PHOTOCATALYTIC INACTIVATION OF HOSPITAL-ASSOCIATED BACTERIA USING TITANIA NANOPARTICLE COATED TEXTILES

By

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This thesis is dedicated to my beloved parents. Without their faith, support, guidance and constant encouragement, this work would not have been possible.

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LIST OF ABBREVIATIONS

Ag	Silver
CFU	Colony Forming Unit
CPS	Counts per Second
CuO	Copper II Oxide
CVA	Cerebral Vascular Accident
DM	Diabetes Mellitus
EDS	Energy Dispersive Spectroscopy
ED-XRF	Energy Dispersive X-ray Fluorescence
H_2O_2	Hydrogen Peroxide
HTN	Hypertension
IESE	Institute of Environmental Sciences and Engineering
MgO	Magnesium Oxide
MICU	Medical Intensive Care Unit
MRSA	Methicillin Resistant *Staphylococcus aureus*
NaOH	Sodium Hydroxide
NaH_2PO_2	Sodium Dihydrogen Phosphate
NPs	Nanoparticles
$O_2^{\bullet -}$	Superoxide radical

$OH^{\bullet}$	Hydroxyl radical
PET	Polyethylene Terephthalate
ppm	Parts per million
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscopy
SiO_2	Silicon Dioxide
TiO_2	Titanium Dioxide
TFTC	Too Few To Count
TNTC	Too Numerous To Count
TTIP	Titanium Tetra Isopropoxide
UV	Ultra Violet
VOCs	Volatile Organic Compounds
VRE	Vancomycin Resistant Enterococci
XRD	X-Ray Diffraction
ZnO	Zinc Oxide

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ABSTRACT

Modification in hospital textiles to include disinfection properties may help in the reduction of nosocomial infections. Antibacterial properties were imparted to cotton fabric by modifying it with pure and (1%) silver doped titania nanoparticles (NPs) prepared through liquid impregnation process. These NPs 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 NPs to fabric. Scanning Electron Microscopy and Energy Dispersive X-ray fluorescence spectrometer were used for the characterization of coated fabric. Catalytic spectrophotometry using UV/visible spectrophotometer was applied to determine titanium dioxide concentration in washing effluent. The antibacterial activity of the fabric was examined against methicillin resistant *Staphylococcus aureus* (MRSA) under UV and fluorescent light. The maximum durability of NPs to the fabric was retained after washing without detergent at 30°C. Coating of NPs on fabric was found durable against washing, hence suitable from an environmental perspective. Antibacterial testing showed 100% photocatalytic inactivation of MRSA after 4 and 24 hours of UV and fluorescent light exposure respectively. The potential of using such textiles in hospital environment was validated through the use of modified bed linens in a local hospital. The viable count indicated significantly lower loads of bacterial contamination on nano-coated fabric as compared to uncoated fabric. Bed linens, curtains, staff uniforms, lab coats and medical garments developed from titania nanoparticle coated fabric may improve hospital environment against antibiotic resistant micro-organisms.

Chapter 1

INTRODUCTION

1.1 Background

The dynamic hospital environment may contain significant number of micro-organisms including viruses, fungi and bacteria. These micro-organisms originate not only from humans (patients, visitors and staff) but may also be spawned by various indoor hospital characteristics and outdoor environmental sources. Poor hospital conditions may lead to the spread of pathogenic micro-organisms that consequently leads to the acquisition of nosocomial infections (Verde *et al*., 2015).

With the emergence of antibiotic-resistant bacteria, nosocomial infections have become a public health problem, creating a new burden on medical care in hospitals (Cornejo-Juarez *et al*., 2015). These nosocomial infections may be transmitted through various modes like direct-contact, airborne transmission, endogenous transmission or transmission via inanimate environment. The textiles used in the hospital environment play an important role in the transmission of pathogenic bacteria (Lax & Gilbert, 2015).

1.2 Hospital textiles and transmission of pathogenic bacteria

Experts believe that hospital textiles with bacterial contamination make an important contribution to the epidemic and endemic transmission of antibiotic resistant pathogenic bacteria like vancomycin resistant enterococci (VRE), methicillin resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. These textiles including bed linens, staffs uniform, lab coats and medical garments, as in close contact with patients and healthcare workers, represent better substrates for bacterial growth due to moisture and protein-rich soil or dirt that may be found on such materials (Mitchell *et al*., 2015). The spread of nosocomial infections by contact of contaminated textiles has created increased pressure for disinfection of textiles used in the hospital environment (Arain *et al*., 2013).

1.3 Methods for textiles disinfection

In order to ensure healthy conditions and protect patients and healthcare workers from hospital-acquired infections to some extent, special measures are required to be taken for disinfection and sanitization of textiles used in the hospital environment (Wilson *et al*., 2007).

Personal Protective Equipment (like plastic apron) has a clear place in protecting staff uniforms and clothing while performing tasks that may involve anticipated exposures to blood and body fluids (Wiener-Well *et al*., 2011). Laundering of textiles is a common method and considered as a cost-effective measure for sanitization of textile materials using thermal disinfection or chemicals suitable for low-temperature washing. However, freshly laundered textiles have also been found to be contaminated with pathogenic bacteria like *Staphylococcus spp*., *Bacillus spp.* and *Corynebacterium spp* (Nordstrom *et al*., 2012).

Production of antibacterial fabrics is considered as a more reliable option for disinfection. In practice, the antibacterial effect may be obtained through the application of specific chemical products during the finishing stage or through the incorporation of these substances into fibres during the spinning process. These substances may include quaternary ammonium, triclosan, chitosan, metals like silver (Ag) or metallic oxides like titania (TiO_2) (Shahidi and Weiner, 2012).

1.4 Proposed solution

TiO_2, due to its excellent photocatalytic properties, is gaining importance in a wide scenario of environmental applications (Uddin *et al*., 2008). Light exposure of TiO_2 excites electrons from the valence band to the conduction band, leaving holes in the valence band. The electrons then react with oxygen molecules to produce superoxide anions whereas the holes react with water to produce hydroxyl radicals. These two highly reactive oxygen species (ROS) are able to decompose a variety of toxic materials and damage bacterial cell structure ultimately to cell death (Pant *et al*., 2011).

Extraordinary photocatalytic properties, non-toxicity, high availability, biocompatibility, high stability and low price make TiO_2 nanoparticles (NPs) particularly attractive to be applied onto textile materials with an aim to produce fabric with multifunctional properties such as UV protective, self-cleaning and antibacterial (Pasqui and Barbucci, 2014; Radetić, 2013; Wu & Long, 2011).

1.5 The present study

Based on the above discussion, it may be assumed that disinfection of hospital textiles by modifying with TiO_2 NPs may be an effective method. Therefore it is hypothesized that titania nanoparticles coated textiles have effective antibacterial properties in hospital environment. The study was structured around three major objectives:

i. Development of pure and silver doped titania nanoparticle coated cotton fabric.

ii. Testing antibacterial effectiveness of such modified cotton fabric against selected bacteria.

iii. Investigating the potential of using such modified cotton fabric in hospitals for photocatalytic inactivation of hospital-associated bacteria.

Prepared pure and Ag doped TiO_2 NPs were characterized through X-ray diffraction (XRD) spectroscopy, scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS). Cotton fabric was modified with pure and Ag doped TiO_2 NPs using succinic acid as a cross linking agent. In order to assess the durability of modified cotton fabric, washings of fabric and analysis of this fabric along with washing effluent was performed. The disinfection efficacy of modified textile was investigated by studying its ability to inactivate methicillin resistant *Staphylococcus aureus* (MRSA) under visible and ultraviolet light. Potential of using such modified fabric in hospitals was investigated through an experiment conducted in a local hospital where titania nanoparticle coated fabric in the form of bed linens were used by patients and monitored for the level of bacterial contamination as compared to uncoated fabric used in the same manner.

Chapter 2

LITERATURE REVIEW

2.1. Nosocomial infections

Despite many recent advances in medical science, nosocomial infections are recognized as the most frequent adverse events affecting the quality of healthcare all over the world. Because of growing awareness in recent years, the burden caused by such infections is receiving a lot of attention (Kaier *et al*., 2012).

Nosocomial infections are those that are acquired by a patient already under medical care in health facility. These infections occur in patients during hospitalization, not present, or in the state of incubation, at the time of admission. In addition to vulnerable patients, such infections may also be acquired by hospital staff, visitors or other healthcare personnel (Khan *et al*., 2015).

These infections include urinary tract infections, bloodstream infections, eye, ear, nose or throat infections, secondary skin infections, soft tissue infections, pneumonia, liver abscess and meningitis (Mohammed & El Seifi, 2014). The hospital acquired infections have an impact on the healthcare system as it increases the rates of morbidity and mortality, use of medical resources, duration of hospitalization as well as the cost of treatment (Cornejo-Juarez *et al*., 2015).

The causative organisms for these infections may be bacterial, viral or fungal in origin. Many of these pathogenic micro-organisms are becoming more difficult to be treated due to their growing level of antibiotic resistance, creating a serious threat to the spread of infectious diseases (Borkow & Gabbay, 2008).

2.2. Nosocomial infections causing bacteria

Bacteria are more responsible as compared to viruses and fungi contributing to about 90% for nosocomial infections. The pathogenic bacteria that are usually involved in hospital-acquired infections include *Streptococcus spp.*, *Acinetobacter spp.*, *Enterococcus spp.*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Klebsiella pneumonia* (*K. pneumonia*) and *Escherichia coli* (*E. coli*) (Khan *et al*., 2015).

i. *Streptococcus pneumoniae* is the cause of pneumonia, acute otitis media, sinusitis and meningitis (Esel *et al*., 2001).

ii. The genus *Acinetobacter* is a major cause of nosocomial infections and has become a widespread concern in a variety of hospitals worldwide. Antibiotic resistant *Acinetobacter baumannii* has now emerged as an important nosocomial pathogen. It causes pneumonia, blood stream infection, wound infection, urinary tract infection and meningitis (Almasaudi, 2016; Eveillard *et al*., 2013).

iii. *Enterococcus spp.* causes surgical-site and blood stream infections (Billington *et al*., 2014).

iv. *P. aeruginosa* causes 11% of all nosocomial infections which are evenly distributed to the entire body sites. It is a cause of surgical and wound infections, urinary tract infections, pneumonia and cystic fibrosis (Khan *et al*., 2015).

v. *S. aureus* is considered as one of the most important pathogens, responsible for nosocomial infections. Hospitalized patients with decreased immunity are more prone to *S. aureus* infections. Methicillin resistant *S. aureus* (MRSA) have a high survival rate on environmental surfaces such as door handles, computer keyboards, soap dispensers and sink taps, and sites where dust is allowed to accumulate in healthcare facilities. MRSA may survive on surfaces or skin scales for up to 80 days. It causes blood stream infections, food poisoning, toxic shock syndrome and staphylococcal scalded skin syndrome (Breathnach, 2013).

vi. *B. cereus* is another important pathogen in clinical settings. It causes infections in wounds, panophthalmitis, pneumonia, meningitis and bacteremia (Hernaiz *et al*., 2003).

vii. 3-7% of hospital acquired infections are related to *K. pneumonia*. *It* infects gastrointestinal tract, urinary tract, pharynx and skin and causes pneumonia and liver abscess (Melot *et al*., 2015).

viii. *E. coli* is an emerging nosocomial pathogen causing problems in health care settings. It is responsible for urinary tract infections, septicemia, pneumonia, neonatal meningitis, peritonitis and gastroenteritis (Khan *et al*., 2015).

2.3. Modes of transmission

The nosocomial infection causing bacteria have various modes of transmission. The most important and recognized transmission is via direct-contact between two persons with one serving as the source of the infectious bacteria and the other as a susceptible host. Airborne transmission is another known route of nosocomial infections. The infection causing bacteria become airborne usually through coughing or sneezing (Borkow and Gabbay, 2008).

Airborne transmission may also originate by various indoor hospital characteristics and outdoor environmental sources. Hospital buildings may be regarded as dynamic environments affected by season, weather conditions, indoor ventilation system design and operation, intrusion of moisture, outdoor microbial load and the number of occupants, visitors and human activities. These factors may be associated with conditions for bacterial growth (Verde *et al*., 2015).

Another risk factor is endogenous transmission of the host's own microbiota from one part of the body to another (Lax and Gilbert, 2015). Additionally, infections may be transmitted indirectly through contaminated intermediate objects such as instruments (catheters, needles and sensors), surfaces (handles, platforms, panels, floor and over-bed tables), phones, computer keyboards, gloves and textiles (Habib, 2013; Kowal *et al*., 2014; Shahid, 2015; Wendler *et al*., 2014).

2.4. Bacterial contamination on hospital textiles

Textiles used in hospitals such as bed linen, curtains, staff uniforms, lab coats and medical garments may be both, the mode of transmission and reservoir, for pathogenic bacteria, thus may be a potential source of cross-infection (Attaway *et al*., 2012; Freeman *et al*., 2012; Lazary *et al*., 2014; Loh *et al*., 2000; Munoz-Price *et al*., 2012; Perry *et al*., 2001; Treakle *et al*., 2009). Medical textiles are often contaminated by bacteria originating from body substances, including blood, skin, stool, urine, vomits and other body fluids (Perelshtein *et al*., 2015).

Textiles in the hospitals may be contaminated with vancomycin-resistant enterococci (VRE), MRSA, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Nordstrom *et al*., 2012). *Enterococcus spp.* and *S. aureus* may survive for more than 90 days on lab coats and staff uniform (Wiener-Well *et al*., 2011). The maximal contamination occurs in areas of greatest hand contact (such as pockets and cuffs), allowing recontamination of already washed hands and may be transferred to patient bedding (Freeman *et al*., 2012; Loh *et al*., 2000).

