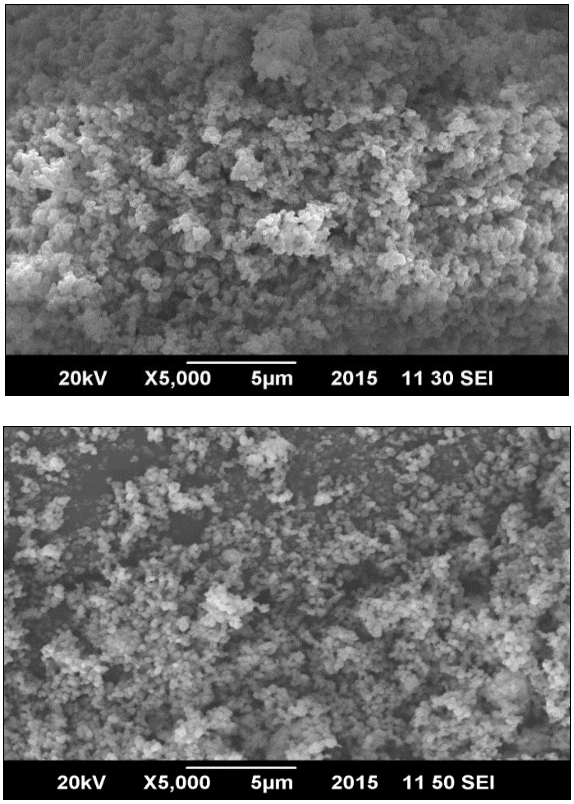

Fig. 5: SEM micrograph for pure titania nanoparticles and 1% silver doped titania nanoparticles at ×5000.

EDS analysis: Fig. 6 depicts the EDS patterns of pure and silver doped titania nanoparticles. Pure titania nanoparticles were composed of ~54% titanium and ~46% oxygen whereas 1% silver doped titania nanoparticles comprised of ~59% titanium, ~40% oxygen and ~1% silver. EDS results confirmed that 1% silver doping of nanoparticles has been successfully achieved. No impurities were detected in the prepared nanoparticles.

Characterization of Titania Nanoparticle Coated Cotton Textile

SEM observations: Titania nanoparticles were successfully deposited on cotton fabric as shown in Fig. 7. These SEM micrographs represent the pure (blank) and modified (titania coated) cotton fabric. The high surface coverage of nanoparticles on modified cotton fabric provides suitable surfaces for photocatalytic activity. Nevertheless this coverage is non-homogenous and inconsistent, somehow due to non-homogenous surface of cotton fabric, also observed and reported in previous studies [7, 32].

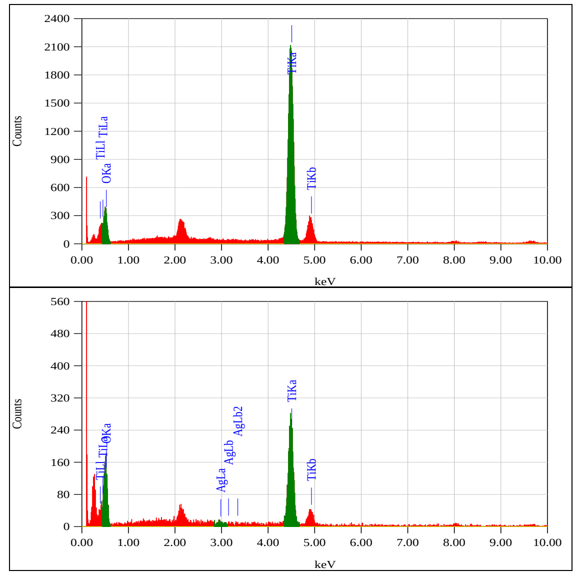

Fig. 6: EDS patterns for elemental compositions of pure titania nanoparticles and 1% silver doped titania nanoparticles.

