DEVELOPMENT OF TITANIA (TiO_2) EMBEDDED POLYMER FOR SELF SANITIZING COMPUTER KEYBOARDS

By

Aashar Habib

(2011-NUST-MSPhD-EnvS-16)

A thesis submitted in partial fulfillment of

the requirements for the degree of

Master of Science

In

Environmental Science

Institute of Environmental Sciences and Engineering (IESE)
School of Civil and Environmental Engineering (SCEE)
National University of Sciences and Technology (NUST)
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It is certified that the contents and forms of the thesis entitled

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Aashar Habib

Has been found satisfactory for the requirements of the degree of
Master of Science in Environmental Science

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Dedicated to…..

My Beloved Parents and Aunt (late)

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Aashar Habib

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LIST OF ABBREVIATIONS

ABS	Acrylonitrile Butadiene Styrene
$Ag\text{-}TiO_2$	Silver doped Titanium Dioxide
CFU/ml	Colony Forming Unit per milli liter
e^-	Electron
EDS	Energy Dispersive Spectroscopy
eV	Electron Volt
FWHM	Full width of a diffraction line at half of maximum intensity
GPR	General Purpose Reagent
h^+	Hole
IESE	Institute of Environmental Sciences and Engineering
LI	Liquid Impregnation
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscopy
TiO_2	Titanium Dioxide
UV	Ultra Violet
XRD	X - Ray Diffraction
λ	Wavelength

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ABSTRACT

Computer keyboards in general, but those having multiple users in particular are likely to be severely contaminated and could serve as a source of infection. The goal of this research was to develop a polymer for computer keyboards which could disinfect their surfaces in the presence of fluorescent room light. Pure TiO_2 and Ag doped TiO_2 nanoparticles, having average particle size of 69 nm, were prepared by using calcination and liquid impregnation methods respectively. These nanoparticles were characterized by using Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and X - Ray Diffraction (XRD) techniques which revealed the surface morphology, elemental composition, size and crystalline structure of nanoparticles. A coating of Acrylonitrile Butadiene Styrene (ABS) film having 2 % Ag - TiO_2 nanoparticles was applied on keyboard buttons. This surface while being found fairly effective against the general aero-flora was very effective against *Pseudomonas aeruginosa*, taken as a model pathogenic microorganism, which was 100 % disinfected after 2 hours with 2 % Ag - TiO_2 nanoparticles embedded ABS polymer film. Coating of ABS polymer having 2 % Ag - TiO_2 nanoparticles on computer keyboards could render their surface to be self-sanitizing.

Chapter 1

INTRODUCTION

Use of computers in every field of life shows boost of technology but at the same time it also helps to spread infectious diseases in the society. This is due to the fact that computers usually have multiple users so keyboards act as a potential reservoir for pathogenic microorganisms. Computer keyboards may have major threats in computer laboratories (schools, universities, etc.), internet cafes or hospital environment. This may be due to poor hygienic practices, lack of awareness or lack of policy implementation in work stations. A number of efforts have been made by scientists to develop methodologies or techniques to disinfect keyboard surfaces. This research study provides a solution in this regard by exploiting the advantages of nanotechnology.

1.1 Scope of Work

Computer keyboards are made up of Acrylonitrile Butadiene Styrene (ABS) polymer. The current study is focused at the development of polymer for computer keyboards which could disinfect itself against pathogenic microorganisms by using photocatalytic activity of TiO_2 nanoparticles. Although, a variety of microorganisms are present in the environment that can cause infection, *Pseudomonas aeruginosa* was selected and used as a model pathogenic microorganism for this research work.

1.2 Significance of Work

Infectious agents are present almost everywhere around us. These may be present on everything which is in our daily use e.g. computer keyboards, mouse, remote controls, mobile phones, laptops, and other electronic devices. Infectious agents have three main roots of entry in human body (dermal, ingestion and inhalation). If a computer keyboard is used by any infected person, the keyboard buttons may get contaminated by the infectious agents like bacteria, virus, etc. These contaminated buttons may infect a healthy subsequent user of this keyboard. There may, thus be chances of spread of a particular disease which can be controlled by developing self-sanitizing computer keyboard materials. The present study provides a solution by developing TiO_2 embedded polymer for computer keyboards.

1.3 Objectives

Objectives of the research study are:

i. To synthesize TiO_2 nanoparticles;

ii. To develop TiO_2 nanoparticles embedded polymer film of Acrylonitrile Butadiene Styrene (ABS) for computer keyboards;

iii. To observe effectiveness of TiO_2 embedded polymer film of ABS.