Similarly bed linen and pillows have higher bacterial contamination due to their direct contact with the patient's skin and also incidentally with body fluids (blood or urine), thus becoming a source of infection (Creamer & Humphreys, 2008). Bed linen contaminated with pathogenic bacteria may also increase bacterial load in air through bed making (Shiomori *et al*., 2002).

In comparison to the effort placed on cleaning and disinfection of hospital environment, much lesser effort is placed on the cleaning and decontamination of hospital textiles. The complex role that these textiles play in transmission of pathogens is further complicated by varied laundering conditions. With an increasing level of antibiotics resistance, textile contamination is a significant environmental factor in the spread of nosocomial infection (Mitchell *et al*., 2015).

2.5. Antibacterial textiles

In order to reduce the spread of nosocomial infections to some extent, antibacterial textiles are receiving a lot of attention (Bauer *et al*., 2012). Various materials can be applied for the production of antibacterial fabrics.

2.5.1. Quaternary ammonium: Quaternary ammonium compounds are suitable for immobilization on polymer surfaces. Also on cotton cellulose, these compounds are an effective method to incorporate antibacterial properties. These compounds may be applied either during the fibre-forming process or during the finishing process (Messaoud *et al*., 2014).

2.5.2. Triclosan: Triclosan (2, 4, 4-hydrophenyl trichloro (II) ether), a halogen compound, is a significant member of the antiseptic and disinfectant family. It has a wide range of action against gram-negative and gram positive bacteria. Due to its antibacterial properties, triclosan has found widespread use in a variety of consumer products including toothpastes, cosmetics, deodorants, soaps, polymers and fibres (Guo *et al*., 2016).

2.5.3. Chitosan: Chitosan has attracted a great deal of interest recently due to its antibacterial properties. The limitation in using chitosan is that it shows antibacterial activity at relatively high concentration which adversely affects the fabric. Another major problem of chitosan is its reduced antibacterial activity under alkaline conditions due to its loss of the cationic nature and its poor durability on textile fabrics due to its poor adhesion to fabric (Arain *et al*., 2013).

2.5.4. Nano-sized metals and metal oxides: Many metals are toxic to microbes at very low concentrations either in free or compound state. Preparation of nano-sized metals and metal oxides mainly silver (Ag), zinc oxide (ZnO), copper II oxide (CuO), magnesium oxide (MgO) and titanium dioxide (TiO_2) has enabled the development of a new generation of disinfectants for textiles (Singh *et al*., 2012; Kowal *et al*., 2014).

2.6. Role of nanotechnology in bacterial disinfection

Nanotechnology may offer new possibilities in the area of bacterial disinfection. Nanomaterials have attracted attention as effective antibacterial agents due to their unique physical and chemical properties (Khan *et al*., 2013). Nanomaterials, especially nanoparticles (NPs), show completely unique properties in comparison with their bulk sized counterparts. The antibacterial effect of NPs has been attributed to their small size and high surface to volume ratio, which allows them to attach closely with bacterial membranes and cause metabolic disruption (Anandgaonker *et al*., 2015).

It has been demonstrated that the disinfection properties of NPs depends on their size, shape, morphology, surface functionalization and their stability. Additionally, the use of inorganic NPs as antibacterial agents has several benefits such as improved stability and safety in comparison with the organic antibacterial agents. Antibacterial NPs can be composed of metals, metal oxides, metal salts, metal hydroxides, organic nanocarriers loaded with antibacterial agents, hybrid materials, and polymers exhibiting antibacterial properties (Moritz & Geszke-Moritz, 2013).

Among the various types, TiO_2 NPs are found to be one of the most suitable disinfectants. Although nanomaterials may also pose hazards when released to the environment, it has been reported that among seven most widespread nanomaterials (TiO_2, ZnO, CuO, Ag, single wall carbon nanotubes**,** multiwall carbon nanotubes and C60-fullerenes), TiO_2 NPs proved to be the least environmentally threatening (Joost *et al*., 2015).

2.7. TiO_2 as a photocatalyst

TiO_2 has become the focus of many research groups worldwide and one of the most widely used photocatalysts because it is cost effective, highly efficient, stable to light and chemically inert (Latif *et al*., 2014).

2.7.1. Photocatalysis: It is actually an oxidation reaction in the presence of light and oxygen. A photocatalyst is the agent capable of combining light and oxygen efficiently in order to enhance the degradation process. The overall process takes place in five independent steps (Khan, 2012).

i. Reactant diffusion from bulk phase to catalyst surface
ii. Adsorption of at least one of the reactants on the catalyst surface
iii. Reaction in adsorption phase
iv. Desorption of the product
v. Removal of the product from the interface region

2.7.2. Photocatalytic activity of TiO_2: The photocatalytic effect of TiO_2 was discovered by Fujishima and Honda in 1972 (Wang *et al*., 2014). TiO_2 as a semiconductor has a band structure which is characterized by the existence of energy gap that extends from the top of the occupied valence band to the bottom of the unoccupied conduction band (Leong *et al*., 2014). As a photocatalyst, when TiO_2 is exposed to UV light whose energy is higher than the band gap energy of TiO_2, electrons at the valance band are excited to the conduction band, leaving positively charged holes in the valance band. The electron and hole pairs then migrate to the surface of TiO_2 to participate in a series of redox reactions and produce reactive oxygen species (ROS). The electrons reduce Ti(IV) into Ti(III) and subsequently react with the absorbed oxygen (O_2) on TiO_2 surface to produce superoxide radical anions ($O_2^{\bullet -}$). At the same time, the holes react with absorbed water molecules (H_2O) on TiO_2 surface to generate hydroxyl radicals ($OH^{\bullet}$). These ROS are responsible for degradation of organic compounds and antibacterial activity (Latif *et al*., 2014).

2.7.3. Environmental applications of TiO_2 NPs as a photocatalyst: TiO_2 NPs have been proven to be most suitable for environmental applications because of their biological and chemical inertness, heat resistance, strong oxidizing power, cost effectiveness and high chemical stability. TiO_2 NPs have been applied in various photocatalytic environmental applications such as water purification, air pollution control, CO_2 conversion and corrosion resistance with promising results.

TiO_2 NPs have been extensively and successfully used in the wastewater treatment and water purification (Mehmood *et al*., 2015; Pelaez *et al*., 2012). Similarly the use of TiO_2 NPs for air quality control may be highly effective, relatively inexpensive and safe technique. Photocatalytic oxidation of volatile organic compounds (VOCs) has been achieved by TiO_2 NPs in different indoor air environments (Aghighi & Haghighat, 2015; Tejasvi *et al*., 2015). TiO_2 NPs have also been used for photocatalytic conversion of greenhouse gases such as CO_2 and CH_4 into energy-bearing products such as methanol, formic acid, formaldehyde and acetic acid

(Delavari & Amin, 2015; Merajin, *et al*., 2013; Liu *et al*., 2012). TiO_2 NPs deposited on steel surfaces also prevents corrosion due to the photocatalytic effect (Ćurković et al., 2013; Barati et al., 2014).

2.7.4. Photocatalytic disinfection property of TiO_2 nanoparticles: Various studies have indicated that TiO_2 NPs are very effective in photocatalytic inactivation of *E. coli*, *S. aureus* and *P. aeruginosa* (Alrousan *et al*., 2009; Anandgaonker *et al*., 2015; Wang *et al*., 2013). TiO_2 NPs may be used as an antibacterial agent in water treatment, construction, hospital environment, food packaging and textile industry. ROS generated by TiO_2 play a role in inactivating pathogenic bacteria by oxidizing the polyunsaturated phospholipid component of the cell membrane of bacteria (Xing *et al*., 2012).

Initially, photogenerated ROS first attack on cell wall and proceed into leakage of potassium ions rapidly from cell (Younas, 2011). These ROS then slowly react with outer cell membrane causing its partial decomposition. During this initial period of photocatalytic action of TiO_2 NPs, the release of lipopolysaccharides as one of the major components of outer membrane occurs. Longer times are needed for changes in peptidoglycan layer. These changes in the outer membrane result in increase of its permeability enabling the ROS to reach the cytoplasmic membrane. The attack of cytoplasmic membrane by ROS is followed by peroxidation of membrane lipids, leading to DNA damage and disruption of cell membrane morphology as well as the electron transport chain and eventually to a loss of cell viability and cell death (Radetić, 2013).

2.8. Polymorphs of TiO_2

TiO_2 exists as three different polymorphs which are known as anatase, rutile and brookite. Photocatalysis with anatase is more efficient than rutile or brookite (Mejía et al., 2009). The anatase phase appears to be the most preferred polymorph as it is most photoactive and stable for wide spread practical applications, whereas rutile and brookite are photocatalytically less active (Dhandapani *et al*., 2012).

2.9. Modifications in TiO_2 nanoparticles

In order to act as a photocatalyst, TiO_2 requires energy of 3.2 eV which can only be provided by ultraviolet (UV) light region of the solar spectrum, making the photocatalysis an inconvenient and expensive process. To overcome this problem, modification of TiO_2 NPs may be done through introduction of a metal ion dopant into the matrix of TiO_2 that induces low energy levels within the band gap of TiO_2. Studies have shown the effective results of these modified TiO_2 NPs (Sood *et al*., 2015; Zhang & Zhu, 2012). Various elements can be used as dopants for TiO_2, including metal dopants like iron, silver, manganese and copper or non-metallic dopants such as carbon, boron and nitrogen (Latif, 2013).

2.10. The effect of silver doping on TiO_2 nanoparticles

Doping of silver (Ag) on TiO_2 NPs allow the extension of the light absorption to the visible light. The positive effect of Ag on the photoactivity of TiO_2 is that it may trap the excited electrons from TiO_2 and reduces the recombination of light generated electron and hole at TiO_2 surface. Consequently, the charge separation occurs and hence, recombination of electron–hole pairs is inhibited. Therefore a more effective electron transfer occurs on the surface of the

TiO_2 NPs producing more ROS than in the case of pure TiO_2 (Albiter *et al*., 2015; Khan, 2012).

2.11. TiO_2 nanoparticle coated fabrics

Due to the photocatalytic disinfection property, TiO_2 NPs may be used to impart antibacterial properties to textiles. Other than disinfection, there are multifunctional properties of TiO_2 NPs coated fabrics such as:

2.11.1. UV protection: The ability of TiO_2 NPs to act as an UV blocker is particularly important for textile industry since the requirements for UV protective garments are rapidly growing due to excessive UV irradiation caused by depletion of ozone layer. Textiles with coated TiO_2 NPs provide desirable level of UV protection (Radetić, 2013).

2.11.2. Self-cleaning: It is a major property of TiO_2 NPs coated fabric that involves photodegradation of oils, dirt, odours and aromatic and aliphatic hydrocarbons. Self-cleaning also exploits the generated ROS to decompose undesirable stains (Bozzi *et al*., 2005; Qi *et al*., 2006; Wu *et al*., 2009).

2.11.3. Flame resistance: TiO_2 NPs coated on textiles increase the stability of its structure which enhances the thermal stability of fabrics (El-Shafei *et al*., 2015).

Thus TiO_2 NPs coated textiles have multiple applications, beneficial for the textile industry. In the hospital environment, textiles modified with TiO_2 NPs may reveal disinfection through inhibition in growth of against *E. coli* and MRSA. Medical garments produced from such fabrics may effectively reduce the rate of infections acquired in hospitals (El-Shafei *et al*., 2015; Kowal et al., 2014; Morris, 2011).

2.12. Methods for the development of TiO_2 nanoparticle coated cotton fabric

The technological mechanisms for the development of nano-related textiles are based on the following three processes (Mantovani *et al.*, 2010).

i. Introduction of nanomaterials into raw fibre materials, which can combine the original features of the fibre with nanomaterial functionality.

ii. Coating the surface of fibres or textiles with nanomaterials to produce functional textiles with greater added value.

iii. Electrospinning of polymers for the production of nanometric fibres leading to nonwoven fabrics with improved or new characteristics with multiple applications.

Cotton is a natural cellulosic fibre widely used for about 7000 years (Mejía *et al.*, 2009). It has maintained its unique share in the textile market due to its prominent physical properties such as comfort, softness and high water absorbency. Promoting such intrinsic fatures with nanotechnology has attracted great attention by scientists and manufacturers leading to a new generation of textiles in recent years. So the major research so far on the application of TiO_2 NPs to textile materials has been performed on cotton fabric (Pakdel *et al*., 2014). Different approaches have been reported for the attachment of TiO_2 NPs to cotton fabric.

i. TiO_2 NPs may be coated on cotton fabric through sonochemical method. TiO_2 NPs may be synthesized and loaded onto the cotton simultaneously by hydrolysis of titanium tetra isopropoxide (TTIP) in the presence of distilled water, acetic acid as a dispersant and the cotton fabric under ultrasonic irradiation. The suitability of this method is proved through the reasonable washing durability confirming the covalent bonding between the hydroxyl groups of cotton and TiO_2. Also the employed method does not affect the tensile strength of the fabric (Sadr and Montazer, 2014).

ii. Pad-dry-cure method may also be used for the development of TiO_2 NPs coated cotton fabric. In this process, cotton fabric is treated with TiO_2 NPs in presence of polycarboxylic acid with sodium hypophosphite as catalyst and chitosan phosphate (El-Shafei *et al.*, 2015).

iii. Cotton fabric may also be modified with TiO_2 NPs through an aqueous TiO_2 nanosol, which was obtained via hydrolysis and condensation of tetrabutyl titanate in water (Wu *et al.*, 2009).

iv. The surface pretreatment of the cotton textile with RF-plasma and vacuum-UV may be used to attach TiO_2 directly on the textile by functionalization of the cotton textile with a variable density of functional groups negatively charged (Bozzi *et al.*, 2005; Mejía *et al.*, 2009).

v. Another method to develop TiO_2 NPs coated cotton fabric is by dipping the fabric in the TiO_2 sols from both peptizing and hydrothermal methods (Tan *et al.*, 2013).

vi. TiO_2 NPs may be attached on cotton surfaces using chemical spacers that act as a cross-linking agent between cellulose and TiO_2 NPs.

