Application of nanotechnology for self-sanitizing keyboards in an academic institution

By

Sehrish

NUST-201261462MSCEE65212F

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Science

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It is certified that the contents and form of the thesis entitled

“Application of nanotechnology for self-sanitizing keyboards in an academic institution”

Submitted by

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has been found satisfactory for the requirement of the degree of

Master of Science in Environmental Science

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Dedicated to

My beloved parents

For their endless affection, support and encouragement

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Sehrish

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List of Abbreviation

TNT	Titania nanotube
TNP	Titania nanoparticle
UV	Ultraviolet light
CK	Computer keyboard
CM	Computer mouse
ROS	Reduced oxidative species
NPs	Nanoparticles
NTs	Nanotubes
ABS	Acrylonitrile butadiene styrene
GPR	General purpose reagent
NaOH	Sodium hydroxide
SEM	Scanning electron microscopy
EDS	Electron dispersive spectroscopy
XRD	X-ray diffraction
MRSA	Methicillin resistant *staphylococcus aureus*
RNS	Reactive nitrogen species
NO-NPs	Nitric oxide producing nanoparticles
CFU	Colony forming unit
ZnO	Zinc oxide
Fe_2O_3	Ferric oxide
TEM	Transmission electron microscopy
PCR	Polymerase chain reaction

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Abstract

Keyboards and mice, being the potential reservoir of fungi, pathogenic and non-pathogenic bacteria, are constantly in contact with humans and may serve as a source of nosocomial infections. The present study was designed to develop self-sanitizing computer keyboards and mice to reduce such incidences in the presence of fluorescent light. Titania Nanotubes (TNTs) were fabricated from already prepared Titania Nanoparticles (TNPs) using the hydrothermal treatment method. TNTs and TNPs were characterized using SEM, EDX and XRD. Resulting Titania nanostructures were in the range of 24 to 35 nm. Selected keys of standard computer keyboards, and mice surfaces, were coated with a commercial adhesive containing 1% TNT and TNP to develop a disinfecting surface. These coated keyboard and mouse surfaces were found effective in reducing important airborne bacterial species present in indoor air. The effectiveness of the technique was checked by taking microbial samples at specific time intervals and disinfection rate was checked against each identified species. Results indicate that the Titania Nanotubes showed a much better disinfection capability compared to Titania Nanoparticles.

Chapter 1

INTRODUCTION

1.1. Background

Over the last few decades, the world is intensively being shifting to E-technology. Today, computer is the basic need of every individual starting from university environment to occupational, residential and recreational settings. In the university environment, students have 100% access to computers, 92.1% regularly use the Internet, and 73.3% regularly use e-mail. To deal with the extensive use of computer technology, universities have designed multiple-user ‘‘computer laboratories’’ on campus for general student’s access.

Indoor air quality is one of the most important factors that influences our general life quality. We breathe almost 10 m^3 air every day and spend 80–95% of our lives in indoor environment. Poor indoor air quality can result in surface contamination of every object including computers, door knobs, mobile phones, tables and other utensils. All of these objects, and contaminated surfaces, can be potential sources for cross-contamination leading to a number of potential health problems.

The fundamental driver of bacterial proliferation on keyboard and mouse surface is eating lunch during office hours on work station which results in trapped food deposits on and in between the keys leading to growth of millions of bacteria. Another reason is thought to be the poor individual hygienic condition, for example, person failing to wash hands after visiting the washroom. Dust, likewise can also trap

dampness and empower microscopic organisms, which are already present on the keyboard, to nourish and multiply.

1.2. How environmental surfaces get contaminated?

Individuals can encounter distinctive bacterial infections living in closed and densely populated areas where daylight has poor access. It has been indicated, with evidence, that the number of bacterial species is considerably higher in closed environment (like conference rooms, class rooms, offices and apartments etc.) because of the near vicinity of more individuals and poor hygienic conditions compared with spacious, ventilated and sterilized areas (Sessa *et al.*, 2002).

Patients at clinics and school going kids are more vulnerable to indoor bacteriological toxins and contagious attacks due their physical growth and suppressed immune system (Daisey *et al.*, 2003; Karwowska, 2003)

In clinical situations microbial contamination (Nosocomial Infections) gets to be more prevalent due to presence of immune-depressed patients. Along with nosocomial contaminations parasitic spores, propagates and metabolites resulting in critical health effects for patients with weak immune system, additionally to the typical guests/attenders of patients (Ortiz *et al.*, 2009).

1.3. Effects of indoor air pollution

Researches have shown that spending more time indoors is giving rise to many common diseases like sick building syndrome, asthma, bronchitis, lung cancer, flu-like symptoms, headaches, nausea etc. In addition, deterioration of indoor air quality can result in ‘‘multiple chemical sensitivity’’ (Shinohara *et al.*, 2004) and a cross-section of physical symptoms for those exposed including allergies, asthma, and headache

(Kostiainen, 1995; Jones, 1998). Some of these effects are immediately noticeable while some pose long term harm.

Modern Building structures that are more energy efficient, being more airtight, get chemically contaminated too. Use of air fresheners and other fragranced products are of no help but are adding more contamination to the air (Ahmad *et al.,* 2006).

1.4. Key methods for bacterial disinfection

The number of breathable pathogens is continuously increasing in indoor air along with substantial increase in nosocomial infections. Efforts have been made by scientists to develop a variety of disinfection strategies and protocols which can be utilized to minimize the microbial pathogens in residential, hospital and university settings or at least they can be kept at a level that is not able to induce infections in people. Indoor contamination control can be attained by applying different methods including, air filtration, Ultraviolet Germicidal Irradiation (UVGI), disinfectants, plasma-group particles, routine cleaning, essential oils and air dilutions etc.

These methods, however, have certain drawbacks i.e. it is extremely hard to maintain the air cleaning systems like ventilating frameworks. Furthermore, these systems are expensive to install and operate. Use of alternative technique can be a viable solution to sanitize the indoor atmosphere.

1.5. Proposed disinfection technique

Keeping in mind all of the situations discussed above, sanitation of air by killing pathogenic forms of bacteria using TiO_2 Nanotube coated surfaces is an effective, energy efficient and relatively low-cost method. Bacterial disinfection through Photo Catalytic Oxidation (PCO) is gaining popularity in the recent times. When Ultra Violet

light strikes a photo-catalytic substance like Titania, it excites electron in the valence band to jump into the conduction band of TiO_2 leaving behind a hole. The hole can react with the water molecules present in the air to form the hydroxyl radical (OH^-). On the other hand, the electron in the conduction band can react with the surrounding oxygen to form super oxide radical (O^{2-}). These two radicals can react with bacteria present in the surrounding air to split them into Potassium ion, RNA, Protein and other components leading to the ultimate death of bacteria (Wamer *et al.,* 1997). Photo-catalytic ability of Titania can thus be used to disinfect bacteria especially in indoor air by fixing TiO_2 on different substrates. For example Goswami and coworkers in 1997 prepared Titania coated fiber glass air conditioning filters to reduce air borne bacteria. Titania coated HEPA filters were demonstrated to be effective to eliminate 60%-80% spore forming air borne bacteria and fungi retained on HEPA filter (Chuaybamroong *et al.,* 2010).

1.6. The Present Work

The current work is focused on the development of self-sanitized surface for computer keyboards and mice which can disinfect pathogenic microorganisms by using photocatalytic activity of TiO_2 nanoparticles and nanotubes. Titania Nanotubes have distinctive property of high surface area to volume ratio; this factor makes Titania Nanotubes more efficient (than simple Titania Nano particles and modified Titania Nano Particles) in photo catalytic disruption of airborne pathogenic bacteria.

The study was designed around two key objectives i.e.

- Immobilization of prepared TNP and TNT on keyboard and mice surfaces
- Bacterial disinfection efficiency testing of TNP and TNT coated keyboard and mouse surfaces exposed to general microflora.

The present study also demonstrated the comparative photo-catalytic ability of Titania particles and Titania Nanotubes coated keyboard and mice surfaces to disinfect airborne bacteria. Prepared Nanomaterials (both nanoparticles and nanotubes) were characterized by SEM, EDS, XRD, BET and coated on keyboard and mice surfaces using a novel and green method of coating (GMSA as a binding agent). Bactericidal properties of prepared coatings were tested by exposing these to general microbial flora of indoor air.

Chapter 2

LITERATURE REVIEW

2.1. Indoor Air Quality

Man's exposure to indoor air pollution is not new; evidence of soot, found in the ceilings of caves of prehistoric times, clearly indicates that the level of pollution at that time was quite noticeable as the soot is an indicator of poor ventilation of open fires (Spengler and Sexton, 1983). With increasing awareness about the effects of outdoor air pollution, people also started taken indoor air pollution seriously. Thus, small and congested rooms were thought to be the cause of different diseases and Gauger (1714) concluded that it was not the warmth of a room that causes disease but the lack of ventilation and impure air is responsible for the spread of ailments (Sundell, 2004). The modern building design, where "energy efficiency" and insulation is a major consideration, however, contributes to the indoor air pollution problem due to poor ventilation (Jones, 1998).

With the evolution of modern techniques scientists have established the real meaning of "impure air" as a mixture of particulate matter (PM); gases such as ozone (O_3), nitrogen dioxide (NO_2), carbon monoxide (CO), and sulfur dioxide (SO_2); microbial and chemical volatile organic compounds (VOCs); and passive smoke (Bernstein *et al.,* 2008). It is ironic that at times most of the indoor air quality problems arise due to poor quality of air outdoors. There are other factors, however, in our modern living, whereby a lot of synthetic material is used in furnishing etc. inside a building that directly contributes to increase in indoor air pollution.

2.1.1. Microorganisms and indoor air quality

Human health is severely damaged and building materials are deteriorated when microorganisms grow in indoor environments (Schaechter, 2009). Most commonly associated microorganism resulting in indoor air quality complaints include bacteria and fungi. Bacteria generally grow in moist environments, therefore they are found indoors in areas with stagnant water such as water spray and condensation areas of air handling system components. On the other hand, fungi requirements for survival are less stringent than bacteria, therefore, it tends to grow on a wide range of building materials that are hydrated from various sources such as floods, plumbing leaks or condensation. With the amplification of microbial colonies, the indoor environment poses health problems to the occupants especially when their byproducts are released as bioaerosols in the air (Lighthart and Shaffer, 1995).

Bioaerosols are collections of airborne biological material that generally consist of droplets or particles ranging in size from 0.5 to 30 μm in diameter. Microbes produce a biological aerosol in atmosphere which spreads similarly to particulate matter. The bio-aerosols can penetrate into respiratory system of living organisms depending on their size (smaller the size, greater the possibility to enter) (De Wit and Bouvier, 2006).

Microorganisms dispersed as bioaerosols, using air as the mean of transport, grow on surfaces. If the bioaerosol is generated from dry, dusty surface or soil, the organisms tend to be suspended as single units or raft on larger particles (Lighthart and Shaffer, 1994) Airborne transmission becomes more important in case of medical environment. So, the controls of such transmissions (which are potential cause of infections) also become very important in such indoor environments. For example in dental clinics, several infectious microbial agents may affect medical staff working in the area and patients visiting the dental clinics (Cellini *et al.,* 2001).

2.1.2. Health impacts of poor indoor air quality

Inhalation process can expose the upper and lower respiratory tracts of humans to a variety of particles and vapors. Airborne transmission of microorganism especially pathogenic bacteria and viruses from various sources, like the surrounding environment, animals or humans, can result in a particular disease. Inhalation process is most important route of exposure to various particles and microorganism as the lungs are more prone to infections than the gastro intestinal tract. Ingested microbial population must pass through the acidic environment of stomach in order to infect various body tissues while inhaled microorganisms deposit directly onto the moist surface of the respiratory tract without any significant hindrance. Inhalation of microbiological aerosols can lead to adverse health impacts like: Infection, Allergic Reaction, Inflammation, Respiratory diseases, Legionnaires' disease, Tuberculosis and nontubercule mycobacterial disease.