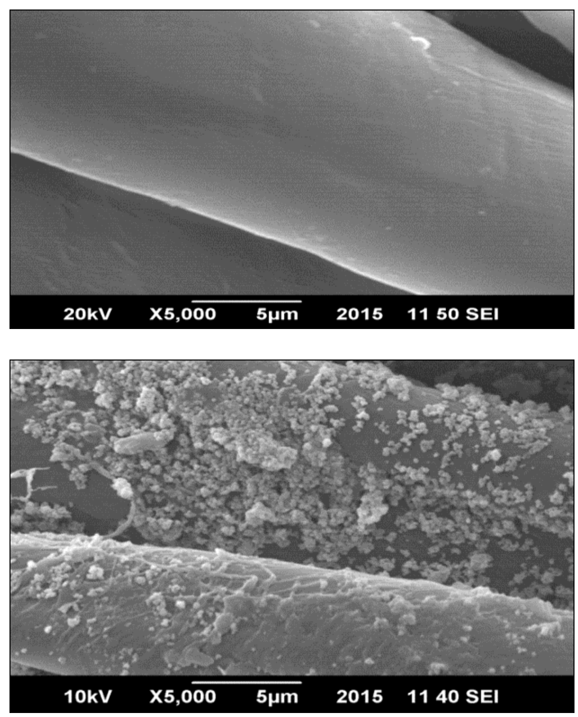

Fig. 7: SEM micrograph for pure cotton fabric (blank) and pure titania nanoparticle coated cotton fabric at ×5000.

ED-XRF analysis: Elemental analysis of modified cotton fabric through ED-XRF analysis shows Ti content confirming presence of titania on modified fabric sample; whereas no Ti was detected in blank sample (Fig. 8).

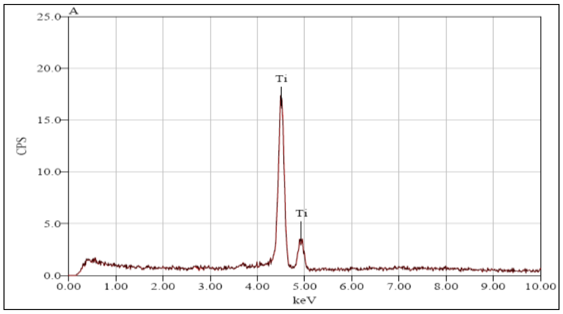

Fig. 8: XRF spectra for titania nanoparticle coated cotton fabric.

Washing Durability

Fabric Surface Characterization: The effect of washing the modified fabric on titania loadings was studied. The experiment confirmed the durability of titania nanoparticle coated fabric against washing at different conditions. The distribution of nanoparticles on the fabrics washed at temperatures of 30, 60 and 90°C; with and without detergent and after 5 and 15 launderings was observed using SEM micrographs (Fig. 9). Micrographs of these washed fabric samples were similar to those of unwashed fabric sample represented in Fig. 7. There was no significant difference among samples washed at different temperatures. Also there was a minor difference between samples after 5 and 15 launderings. No particular temperature presented the greatest release of titania. However, a considerable difference may be seen in samples washed with and without detergent.

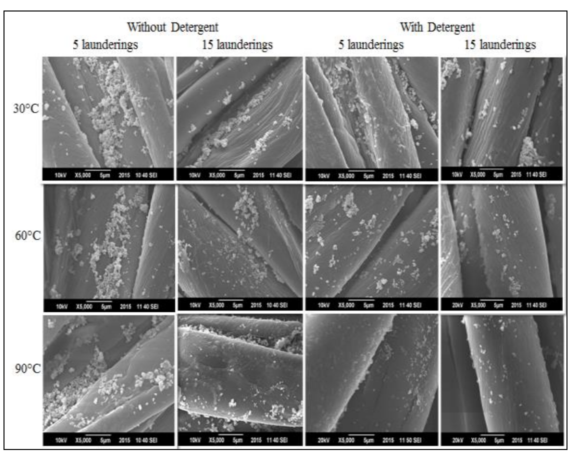

Fig. 9: SEM micrographs of titania nanoparticle coated cotton fabric after washing at 30, 60 and 90°C; with and without detergent and after 5 and 15 launderings at ×5000.