2.13. Cross-link process

In addition to the various methods described above, cross-link process can also be used to develop TiO_2 NPs coated cotton fabrics. This method implies the use of cross-linking agents attached for fixation of NPs on the cotton fabric (Farouk *et al*., 2013; Karimi *et al*., 2014; Xia *et al*., 2012).

Succinic acid can be used as a cross-linking agent having two free carboxylic groups (Fig. 2.1). 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 the cellulose through the other carboxylic group of the attached succinic acid. This method to prepare TiO_2 NPs coated cotton fabric does not employ toxic compounds or solvents. (Meilert *et al*., 2005). Attachment of TiO_2 NPs on the cotton by cross linking is a simple and durable method that exhibits effective antibacterial properties (Farouk *et al.*, 2013).

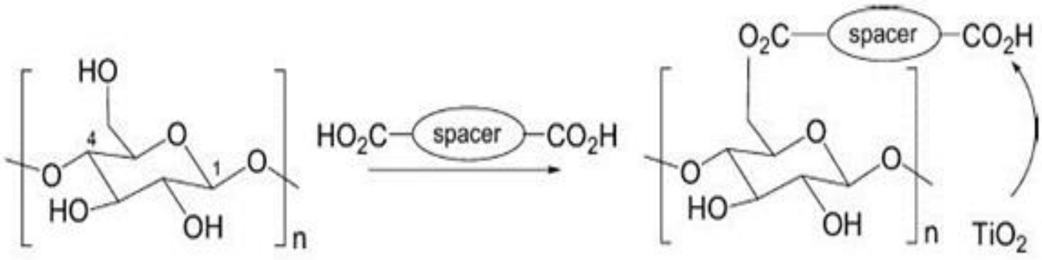


Fig. 2.1 Binding scheme of succinic acid with cotton cellulose and TiO_2.

2.14. Durability of TiO_2 nanoparticle coated fabric

Durability is the most important characteristic to be considered in the use of washable and reusable TiO_2 NPs coated textile. It is relevant to workers and consumer safety from probable hazards associated with NPs exposure along with environmental contamination on possible release of NPs in effluents. It is also significant because antibacterial properties of modified fabric originate from the presence of the TiO_2 NPs on the textile surface. So, testing the durability against washing confirms the suitability of method employed for NPs coatings (Kowal *et al*., 2014; Sadr and Montazer, 2014).

2.15. Related work done at IESE, NUST

A number of studies have been done at the Institute of Environmental Sciences and Engineering (IESE) regarding environmental nanotechnology, especially for the disinfection. These include

i. Water disinfection by metal doped TiO_2 NPs (Younas, 2011).

ii. Decontamination of microbes using metal doped TiO_2 NPs (Khan, 2012).

iii. Development of TiO_2 embedded polymer for self-sanitizing computer keyboards (Habib, 2013).

iv. Development of TiO_2 nanotube coated surfaces for reduction of airborne bacteria (Latif, 2013).

v. Development of TiO_2 embedded polyethylene terephthalate (PET) films for self-sanitizing touch screens (Shahid, 2015).

In the work represented in this thesis, cotton fabric is modified with pure and Ag doped TiO_2 NPs through cross-linking mechanism. Succinic acid is used as a cross linking agent for binding of TiO_2 NPs to the cellulose fiber. Strain of methicillin resistant *Staphylococcus aureus*, a significant nosocomial infection causing bacteria, is used to investigate the disinfection efficacy of modified textile by studying its ability to inactivate (MRSA) under visible and UV light. The effect of doping of NPs in the extension of light absorption spectra is studied through the comparative results of disinfection using pure and Ag doped TiO_2 NPs. Appropriateness of cross-linking method in environmental perspective is confirmed through washings of modified fabric and analysis of this fabric along with washing effluent. Modified cotton fabric may be tested in the hospital environment to investigate its potential for bacterial inactivation. This is done through the application of TiO_2 NPs coated fabric in the form of bed linen, which is one of the significant sources of bacterial contamination in the hospitals.

Chapter 3

MATERIALS AND METHODS

3.1. Reagents and materials

White cotton textile was obtained from the local market. Titanium (IV) dioxide (Sigma-Aldrich Labor chemikalien) was used for the synthesis of pure TiO_2 NPs. 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. Fluorescent lamp and UV lamp were used as light sources to test the photocatalytic activity of modified cotton fabric.

3.2. Synthesis of titania nanoparticles

3.2.1. Pure titania nanoparticles: TiO_2 NPs were prepared through liquid impregnation method (Khan *et al.,* 2013). For preparation, general purpose TiO_2 reagent (50g), after adding to 300 ml distilled water, was stirred on a magnetic plate for 24 hours. The resulting slurry was allowed to settle for another 24 hours and oven-dried at 105°C for 12 hours. Using mortar and pestle, the dried solids were crushed. The powder resulting from this step was calcined at 400°C for 6 hours in a muffle furnace.

3.2.2. Silver doped titania nanoparticles: 1% Ag doped TiO_2 NPs were prepared using $AgNO_3$ (1.05g) with general purpose TiO_2 reagent (48.95g) in 300 ml distilled water placed on a magnetic stirrer for 24 hours. The resulting slurry was then allowed to settle for 24 hours and oven-dried at 105°C for 12 hours. Dried solids were crushed with mortar and pestle. The resulting powder was calcined at 400°C for 6 hours in a muffle furnace.

3.3. Characterization of nanoparticles

3.3.1. X-ray diffraction (XRD) spectroscopy: Crystalline phase and size of synthesized pure and Ag doped TiO_2 NPs were analyzed through the standard technique of XRD spectroscopy. In XRD spectroscopy, X-ray beam strikes the sample, resulting in diffraction of some of the X-rays at different angles. X-rays diffracting from a specific plane at the same angle will reinforce each other giving high peaks indicating the crystallinity of the sample. In the present study, X-ray diffraction patterns were recorded on JEOL JDX-II X-ray diffractometer with Cu-Kα radiation (λ=1.54060 nm; voltage=20 kV; current=5 mA) and range of diffraction angles (2θ) from 20° to 80°.

3.3.2. Scanning electron microscopy (SEM): The morphology of pure and Ag doped TiO_2 NPs was examined through SEM. In this instrumental technique, an electron beam strikes the sample and signals are generated. These signals are collected by electron collector and image of the illuminated sample is formed by magnetic lenses. SEM has a wide range of resolution ranging from 10X to 300,000X and is particularly suitable for examination of nanomaterials. Using JEOL JSM-6460 SEM, the morphology of NPs was determined at an acceleration voltage of 20kV.

3.3.3. Energy dispersive spectroscopy (EDS): For the elemental analysis of pure and Ag doped TiO_2 NPs, EDS system embedded with SEM was used. When an electron beam strikes the sample different elements present in it produce characteristic X-rays having different energies. The elements are identified by collecting and analyzing these characteristic X-rays. In the present study, elemental analysis was done by using EDS Oxford INCA X-sight 200.

3.4. Synthesis of titania nanoparticle coated cotton textile

Cotton fabric was modified with TiO_2 NPs through the cross-linking method which involves three steps represented in Fig. 3.1 (Meilert *et al.*, 2005).

3.4.1. Pre-treatment: To remove stains from the cellulose fibres, the cotton fabric was initially pre-treated. For this purpose, the fabric sample was bleached with hydrogen peroxide (H_2O_2), treated with sodium hydroxide (NaOH) and washed with water. An ammonia treatment was then applied with the excess chemical being removed from the sample fabric through drying in an oven at 100°C.

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

3.4.3. Loading of TiO_2 NPs on cotton fabric: Lastly, in order to load TiO_2 NPs on sample fabric, a 5 g/L TiO_2 NPs suspension was prepared and sonicated for 30 min. Immersed into this aqueous suspension of TiO_2 NPs, fabric sample was heated at 75°C for 30 min with continuous stirring on a 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 TiO_2 NPs were washed out from the modified cotton fabric in distilled water through sonication for 5 min.

1. Pretreatment

1. Bleached with Hydrogen Peroxide

2. Treated with Sodium Hydroxide

3. Washed with water

4. Sprayed Ammonia

5. Dried in oven at 100°C

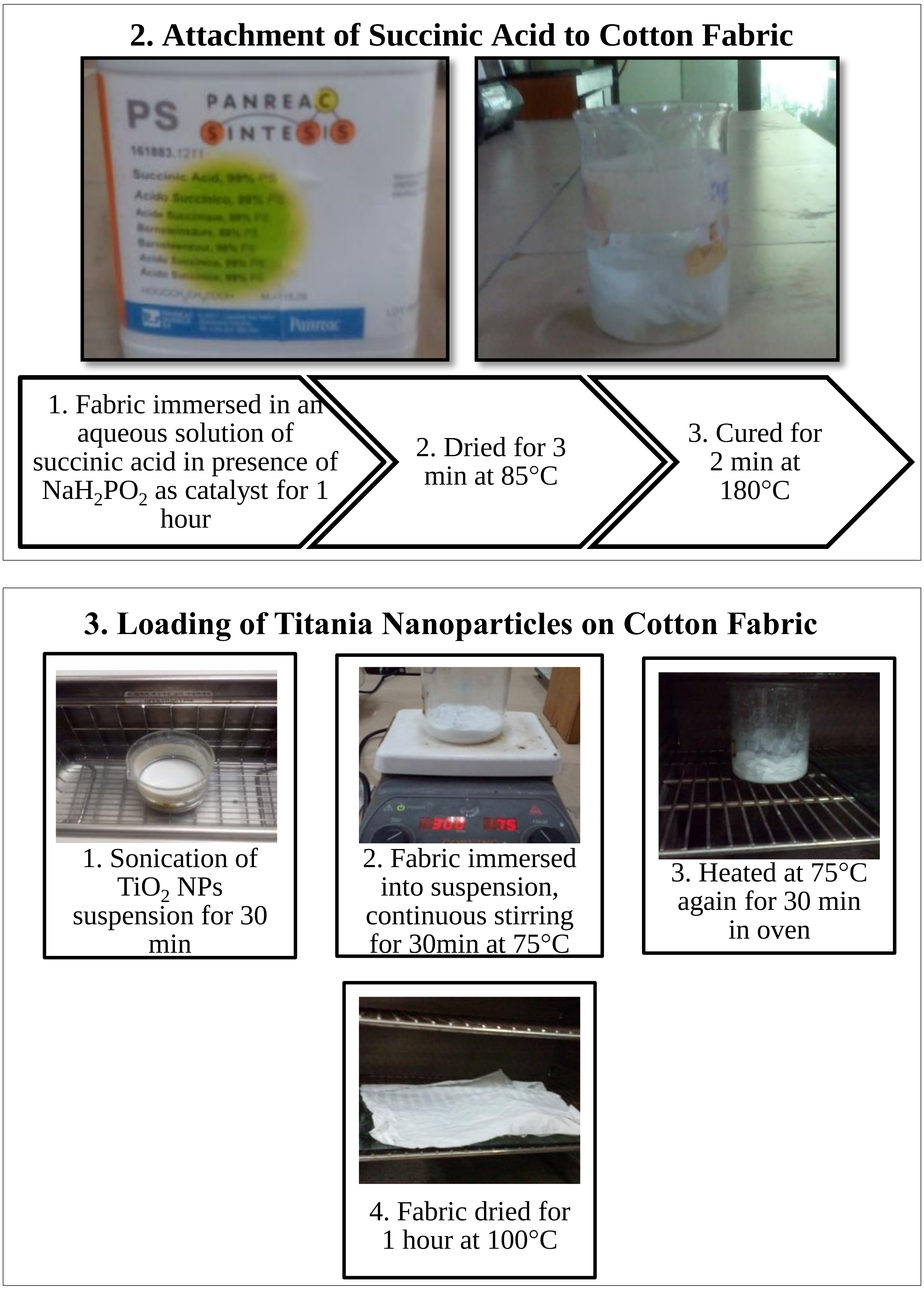


Fig. 3.1: Steps involved in the synthesis of titania nanoparticle coated cotton textile.

3.5. Characterization of titania nanoparticle coated cotton textile

3.5.1. Scanning electron microscopy (SEM): The topography of titania nanoparticle coated cotton textile was studied through SEM. This analysis was done to confirm the adhesion of TiO_2 NPs to cotton textile. Before scanning, the cotton fabric samples were cut to 0.25 inches^2 pieces and sputtered with a layer of gold sputter which increased the focusing potential.

3.5.2. Energy dispersive X-ray fluorescence (ED-XRF) spectrometry: ED-XRF spectrometry was used to analyze the TiO_2 content on modified cotton fabric. In this technique, the sample is irradiated by an intense x-ray beam, which causes the emission of characteristic fluorescent x-rays. The elements are identified by collecting and analyzing these characteristic X-rays. ED-XRF element analyzer JOEL JSX 3202 M was used in this study for the determination of Ti on titania nanoparticle coated textile representing the TiO_2 content.