Viral diseases that may spread due to respiration are:

- Influenza
- Severe Acute Respiratory Syndrome (SARS)
- Norwalk-Like Viruses disease (NLVs)
- Hanta virus disease
- Measles
- Varicella

In general, exposure to some gram negative and gram positive bacteria, endotoxins and actinomycetes during the process of air dispersal and consequent inhalation may lead towards variety of diseases. Diseases caused by air dissemination of fungi include:

- Histoplasmosis

- Coccidiomycosis
- Cryptococcosis
- Aspergillosis

All these microbial agents may be used in bioterrorism also (Schaechter, 2009).

Indoor air quality is also an important factor in preventing infections in occupants (medical staff and patients) of hospital facilities. Poor indoor hospital Indoor Air Quality (IAQ) may lead towards hospital acquired infections and a variety of occupational and biological hazards (Wan *et al.,* 2011). Microbial infection in an operation room may significantly enhance the risk of Surgical Site Infection (SSI). Threshold limit for airborne bacterial concentration in operation rooms is considered to be 180 CFU/m^3 during normal surgery and 10 CFU/m^3 during prosthetic replacement and arthroplasty procedures (Gosden *et al.,* 1998).

2.2. Contamination of Keyboard and Mouse Surface

Surfaces are generally not considered critical in university settings as these surfaces come in contact with intact skin only. Such surfaces, however, may become contaminated when touched by people infected with certain pathogenic bacteria or medical personnel involved with these patients. So it can be concluded that surfaces (which are non-critical in most of the cases) may become critical in specific conditions. It has also been revealed by various studies that important airborne bacteria like MRSA and P. aeruginosa may survive on surfaces even for months spreading infections for long periods of time (Rutala and Weber, 2001).

Keyboard and mouse is an essential part of a computer which remains mostly in touch with human beings. Surfaces of these two electronic gadgets are highly susceptible to be contaminated with different types of harmful microorganisms due to

their constant usage in every field of life e.g. hospitals, offices, homes, industries, educational institutes, laboratories, etc. Multiple user computer keyboards have more number of microorganisms as compared to single user computer keyboards (Anderson and Palombo, 2009). These keyboards have become potential threat for spreading of infectious diseases in schools, universities, hospitals, or anywhere they are going to be shared (Marsden and Eng, 2009).

2.2.1 Disinfection of keyboard and mouse surface

Disinfection and sterilization involving engineering techniques have now gained paramount importance in controlling nosocomial infection, because hospital environments are very prone to disease outbreaks due to the presence of patients with compromised and fragile immune system leading towards increased cost, mortality and morbidity etc. Along with conventional disinfection methods, several kinds of engineering techniques have been employed in modern world so that the microbial pathogens can be kept away from university settings, residential buildings and medical facilities or they may be kept at a level that is unable to induce diseases in humans (Bolashikov and Melikov, 2009).

A number of computer keyboard and mouse disinfection techniques have been recognized so that germ free keyboards and mice can be developed. Some of these are (Marsden and Eng, 2009):

Plastic wraps: When keyboards and mice are not in use they are wrapped up with plastic sheets, the issue with this technique is that of aesthetics.

Rubber caps: Keyboard buttons and mouse surfaces can be covered with rubber caps. These rubber caps are removed, cleaned and disinfected with some sort of disinfectants (i.e. ethyl alcohol, phenol, alcohol, chlorine, quaternary ammonium, etc.) and again put on the buttons (Rutala and Weber, 2001; Fukada *et al.*, 2008). Microorganisms can

reside in crevices and cracks of rubber. If these covers are not washed routinely they become reservoirs for pathogenic microorganisms which pose more threat than ordinary keyboards. In addition, it is a cumbersome process.

UV light: UV light exposure to keyboard also disinfects it by killing microorganisms (Martin *et al.,* 2011). Usually, a UV lamp is installed in computer table which kills bacteria when keyboard and mouse slide back into the table and is not in use. Computer keyboards and mouse are made up of Acrylonitrile Butadiene Styrene (ABS) polymer and exposure of ABS polymer to UV light results change in mechanical properties and appearance of ABS polymer (Loctite, 2011). This makes it an unsuitable option for disinfection of keyboards.

So, there is a need to develop an efficient method which should not be time consuming, cheap and have more efficiency than others. Photocatalytic activity of TiO_2 nanotubes makes it a suitable option to be used for disinfection of microbes, and these could be used for the development of self-sanitizing computer keyboards and mice.

2.2.2. Photocatalytic sterilization

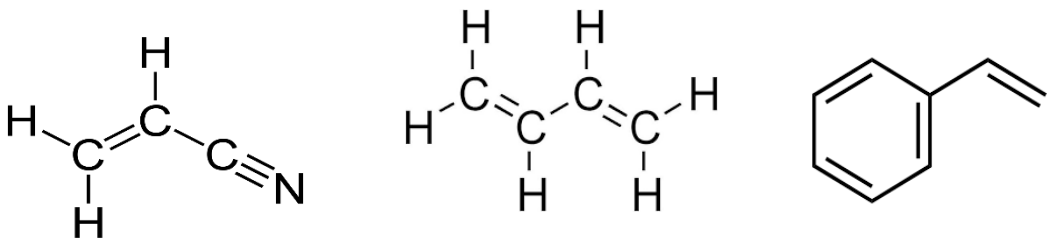
Photocatalytic property of TiO_2 makes it an ideal agent to be used in manufacturing of self-sterilizing surfaces. Oxidative species produced during photocatalysis of TiO_2 interact with cell membrane of microorganisms. Cell membrane oxidizes and gets damaged which ultimately results in the death of microorganisms.

A number of studies have been conducted in this regard. First study of this nature was with *Escherichia coli* by Matsunaga and coworkers in 1985. It was observed that all *Escherichia coli* bacterial species were disinfected after a period of one hour (Fujishima *et al.,* 2000). Another study reveals that 96% and 100% *Escherichia coli* species died after 30 min and 60 min exposure to TiO_2 respectively (Blake *et al.,* 1999). *Streptococcus mutants* is a pathogenic microorganism which causes infection during

dental surgery. Its 15 min exposure to TiO_2 results in death (Kim *et al.*, 2007). TiO_2 also shows high photocatalytic efficiency against *Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa and Salmonella typhimurium* bacterial species (Ibáñez *et al.*, 2003). As Ag-TiO_2 has more photocatalytic activity than pure TiO_2 so Ag-TiO_2 powder coating of keyboard surfaces reveals high sterilizing efficiency by killing *Pseudomonas aeruginosa* (Habib, 2013). TiO_2 nanotube coated surfaces also proved effective regarding disinfection of *Pseudomonas aeruginosa* and *Staph aureus* (Latif, 2013).

2.2.3. Acrylonitrile Butadiene Styrene (ABS)

Acrylonitrile Butadiene Styrene (ABS) polymer is an amorphous thermoplastic which can be molded easily after attaining certain temperature. Basically, ABS polymer is a combination of three monomers named as acrylonitrile, butadiene and styrene (Rutkowski and Levin, 1986). Chemical structures of above mentioned monomers are shown in Figure 1.



Acrylonitrile **Butadiene** **Styrene**

Figure 1: Chemical Structure of Acrylonitrile Butadiene Styrene (ABS) Polymer

Each monomer has its own specific properties while the ratio of these monomers (acrylonitrile, butadiene and styrene) decides the properties and chemical structure of the resulting polymer. Thermal stability and chemical resistance of ABS polymer is due to acrylonitrile. Butadiene provides toughness to the resulting polymer while styrene contributes towards rigidity and surface appearance of ABS polymer. Some important physical properties of ABS polymer are mentioned in Table 1 (Loctite, 2011).

Table 1: Physical Properties of ABS

Boiling Point	105^{o}C
Tensile Strength	2.8-8.4Kg/cm^2 x 10^2
Density	1.02-1.28g/cm^3
Hardness	96-125 Rockwell
Thermal Conductivity	0.17-0.23W/m -K^{o}

ABS polymer has wide range of applications in our daily life. Above mentioned properties of ABS polymer enables it to be used in various industrial applications. It is frequently used for the manufacturing of computer keyboards, telephones, bath tubs, toys, sports goods, etc.

2.3. What is Photocatalysis?

Photocatalysis can be defined as the change in the rate of a chemical reaction in the presence of a substance (Photocatalyst) that absorbs UV, visible or infrared light (Braslavsky, 2007).

2.3.1. Ideal photocatalyst

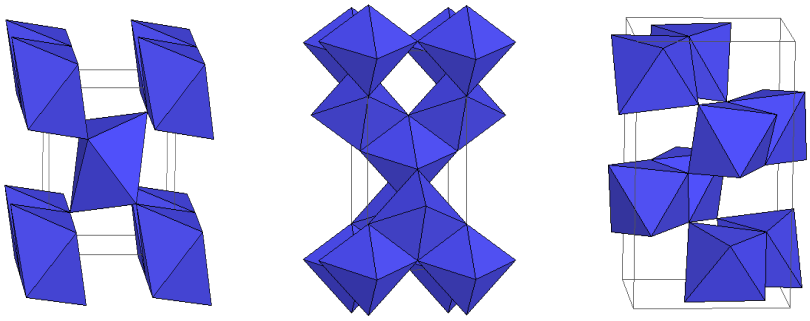
Photocatalyst is usually solid and semiconductor in nature, not consumed during a reaction. Some of the semiconductors which have been investigated for use during heterogeneous photacatlytic reactions are CdS, CdSe, CdTe, ZnS, ZnSe and some metal oxides like TiO_2, Fe_2O_3, ZrO_2, ZnO, SnO_2 and WO_3 (Benabbou *et al.,* 2007). An ideal photocatalyst should be inert chemically as well as biologically, and also stable under light. It should exhibit a narrow band gap thus being photoactive under visible light. Most importantly, it should be non-toxic to both man and environment (Bhatkhande *et al.,* 2002).

2.3.2. Titania as a photocatalyst

Titanium is the ninth most plentiful element on the earth's crust and Titania is its most stable oxide with high photocatalytic activity and no toxicity. Due to its abundance on earth's crust, it is not very costly. These properties are responsible for the extensive use of Titania as photocatalyst in the world of nanotechnology (Herrmann, 2005; Benabbou *et al.*, 2007).

2.3.3. Polymorphs of Titania

Titanium dioxide commonly known as Titania is basically present in three polymorphic forms; Anatase, Rutile and Brookite as shown in Figure 2. Anatse has the highest photocatalytic activity among all the polymorphs of titania (Carp *et al.*, 2004; Malato Rodrıguez *et al.*, 2004). Some studies have reported better results for the photocatalytic reactions by using a mixture of anatase and rutile polymorphs (Giolli *et al.*, 2007; Sun *et al.*, 2011).



Anatase **Rutile** **Brookite**

Figure 2: Three polymorphs of Titania

2.3.4. Technical limitations of TiO2 and its solution

TiO_2 is an effective photocatalyst but its band gap energy of 3.2 eV, equivalent to 388 nm wavelength restricts its use in only UV region (Linsebigler *et al.*, 1995). This issue has been addressed with the help of doping of Titania (Gaya and Abdullah, 2008). Various metals (Iron, Silver and Copper etc) and non-metals (Carbon, Nitrogen and Boron etc) have been used as dopants to reduce the band gap energy of Titania (Arana

et al., 2004; Wu *et al.*, 2004; Hamal and Klabunde, 2007). Doping must be controlled carefully as high dopant concentrations may result in electron and hole recombination (Carp *et al.*, 2004; Coleman *et al.*, 2005).

2.3.5. Titania nanotubes (TNTs)

Titania nanotubes are widely used in photo catalysis due to their exceptional physical and chemical properties like high surface area, photo catalytic activity and widespread accessibility. TNTs could be prepared by the hydrothermal method under mild conditions of temperature and pressure (Lee *et al.*, 2011).

2.3.6. Hydrothermal method

Wang and coworkers carried out an in-depth study of the TNT formation mechanism (Wang *et al.*, 2002). The three-dimensional TiO_2 structures (anatase phase), were transformed into 2-dimensional layered structures after reacting with aqueous solution of NaOH. These structures then scrolled to form TNT. According to them, the two-dimensional lamellar structure was in the formation of TNT.