1.4 Letter Count - An Overview

Letter frequency depends on the language and has major effect on cryptography and many puzzle games e.g. Scrabble, Hangman, etc. Letter frequency also has strong impact on design of computer keyboard layouts. In previous times, letter counts were done manually. In modern era of technology, different softwares have been developed which can count keystrokes of a computer keyboard easily. In this study, ‘Keystroke Counter and Frequency Recorder Software (Trial Version)' was used to find the frequency of keystrokes on computer keyboards.

Chapter 2

LITERATURE REVIEW

2.1 Nanotechnology

Prefix ‘nano’ signifies 10^{-9} (one billionth or 0.000000001) of anything. Nanotechnology, thus refers to working with atoms or molecules at nanoscale. Nanoscale ranges from 1 – 100 nm. The U.S Government defines nanotechnology as (Davies, 2009);

“the way discoveries made at the nanoscale are put to work”

Nanoscale materials have different physical, chemical and biological properties as compared with their larger structures (e.g., titania, carbon, iron, etc.). Nanomaterials are more reactive because they have greater surface area to mass ratio as compared to their bulk materials. As most of the biological and chemical reactions take place at surface so nanoscale particles are considered more reactive than bulky materials (Davies, 2009).

2.1.1 Applications of Nanotechnology

Nanotechnology has its wide applications almost in every field of life while next industrial revolution is based on nanotechnology. Advance version of nanotechnology will have significant impacts on all industries so it is also referred as a general purpose technology (Bhattacharyya *et al*., 2009). It is going to be used in environmental cleanup processes, food technology, medical applications, material science, manufacturing processes, national security, electronics, information technology, energy generation and storage, etc.

Environmental damage and pollution can be repaired with the help of nanotechnology. This is so because of photocatalytic action of nanoparticles especially TiO_2 nanoparticles. Many nanotechnology related products have been launched in market e.g., bandages, heart valves, scratch less glass and paints, sunscreens, electronic components, nano films, sports equipments (European Commission, 2004; Bhattacharyya *et al*., 2009).

2.1.2 Titanium Dioxide (TiO_2)

In nature, TiO_2 is normally found in abundance in the form of ilmenite ($FeTiO_3$), rutile (TiO_2) and sphene ($CaSiTiO_5$). Generally, TiO_2 has three crystalline forms named as anatase, rutile and brookite (US-EPA, 2010). Anatase has higher photocatalytic activity due to which it is used for photocatalysis while rutile has more thermodynamic stability (Stamate *et al*., 2007; Kumar *et al*., 2012). Amorphous phase of TiO_2 starts converting into anatase phase above 250 ^{0}C and anatase phase remains stable up to 650 ^{0}C. Above 650 ^{0}C anatase phase starts converting into rutile phase which melts at 1850 ^{0}C. TiO_2 is relatively cheap, recyclable, reusable and simple to produce. In addition to this, synthesis of TiO_2 nanoparticles is also very simple and easy as compared to others (Ahmad *et al*., 1994).

2.1.3 Photocatalytic Activity of TiO_2

Photocatalytic activity of TiO_2 was first discovered by Fujishima and Honda in 1972 (Fujishima *et al*., 1972). They discovered that in the presence of light, electrons from valence band of TiO_2 are promoted to conduction band. This movement of electrons creates an electron-hole pair (e^- - h^+).

$$TiO_2 + h_v \rightarrow e^- + h^+ \qquad \text{(Tan } \textit{et al.}\text{, 2011)}$$

This electron-hole (e^- - h^+) pair can recombine or interact with other molecules (Behnajady *et al.*, 2008). Holes created in valance band take part in oxidation reactions while electrons in conduction band take part in reduction reactions of interacting molecules (Stamate *et al*., 2007).

Oxidation reaction;

$$h^+ + H_2O \rightarrow {}^{\bullet}OH + H^+ \qquad \text{(Tan } et\ al.\text{, 2011)}$$

Reduction reaction;

$$e^- + O_2 \rightarrow O_2^{\bullet -} \qquad \text{(Tan } et\ al.\text{, 2011)}$$

Holes created at valence band can interact with water molecules and hydroxyl ions (OH^-) and other Reactive Oxygen Species (ROS) can be generated. These hydroxyl (OH^-) or Reactive Oxygen Species (ROS) interact with any organic molecule present at or near the surface of TiO_2 and oxidizes it leaving behind CO_2 and H_2O as an end product (Osburn, 2008).

$${}^{\bullet}OH + \text{Pollutant} + O_2^- \rightarrow CO_2 + H_2O \quad \text{(Tan } et\ al.\text{, 2011)}$$

2.1.4 Photocatalytic Activity of Ag-TiO_2

Chances exist for the recombination of electron-hole (e^- - h^+) pair which may reduce the photocatalytic activity of TiO_2. Metal (Ag, Pt, Fe, etc.) doping of semiconductor (TiO_2) reduces recombination of these electron-hole (e^- - h^+) pair. Studies have shown that doping of silver metal with TiO_2 has reduced recombination of electron-hole (e^- - h^+) pair significantly and Ag doping of TiO_2 nanoparticles increases photocatalytic ability (Behnajady *et al.*, 2008).