3.6. Durability testing

The durability of titania coated textile was tested through a modified version of standard method of AATCC Test Method 61-2013 (American Association of Textile Chemists and Colorists, 2013). In this method, 45 minutes 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 (Kowal *et al.*, 2014;

Sadr & Montazer, 2014). The effect of detergent was also analyzed by washing the fabric with and without the addition of detergent.

Coated samples with size 10cm × 10cm 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 100 rpm. Wash water was stirred and the required temperature was maintained using a magnetic hot plate. Washing was done for 45 minutes (equal to five home launderings) and 135 minutes (equal to fifteen home launderings). After washing, each fabric piece 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 and ED-XRF spectrometer to determine their TiO_2 content after washing process.

The concentration of TiO_2 in the washing effluent was analysed through catalytic spectroscopy using UV–Vis spectrophotometer (T-60U) (Mousavi & Pourreza, 2008). In this method, concentration of the Ti was determined through its catalytic effect on methylene blue-ascorbic acid redox complex. 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 the 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 minutes, a portion of the 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.

3.7. Antibacterial testing

3.7.1. Bacterial culture preparation: Antibacterial test was performed on modified cotton fabric using a culture of methicillin resistant *S. aureus* (MRSA). MRSA strain was selected as was common in other nosocomial infection studies. Liquid culture of MRSA was grown aerobically in Luria-Bertani (LB) broth at 37°C for 16 hours. The cell suspensions used for antibacterial testing were in the range of 1×10^9 to 2.5×10^9 colony forming units (CFU)/ml.

3.7.2. Nutrient agar plates preparation: Nutrient agar (14g) was mixed in 1 litre warm distilled water containing flask with slow mixing by a glass rod. When nutrient agar was completely dissolved, it was sterilized in an autoclave at 121^{o}C for 15 minutes. After sterilization, the prepared molten agar was poured into autoclaved petri plates under sterile laminar flow hood and allowed to cool down. Prepared nutrient agar plates, after solidification, were transferred into incubator for 24 hours at 37^{o}C to check their sterility.

3.7.3. Saline blank preparation: Test tubes were washed thoroughly first with water containing detergent and then with distilled water. These test tubes were filled with 9 ml saline solution (0.85% NaCl) and then autoclaved at 121^{o}C for 15 minutes for sterilization.

3.7.4. Experimentation: Fabric samples were sterilized in an autoclave before testing. Inside a sterile laminar flow hood, fabric samples were transferred to test tubes containing 10 ml of freshly prepared culture as depicted in Fig. 3.2. These test tubes were subjected to UV light for 4 hours. Samples were collected at 0, 2 and 4 hours interval. Serial dilutions of each bacterial sample were made in saline solution in order to obtain a countable range (25-250 CFU/ml). Countable range was achieved after seven dilutions. During each dilution, the bacterial suspension was mixed well with the help of the vortex mixer in order to achieve a uniform suspension. Bacterial load was analyzed in each sample through viable count on nutrient agar media after serial dilutions of the sample. After incubation for 24–48 hours at 37°C, CFU were counted with the help of a colony counter 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 hours.

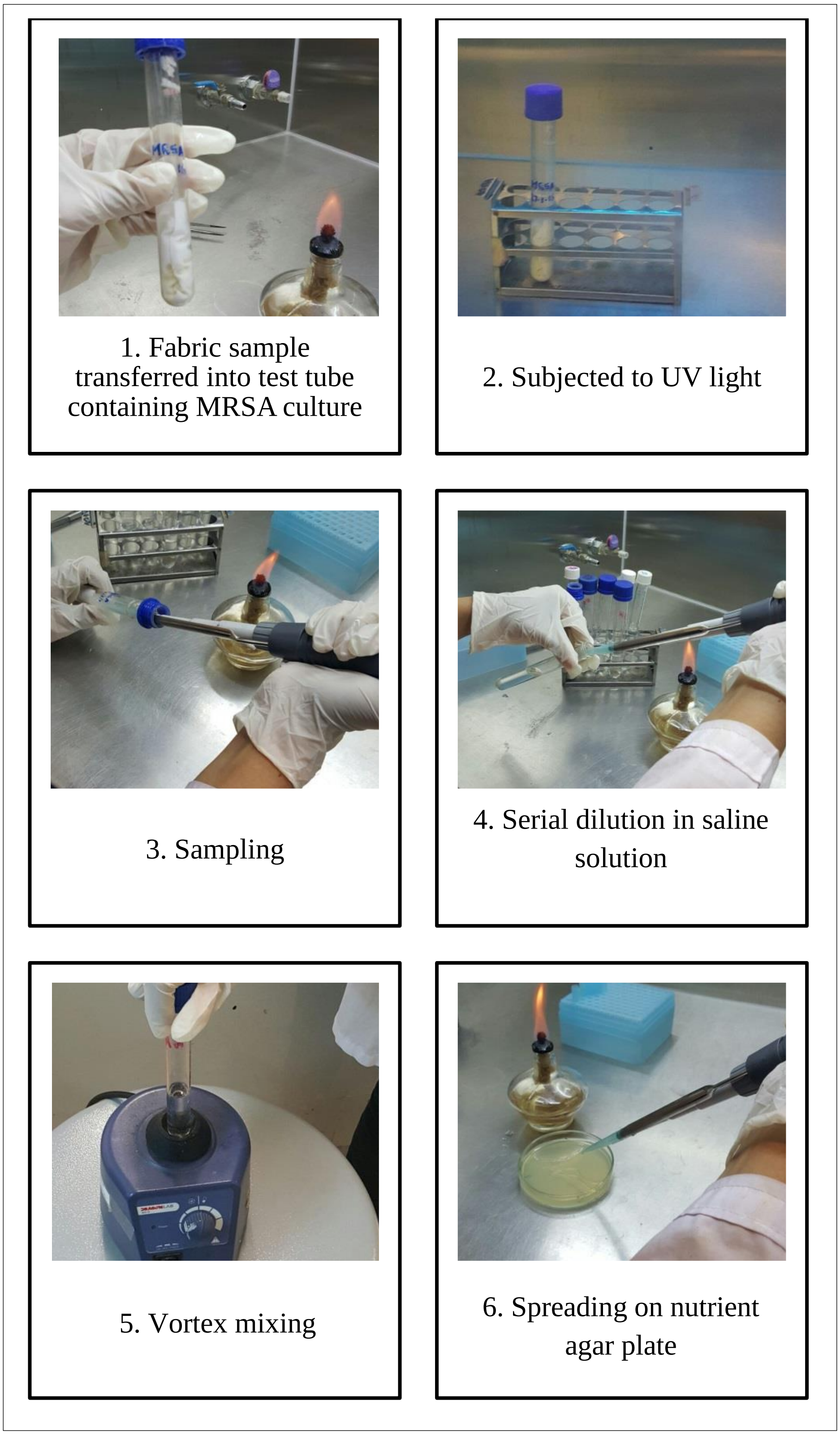


Fig. 3.2: Pictorial explanation of antibacterial testing.

3.8. 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 an hospital environment. For this purpose white bed linens were coated with TiO_2 NPs through the mechanism explained in 3.4, above. Each bed linen was divided into three sections including uncoated (control), pure titania nanoparticle coated and Ag doped titania nanoparticle coated as shown in Fig. 3.3.

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 3.1. These patients were in a state of low consciousness and completely reliant on medical personnel for all necessities. Modified autoclaved bed linens were placed on the beds of these patients consecutively for a period of 3 days (Fig. 3.4).

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 $10cm^2$) of every section. These samples were collected using sterile cotton-tipped swabs, pre-moistened with sterile saline solution. The viable count was executed on prepared nutrient agar plates after directly spreading the sample through rubbing the cotton swabs on agar plates and also after the serial dilutions (upto the dilution factor of 10^{-2}) in saline solution. The entire procedure was performed in a laminar flow hood. Colonies were counted after 24 hours of incubation at 37°C to compare the level of bacterial contamination among different sections of bed linens.

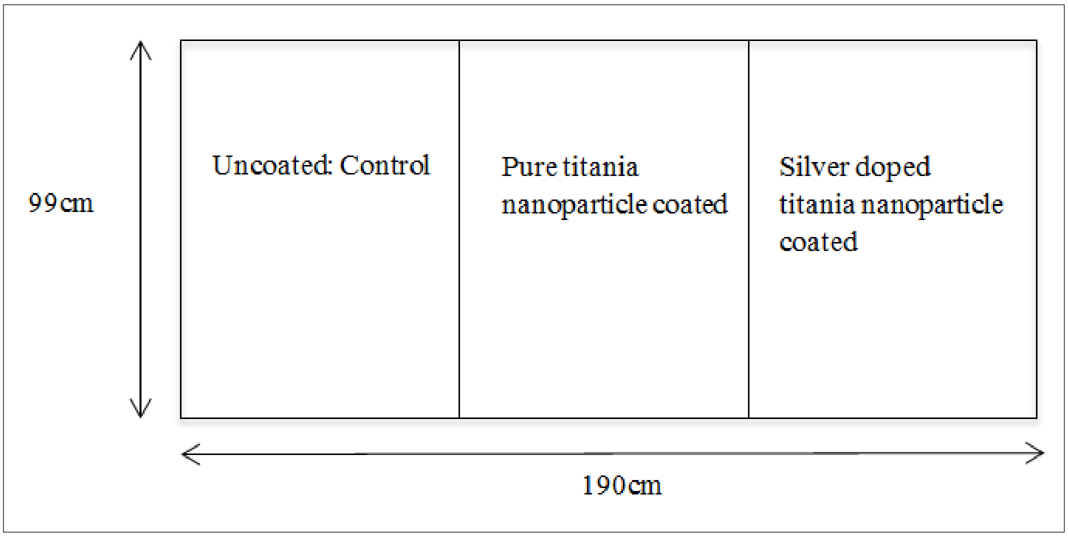


Fig. 3.3: Coating pattern and dimensions of bed linen.

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

Table 3.1: Clinical characteristic of patients.

Fig. 3.4: Placement of bed linen in MICU of a local hospital.

Chapter 4

RESULTS AND DISCUSSION

4.1. Characterization of titania nanoparticles

4.1.1. XRD patterns: X-ray diffraction spectroscopy was carried out using Cu-Kα radiations at an angle 2θ ranging from 20° to 80°.

In case of pure TiO_2 NPs, Fig. 4.1 shows peaks at 25°, 37°, 38°, 48°, 54°, 55°, 63°, 68°, 71°, 76° and 77°. These peaks characterize that TiO_2 NPs were in the anatase phase confirming the crystalline nature of NPs. This crystallinity is due to heat treatment of TiO_2 during calcination at 400°C for 6 hours. The average sizes of pure TiO_2 NPs determined through this technique was 43.1 nm.

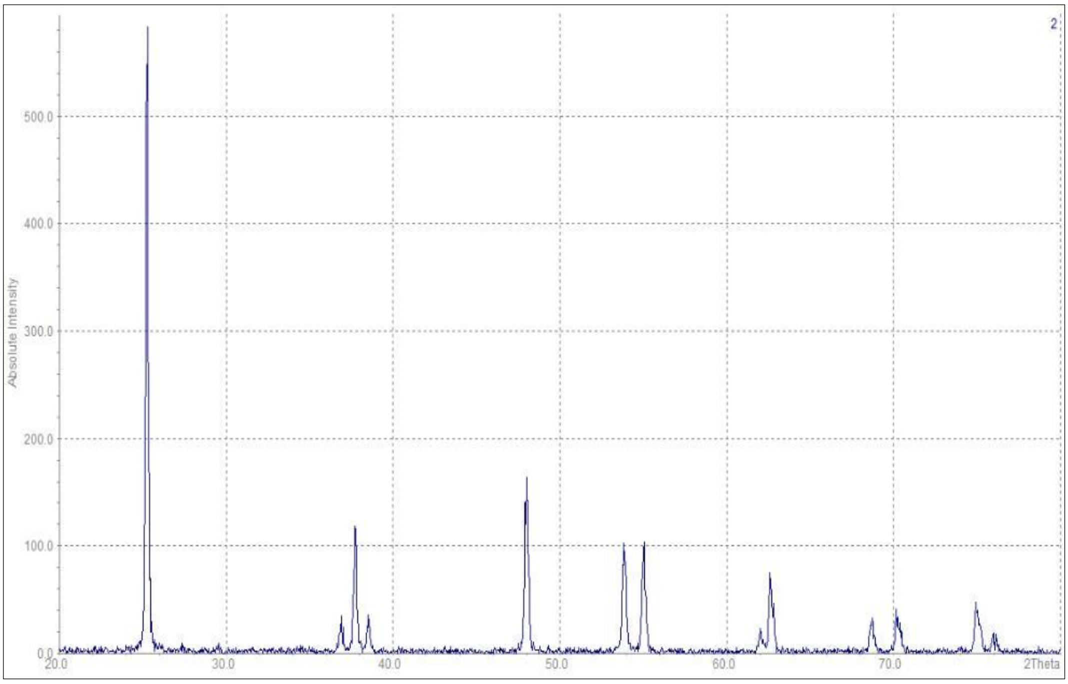


Fig. 4.1: XRD intensity plot for pure TiO_2 NPs.

In case of 1% Ag doped TiO_2 NPs, peaks may be seen at 25°, 28°, 36°, 37°, 43°, 48°, 54°, 55°, 61°, 63°, 68° and 75° in Fig. 4.2. Similar to pure TiO_2 NPs, these peaks confirm that Ag doped TiO_2 NPs were highly crystalline in nature, due to the calcination at 400°C for 6 hours. The average sizes of Ag doped TiO_2 NPs calculated through this technique was 54.6 nm.