These washed fabric samples were also characterized through XRF analysis. Fig. 8 represents the XRF graph for control fabric (coated and unwashed) with Ti peak at 17 counts per second (CPS). Table-2 shows that there is an insignificant decrease of CPS of Ti representing a negligible decrease in titania content on fabric samples after washing at increasing temperature conditions. But sample washed at 30°C showed CPS of Ti equal to control sample, comparatively higher than other samples, proving that the titania loadings on textiles were highly maintained after washing at 30°C. Similar to the SEM micrographs, only a minor difference can be seen in CPS values of samples after 5 and 15 launderings but a significant difference may be seen in CPS values of samples washed with and without detergent.

Table-2: XRF analysis representing counts per second (CPS) of titania nanoparticle coated cotton fabric after washing **at** 30, 60 and 90°C; with and without detergent and after 5 and 15 launderings at ×5000.

Washing	Launderings	Temperature (°C)	CPS
Without detergent	**5**	**30**	**17**
		60	**16**
		90	**13**
	15	**30**	**17**
		60	**15**
		90	**12**
With detergent 2g/L	**5**	**30**	**15**
		60	**12**
		90	**8**
	15	**30**	**10**
		60	**12**
		90	**6**
Control			**17**

The results of SEM and XRF analysis confirm that nanoparticles were strongly adhered to surface of cotton fabric through a cross-linkage of succinic acid. Loosening of certain nanoparticles during washing is due to multiple factors. During washing process, the motion of fabric occurs in the form of tumbling, rubbing, agitation, abrasion and dragging which induces mechanical forces on loosely bonded nanoparticles leading to their slackening from fabric [7]. The increase in temperature also increase kinetic energy of these loosely bonded nanoparticles that get detached from fabric which might be the reason for slight decrease in titania content with an increase in temperature. Detergents used for washing were composed of various active ingredients such as surfactants, enzymes, fillers, polycarboxylates, builders, corrosion inhibitors, optical brighteners, foam regulators and bleaching agents. These constituents act together to provide best laundering effect. Absorbed surfactant molecules in detergents enhance removal of nanoparticles from textile surface by weakening electrostatic forces between titania nanoparticles and succinic acid bonded with cotton textile [38]. Adherence of nanoparticles on fabric even after numerous washing cycles is probably because only loosely bonded nanoparticles were removed from the fabric in primary washing process whereas strongly bonded nanoparticles remained attached even after subsequent washings.

Washing effluent analysis: Analysis of washing effluent is considered as a direct approach for estimation of durability against washing. Catalytic spectrophotometry was applied for determination of Ti in washing effluent that represents concentration of titania. The absorption spectrum of the reduction product of methylene blue was determined by UV-Vis spectrophotometer using a standard solution (120 ppm) of titanium prepared as described above. The spectrum shows a peak at 665 nm (Fig. 10).

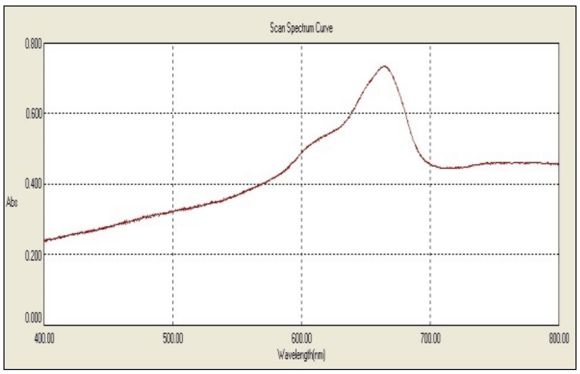

Fig. 10: Absorption spectrum for 120 ppm standard titanium solution.