Photocatalytic activity only triggers when incident light has energy greater than band gap of the semiconductor (Fujishima *et al*., 2000; Qureshi, 2012). TiO_2 has band gap of 3.2 electron volts (E = 3.2eV) which requires incident light having wavelength of lower than 388 nm for photocatalytic activity (Stamate *et al*., 2007; Blake *et al*., 1999). In ordinary circumstances, photocatalytic activity of TiO_2 begins when UV radiation falls on it while sunlight has only 5 % UV radiation (Osburn, 2008). So, band gap of TiO_2 has to be reduced to start photocatalytic activity even in ordinary sunlight. For this purpose, doping of TiO_2 is done with some suitable metal which ultimately narrow downs band gap.

2.1.5 Applications of TiO_2 Nanoparticles

TiO_2 nanoparticles have emerged as an excellent photocatalyst semiconductor which is being used in many fields of life. Especially, it has wide applications in the field of environment (Fujishima *et al*., 2000).

Some practical applications of TiO_2 photocatalysis are mentioned below (Stamate *et al*., 2007):

- Anti bacterial effect;
- Self cleaning effect;
- Air cleaning effect;
- Anti fogging effect;
- Water treatment.

2.2 Keyboards

Computers are being used in every field of life e.g. hospitals, offices, homes, industries, educational institutes, laboratories, etc. Keyboard is an essential part of a computer which remains mostly in touch with human beings. It is susceptible to be contaminated with different types of harmful microorganisms. Multiple user computer keyboards are likely to have a higher number of microorganisms as compared to single user computer keyboards and are thus considered as potential reservoir for microorganisms (Anderson *et al*., 2009). Now a days, keyboards have become potential threat for spreading of infectious diseases in schools, universities, hospitals, or anywhere they are going to be shared (Marsden, 2009).

2.2.1 Disinfection of Keyboards

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

Plastic wraps: When keyboards are not in use they are wrapped up with plastic sheets while issue associated with this technique is that it looks unaesthetic.

Rubber caps: Keyboard buttons are 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 (Fukada *et al*., 2008; Rutala *et al*., 2006). 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 tedious process requiring a lot of time.

Touch sensitive: A glass or acrylic top having touch sensitive keys is considered as an effective solution in this regard. It only requires less than 15 seconds for cleaning and disinfection. Although, it saves time but it is an expensive gadget.

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 is not in use and is slid back into the table. Computer keyboards are made of Acrylonitrile Butadiene Styrene (ABS) polymer. 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.

In our daily busy routine of life, use of above mentioned disinfection techniques are very much time consuming. So, this is need of the hour to develop such an efficient method which should not be time consuming, cheap and have more efficiency than others. Nanotechnology provides us a solution in this regard and TiO_2 nanoparticles are going to be used for development of self-sanitizing computer keyboards. Photocatalytic activity of TiO_2 nanoparticles makes it a suitable option to be used for disinfection of microbes. This research work develops a polymer which can be used for manufacturing of self-sanitizing computer keyboards.

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 death of microorganisms. A number of studies have been conducted in this regard. First bactericidal study of this nature was studied with *Escherichia coli* and 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 (Haung *et al*., 1999). *Streptococcus mutans* is a pathogenic microorganism which causes infection during dental surgery. It's 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 (Ibanez *et al.*, 2003).

Ag-TiO_2 has more photocatalytic activity as observed during a study comparing the efficiency of pure and Ag doped titania coated surfaces by killing *Pseudomonas aeruginosa* and *Bacillus subtilis* (Khan *et al*., 2013). Fe nanotube coated surfaces also proved effective regarding disinfection of *Pseudomonas aeruginosa* and *Staph aureus* (Latif, 2013).

2.2.3 *Pseudomonas aeruginosa* - A Pathogenic Microorganism

A variety of pathogenic microorganisms can stick on computer keyboard buttons from atmosphere or hands of the computer users. *Pseudomonas aeruginosa* is considered as a model pathogenic microorganism which was used in the proposed research work. It causes 10 - 20 % nosocomial infections (Leclerc *et al.*, 2002).

Pseudomonas aeruginosa is also known as an opportunistic microorganism which attacks through susceptible tissues, wounds, mucous membranes, burns, surgical wounds, etc. Health impacts of *Pseudomonas aeruginosa* are headache, skin infection, pneumonia and even death in severe cases (Angela *et al*., 2010).