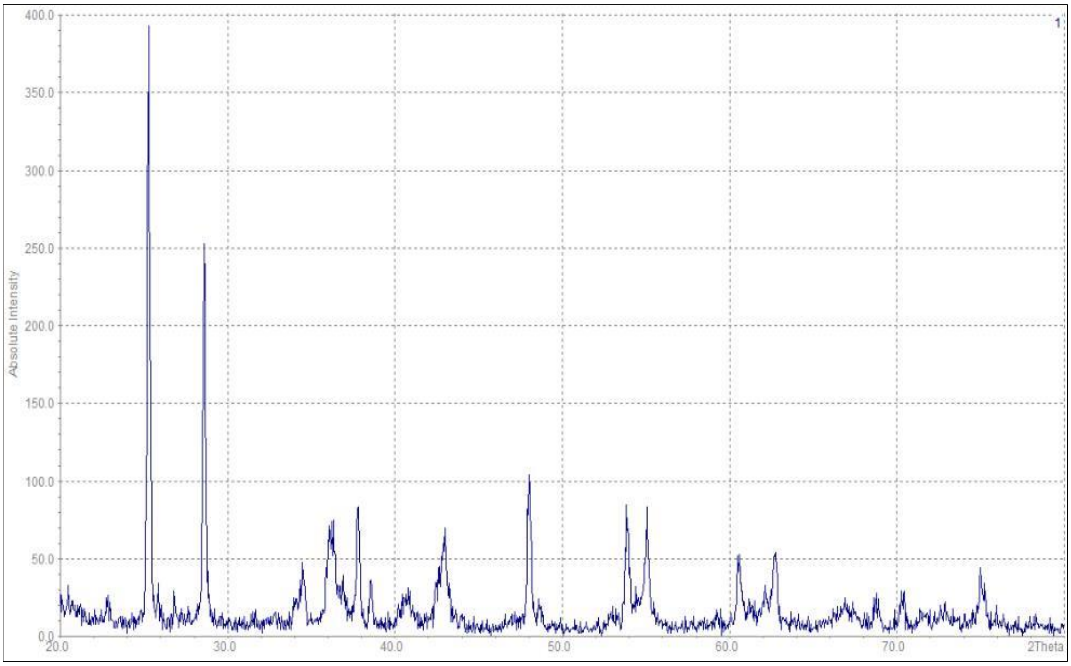


Fig. 4.2: XRD intensity plot for 1% Ag doped TiO_2 NPs.

TiO_2 NPs in anatase phase depicts better photocatalytic results than in other phases (rutile and brookite) because of its optimal performance under UV irradiation (Leong *et al.*, 2014). The anatase phase is also preferred due to its higher potential to produce ROS (Joost *et al.*, 2015). It has also been observed that TiO_2 in the crystalline form is a strong antibacterial agent when exposed to UV light (Marciano *et al.*, 2009).

4.1.2. SEM observations: SEM was used for observing the morphology of NPs. Fig. 4.3 and 4.4 show the SEM micrographs of pure and 1% Ag doped TiO_2 NPs respectively at 5000 magnification. Most of these pure and 1% Ag doped TiO_2 NPs were spherical in shape and occur in the form of micro-sized aggregates. This aggregation is advantageous for their removal from the aqueous environment after treatment. Such structure also indicates the high surface area which has been proven to be efficient for photocatalytic activity (Khan *et al.*, 2013).

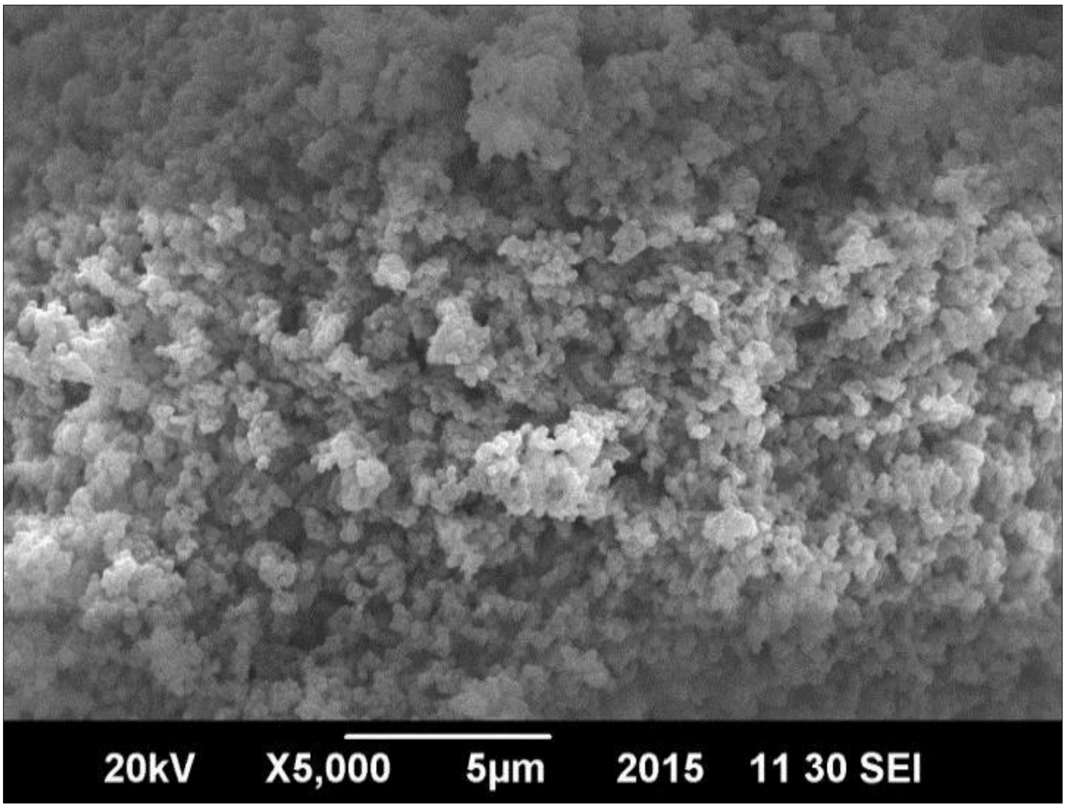


Fig. 4.3: SEM micrograph for pure TiO_2 NPs at ×5000.

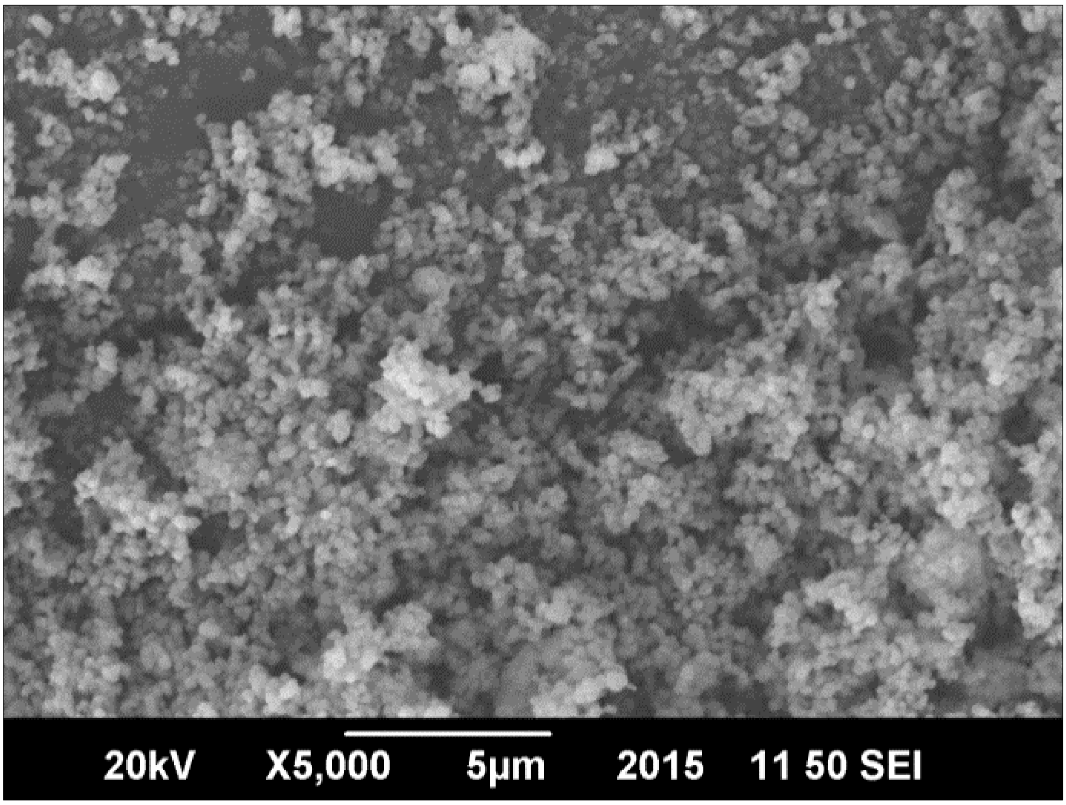


Fig. 4.4: SEM micrograph for 1% Ag doped TiO_2 NPs at ×5000.

4.1.3. EDS analysis: Fig. 4.5 and 4.6 depict the EDS patterns of pure and 1% Ag doped TiO_2 NPs. Pure TiO_2 NPs were composed of ~54% Ti and ~46% O (Table 4.1) whereas 1% Ag doped TiO_2 NPs comprised of ~59% Ti, ~40% O and ~1% Ag (Table 4.2). EDS results confirmed that 1% Ag doping of NPs has been successfully achieved. No impurities were detected in the prepared NPs.

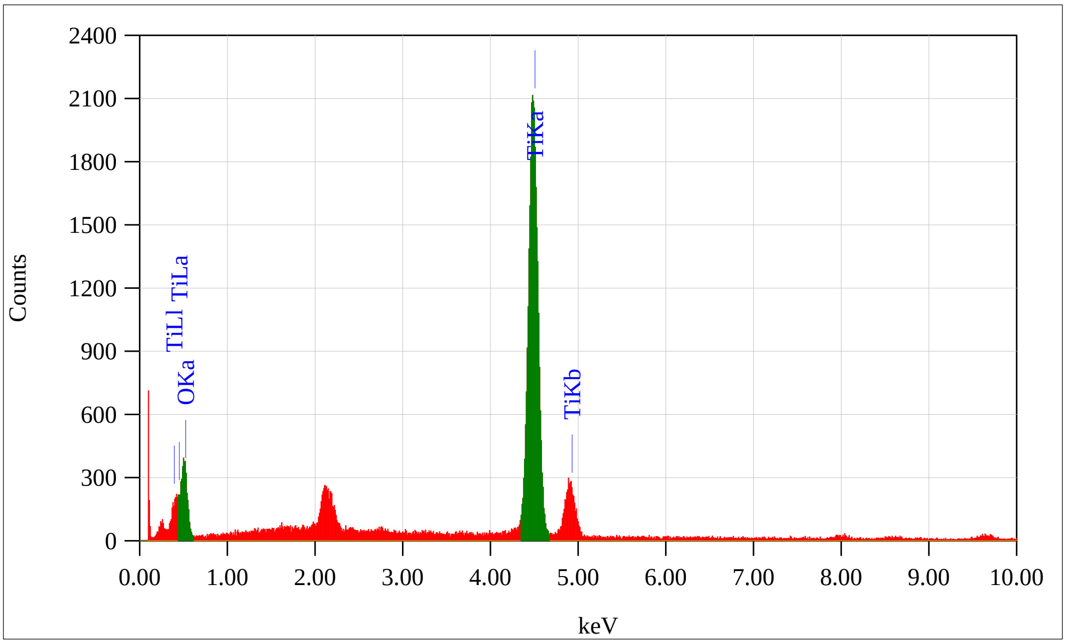


Fig. 4.5: EDS patterns for elemental compositions of pure TiO_2 NPs.

Element	Mass %
Ti	54.33
O	45.67

Table 4.1: Mass % of elements in pure TiO_2 NPs.

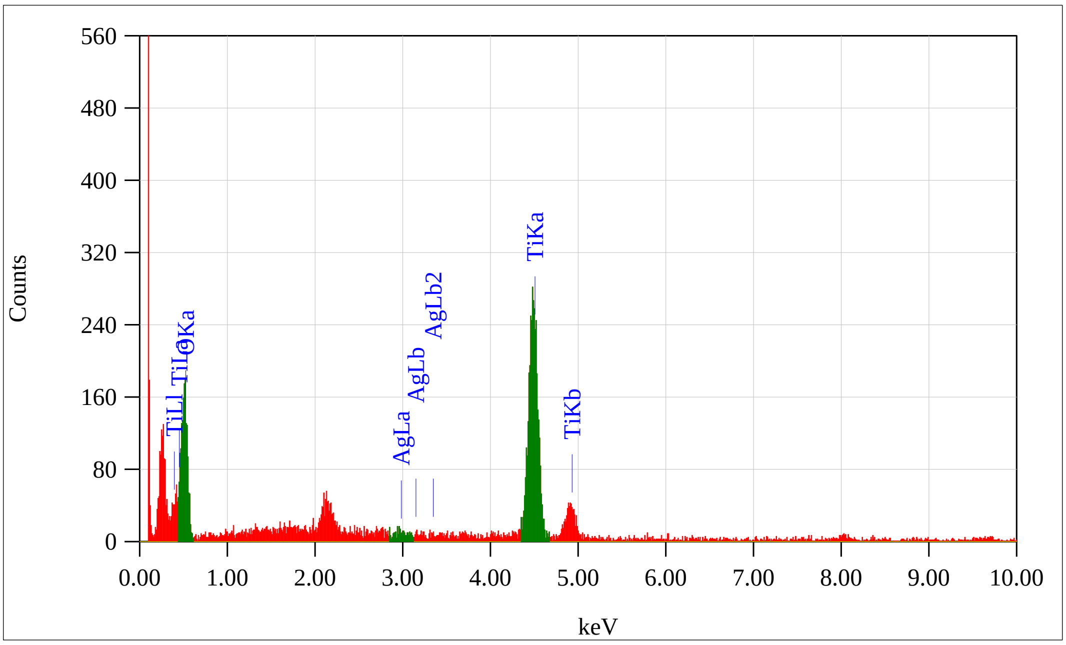


Fig. 4.6: EDS patterns for elemental compositions of 1% Ag doped TiO_2 NPs.