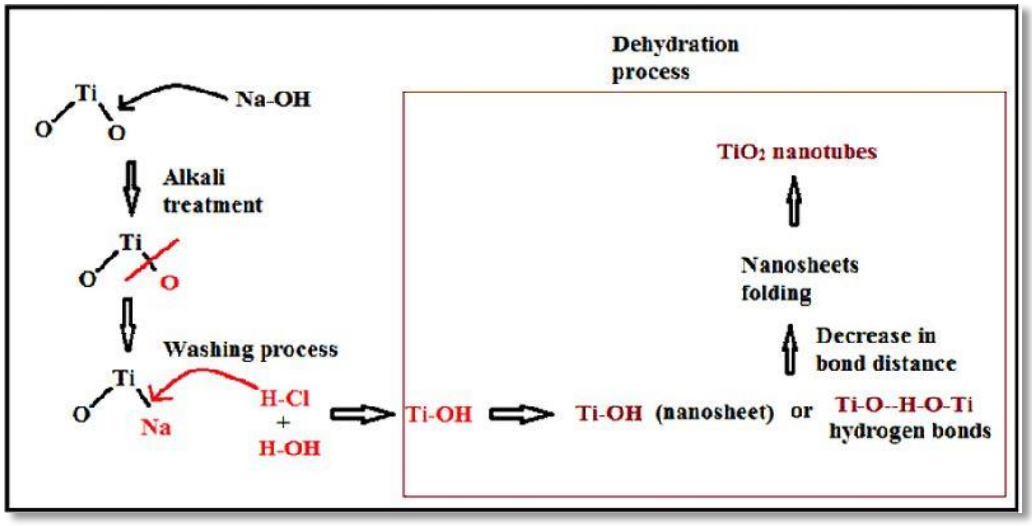


Figure 3: Hydrothermal method of Titania Nanotubes

Hydrothermal reaction of NaOH as a strong base broke Ti-O-Ti bonds. The hydroxyl bridges were formed by sharing edges of free octahedral shapes Ti ions. This results in

a zig-zag structure. Further rolling of crystalline sheet help to saturate these surface dangling bonds, which lower total energy and TiO_2 nanotubes were formed (Chen and Mao, 2007).

2.3.7. The toxicity mechanisms of Nanomaterials (NPs & NTs) against bacteria

The proper and complete mechanism of Nanoparticles and Nanotubes toxicity against microbial species is yet to be established. In several cases, Nanomaterials have the great ability to adhere themselves to the walls of bacteria by electrostatic interactions and degrade the structure and integrity of membrane (Zeyons *et al.*, 2009). In other cases, when light strikes the Nano species, they produce Reactive Oxidation Species (ROS). These ROS react with bacteria and disrupt their cell wall (Soenen *et al.*, 2011).

Affectivity of Nanomaterials depends on several factors like:

- Composition of Nanomaterials
- Surface modification due to doping
- Intrinsic properties of Nanomaterials
- Type of Nanomaterials

TiO_2 Nanomaterials are toxic towards bacterial species like *E. hire* (gram positive), *Staph. aureus* (gram positive), *P. aeruginosa* (gram negative), *E. coli* (gram negative), and *B. fragilis* (gram negative) under UV light killing all these species within 60 minutes. These Titania NPs and NTs don't show any antimicrobial activity under dark conditions. Photo-catalysis of Titania Nanoparticles under UV light disrupts the polyunsaturated phospholipid constituent of lipid membrane leading towards the degradation of cell (Wan *et al.*, 2011).

The antimicrobial effect of Copper Nanoparticles depends on sever factors such as:

- pH (Low pH increase the toxicity of NPs)
- Temperature (High temperature help increasing the efficiency of NPs)
- Aeration (High aeration rate is suitable for NPs functioning)
- Nanoparticles Concentration (greater the concentration of NPs, greater will be the efficiency)
- Concentration of Bacteria (Lower the concentration of bacteria, greater will be the efficiency of NPs) (Pramanik *et al.*, 2012).

Different Nanoparticles have different efficiencies towards bacterial species like *Staph. aureus*, *B. subtilis* and *E. coli*. Among NiO, CuO, ZnO, and Sb_2O_3, Nanoparticles of Copper Oxide are more efficient in removing these bacterial species followed by ZnO, NiO and Sb_2O_3 Nanoparticles (Baek and An, 2011). Additionally, Nanoparticles containing Oxide (Like ZnO and NiO) doesn't always disrupt the bacterial growth by entering the cell. In fact, these types of Nanoparticles create microenvironments near their surface and produce Reactive Oxidizing Species (ROS), which results in bacterial death (Heinlaan *et al.*, 2008).

Silver Nanoparticles combined with antibiotics is a good combination for killing several species of bacteria. For example, ampicillin (an antibiotic) damages the cell wall thus making it easy for Silver Nanoparticles to enter into the bacterial cell. After entering the cell, these Nanoparticles attack the DNA of the cell inhibiting its unwinding, consequently leading to cell death. Additionally, Silver doped Titania Nanoparticles are efficient in killing *E. coli* and *Staph. aureus*. Silver Nanoparticles bind themselves to the cell membrane of bacteria and damage it, then Silver ions bind themselves with the Oxygen, Sulphur and Nitrogen (components of all major molecules present within cell) thus inhibiting cell growth, ultimately leading to cell death (Juan *et al.*, 2010). Selecting suitable Nanoparticles for certain kind of bacterial species in order

to kill them is a sound option as this phenomenon will minimize the side effects of Nanoparticles and will enhance the bactericidal effect of NPs (Sondi and Salopek-Sondi, 2004).

Nitric Oxide producing Nanoparticles (NO-NPs) are also toxic towards many bacterial species of resistive nature and that are clinically very sensitive. Some of these species include *E. faecalis, Str. pyogenes, E. coli, P. aeruginosa* and *K. pneumoniae*. The working mechanism of these types of Nanoparticles depends on delivery of NO to target bacterial specie. NO released from these NPs has the ability to chemically modify the structure of bacterial membrane. These NPs also produce Reactive Nitrogen Species (RNS) which have the same ability (Martinez *et al.,* 2009). Zinc Oxide Nanoparticles are also able to kill bacteria like *Methicillin Resistant Staph. aureus (MRSA)*. ZnO Nanoparticles have the ability to damage and rupture cell membrane that ultimately leads towards the cell death.

2.3.8. Coating of Titania on surfaces

Coatings can be prepared by embedding synthetic Nano materials in the matrix of coating material. Such type of coating is usually a thin layered composite present on a substrate. Water and ethanol based glass and plastic venetian blinds Ag-TiO_2 coatings were prepared by Khan and coworkers in 2013 to disinfect important indoor bacterial species *P. aeruginosa* and *B. subtilis*. Coatings were efficient enough to kill these pathogenic bacteria just within 2 hours under visible light. Glass beads were also coated by Titania Nanoparticles through the process of calcination in order to remove arsenic from water (Danish *et al.,* 2012).

Most important issue is that whether these coatings are reliable or not. For this purpose, several studies have been performed to check the stability of such coatings that whether they are harmful or not for environment and humans. These tests may

include but are not limited to abrasion, scraping, and sanding. Apart from determination of particle size distribution and concentration, chemical and morphological analysis like SEM, TEM, EDS and ICP are also important in order to determine any unintentionally produced particles (which got free from coatings) in Nano range in airborne emission (Kuhlbusch *et al.,* 2011). Specific arrangements of Titania Nanotubes, thin films and coatings of TNTs will have potential applications in near future in areas such as

- Filtration
- Catalysis
- Sensing
- Photovoltaic cells
- High surface area electrodes (Tian *et al.,* 2003).

2.3.9. Nano-materials against environment and ecosystems

Nano materials are being applied in several fields like biological science, medical science and commercial products thus leading towards the leakage and accumulation of these Nano-materials in our environment mostly in soil and water. It is very important to save our environment and bacteria (beneficial for environment and humans) from hazardous impacts of indiscriminate use of Nano materials. For example use of Silver doped Nano particles causing accumulation of Silver in environment as a toxicant. The leakage of the Nanoparticles in environment is especially hazardous for many beneficial microbes, bacterial populations and public health (Gajjar *et al.*, 2009). Several species of microbes are beneficial to environment and ecosystem, as they play an important role in processes like bioremediation, element cycling and nitrogen fixation (essential for plant growth) (Molina *et al.*, 2006; Van Wees *et al.*, 2008; Kumar *et al.,* 2011). During the process of nitrification, Ammonia nitrogen is first converted

to nitrite by Ammonia fixing bacteria and then nitrate by nitrite fixing bacteria. The nitrifying bacteria are present in the regions with high concentrations of ammonia. Silver nanoparticles disrupt the membrane of these bacteria which contain ammonia oxidation enzyme by producing ROS. The killing of such bacterial species leads towards the decreased nitrogen removal from environment which disturbs plant growth (Choi and Hu, 2008). Nano sized materials can also help different viruses to penetrate into bacterial cells.

For example, Silver and Zinc Nanoparticles disrupt the cell wall of *E. coli* and mediate the internalization of MS_2 phage into bacterium by 2 to 6 folds increased magnitude. This is a serious problem, as NPs make internalization easy for MS_2 phages carrying drug resistant genes thus facilitating development of multidrug resistant in *E. coli* (You *et al.,* 2011). Nanoparticles antimicrobial activities depend on two major factors:

1. Physiochemical properties
2. Type of bacteria being exposed

Result reported by different studies are contradicting regarding bacterial killing rate and functioning of Nanoparticles because most of the researchers used as present NPs and bacterial species rather than using specific bacteria and NPs (Hajipour *et al.*, 2012).

Chapter 3

METHODOLOGY

3.1. Materials

Acrylonitrile Butadiene Styrene (ABS) polymer for research work was obtained from old computer keyboards, Chloroform (Merck, Germany) as solvent and GMSA (commercial binding agent) was used to make coating material while Acetone and Ethanol (Merck, Germany) was used to disinfect keyboards and mice surfaces. Titanium Dioxide (GPR, BDH Chemicals Ltd. England) was used for the fabrication of Titania nanoparticles and titania nanotubes. Solidified agar plates were prepared using Nutrient Agar (Merck, VM 100650 943). Six black keyboards and mice were used for this research study, API 20E kit (BioMerieux, Canada) was used for biochemical identification.

Software named as "Keystroke Counter and Frequency Recorder" was used to find the frequency of keystrokes on computer keyboards.

3.2. Methods

3.2.1. Synthesis of Titania Nanoparticles (TNPs)

Titania nanoparticles were prepared by liquid impregnation method. For this purpose, solution of 25g TiO_2 (GPR, BDH Chemicals Ltd. Poole England) was taken in distilled water and stirred for 6 hours on a magnetic plate. The resulting solution was allowed to settle overnight. The sample was then dried in oven at 105^{o}C for 12 hours. The dried sample was then crushed and calcined in a muffle furnace at 550^{o}C for 6 hours to obtain nanoparticles in anatase phase.

3.2.2. Synthesis of Titania Nanotubes (TNTs)

A sequence of steps was performed for the synthesis of Titania nanotubes in Teflon lined autoclave. According to the capacity of Teflon lined autoclave; the concentration, volume and conditions were optimized by carrying out a number of experiments in order to get the required results. Titania Nanotubes were synthesized by hydrothermal method (Asapu *et al.*, 2011) with some modifications. For this purpose, 2.5g prepared Titania Nanoparticles were added in to 100ml of 10M NaOH solution with 2 and 1 hour of stirring and sonication respectively. The resulting suspension was placed in a Teflon lined steel autoclave. The autoclave was maintained at a temperature of 135^{o}C for 24 hours with continuous stirring at 300rpm. Then the treated solution was allowed to cool to room temperature. The solution obtained was washed with 0.1M HCl and distilled water numerous times, until the pH was around neutral. The sample was dried at 105^{o}C for 24 hours. Finally the sample was calcinated in a muffle furnace at 500^{o}C for 1 hour in order to obtain TNTs in anatse phase.

3.3. Characterization of prepared Nanomaterial (TNP and TNT)

3.3.1. Morphology of nanomaterials (TNP and TNT)

Scanning Electron Microscopy (SEM) is a powerful tool, being used now-a-days, in place of optical microscope. An electron beam is produced from electron gun, and accelerated by high voltage in vacuum. The electron beam then strikes the sample and signals are generated. These signals are detected by electron collector and image of illuminated sample is formed by magnetic lenses. SEM has a wide range of resolution ranging from 10X to 300,000X and can resolve even few nanometers. In the present study, morphology of samples was observed by using JEOL JSM-6460 SEM. SEM examined the prepared samples of TNP and TNT at an acceleration voltage of 20 kV.