The calibration curve obtained for Ti spectroscopy was linear representing a straight line with an equation for the line y=0.006x+0.0167 where x and y are the concentration and absorbance respectively (Fig. 11). Using this equation, concentration of Ti in washing effluents was determined (Table-3). Average of three replicates for each different condition was noted.

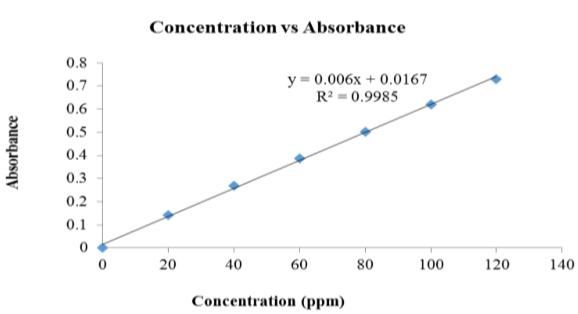

Fig. 11: Calibration curve for titanium spectrophotometry.

High concentrations of Ti in washing effluents represent poor durability. Based on the results shown in Table-3, it is evident that there were very less concentrations of Ti in wash water showing high durability and confirming the resulting of SEM and XRF analysis. The concentrations show negligible variations after different number of launderings as concluded previously [7, 23]. This is because unstable titania nanoparticles which were physically bonded to fibre (not chemically attached through succinic acid), were released into wash water through primary washing cycles. During the subsequent washings, the absence of Ti in washing effluents indicate that loosely attached titania nanoparticles were already detached from the fabric in primary washing. There is a visible difference in Ti concentrations of washing effluents without and with detergents. This is because of the highly efficient removal properties of surfactants that a greater amount of titania is removed from the fabric. Also the Ti concentration released from pure detergent solution creates a difference between Ti concentrations of washing effluents without and with detergents. In a pure detergent, Ti compounds could be present in the form of corrosion inhibitors contributing to the release of Ti in washing effluents [39, 40]. In comparison, the highest titania concentration was obtained in effluent after washing at 90°C as previously reported [7]. Therefore, in terms of environmental safety, low temperatures and least detergents are suitable to be applied for washing titania coated fabric. In terms of temperature, maximum durability of titania on textiles was examined after washing at 30°C. Nevertheless, washing of titania coated fabric does not significantly affect the coating of titania nanoparticles. Therefore it is concluded that titania nanoparticle coated textile have high durability against washing even at high temperature and with detergents. This stability proposed the formation of strong linkages between cotton and titania nanoparticles through succinic acid [32]. Hence the development of titania nanoparticle coated fabric through cross linking phenomena is an effective and reliable method.

Table-3: Concentrations of titanium in washing effluents.

Washing	Launderings	Temperature (°C)	Average Concentration (ppm)
Without detergent	5	30	11
		60	17
		90	24
	15	30	11
		60	17
		90	26
With detergent 2g/L	5	30	139
		60	140
		90	140
	15	30	146
		60	140
		90	156
Detergent Solution			27

Antibacterial Testing

After the exposure of titana nanoparticle coated cotton fabric placed in bacterial culture to UV light for 4 h; flourescent light and dark for 24 h, it was observed that the modified textile has photocatalytic bacterial inactivation properties.