Element	Mass %
Ti	59.10
O	39.58
Ag	1.33

Table 4.2: Mass % of elements in Ag doped TiO_2 NPs.

4.2. Characterization of coated cotton textile

4.2.1. SEM observations: Comparison between the SEM micrographs of pure cotton fabric (blank) and pure and Ag doped titania nanoparticle coated cotton fabric helps to investigate the topography of modified textile. The SEM micrograph in Fig. 4.7 represents a clean and smooth surface on pure cotton fabric (blank). Topography of pure and Ag doped titania nanoparticle coated cotton fabric is represented in Fig. 4.8 and 4.9 respectively. It is clearly visible that NPs were successfully deposited on cotton fabric through the mechanism of cross-linking. The high surface coverage of NPs on modified cotton fabric provides suitable surface for photocatalytic activity. Nevertheless this coverage is non-homogenous and inconsistent, somehow due to roughness and non-homogenous surface of cotton fabric, also observed and reported in previous studies (Kowal *et al*., 2014; Meilert *et al*., 2005; Sadr & Montazer, 2014).

4.2.2. ED-XRF analysis: For the detection of TiO_2 on modified cotton fabric, ED-XRF spectrometry was applied. Elemental analysis of modified cotton fabric through ED-XRF analysis shows Ti content confirming the presence of TiO_2 on modified fabric sample (Fig. 4.10); whereas no Ti was detected in blank sample.

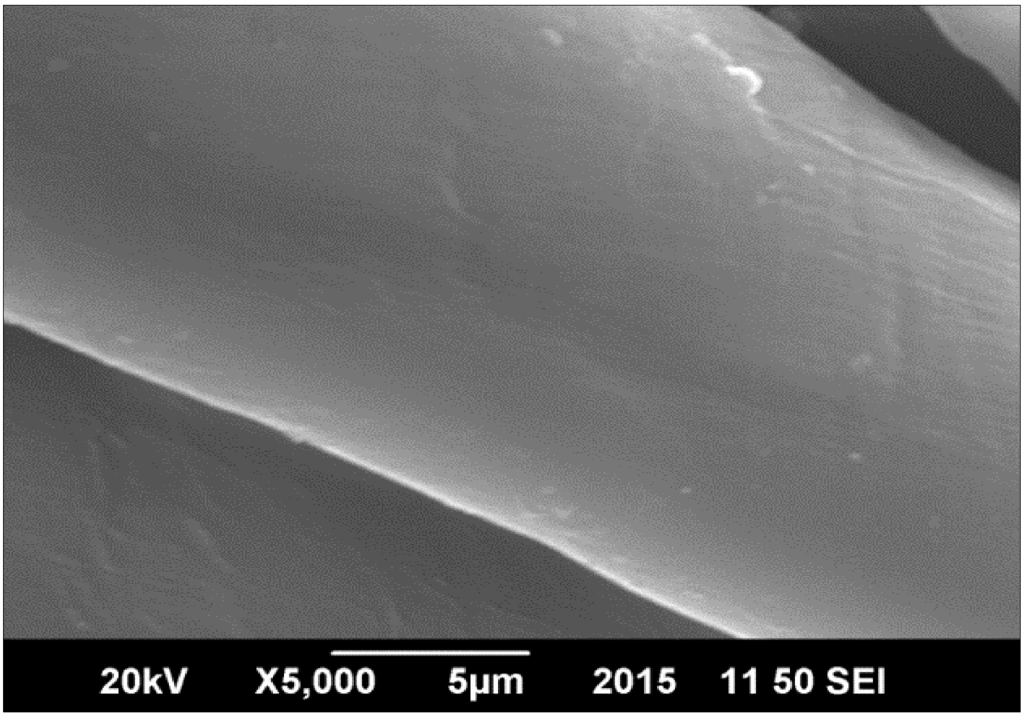


Fig. 4.7: SEM micrograph for pure cotton fabric (blank) at ×5000.

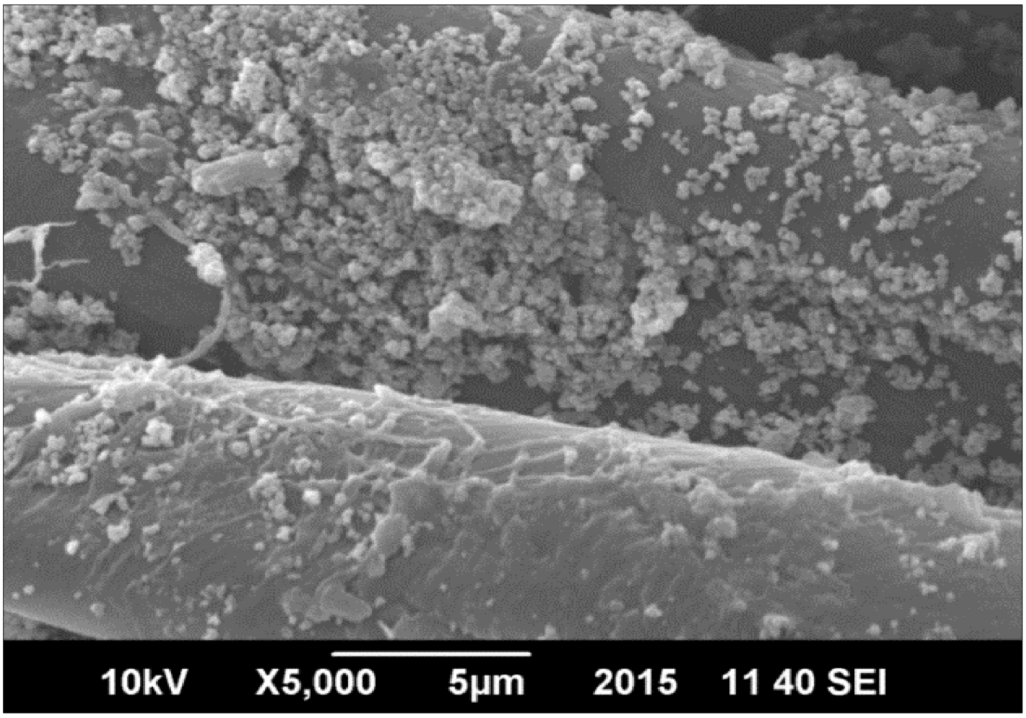


Fig. 4.8: SEM micrograph for pure titania nanoparticle coated cotton fabric at ×5000.

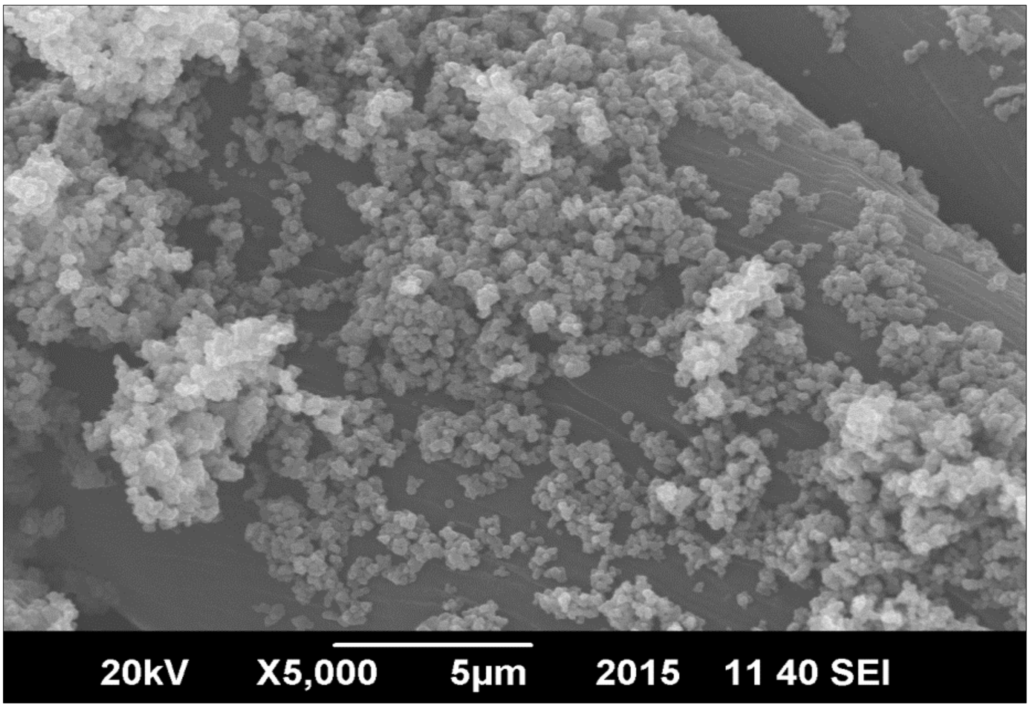


Fig. 4.9: SEM micrograph for Ag doped titania nanoparticle coated cotton fabric at ×5000.

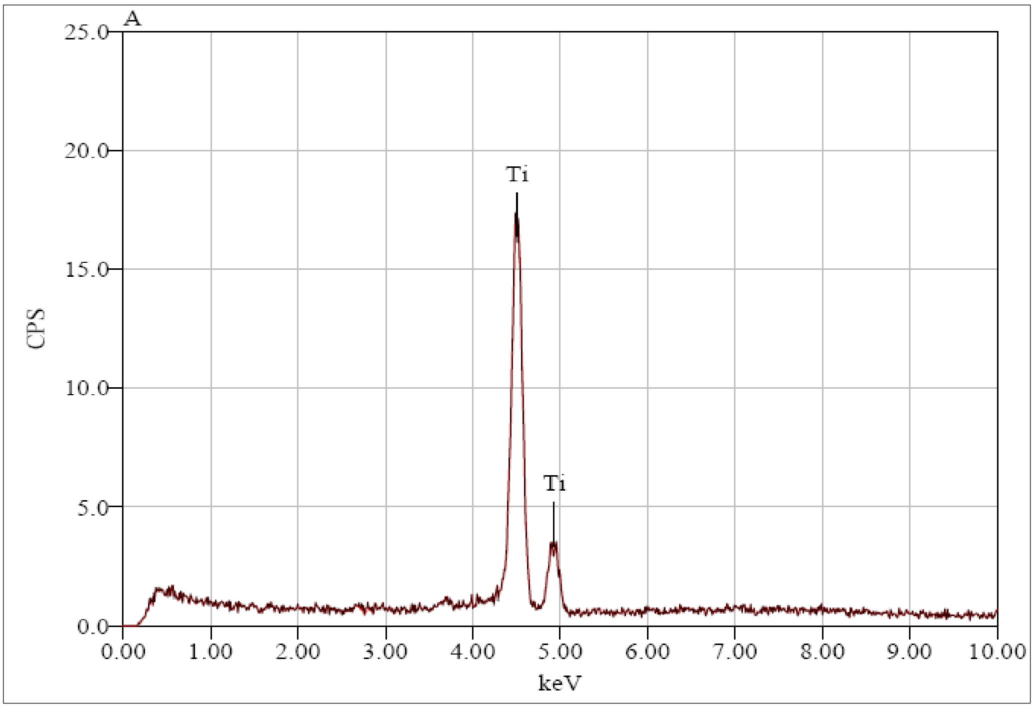


Fig. 4.10: XRF spectra for titania nanoparticle coated cotton fabric.

4.3. Washing durability

4.3.1. Fabric surface characterization: Through durability testing, the effect of washing the modified fabric on TiO_2 loadings was studied. The experiment confirmed the durability of titania nanoparticle coated fabric against washing at different conditions. The distribution of NPs 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. 4.11). Micrographs of these washed fabric samples were similar to those of unwashed fabric sample represented in Fig. 4.8. 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 observed in samples washed with and without detergent.

These washed fabric samples were also characterized through ED-XRF analysis. Fig. 4.10 represents the ED-XRF graph for control fabric (coated and unwashed) with Ti peak at 17 counts per second (CPS). Table 4.3 shows that there is an insignificant decrease of CPS of Ti representing a negligible decrease in TiO_2 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 TiO_2 loadings on textiles were retained to a considerable degree even after washing at 30°C. Similar to the SEM micrographs, only a minor difference may 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.