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 *et al.*, 1986). Chemical structures of above mentioned monomers are shown in Figure 2.1.

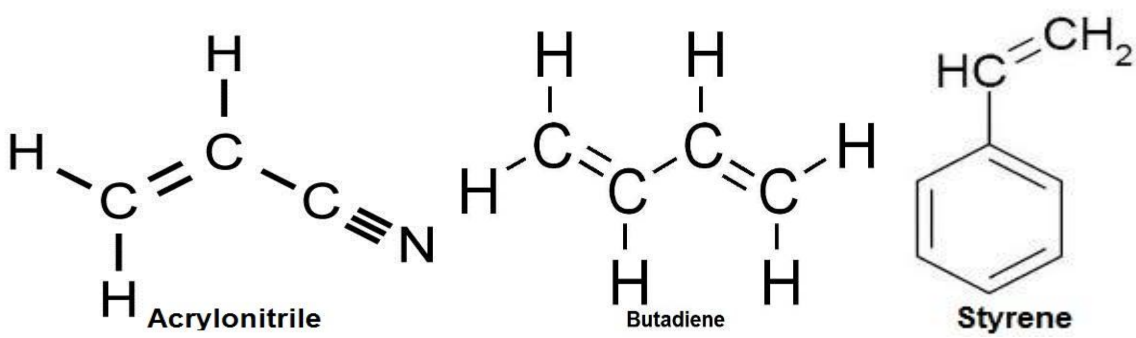

Figure 2.1: Chemical Structures of Acrylonitrile Butadiene Styrene (ABS) Monomers

Each monomer has its own specific properties while ratio of these monomers (acrylonitrile, butadiene and styrene) decides 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 2.1 (Loctite, 2011).

Table 2.1: Physical Properties of ABS

Melting Point	105^0C
Tensile Strength	2.8-8.4kg/$cm^2 \times 10^2$
Density	1.02 to 1.28 g/cm^3
Hardness	96-125 Rockwell
Thermal Conductivity	0.17-0.23W/m -^{0}K

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 going to be used for the manufacturing of computer keyboards, telephones, bath tubs, toys, sports goods, etc.

Chapter 3

MATERIALS AND METHODS

3.1 Materials

Computer keyboards are made up of Acrylonitrile Butadiene Styrene (ABS) polymer so ABS polymer for research work was obtained from old computer keyboards. Chloroform (Merck, Germany) was used as a solvent for development of Acrylonitrile Butadiene Styrene (ABS) polymer films while acetone (Merck, Germany) was used in cleaning and film pasting process on buttons. Titanium Dioxide (GPR, BDH Chemicals Ltd. England) was used for the synthesis of pure TiO_2 nanoparticles while Silver Nitrate (Merck, Germany) was used for synthesis of Ag doped TiO_2 nanoparticles. Solidified agar petri plates were prepared with the help of Nutrient Agar (Merck, VM 100650 943).

Software named as 'Keystroke Counter and Frequency Recorder (Trial Version)' was used to find the frequency of keystrokes on computer keyboards developed by 'Sobolsoft'.

3.2 Methods

3.2.1 Synthesis of Pure TiO_2 Nanoparticles

Calcination method (a physical method) was adopted for the synthesis of pure TiO_2 nanoparticles. TiO_2 nanoparticles were synthesized after placing Titanium Dioxide General Purpose Reagent (50 g) in a muffle furnace for 6 hours at 400 ^{0}C.

3.2.2 Synthesis of Ag - TiO_2 Nanoparticles

Liquid Impregnation (LI) method was used for the synthesis of Ag - TiO_2 nanoparticles. It involves following major steps:

Mixing: Slurry of 1 % Ag - TiO_2 nanoparticles was prepared in water by mixing 48.95 g of TiO_2 GPR and 1.05 g of $AgNO_3$ in a beaker and continuous stirring for 24 hours so that proper mixing of TiO_2 GPR and $AgNO_3$ could take place. Similarly, slurry of 2 % Ag - TiO_2 nanoparticles was prepared in water by mixing 47.9 g of TiO_2 GPR and 2.1 g of $AgNO_3$ and stirring for 24 hours so that proper mixing of said chemicals could take place.

Settling: These solutions were allowed to settle for another 24 hours in separate beakers so that proper settling of solutions could take place.

Drying: After removing of the supernatant the solid material was placed in oven for 12 hours at 105 ^{0}C so that water can be evaporated.

Calcination: After drying, dried materials were crushed properly in mortar and pastel and placed in china dishes separately. These china dishes were placed in muffle furnace for 6 hours at 400 ^{0}C.