3.3.2. Elemental analysis of nanomaterials (TNP and TNT)

In order to determine the chemical composition (in percentage) of the prepared TNP and TNT, Energy Dispersive Spectroscopy (EDS) coupled with SEM was used. When electron beam strikes the sample, different elements present in the sample produced characteristic X-rays having different energies. Composition of the elements is found out by collecting and analyzing these characteristics X-rays. In the present study, Elemental analysis of prepared TNP and TNT was done by using EDS Oxford INCA X-sight 200.

3.3.3. Structure analysis of prepared nanomaterials (TNP and TNT)

X-Ray Diffraction (XRD) is an established technique used to determine the crystalline phase of any powdered sample. In XRD, a coherent electron beam strikes the compact sample. After striking the sample, some of the X-rays will diffract at different angles. X-rays diffracting from a specific plane at same angle will reinforce each other giving high peaks indicating the crystallinity of sample. In the present study, JEOL JDX-II X-ray diffractometer was used to analyze the crystalline phase of the prepared Titania nanoparticles and Titania Nanotubes.

3.3.4. Surface area measurement of prepared nanomaterial (TNP and TNT)

Brunauer, Edward, Teller (BET) Analyzer is used to measure the surface areas of powders, solids and granules. In BET surface analyzer, Nitrogen (N_2) in liquid form is adsorbed on surface of pure solid having no other gas molecules (this is achieved by degassing of sample prior to surface area estimation) in monolayer at very low pressure. Surface area is calculated by measuring the number of molecules present in monolayer and by using the dimensions of the gas molecule. In the present study, Micromeritics Gemini VII BET surface area Analyzer was used to calculate the specific surface area of Titania nanoparticles and Titania Nanotubes. For BET analysis TNTs samples were first degassed at 130^{o}C for 4 hours under vacuum (Akarsu *et al.*, 2006).

3.4. Coating of computer keyboard and Mouse

3.4.1. TNP embedded coating for keyboard and mouse surface

In this method, 1.5g of ABS polymer was mixed thoroughly in ethanol solvent after providing continuous stirring of 15 minutes at room temperature. To the solution, an optimized quantity of commercial adhesive (GMSA) was added to make it sticky so that it can bind to the substrate surface (CK & CM). Along with continuous mixing, an optimized quantity (0.1g) of TNP was added to the sticky solution and this solution was then sonicated for 30 minutes in in order to get uniformly dispersed particles. A smooth thin layer of prepared homogenized slurry was immobilized on selected CK & CM surface with the help of a brush. At the end the coated surfaces were air dried.

3.4.2. TNT embedded coating on keyboard and mouse surface

Similar procedure was repeated for coating with TNT as mentioned for TNP just by adding 0.1g of TNT in the sticky solution followed by 30 minutes sonication. At the end homogenized slurry was immobilized on selected CK & CM surface in order to get a thin, smooth and fine layer after air drying for 24 hours.

3.5 Experimental Setup

An experimental setup was designed to check the effectiveness of nanoparticle and nanotube coated buttons against non-coated buttons. A set of six keyboards and mice were chosen for this research study. A computer keyboards consists of three main sections i.e. alphabetic, numeric and functional. Keystroke frequencies were checked using “Keystroke Counter and Frequency Recorder Software” on computer keyboards. Based on the recorded frequencies, each section was further subdivided into three major groups named as “Group I” “Group II” and “Group III”, in a manner so that all the groups have almost same number of hits as shown in Figure 6. In order to carry out the

comparative study, buttons belonging to Group I were kept as control, buttons belonging to Group II were coated with TiO_2 nanoparticles while buttons belonging to Group III were coated with TiO_2 nanotubes using commercial binding agent named GMSA. Same procedure was applied for computer mice. The coated computer keyboards and mice were installed at different labs in premises of author's (Mustafa) institute. These were then exposed to prevailing indoor air conditions for one month and were also observed bacterial contamination due to continuous hand contact of multiple users. At defined time intervals, samples were collected from each experimental section.

3.6. Isolation and Identification of Bacterial Strains

3.6.1. Sampling of bacterial strains

After a period of one week, sample from each button was taken with the help of a sterilized cotton swab by placing keyboard in laminar flow hood. Each swab was streaked on a separate petri plate and each plate was incubated for a period of 24 hours at 37^0C. After 24 hours of incubation, colonies of microorganisms were counted with the help of a colony counter in terms of Colony Forming Units (CFU).

3.6.2. Identification

Identification of isolated strain was performed with the help of colony morphology, gram-staining, biochemical test (Collins and Uttley, 1985) and growth on selective media. Species level identification was done through polymerase chain reaction.

3.6.2.1 Morphological characteristics

Biochemical and physiological characteristics were determined according to the procedures outlined in Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994). Colony morphological characteristics of isolated strain grown on nutrient agar

plates were observed to identify and classify the strain. Following morphological characteristics were observed;

Table 2: Colony Morphology

Morphological Characteristics	Description
Colony size	Punctiform, Small, Large
Margins	Entire, Undulate, Filiform, Curled, Lobate
Forms	Circular, Irregular, Filamentous, Rhizoid
Texture	Creamy, Mucoid, Dry
Elevation	Raised, Convex, Flat, Umbonate
Colour	Yellow, Orange, Pale Yellow, Off-White

(Pelczar and Bacteriologists, 1957)

Cell morphology was observed through Gram staining method under oil immersion at 100X resolution using light microscope.

3.6.2.2. Biochemical Characterization

Biochemical characterization and identification was conducted using different standard biochemical tests and API20E identification kit as given below.

3.6.2.3. Analytical Profile Index-20E

This API-20E test strip (BioMerieux, Canada) is used to identify the enteric gram negative rods. There are 20 separate small capsules on the strip in dehydrated form each responding to a biochemical test. A bacterial suspension is used to rehydrate each of the wells. Single colony of the isolated bacterial strains was inoculated into the 0.85% NaCl solution, ensuring that the suspension was homogenous and without clumps of floating bacteria. Capsules of LDC, ODC, ADH, H2S, and URE are half filled. These capsules were then filled up to the top with sterile mineral oil. CIT, VP, and GEL capsule were filled up-to the top of the well. Strip was labelled and kept in an

incubator at 37ºC for 24 hours. After 24 hours reagents were added in IND, VP and TDA chambers.

Table 3: API 20E Interpretation

Tests	Substrate	Reaction Tested	- Results	+ Results
ONPG	ONPG	Beta-galactosidase	Colorless	Yellow
ADH	Arginine	Arginine dihydrolase	Yellow	Red
LDC	Lysine	Lysine decarboxylase	Yellow	Red
ODC	Ornithine	Decarboxylase	Yellow	Orange
CIT	Citrate	Citrate utilization	Pale green	Blue-green
H_2S	$Na_2S_2O_3$	H_2S production	Colorless/gray	Black
URE	Urea	Urea hydrolysis	Yellow	Red
TDA	Tryptophan	Deaminase	Yellow	Brown-red
IND	Tryptophan	Indole production	Yellow	Red
VP	Na pyruvate	Acetoin production	Colorless	Pink/red
GEL	Gelatin	Gelatinase	No diffusion	Black
GLU	Glucose	Fermentation/oxidation	Blue/blue-green	Yellow
MAN	Mannitol	Fermentation/oxidation	Blue/blue-green	Yellow
INO	Inositol	Fermentation/oxidation	Blue/blue-green	Yellow
SOR	Sorbitol	Fermentation/oxidation	Blue/blue-green	Yellow
RHA	Rhamnose	Fermentation/oxidation	Blue/blue-green	Yellow
SAC	Sucrose	Fermentation/oxidation	Blue/blue-green	Yellow
MEL	Melibiose	Fermentation/oxidation	Blue/blue-green	Yellow
AMY	Amygdalin	Fermentation/oxidation	Blue/blue-green	Yellow
ARA	Arabinose	Fermentation/oxidation	Blue/blue-green	Yellow
OX	Oxidase	Oxidase	Colorless	Violet

The positive and negative results of API E20 translated into numerical profiles (as shown in Figure 4) and interpreted with the API web software.

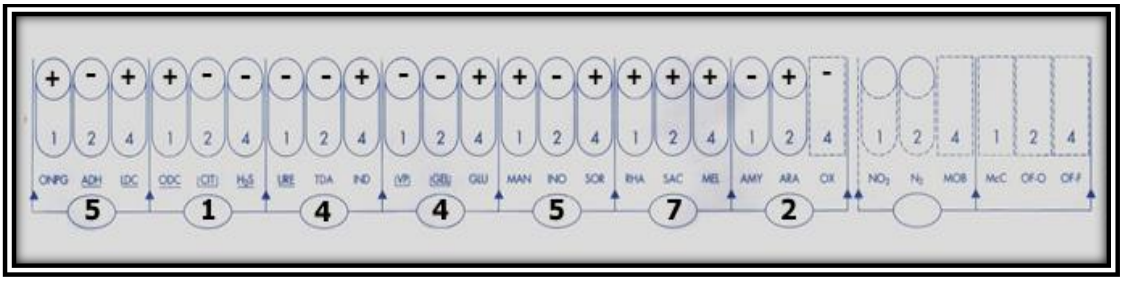


Figure 4 : API E20 Numerical Code

3.6.2.4. Oxidase test

A loop full of inoculum was taken from 24 hour fresh culture and placed on a piece of filter paper. One drop of 1% N, N-dimethyl-p-phenylenediamine dihydrochloride solution was added. The appearance of blue or purple colour formation within a few seconds is indicative of oxidase positive showing the presence of enzyme cytochrome oxidase.

3.6.3. Polymerase Chain Reaction (PCR)

3.6.3.1. DNA extraction

Bacterial template DNA was extracted from the colony of an overnight streaked plate. The colony was picked with sterile pipette tip and suspended in 100 µl of NF water in PCR tube and mixed thoroughly to form a turbid solution. The tube was then given a heat shock of 20 minutes at 85 degrees, and was then spun for 3-4 minutes until a pellet was formed. The supernatant was collected in a separate sterile tube while discarding the pellet. The supernatant contained the bacterial chromosomal and plasmid DNA.

3.6.3.2. Molecular identification

The Polymerase Chain Reaction (PCR) is an enzyme catalyzed biochemical reaction in which small amounts of a specific DNA sequence is amplified into large amounts of linear double stranded DNA (Mullis, 1990). It comprises of numbers of cycles and each cycle consists of denaturation, annealing and extension step (Figure 5).

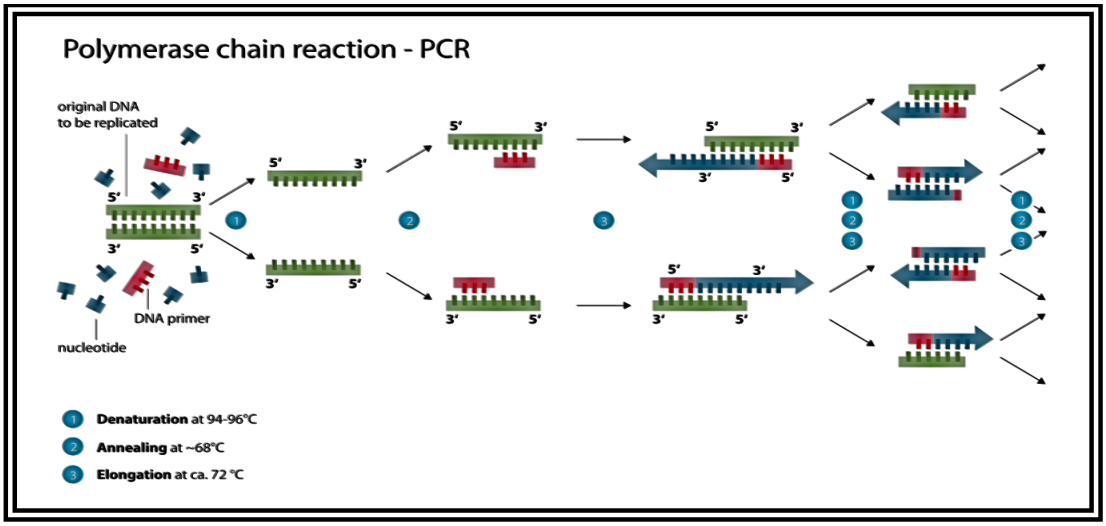


Figure 5: Mechanism of Polymerase Chain Reaction (PCR)

PCR reactions were carried out using TE Thermocycler (Extragene). For the amplification, an initial denaturation step of 10 min at 95°C was followed by 40 cycles of amplification consisting of 1 min at 95°C, 1 min at 58°C and 10 min at 72°C. Then PCR product and 100bp DNA ladder was run on Gel Electrophoresis. For gel electrophoresis, 1% agarose gel was prepared in TBE buffer. Ethidium bromide (3 µl) was added in agrose gel as this is a chemical that intercalates DNA and makes it visible under UV light (Parveen and Khan, 2012).