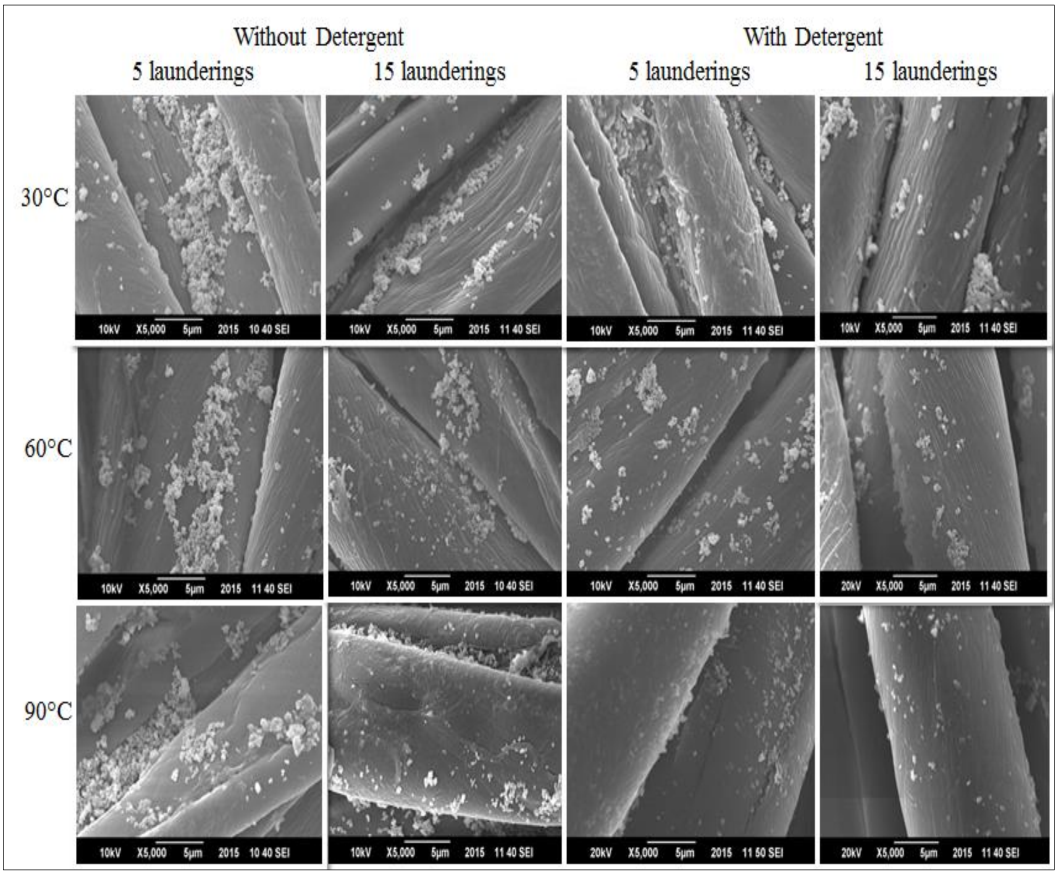


Fig. 4.11: SEM micrographs of titania nanoparticle coated cotton fabric after washing at 30, 60 and 90°C; with and without detergent and after 5 and 15 launderings at ×5000.

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

Table 4.3: 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.

The results of SEM and XRF analysis confirm that NPs were strongly adherent to the surface of cotton fabric through a cross-linkage of succinic acid. Loosening of certain NPs during washing is due to multiple factors. During the washing process, the motion of fabric occurs in the form of tumbling, rubbing, agitation, abrasion and dragging which induces mechanical forces on loosely bonded NPs leading to their removal from the fabric (Kowal *et al*., 2014). The increase in temperature also increase the kinetic energy of these loosely bonded NPs that get detached from the fabric which might be the reason for slight decrease in TiO_2 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 suitable laundering effect. Absorbed surfactant molecules in the detergents enhance the removal of NPs from textile surface by weakening of the electrostatic forces between TiO_2 NPs and succinic acid bonded with the cotton textile (Free, 2016). Adherence of NPs to the fabric, even after numerous washing cycles, is probably because only loosely bonded NPs were removed from the fabric in primary washing process whereas strongly bonded NPs remained attached even after subsequent washings.

4.3.2. Washing effluent analysis: Analysis of the 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 TiO_2. 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. 4.12).

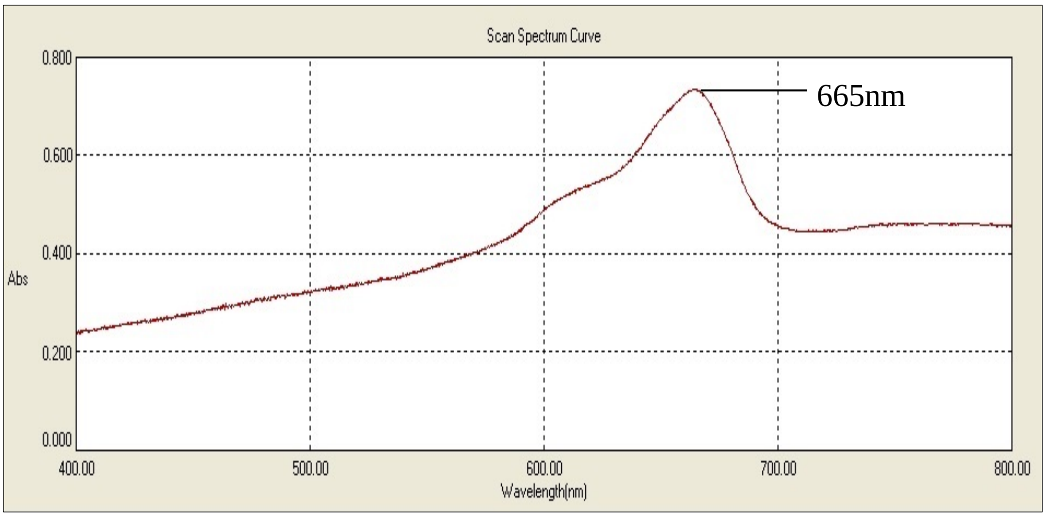


Fig.4.12: Absorption spectrum for 120 ppm standard Ti solution.

The calibration curve obtained for Ti spectroscopy was linear representing a straight line with the following equation for the line (Fig. 4.13).

$$y = 0.006x + 0.0167$$

Here,

x = Concentration

y = Absorbance

Using this equation, the concentration of Ti in washing effluents was determined (Table 4.4). Average of three replicates for each different condition was noted.

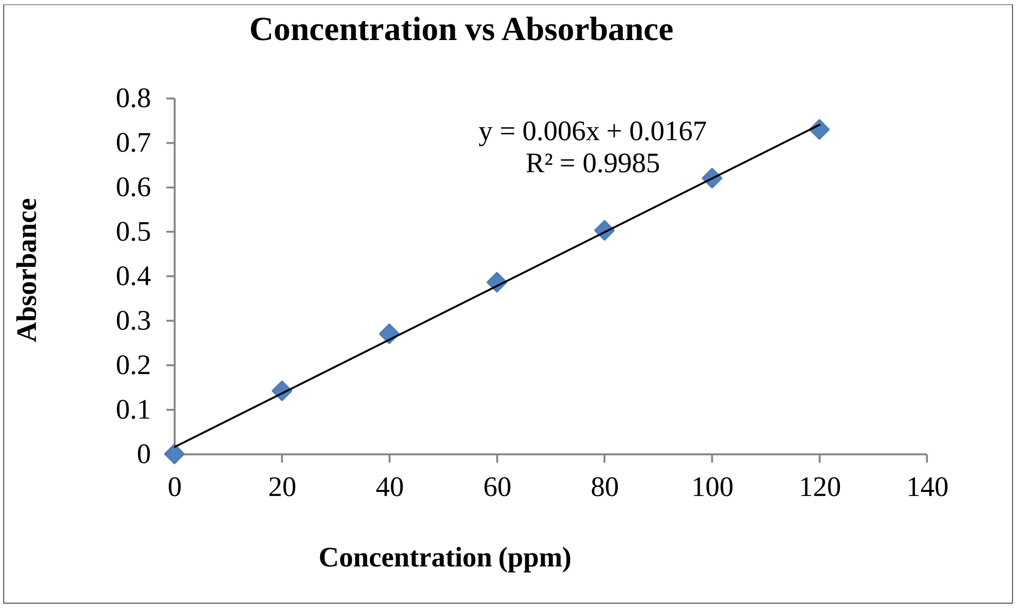


Fig. 4.13: Calibration curve for titanium spectrophotometry.

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

Table 4.4: Concentrations of Ti in washing effluents.

High concentrations of Ti in the washing effluents represent poor durability. Based on the results shown in Table 4.4, it is evident that there were very low concentrations of Ti in wash water showing high durability confirming the results of SEM and XRF analysis. The concentrations show negligible variations after different number of launderings as concluded previously (Kowal *et al.*, 2014; Sadr & Montazer, 2014). This is because unstable TiO_2 NPs which were physically bonded to the cotton fiber (not chemically attached through succinic acid), were released into the wash water through primary washing cycles. During the subsequent washings, the absence of Ti in the washing effluents indicates that the loosely bonded TiO_2 NPs were already detached from the fabric in the 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 TiO_2 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 (Ćurković *et al.*, 2013; Petit *et al.*, 1981). In comparison, the highest TiO_2 concentration was obtained in effluent after washing at 90°C as previously reported (Kowal *et al.*, 2014). Therefore, in terms of environmental safety, low temperatures and lower amount of detergents are suitable to be applied for washing the TiO_2 coated fabric. In terms of temperature, maximum durability of TiO_2 on textiles was examined after washing at 30°C. Nevertheless, washing of TiO_2 coated fabric does not significantly affect the coating of TiO_2 NPs. 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 TiO_2 NPs through succinic acid (Meilert *et al*., 2005). Hence the development of titania nanoparticle coated fabric, through cross linking phenomena, is an effective and reliable method.

4.4. Antibacterial testing

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

4.4.1. Disinfection under UV radiations: The percentage reduction of MRSA with the sample fabric on exposure to UV radiations is shown in Fig. 4.14. 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 hours and 100% reduction (cell count decreased from 2.1×10^9 to Too Few to Count (TFTC) CFU/ml) after 4 hours of UV light exposure through pure titania nanoparticle coated fabric. Such modified fabric also retained the antibacterial properties even after the 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 hours respectively with the 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 hours and 4 hours 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 finest results with 100% inactivation (cell count decreased from 1.3×10^9 to TFTC CFU/ml) of MRSA within 2 hours. 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 hours of UV light exposure.

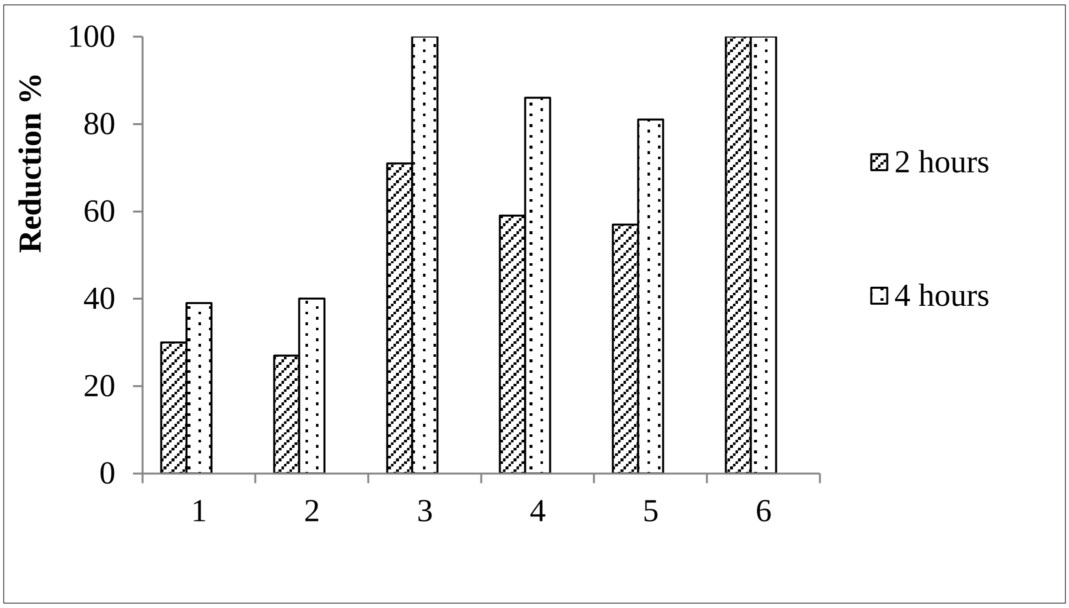


Fig. 4.14: Antibacterial activity results in UV light.

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.

4.4.2. Disinfection under fluorescent light: Fig. 4.15 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 hours and almost complete reduction (cell count decreased to TFTC CFU/ml) after 24 hours 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 hours 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 hours and 24 hours respectively. Similar to the results of UV experiment, silver doped titania nanoparticle coated fabric showed complete sterilization in just 12 hours (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 hours under fluorescent light.