3.2.3 Preparation of ABS Film

1.5 g of ABS polymer was dissolved in 50 ml of chloroform and stirring for 15 minutes. The solution was poured into a clean petri plate and allowed to evaporate for 24 hours. After evaporation, a thin, fine and smooth film of ABS polymer was obtained.

3.2.4 Preparation of Nanoparticles (Ti O_2, 1% Ag - TiO_2, 2% Ag - TiO_2) Embedded ABS Films

An optimized quantity (0.075 g) of nanoparticles (TiO_2, 1% Ag - TiO_2, 2% Ag - TiO_2) was added in the above mentioned solution of ABS polymer and chloroform. Stirring of 15 minutes was allowed and solution was placed in sonicator for 30 minutes so that nanoparticles can spread uniformly in the said solution. Finally, this solution was poured into petri plate and solvent (Chloroform) was allowed to evaporate for 24 hours. In this way, nanoparticles (TiO_2, 1% Ag - TiO_2, 2% Ag - TiO_2) embedded ABS films were prepared.

3.2.5 ABS Film Coating on Buttons

Keyboard buttons were dipped in acetone just for 5 to 10 seconds so that ABS polymer surface of buttons becomes active. After this, buttons were dipped in a solution of ABS polymer and chloroform having 2 % Ag - TiO_2 nanoparticles (as selected for coating, discussed in up-coming section) so that a thin and fine layer of ABS polymer having 2 % Ag-TiO_2 nanoparticles could stick to these. At the end, these buttons were dried in open air.

3.2.6 Selection of Nanoparticles for Button Coating

TiO_2, 1 % Ag - TiO_2 and 2 % Ag - TiO_2 nanoparticles embedded ABS films were prepared for the selection of efficient nanoparticle coating on computer keyboard buttons. For this purpose, one pure ABS film and three nanoparticles (TiO_2, 1 % Ag - TiO_2 and 2 % Ag - TiO_2) embedded ABS films were exposed to ambient air for 48 hours and results were obtained by using streak plate method.

3.2.7 Culture Preparation for *Pseudomonas aeruginosa*

Sterilized solution of 200 ml of nutrient broth (Merck, VM 747243 703) was taken in a flask and 1 ml of already existing *Pseudomonas aeruginosa* culture was mixed with it. This solution was incubated in an incubator having orbital shaker for 24 hours at 37 ^{0}C.

3.2.8 Preparation of Nutrient Agar Plates

Preparation of solidified plates of nutrient agar involved following major steps:

- Washing and drying of petri plates;
- Sterilization of petri plates for 15 min at 121 ^{0}C;
- Oven drying of petri plates at 105 ^{0}C;
- Pouring of sterilized molten nutrient agar in petri plates;
- Incubation of petri plates for 24 hours at 37 ^{0}C.

3.2.9 Streak Plate Method

In this method, a microbial sample is taken with the help of a sterile cotton swab and streaked over a solidified agar plate. These plates are incubated in an incubator for 24 hours at 37 ^{0}C. Colonies of bacteria can be observed after incubation and each colony is considered as a single microbial cell (Prescott, 2002). Figure 3.1 shows schematic of streak plate method.

3.2.10 Serial Dilution Method

A microbial sample is taken and diluted up to desired concentration (in current scenario *Pseudomonas aeruginosa* was diluted up to 10^{-5} times). 1 ml of diluted sample is taken and spread over solidified petri plate with the help of sterilized spreader and incubated (Prescott, 2002). Figure 3.2 shows schematic of serial dilution method.