Table 4: Composition of PCR Master Mix

Ingredients	Concentration	Volume (µl)
MgCl2	20mM	2.5
dNTPs	2.5mM	2
Taq buffer	10 X	2.5
Forward Primer	10 µM	1
Reverse Primer	10 µM	1
DNA Template	1-100 ng/ µl	3
Taq Polymerase	500U/ µl	0.3
PCR Water	----	12.7

3.7. Bacterial Cultures Preparation

1 ml of pure bacterial cultures were inoculated in 100 ml of Nutrient Broth and Luria Bertani Broth (Sigma Aldrich) respectively and incubated in a shaker incubator at 37^{o}C for 24 hours and 16 hours respectively. The density of the bacterial suspensions was estimated using optical density at 600 nm. A range of 0.8-1.0 was chosen as optimal density for subsequent experimental testing. Colony range of 25-250 was taken as a standard during viable count procedure.

3.7.1. Agar plates preparation

14g of Nutrient Agar (Sigma Aldrich) mixed in 1 liter warm distilled water with slow mixing by glass rod. When nutrient agar was completely dissolved, flask containing agar solution was transferred to autoclave for complete sterilization at 121^{o}C for 15 minutes. After sterilization, prepared molten agar was poured into petri plates (autoclaved at 121^{o}C for 15 minutes) under laminar flow hood cabinet and allowed to cool down. Prepared petri plates, after solidification were transferred into incubator (for 24 hours at 37^{o}C) to check their sterility.

3.7.2. Saline blanks preparation

Six test tubes were taken and washed thoroughly first with water containing detergent and then with distilled water. The test tubes were filled with 9 ml solution of 0.85% NaCl (prepared in distilled water). Test tubes were then autoclaved at 121^{o}C for 15 minutes for complete sterilization.

3.7.3. Serial dilution technique

Serial dilutions of freshly prepared cultures were made by adding 1ml bacterial culture in 9 ml in 0.85% saline solution in order to obtain a countable range (25-250 CFU/mL) of bacterial colonies. Countable range was achieved in approximately 5th

dilution. During each dilution, the bacterial suspension was well mixed with the help of vortex mixer in order to achieve uniform suspension.

3.8. Degradation Experiments

3.8.1. Bactericidal effect of TNP and TNT on isolated strains

Spray of each bacterial stain was made on titania nanoparticles and nanotubes coated sections of keyboard and mouse surface with the help of atomizer. Samples were taken with the help of sterilized cotton swabs after regular intervals of 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes. Serial dilution of each bacterial sample was done up to 10-5. 1ml of each sample was taken from 10-5 times diluted sample with the help of a pipette and poured on solidified agar petri plate. Sample was spread over solidified agar petri plate with the help of sterilized spreader. After spreading of sample, petri plates were incubated in incubator for 24 hours at 37^0C and CFU/ml were counted on a colony counter.

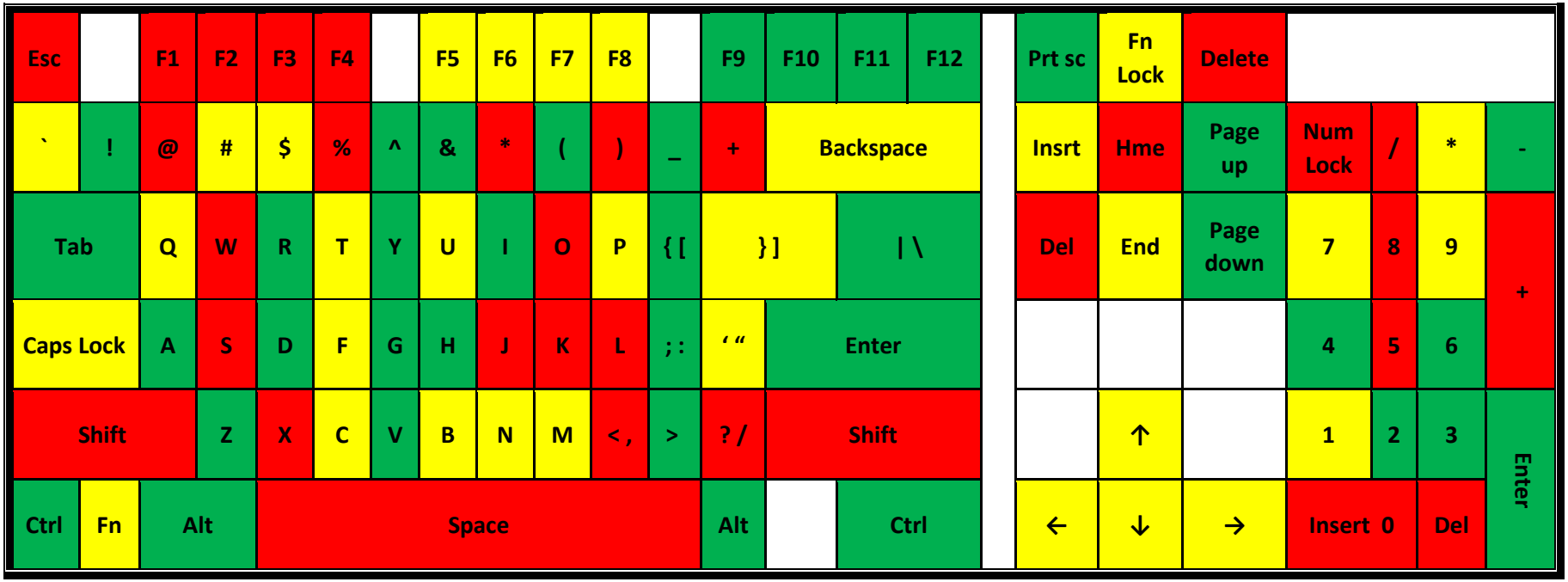


Figure 6: Schematic diagram of Keyboard

- **Group I (control; red)**
- **Group II (coated with TNPs; yellow)**
- **Group III (coated with TNTs; green)**

Chapter 4

RESULTS AND DISCUSSION

4.1. Characterization of Nanoparticles and Nanotubes

4.1.1. SEM Analysis

Scanning Electron Microscopy (SEM) was used in order to observe the morphology and diameter of Nanotubes. Figure 7 and 8 show the images of Pure Titania Nanoparticles by JEOL JSM-6460 at 10,000 and 50,000 magnifications. SEM images prove that spherical structure of nanoparticles have been formed with diameters ranging from 39 nm to 57 nm.

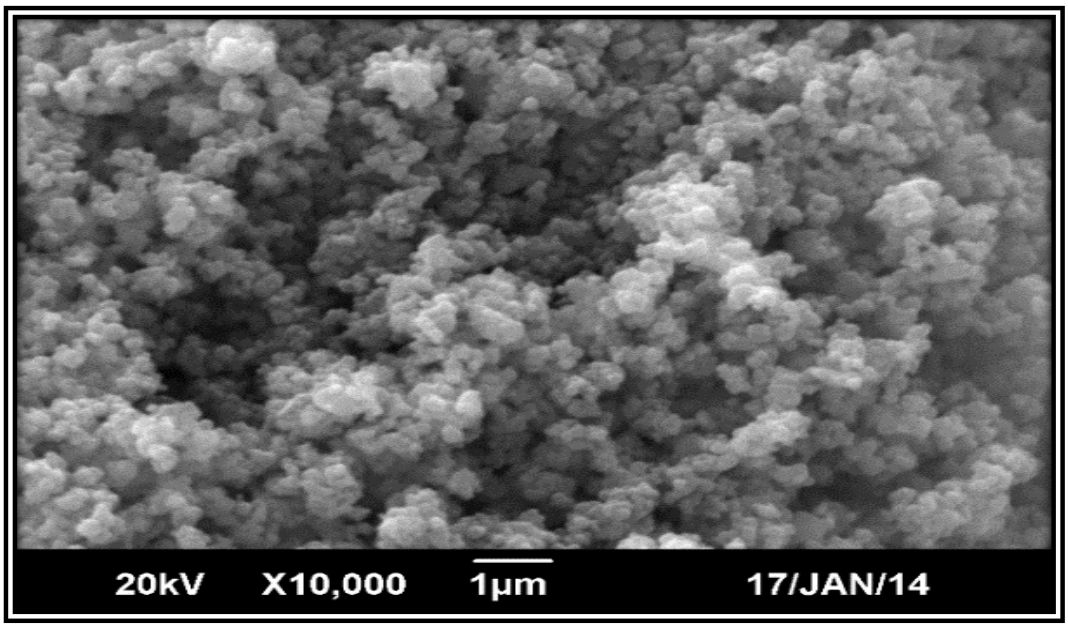


Figure 7: SEM images of Pure Titania Nanoparticles (TNPs) at X 10,000

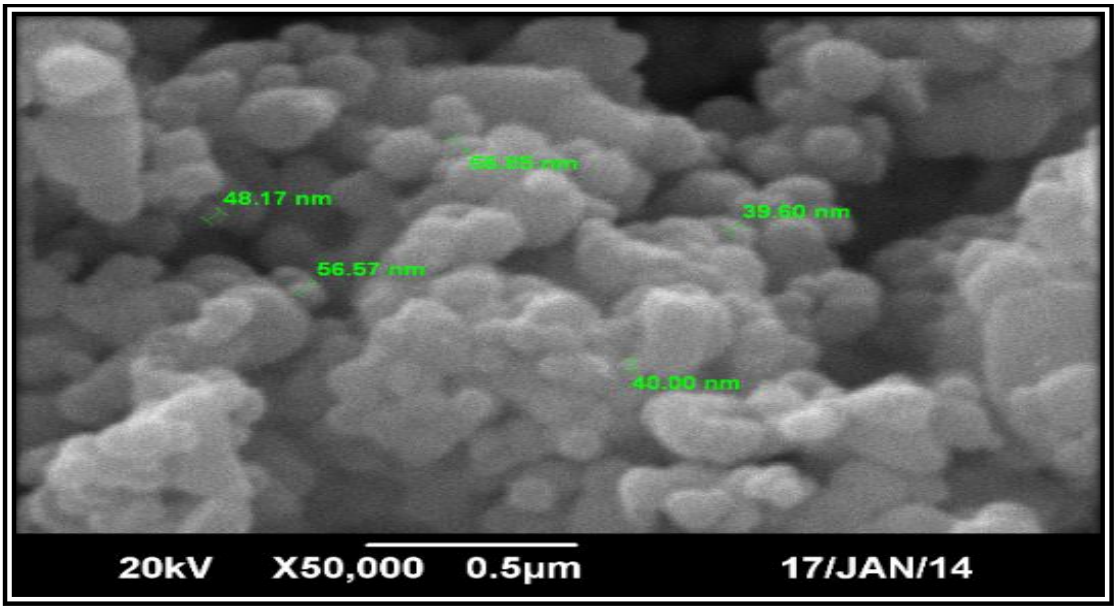


Figure 8: SEM images of Pure Titania Nanoparticles (TNPs) at X 50,000

Figure 9 and 10 shows the images of Titania Nanotubes by JEOL JSM-6460 at 10,000 and 20,000 magnifications. SEM images prove that very fine tubular structures i.e. Nanotubes have been formed with diameters ranging from 41 nm to 82 nm. These tubular structures exhibit high surface area than nanoparticles with expected high photocatalytic activity.