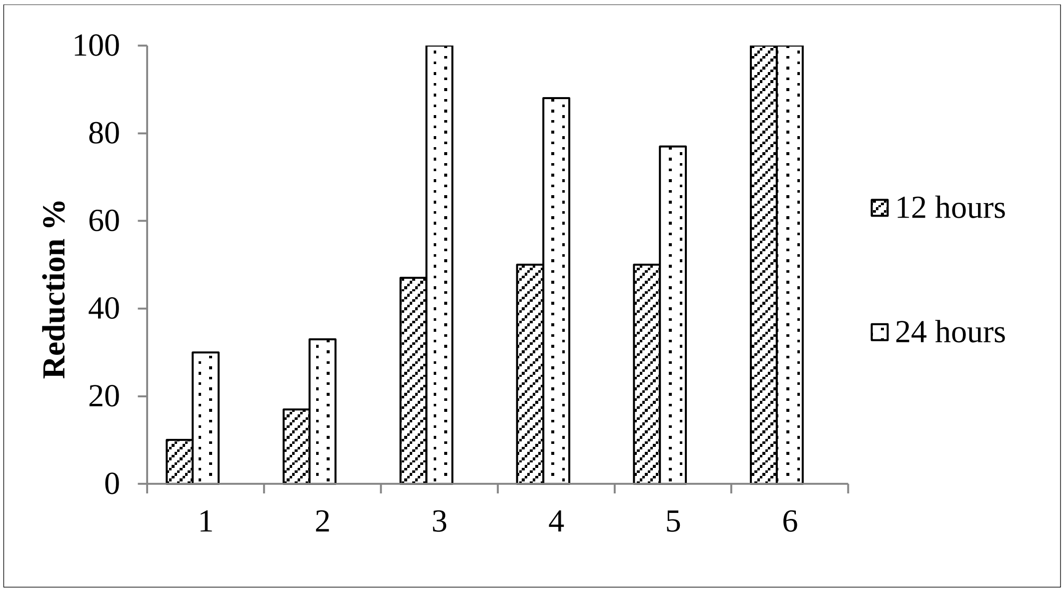


Fig. 4.15: Antibacterial activity results in fluorescent light, 1- control-1 pure cotton fabric, 2- control-2 pretreated & succinic acid attached cotton fabric, 3- titania coated, 4- titania coated washed without detergent, 5- titania coated washed with detergent, 6- silver doped titania coated.

4.4.3. Disinfection under dark conditions: Similar experiment was also performed under dark conditions for 24 hours. Results (Fig. 4.16) showed that limited bacterial inactivation occurred in the dark conditions. After 24 hours 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.

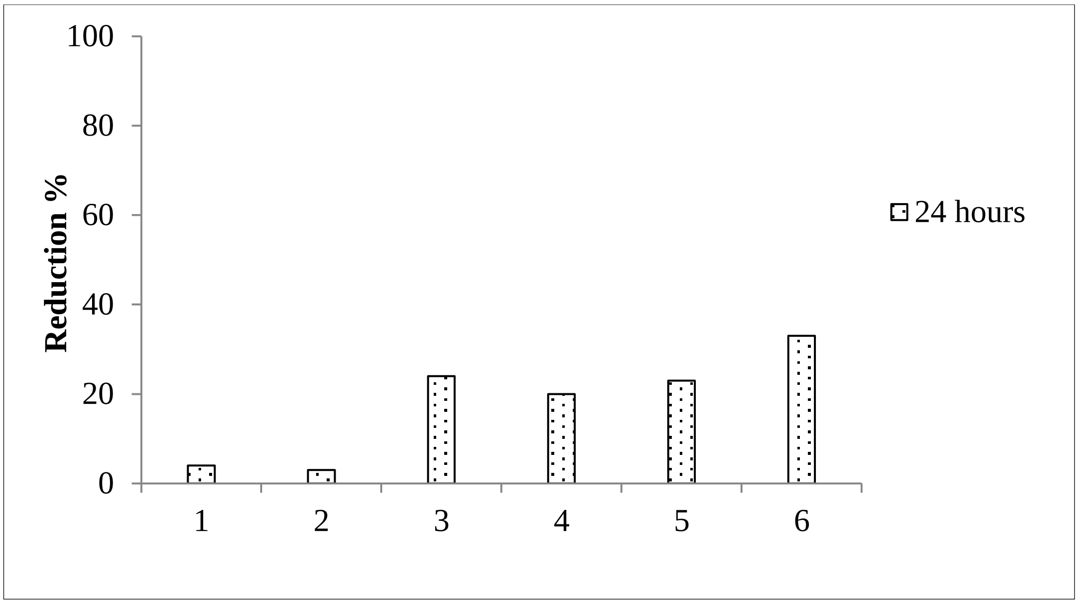


Fig. 4.16: Antibacterial activity results in dark conditions, 1- control-1 pure cotton fabric, 2- control-2 pretreated & succinic acid attached cotton fabric, 3- titania coated, 4- titania coated washed without detergent, 5- titania coated washed with detergent, 6- silver doped titania coated.

The cell wall of MRSA has a complex structure that requires a large amount of energy to break. The ROS provided by TiO_2 NPs are involved in damaging the cell wall as well as the catalytic properties, optical properties, and electrical conductivity of the bacterial cells. Though TiO_2 NPs 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 TiO_2 when exposed to UV light resulting in the 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. Photo radiations 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 (Mathur *et al.*, 2015). The disinfection also occurred to some extent in samples placed in dark conditions. Pre-activation of TiO_2 NPs by exposure to light before exposure to dark is the likely cause of MRSA reduction in dark conditions (Tsuang *et al*., 2008). Also this shows that textiles have an inherent antibacterial activity which is enhanced through NPs and light source (Kowal *et al*., 2014). Similarly complete disinfection occurs with Ag doped TiO_2 NPs both in UV and fluorescent light because doping of Ag on TiO_2 NPs allows the extension of light absorption to the visible light. Also when light strikes the doped NPs, due to metal doping, it produces electron–hole pairs easily to form more ROS involved in bacterial destruction (Khan *et al*., 2013; Latif *et al*., 2014). The antibacterial effect was decreased after washing due to NPs 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.

4.5. Field testing

Although the results of the antibacterial properties of titania nanoparticle coated fabric had been very encouraging, whether these will function in the same way, in the actual hospital environment, remained unclear. For this purpose an experiment was conducted in MICU of a hospital to investigate whether the use of such antibacterial textiles in the form of bed linens may reduce the bacterial contamination. The results of this experiment are shown in the Fig. 4.17.

Countable range of CFU, obtained in agar plates incubated after directly spreading the sample without serial dilutions, are only represented here. Bacterial count was, in general, below the countable range (TFTC) after serial dilutions of the sample. Fig. 4.17 represents the average number of CFU from three similar sites of each section. The results are almost similar for all bed linens. It may be noted 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 (Fig. 4.18).

Significantly lower loads of bacteria found on TiO_2 and Ag doped TiO_2 coated fabric than on uncoated (control) fabric confirmed the disinfection potential of these modified textiles. Comparing the effectiveness of nano-coatings, Ag doped TiO_2 is more effective than pure TiO_2 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 section 4.4.

Bacterial count on uncoated section of each bed linen may be correlated to the time period of that patient in hospital given in Table 3.1. First bed linen with 209 CFU/10cm^2 of uncoated section was used by patient who had been in hospital for 126 days till experiment. Second bed linen with 177 CFU/10cm^2 of uncoated section was in use by patient who had been in hospital for 24 days till experiment. Third bed linen with 189 CFU/10cm^2 of uncoated section was used by patient who had been 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 TiO_2 coated and Ag doped TiO_2 coated sections.

The study proves that bed linens in hospitals are associated with bacterial contamination as previously reported (Mitchell *et al*., 2015; Perelshtein *et al*., 2015; Creamer & Humphreys, 2008; Lemmen *et al*., 2004; Sexton *et al*., 2006; Lazary *et al*., 2014). These bed linens are a potential source of pathogenic bacteria for the immediate and distant environment. It has been reported that 42% of personnel are affected with bacterial contamination who had only touched the bed linens, even though had no direct contact with patients (Lazary *et al*., 2014). Although the bed linens 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 (Attaway *et al*., 2012). The results clearly prove that the use of such titania nanoparticle coated textiles may control the bacterial contamination and reduce the risk of nosocomial infections.

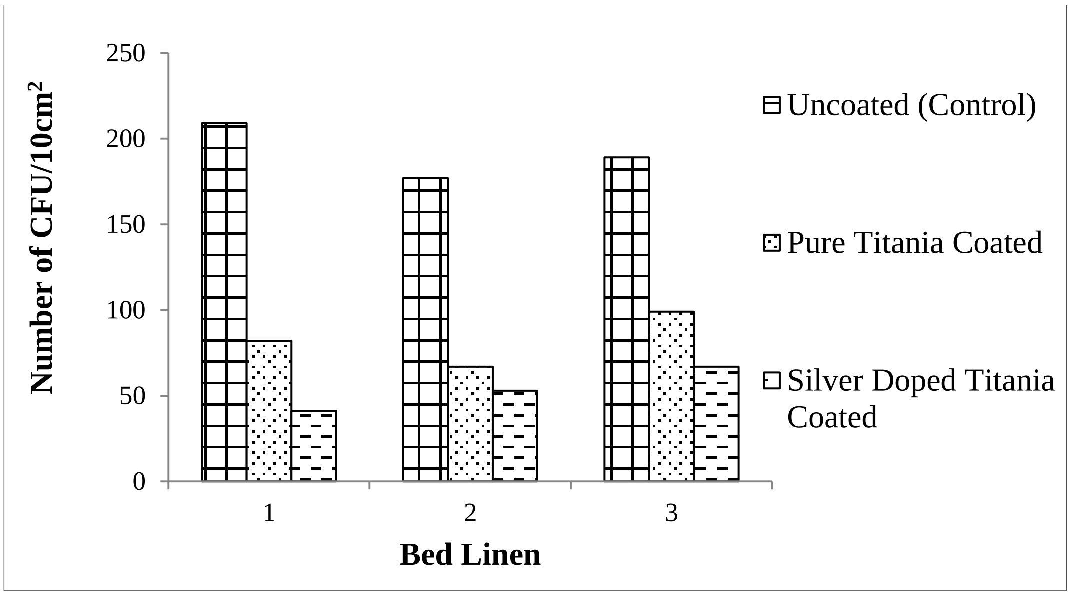
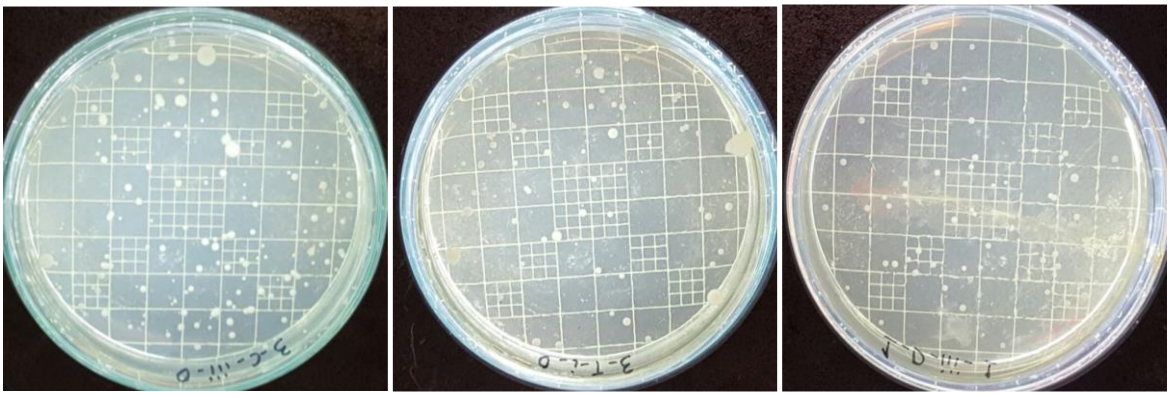


Fig. 4.17: Summary of bacterial count from hospital used bed linen.



Uncoated (control)	Pure Titania coated	Silver doped titania coated
CFU/10cm^2 = 177	CFU/10cm^2 = 67	CFU/10cm^2 =53

Fig 4.18: Bacterial colonies from uncoated (control), pure TiO_2 coated and Ag doped TiO_2 coated sections of hospital used bed linen 2.

Chapter 5

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In this study pure and 1% Ag doped TiO_2 nanoparticles (NPs) were synthesized through a simple and inexpensive liquid impregnation method and applied for the development of an antibacterial textile. TiO_2 NPs were successfully applied on cotton fabric surface using a cross-linking agent succinic acid. The durability of modified fabric was encouraging after washing, noting that they were suitable in environmental perspective. This stability also concluded the formation of strong linkage between cotton and TiO_2 NPs through succinic acid proposing this method as an effective and reliable method. In this study attempts were made for the first time to determine the TiO_2 NPs concentration in washing effluents 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. Pure titania nanoparticle coated fabric showed an enhanced photocatalytic inactivation of MRSA in UV light exposure (complete sterilization in 4 hours) as compared to fluorescent light exposure (complete sterilization in 24 hours). Silver doped titania nanoparticle coated fabric was more efficient than pure titania nanoparticle coated fabric under both light sources as it showed 100% killing efficiency of MRSA in a comparatively lesser exposure time (2 hours in UV light and 12 hours in fluorescent light). The results represent that these antibacterial properties of modified cotton fabric were also retained after washing.

The disinfection properties of such fabric were also successfully validated in hospital environment through the application of bed linens in medical intensive care unit (MICU) of a local hospital. Significantly lower loads of bacteria were found on titania and silver doped titania coated fabric (colony forming units less than 100) than on uncoated fabric (colony forming units greater than 100), confirming the disinfection potential of modified textile. Hence it is concluded that titania nanoparticle coated textiles may effectively improve the quality of hospital environment and also protect from nosocomial infections.

5.2. Recommendations

It is recommended that such titania nanoparticle coated cotton textiles should be employed in hospitals and other healthcare facilities to reduce the spread of infectious diseases. These textiles may also be used in homes for the elderly and in other environments where immune-compromised individuals are at high risk of infections. Sport textiles which require high protection and enhanced performance properties should also be developed using titania nanoparticle coated cotton textiles. In addition to disinfection, other properties of titania nanoparticle coated cotton textile such as self-cleaning, flame resistance and UV-blocking should be assessed. For applications in textile industry, titania nanoparticles should be applied on other types of fabric like polyester, nylon and wool. Textiles with titanium based nanocomposites (including metals, chitosan, SiO_2) should also be developed and tested for improved functioning.

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