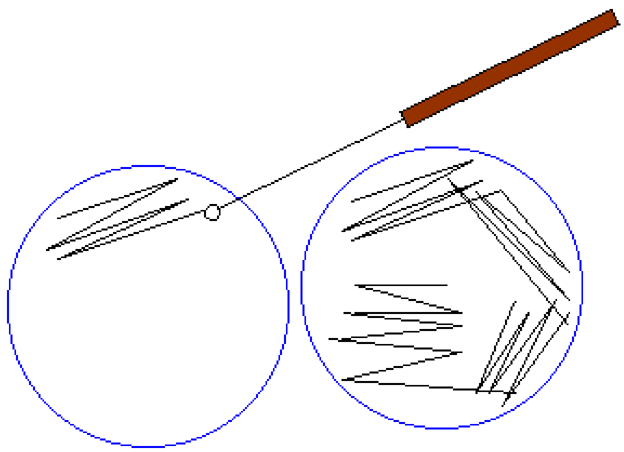

Figure 3. 1: Schematic of Streak Plate Method

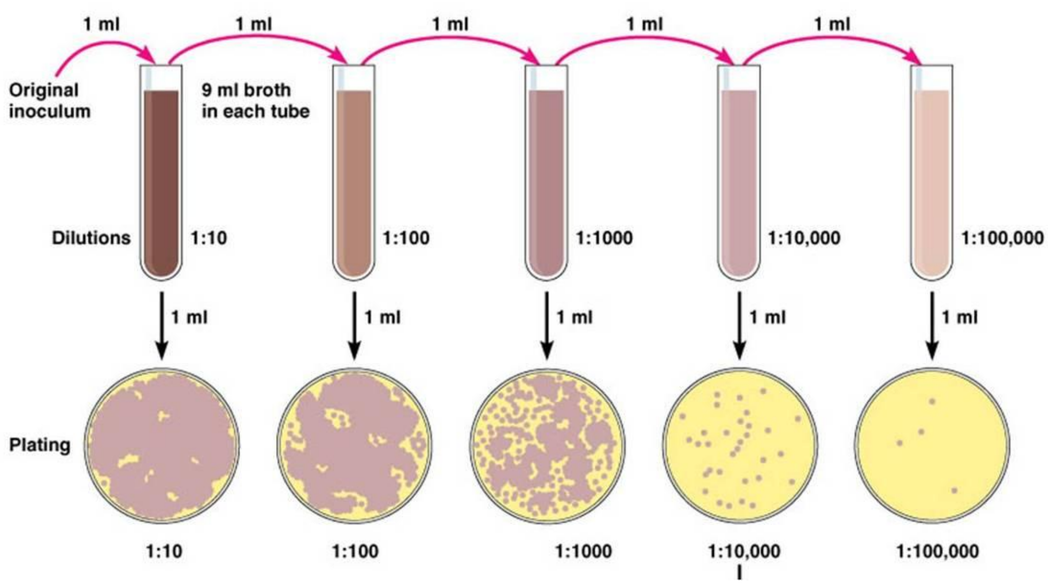

Figure 3. 2: Schematic of Serial Dilution Method

3.3 Characterization of Nanoparticles

X-Ray Diffraction (XRD) analysis of the pure and doped TiO_2 nanoparticles was done to find out the crystalline structure and particle size of the pure and doped TiO_2 nanoparticles. Scanning Electron Microscopy (SEM) analysis revealed the surface morphology of nanoparticles and provided images in this regard. Energy Dispersive Spectroscopy (EDS) analysis provided elemental composition of nanoparticles. It also provided percentages of composition for each element.

3.4 Experimental Setups

3.4.1 Experiment with *Pseudomonas aeruginosa*

Spray of *Pseudomonas aeruginosa* was made on TiO_2, 1 % Ag - TiO_2 and 2 % Ag - TiO_2 nanoparticles embedded ABS films with the help of atomizer. Samples were taken with the help of sterilized cotton swabs after regular interval of 30 minutes (0, 30, 60, 90 and 120 min) for two hours. Serial dilution of each bacterial sample was done up to 10^{-5}. 1 ml 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 ^{0}C and CFU/ml were counted on a colony counter.

3.4.2 Experiment with General Aero-flora

An experimental setup was designed to check the effectiveness of 2 % Ag - TiO_2 nanoparticles coated buttons against uncoated buttons. For this purpose, 'Keystroke

Counter and Frequency Recorder Software (Trial Version)' was used to find out the frequencies of keystrokes on computer keyboards. Based upon recorded frequencies, two major groups were devised named as 'Group I' and 'Group II' in such a way that both the groups have the same frequency as shown in Table 4.3.

Buttons belonging to 'Group II' were coated with ABS polymer layer containing 2 % Ag - TiO_2 nanoparticles while buttons belonging to 'Group I' were left uncoated. Figure 3.3 shows a schematic of a computer keyboard having 2 % Ag - TiO_2 coated and uncoated buttons. Buttons belonging to both groups were re-fixed on the keyboard and this keyboard was replaced with a keyboard which was present on Computer Catalogue of Institute of Environmental Sciences and Engineering (IESE) Library for the period of one week. Computer Catalogue of library was chosen because keyboard of this system is used by a diverse group of users.

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 the period of 24 hours at 37 ^{0}C. After 24 hours of incubation, colonies of microorganisms were counted with the help of colony counter in terms of Colony Forming Units (CFU) and presented in Table 4.3.