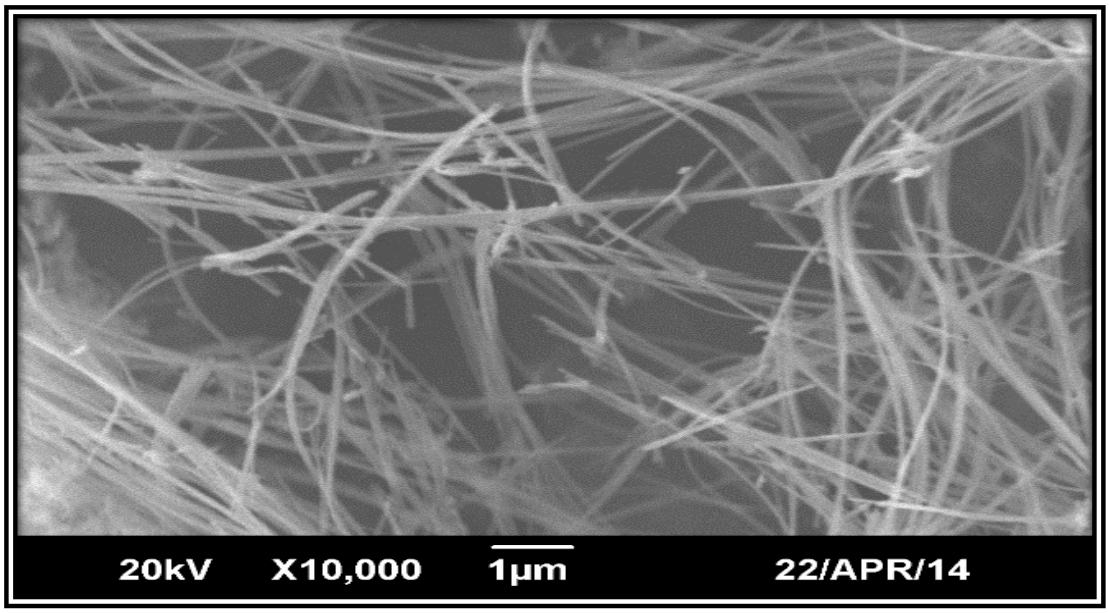


Figure 9: SEM images of Pure Titania Nanotubes (TNTs) at X 10,000

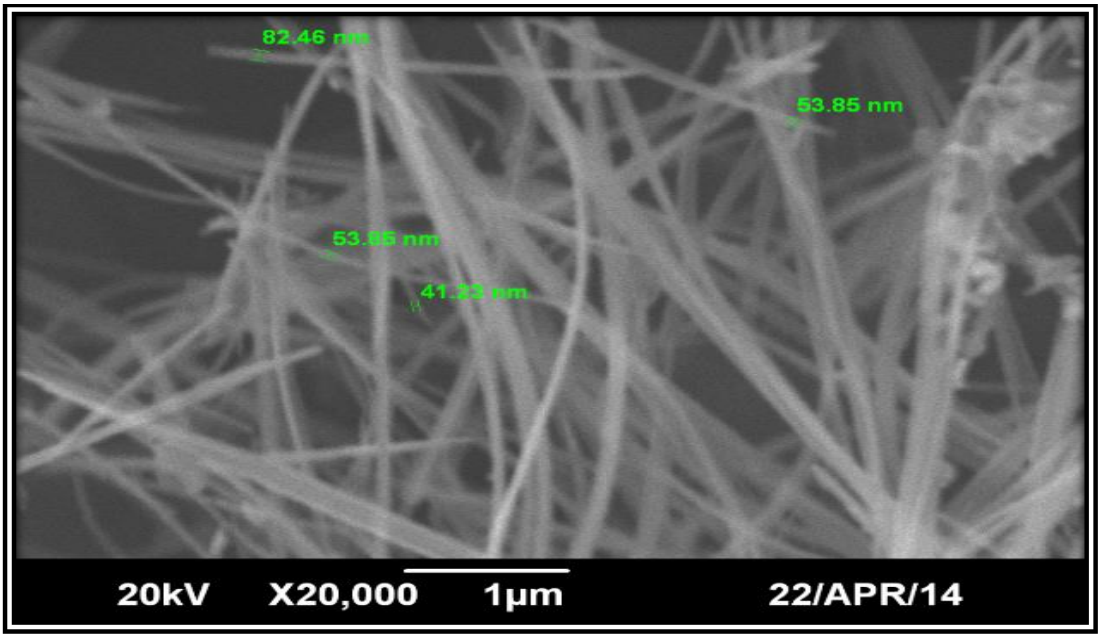


Figure 10: SEM images of Pure Titania Nanotubes (TNTs) at X 20,000

4.1.2 EDS Analysis

The chemical composition of nanostructures was investigated through EDS analysis. All the samples were uniform in composition as shown by all EDS graphs given in Figures 11 and 12. The main elements are titanium and oxygen. The mass percent composition of each sample is given in a table under the figure of the respective sample.

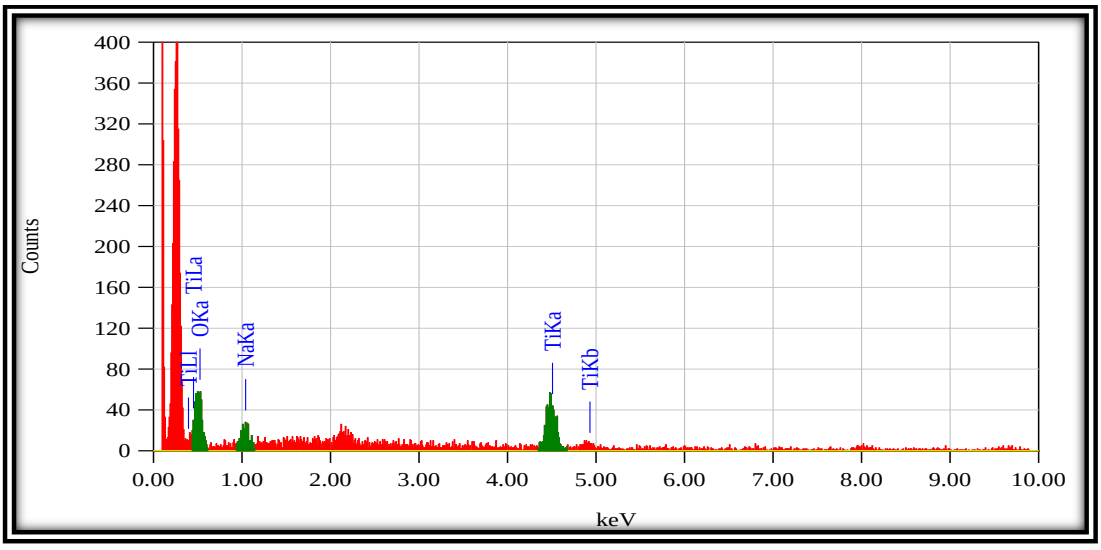


Figure 11: Elemental composition of pure Titania Nanoparticles (TNPs)

The peak in some samples at a position of 2.2 eV on x-axis is indicating carbon as all these samples were applied on carbon tape for EDS analysis. This was because of the fact that sample synthesis mechanism also involves carbon along with other elements. Figure 11 reveals that major constituents for pure Titania Nanotubes are Titanium and Oxygen i.e. approximately 59.79% and 40.21% respectively (Table 5).

Table 5: Mass % of Elements in Titania Nanoparticles

Element	Mass Percent (%)	
	Experimental	Expected
Ti	59.79	60
O	40.21	40

EDS results of Titania Nanotubes in Figure 4.6 and Table 6 showed that product is majorly comprised of Oxygen (61%), Titanium (39%), and Sodium (1.2%). Source of Sodium is NaOH, which was used during synthesis process and small quantity of Sodium was left over after several washings.

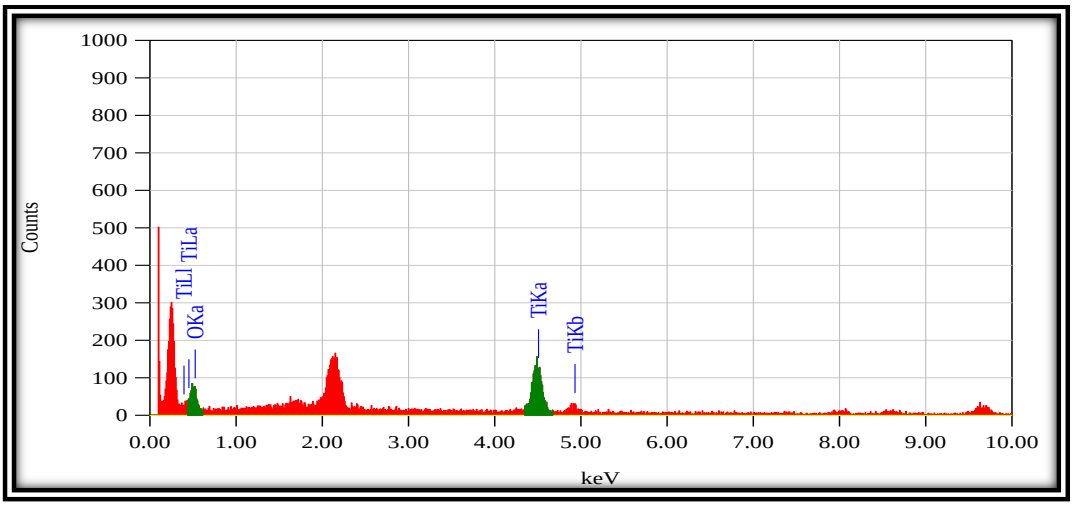


Figure 12: Elemental composition of pure Titania Nanotubes (TNTs)

EDS Results proved pure Nanotubes had been successfully formed without any impurity**.**

Table 7: Mass % of Elements in Titania Nanotubes

Element	Mass Percent	
	Experimental	Expected
Ti	61	60
O	39	40

4.1.3 XRD Analysis

X-Ray Diffraction (XRD) characterization for both pure Titania Nanotubes and 1% Iron doped Titania Nanotubes were carried out using Cu-Kα radiations at an angle of 2θ from 10º to 80º. In case of pure TNTs, peaks can be seen at 25º, 27.5º, 32º, 45.5º, 48º, 54º, 55º, 56.5º, 63º, 66º and 76º in Figure 13.

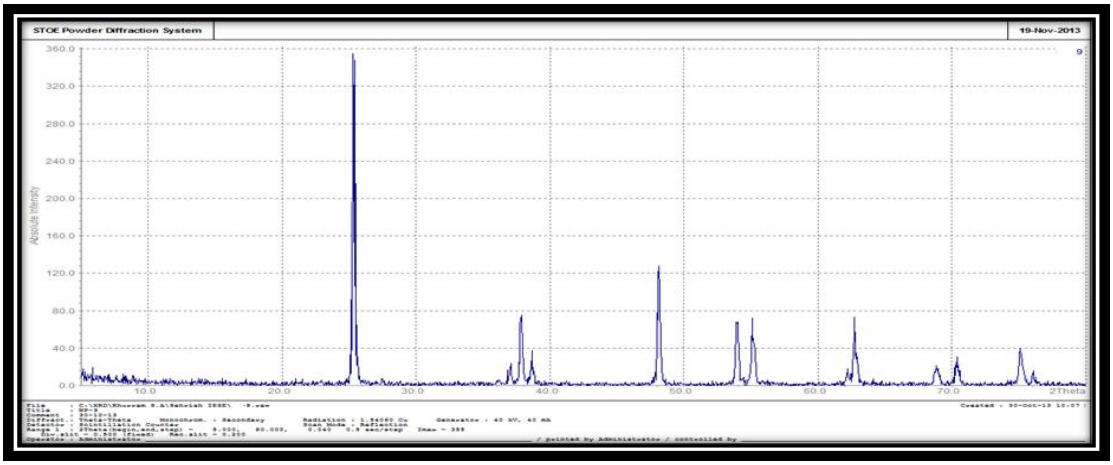


Figure 13: XRD Intensity plot for Titania Nanoparticle (TNPs)

These peaks are characteristics of TiO_2 in anatase phase revealing that synthesized Titania Nanoparticles are spherical in nature and have anatase phase. The main reason behind this anatase phase is calcination of Nanoparticles at 400ºC for 6 hours.

In case of Titania Nanotubes, Figure 14 shows peaks at 25º, 38º, 48º, 55º, 56º, 63º, 68º, 71º and 75º. These peaks strengthen the argument that TNTs are also highly crystalline in nature attributing this characteristic towards calcination of prepared

Nanotubes in muffle furnace for 1 hour and crystalline structure of Nanoparticles used as a starting material for the synthesis of Titania Nanotubes.