Disinfection under UV radiations: The percentage reduction of MRSA with sample fabric on exposure to UV radiations is shown in Fig. 12. It may be seen that MRSA culture exhibited 71% reduction (cell count decreased from 2.1×10^9 to 0.6×10^9 CFU/ml) after 2 h and 100% reduction (cell count decreased from 2.1×10^9 to Too Few to Count (TFTC) CFU/ml) after 4 h of UV light exposure through pure titania nanoparticle coated fabric. Such modified fabric also retained the antibacterial properties even after washing process showing 59% inactivation (cell count decreased from 2.2×10^9 to 0.9×10^9 CFU/ml) and 86% inactivation (cell count decreased from 2.2×10^9 to 0.3×10^9 CFU/ml) after 2 and 4 h respectively with sample washed without detergent whereas 57% inactivation (cell count decrease from 2.1×10^9 to 0.9×10^9 CFU/ml) and 81% inactivation (cell count decreased from 2.1×10^9 to 0.4×10^9 CFU/ml) after 2 h and 4 h respectively with detergent washed sample. Washed samples with maximum number of launderings (15) at highest temperatures (90°C) were tested for antibacterial activity. Silver doped titania nanoparticle coated fabric showed the best results with 100% inactivation (cell count decreased from 1.3×10^9 to TFTC CFU/ml) of MRSA within 2 h. Control samples including pure cotton fabric and pretreated cotton fabric showed around 39% reduction (cell count decreased from 1.8×10^9 to 1.1×10^9 CFU/ml) and 39% reduction (cell count decreased from 1.8×10^9 to 1.1×10^9 CFU/ml) after 4 h of UV light exposure.

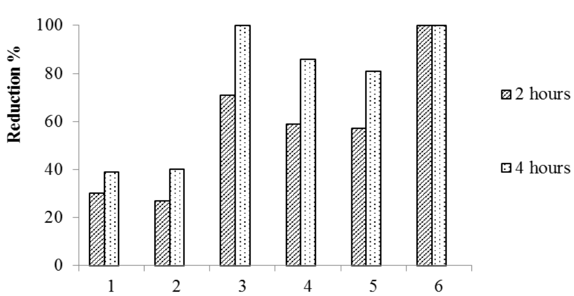

Fig. 12: Antibacterial activity results in UV light.

1- control-1 pure cotton fabric, 2- control-2 pretreated & succinic acid attached cotton fabric, 3- titania coated, 4- titania coated washed without detergent, 5- titania coated washed with detergent, 6- silver doped titania coated.

Disinfection under fluorescent light: Fig. 13 represents the percentage reduction of MRSA with sample fabric on exposure to fluorescent light. With pure titania nanoparticle coated fabric, 47% reduction (cell count decreased from 1.9×10^9 to 1×10^9 CFU/ml) after 12 h and almost complete reduction (cell count decreased to TFTC CFU/ml) after 24 h of fluorescent light exposure was achieved. Also 50% inactivation (cell count decreased from 2.4×10^9 to 1.2×10^9 CFU/ml) and 88% inactivation (cell count decreased from 2.4×10^9 to 0.3×10^9 CFU/ml) was obtained after 12 and 24 h respectively with sample washed without detergent. While with detergent washed sample, 50% inactivation (cell count decrease from 2.4×10^9 to 1.2×10^9 CFU/ml) and 79% inactivation (cell count decreased from 2.4×10^9 to 0.5×10^9 CFU/ml) was detected after 12 h and 24 h respectively. Similar to the results of UV experiment, finest results were observed with silver doped titania nanoparticle coated fabric showing complete sterilization even after 12 h (cell count decreased from 1.5×10^9 to TFTC CFU/ml) of MRSA. Pure cotton fabric and pretreated cotton fabric (control samples) showed 30% reduction (cell count decreased from 2.3×10^9 to 1.6×10^9 CFU/ml) and 33% reduction (cell count decreased from 1.8×10^9 to 1.2×10^9 CFU/ml) after 24 h under fluorescent light.

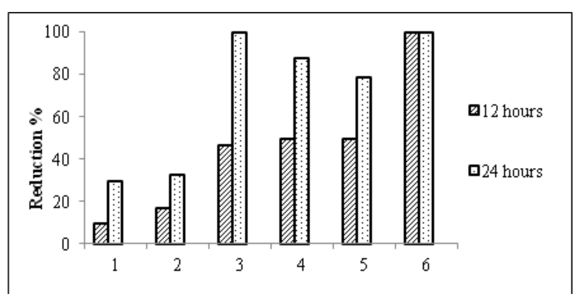

Fig. 13: Antibacterial activity results in fluorescent light.