Figure 3. 3: Schematic of a Computer Keyboard

Chapter 4

RESULTS AND DISCUSSION

4.1 Characterization Results

4.1.1 X - Ray Diffraction (XRD) Analysis

XRD identifies crystalline phase of the nanoparticles by measuring diffraction of X-rays. This also helps to find out particle size of TiO_2 nanoparticles. TiO_2 nanoparticles used in this study ranged from 55 – 78 nm while average particle size of nanoparticles is 69 nm. Average particle size of each category of nanoparticles (TiO_2, 1 % Ag - TiO_2 and 2 % Ag - TiO_2) is shown in Table 4.1. Peaks of XRD results reveal that nanoparticles have crystalline structure (see Figures 4.1 - 4.3).

Table 4.1: Average Particle Size of Nanoparticles

Nanoparticles	Average Size (nm)
Pure TiO_2	55
1 % Ag - TiO_2	75
2 % Ag - TiO_2	78

Average crystalline size of nanoparticles was determined by using Scherer formula (Younas, 2011):

$$L = \frac{k\lambda}{\beta \cos\theta}$$

Where,

L = Average particle size

k = 0.891, a shape factor of spherical particles

λ = 0.1542, wavelength of X-Rays

β = Full width of a diffraction line at half of maximum intensity (FWHM)