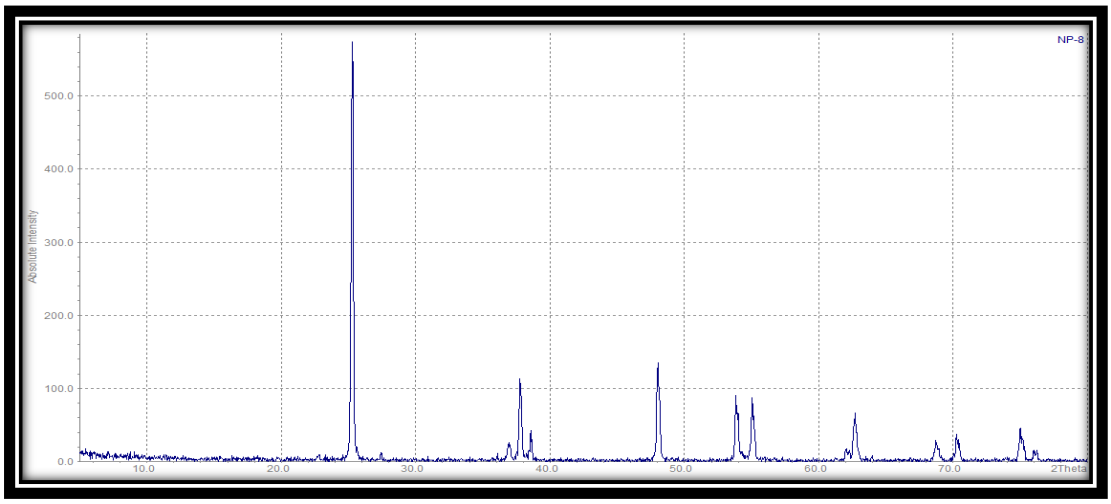


Figure 14: XRD Intensity plot for pure Titania Nanotubes (TNTs)

In short, synthesized Nanotubes are highly crystalline having anatase phase. It has been reported that Nanotubes when annealed or calcinated at high temperature (almost 400^{o}C) are converted to anatase phase having two to three time more photo-catalytic ability than the commercially prepared Titania Nanoparticles (Sekino, 2010). It has also been proved that Titania in anatse form gives better photocatalytic results than other forms of Titania i.e. rutile and brookite (Yao *et al.,* 2006). Anatase phase is also a powerful oxidizing agent having other characteristics like nontoxicity and long term photo-catalytic ability (Hoffmann *et al.,* 1995).

4.1.4. BET Analysis

BET analysis of Titania Nanotubes (Figure 15) revealed that at a relative pressure of 0.25 maximum amount of liquid Nitrogen adsorbed on the surface of Nanotubes is about 80 cm^3/g at Standard Temperature and Pressure (STP). This corresponds to almost 320 m^2/g surface area of Titania Nanotubes that matches well with the surface area limits (300-350 m^2/g) for Nanotubes given by Sekino (2010).

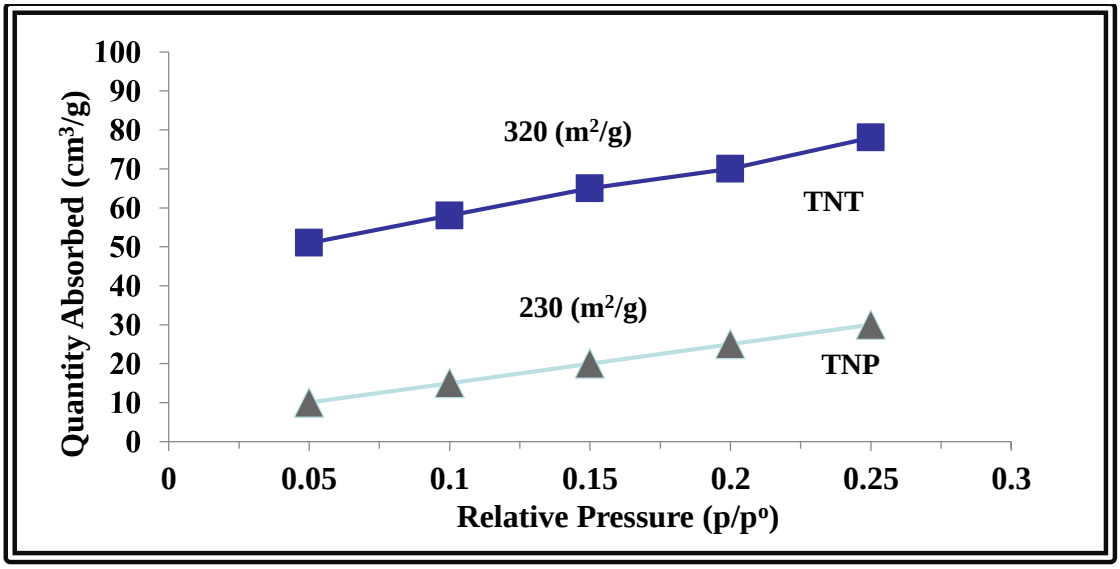


Figure 15: BET Analysis of Titania nanoparticles and Titania nanotubes

While, on the other hand, BET analysis of Titania Nanoparticles (Figure 15) showed that at a relative pressure of 0.25 the amount of liquid Nitrogen adsorbed on the surface of Nanoparticles is about 35 cm^3/g at Standard Temperature and Pressure (STP). This corresponds to almost 230 m^2/g surface area of Titania Nanoparticles.

4.2 Bacterial Disinfection from Keyboard & Mouse Surface

The extent and nature of activity on keyboard and mouse surface is found out by taking into the total number of bacteria and types of bacteria present on it. The CFU/ml, found out by spread plate technique indicates that the bacterial activity was greatest on Keyboard surface compared to mouse surface. Thus it can be anticipated that the usage of keyboard for typing at work station would be greatest and least for mouse surface. The colony count for all three sections (i.e. control, TNP-coated and TNT-coated surface) of keyboard and mouse for 2^{nd}, 4^{th}, 6^{th} and 8^{th} week is given in the Tables 7 and 8.

Table 7: Microbial Count on Control, NPs and NTs Coated Section of Keyboard

Sample Sites	Time Interval											
	2nd week			4th week			6th week			8th week		
	C	NP	NT	C	NP	NT	C	NP	NT	C	NP	NT
Library	25	8	1	22	4	0	20	3	0	21	4	0
Assistant Office	10	6	0	9	5	0	12	8	0	8	5	0
Computer Lab	25	5	0	20	6	0	26	5	0	24	6	0
Chemistry Lab	15	2	0	10	6	0	12	6	0	11	9	0
Biotech. Lab	15	4	0	15	8	0	18	7	1	19	7	0
Reception	20	2	0	10	9	0	16	5	0	12	6	0

These experiments clearly indicated that over all contamination on coated surface is reduced to almost nil within a fortnights time and surface remain self-sanitized thereafter.

Table 8: Microbial Count on Control, NPs and NTs Coated Section of Mouse

Sample Sites	Time Interval											
	2nd week			4th week			6th week			8th week		
	C	NP	NT	C	NP	NT	C	NP	NT	C	NP	NT
Library	15	4	0	10	4	0	12	5	0	11	4	0
Assistant Office	7	4	0	6	2	0	5	1	0	4	1	0
Computer Lab	19	5	0	26	9	0	20	9	0	15	5	0
Chemistry Lab	10	6	1	9	4	0	15	6	0	12	4	0
Biotech. Lab	16	5	0	15	8	0	12	7	1	11	4	0
Reception	10	4	0	6	2	0	9	4	0	3	1	0

The same situation exists for group of mice installed at six different locations as shown in Table 8. Again, disinfection appears after 2nd week. It shows that disinfection property for mice surface coated with Titania nanotubes is also permanent.

4.3. Identification of Microbes on Controlled Surface

At this stage, however to check which microbe was being actually inactivated, bacterial consortium on the control surface which had no coating was observed. As it was not possible to examine each and every type of microbe present on the surface, the most prominent colonies were picked out. These are 8 gram negative bacteria which were identified by Analytical Profile Index shown in Table 9.

Table 9: Bacterial Strains Identified from Control Surface of CK & CM

Isolates	Bacteria	Family
1.	*Serratia rubidaea*	Enterobacteriaceae
2.	*Pantoea spp.*	Enterobacteriaceae
3.	*Pasteurella pneumotropica*	Pasteurellaceae
4.	*Pseudomonas luteola*	Pseudomonadaceae
5.	*Escherichia coli*	Enterobacteriaceae
6.	*Pseudomonas aeruginosa*	Pseudomonadaceae
7.	*Burkholderia cepacia*	Burkholderiaceae
8.	*Proteus vulgaris*	Enterobacteriaceae

4.4. Disinfection Experiments

Once these species had isolated, it was important that the Titania nanoparticle and Titania nanotube disinfection be examined against each of these individually on keyboard and mouse surface. Following the procedure mentioned earlier, the disinfection of each bacteria was monitored for 2 hours at 15 minutes interval.

4.4.1. Bactericidal effect of each bacteria against coated sections of CK and CM

Figure 16 represents the degradation of *Burkholderia cepacia* under visible light. Initially cell count for *Burkholderia cepacia* was 148×10^7 CFU/mL, however the cell count decreased for TNT coated section of computer keyboard (CK) and computer mouse (CM) after exposure to florescent light for almost 30 minutes and found to be

66×10^7 CFU/mL, showing almost 50% removal of *Burkholderia cepacia*. Somewhat similar behavior was observed from TNP section of CK and CM and after 1 hour exposure to visible light, bacterial colonies found to be 75×10^7 CFU/mL. While, complete disinfection of *Burkholderia cepacia* was achieved with TNTs after 1 hour and cell count decreased from 146×10^7 CFU/mL to zero while the degradation efficiency with TNPs was bit slower. It means TNTs (higher surface/volume ratio) thus increased the reaction rate for bacterial killing. While, on the other hand, fluorescent light manage to generate very few electron hole pairs from pure TNPs resulting in incomplete inactivation of *Burkholderia cepacia* within one hour. (Hallmich and Gehr, 2010).

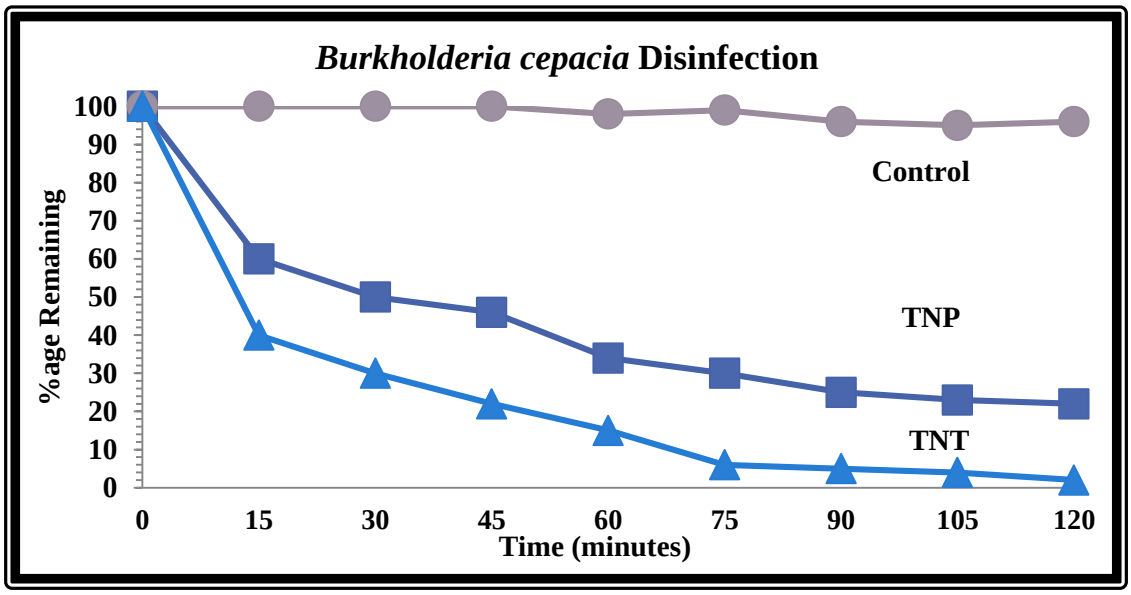


Figure 16: Disinfection of Burkholderia cepacia against TNP and TNT

In case of *Serratia rubidaea* and *Pantoea spp.*, Figure 17 and 18 represents that almost 55% bacterial degradation was achieved under Fluorescent light while complete disinfection was achieved after 60 minutes. Control solution here, under visible light showed no degradation after 2 hours of exposure. While, TNTs coated section of CK and CM showed better and faster disinfection compared to TNPs coated surface of CK and CM. This degradation of bacterial population in TNT section may be attributed

towards bactericidal effect of Titania nanotubes due to their higher surface area. (Juan *et al.*, 2010).