1- control-1 pure cotton fabric, 2- control-2 pretreated & succinic acid attached cotton fabric, 3- titania coated, 4- titania coated washed without detergent, 5- titania coated washed with detergent, 6- silver doped titania coated.

Disinfection Under Dark Conditions: Similar experiment was also performed under dark conditions for 24 h. Results (Fig. 14) showed that limited bacterial inactivation occurred in the dark conditions. After 24 h in dark conditions, MRSA reduced to 24% (cell count decreased from 2.5×10^9 to 1.9×10^9 CFU/ml) with pure titania coated fabric, 20% (cell count decreased from 1×10^9 to 0.8×10^9 CFU/ml) in sample washed without detergent, 23% (cell count decreased from 1.3×10^9 to 1×10^9 CFU/ml) in detergent washed sample and 30% (cell count decreased from 1.3×10^9 to 0.9×10^9 CFU/ml) in silver doped titania nanoparticle coated fabric. Whereas the pure cotton fabric and pretreated cotton fabric showed negligible reduction of MRSA in dark conditions.

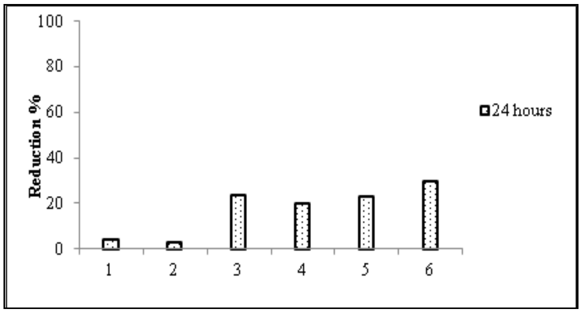

Fig. 14: Antibacterial activity results in dark conditions.

1- control-1 pure cotton fabric, 2- control-2 pretreated & succinic acid attached cotton fabric, 3- titania coated, 4- titania coated washed without detergent, 5- titania coated washed with detergent, 6- silver doped titania coated.

The cell wall of MRSA has a complex structure that requires a large amount of energy to break. The ROS provided by titania nanoparticles are involved in damaging cell wall as well as the catalytic properties, optical properties, and electrical conductivity of bacterial cells. Though titania nanoparticles by themselves are capable of inducing the oxidative stress, the complete disinfection in UV light is because the effect is possibly much stronger under UV light. This is due to the formation of electron–hole pairs on titania when exposed to UV light resulting in generation of additional ROS and hence complete disinfection. The photocatalytic activity is lesser in fluorescent light as compared to UV light as the process depends upon energy of incident photons. Photoradiations themselves may also induce oxidative stress and that will be more in case of UV light. With an increase in time of fluorescent light exposure, photocatalytic inactivation was enhanced [41]. The disinfection also occurred to some extent in samples placed in dark conditions. Preactivation of titania nanoparticles by exposure to light before exposure to dark is the likely cause of MRSA reduction in dark conditions [42]. Also this shows that textiles have an inherent antibacterial activity which is enhanced through nanoparticles and light source [7]. Similarly, complete disinfection occurs with silver doped titania nanoparticles both in UV and fluorescent light because doping of silver on titania nanoparticles allows the extension of light absorption to the visible light. Also when light strikes the doped nanoparticles, due to metal doping, it produces electron–hole pairs easily to form more ROS involved in bacterial destruction [4, 13, 35-37]. The antibacterial effect was decreased after washing due to nanoparticles reduction on fabric sample. Washing textile sample without detergent did not significantly influence the antibacterial activity against MRSA but with detergent has comparatively higher effect on disinfection properties. Hence, these modified cotton fabric are suitable for bacterial disinfection.

Field Testing

Although the results of the antibacterial properties of titania nanoparticle coated fabric have been already obtained, the examination whether these will function in the same way in the actual hospital environment remained unclear. For this reason an experiment was conducted in MICU of a hospital to investigate whether the use of such antibacterial textiles in the form of bed linen may reduce the bacterial contamination. The results of this experiment are shown in the Fig. 15.