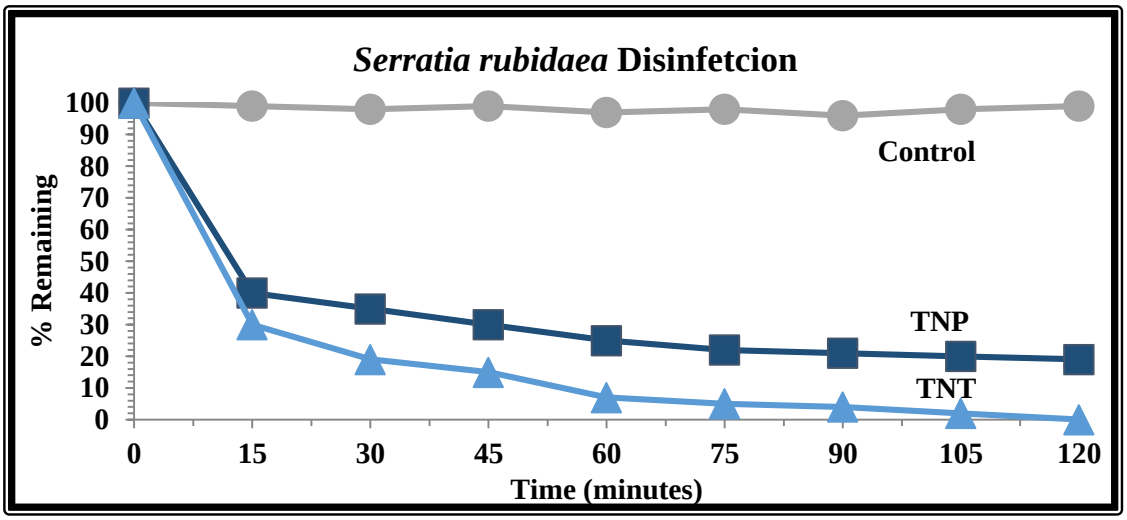


Figure 17: Disinfection of *Serratia rubidaea* against TNP and TNT

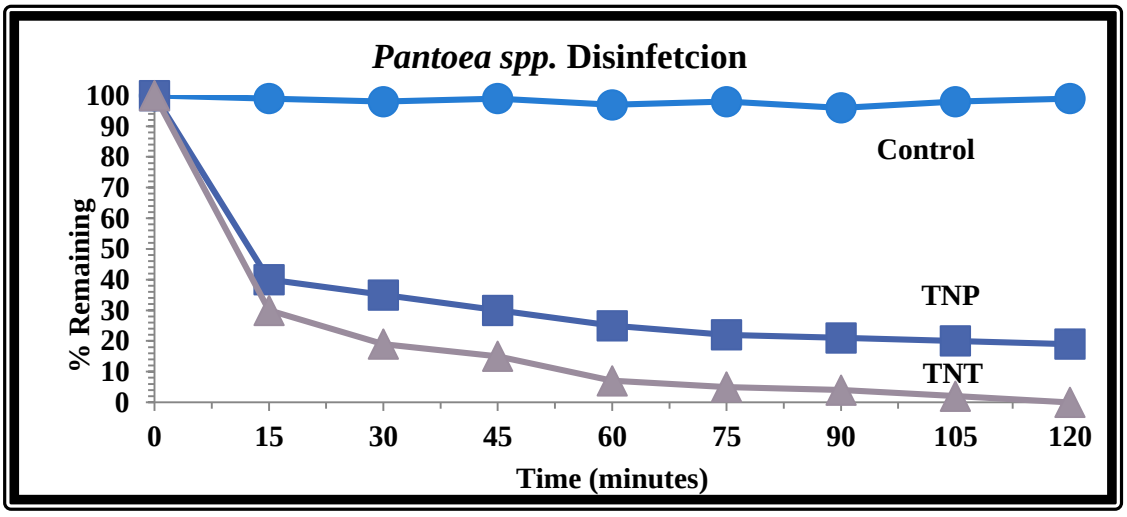


Figure 18: Disinfection of *Pantoea spp.* against TNP and TNT

In case of *Pseudomonas leutela*, initially cell count was 150×10^7 CFU/mL, however a sudden decrease was observed from coated sections (TNT and TNP) of computer keyboard and computer mouse after 15-30 minutes exposure to visible light (Figure 19). Again complete disinfection of *Pseudomonas leutela* was achieved within one hour with TNT coated surface of keyboard and mouse. It confirms the efficacy of TNTs (higher surface/volume ratio) for bacterial killing and producing self- sterilized

surfaces.

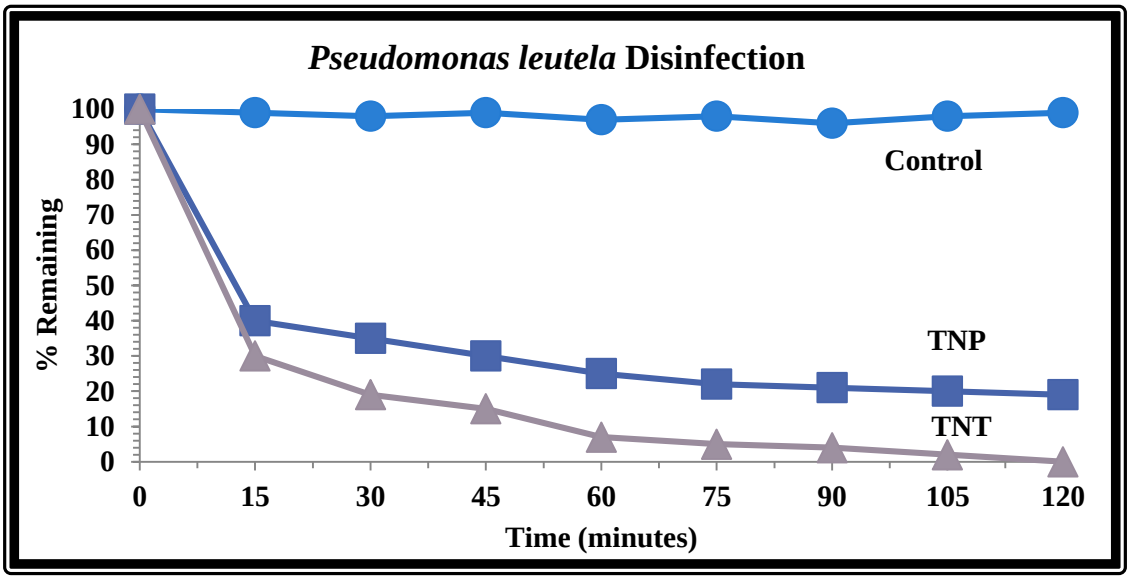


Figure 19: Disinfection of *Pseudomonas leutela* against TNP and TNT

Almost similar disinfection rate of *Pasteurella pneumotropica* was seen with both pure and 1% TNP and TNT coated wood surfaces at exposure to fluorescent light, to that of gram negative bacterial speices (Figure 20). This is most probably because all gram negative species belonging to the same genus having similar cell structure. About 80% and 90% bacterial population still survived at the pure TNP and TNT coated wood surfaces after the 120 minute of fluorescent light exposure, however, 90% of bacterial colonies were inactivated at 60 minutes of exposure to TNT coated surface of keyboard and mouse whereas complete disinfection was observed after 105 minutes of exposure to said surface in fluorescent light. Almost similar disinfection rate was observed in case of each identified strain i.e. *Chryseobacterium indologenes, Pasteurella pneumotropica, Serratia rubidaea, Burkholderia cepacia, Pseudomonas aeruginosa, Pseudomonas leutela, Thermococcus nautilus, Pantoae spp., Escherichia coli, Proteus vulgaris.*

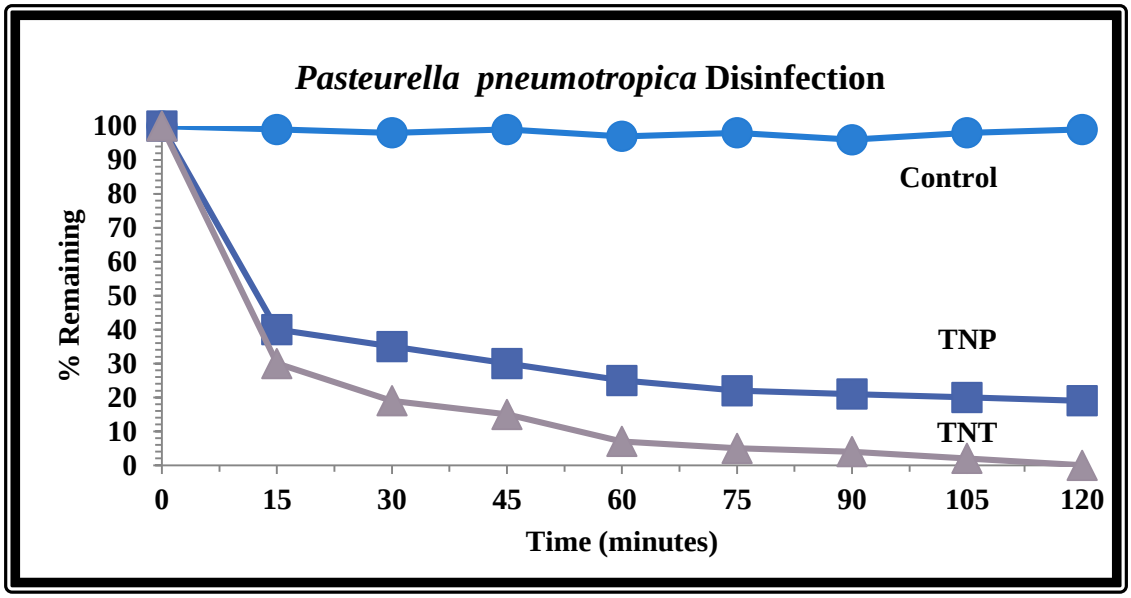


Figure 20: Disinfection of *Pasteurella pneumotropica* against TNP and TNT

Chapter 5

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

- A novel method of coating of keyboard and mouse surface was devised by using a commercial adhesive (GMSA) which is not only cost effective but also easy to apply as this doesn't require any industrial processing except brushing and sonication of slurry.
- Nanotube coated surfaces in our study showed remarkable reduction in airborne bacterial population. Results exhibit that Titania Nanotubes coated section of Keyboard and mouse surface are more effective (complete sterilization in almost 60-105 minutes under normal light) in killing P. aeruginosa and Staph. aureus than pure Titania Nanoparticles coated section of keyboard and mouse surface where almost 20-30% bacteria remain viable even after 2 hours of exposure to fluorescent light.
- Bacterial strains which were completely disinfected after 2 hour exposure to coated TNT surface under fluorescent light include: *Chryseobacterium indologenes, Pasteurella pneumotropica, Serratia rubidaea, Burkholderia cepacia, Pseudomonas aeruginosa, Pseudomonas leutela, Thermococcus nautilus, Pantoae spp., Escherichia coli, Proteus vulgaris*

5.2. Recommendations

1. The proposed coating method (for immobilization of Nanotubes on keyboard and mouse surface) can be implemented in an entire building in order to provide sanitized environment to the occupants.
2. The coating technique can be replaced by incorporating Titania nanotubes in Acrylonitrile Butadiene Styrene (ABS) polymer during process of key and mouse moulding.
3. Titania Nanotubes can be embedded in glass to provide self-sterilizing glass surfaces. Following hydrothermal treatment, Titania Nano-flowers may also be synthesized having potential to disinfect bacterial population.

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