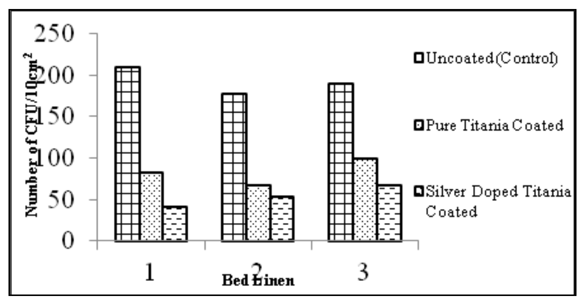

Fig. 15: Summary of bacterial count from hospital used bed linen.

Countable range of CFU, obtained in agar plates incubated after directly spreading the sample without serial dilutions, are only represented here. Bacterial count was majorly below countable range (TFTC) after serial dilutions of the sample. Fig. 15 represents the average number of CFU from three similar sites of each section. The results are much similar for all bed linen. It may be seen that uncoated (control) section has the maximum number of CFU. Titania coated section has lesser number of CFU as compared to uncoated section. Whereas silver doped titania coated section has the lowest number of CFU compared to both uncoated and titania coated sections.

Significantly lower loads of bacteria found on titania and silver doped titania coated fabric than on uncoated (control) fabric confirmed the disinfection potential of these modified textiles.

Comparing the effectiveness of nano-coatings, silver doped titania is more effective than pure titania coated fabric, similar to the results of laboratory analysis. This is because, in the fluorescent light, doped particles are more active in reducing the population of bacteria as explained in 3.4.

Bacterial count on uncoated section of each bed linen may be correlated to the time period of that patient in hospital given in Table 1. First bed linen with 209 CFU/10cm^2 of uncoated section was used by patient in hospital for 126 days till experiment. Second bed linen with 177 CFU/10cm^2 of uncoated section was in used by patient in hospital for 24 days till experiment. Third bed linen with 189 CFU/10cm^2 of uncoated section was used by patient in hospital for 55 days till experiment. Hence it may be anticipated that bacterial contamination is enhanced with the greater exposure period in hospital environment. But no such trend may be seen in case of titania coated and silver doped titania coated sections.

The study proves that bed linen in hospitals are associated with bacterial contamination as previously reported [3, 24, 43-45]. These bed linen may also spread the pathogenic bacteria into the immediate and distant environment. It has been reported that 42% of personnel are affected with bacterial contamination who had only touched the bed linen, even though had no direct contact with patients [46]. Although the bed linen used in the hospitals are regularly changed and sterilized but such high-touch objects which are closest to patients are more likely to have higher bacterial load and turn out to be contaminated within a small period of exposure time [47]. The results clearly prove that the use of such titania nanoparticle coated textiles may control the bacterial contamination and the risk of nosocomial infections.

Conclusions

In this study pure and (1%) silver doped titania nanoparticles synthesized through liquid impregnation method were applied for the development of an antibacterial textile. Titania nanoparticles were coated on cotton fabric surface using a cross-linking agent succinic acid. The durability of modified fabric was encouraging after washing at different conditions, noting that they were suitable in environmental perspective. In this study attempts were made for the first time to determine the titania nanoparticles concentration in washing effluents successfully using catalytic spectrophotometry with UV/visible spectrophotometer. The antibacterial test of these pure and silver doped titania nanoparticle coated fabric using methicillin resistant Staphylococcus aureus (highly significant in hospital acquired infections) showed promising results in both UV and fluorescent light. The disinfection properties of such fabric were successfully validated in hospital environment through the application of bed linen in medical intensive care unit (MICU) of a local hospital. Hence it is concluded that titania nanoparticle coated textiles may effectively protect the hospital environments from nosocomial infections and also improve the textile market.

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