θ = Diffraction angle of crystal phase

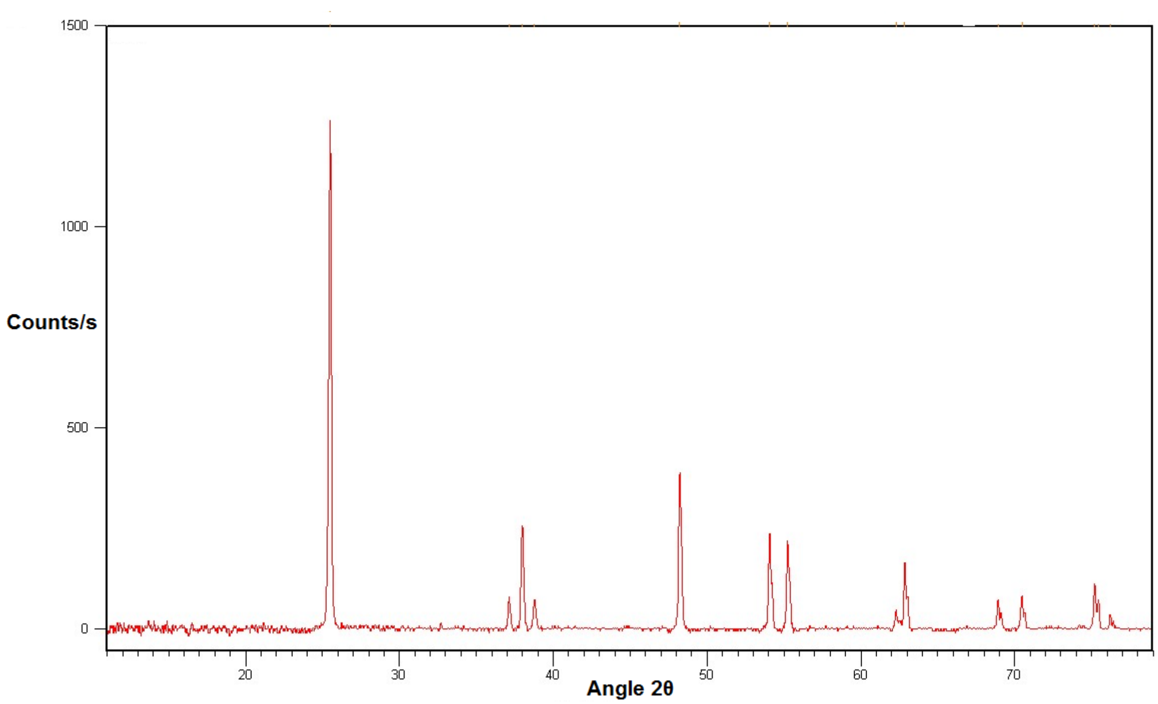

Figure 4.1: XRD Pattern of Pure TiO_2 Nanoparticles

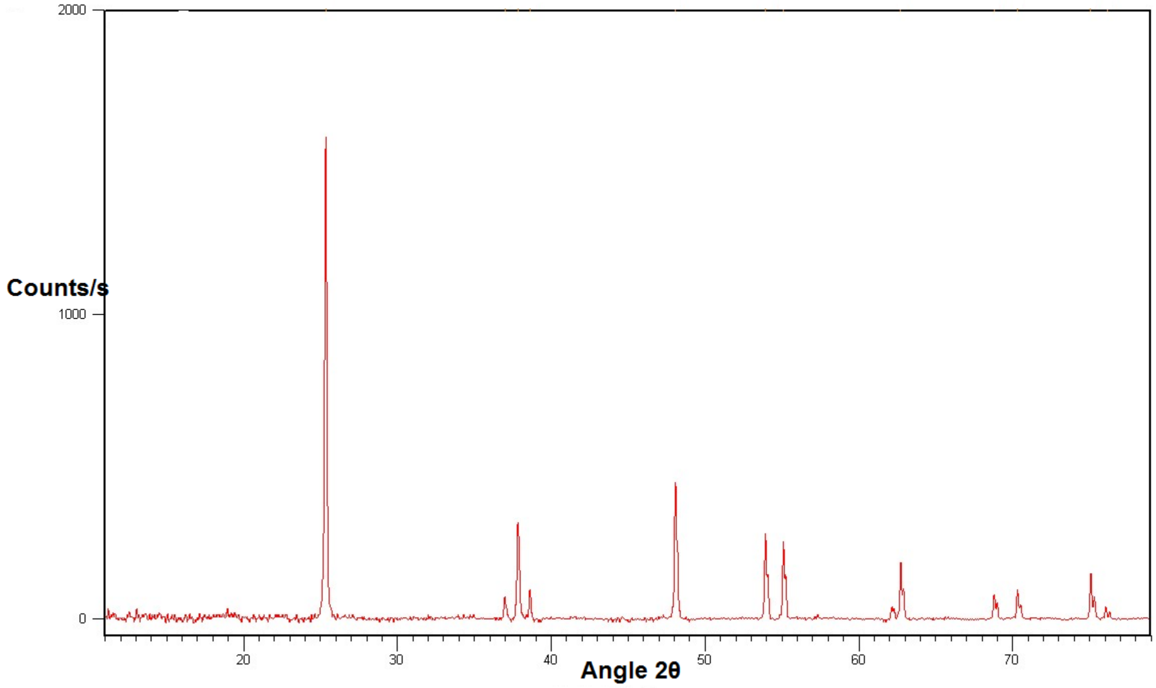

Figure 4.2: XRD Pattern of 1 % Ag - TiO_2 Nanoparticles

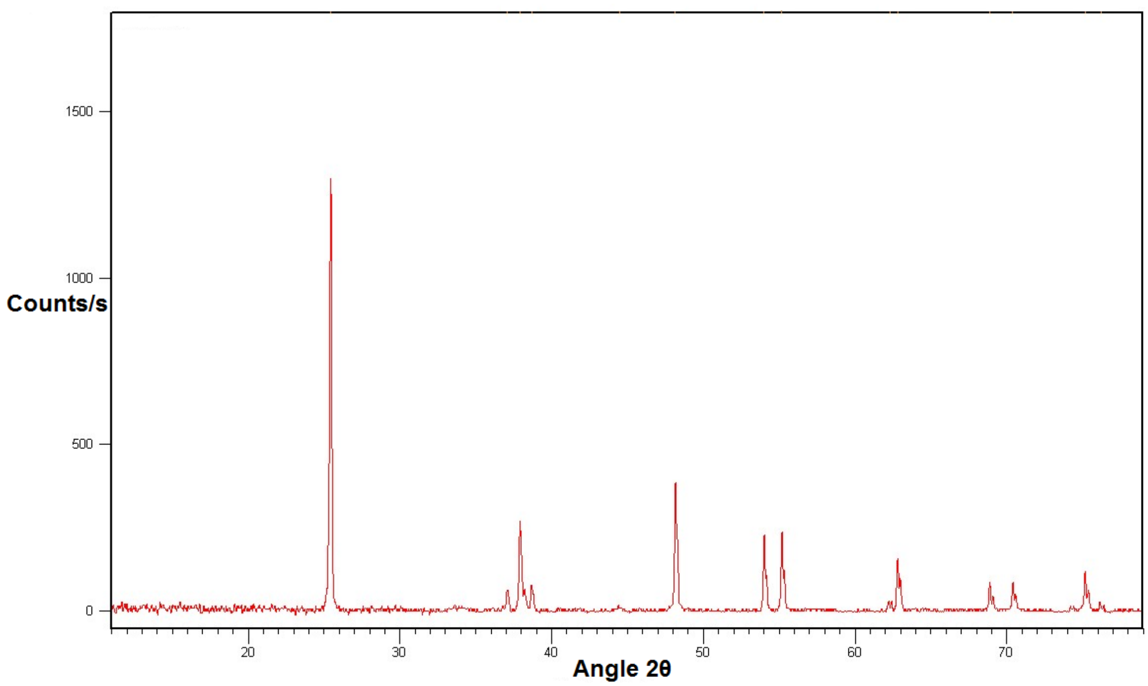

Figure 4.3: XRD Pattern of 2 % Ag - TiO_2 Nanoparticles

4.1.2 Scanning Electron Microscopy (SEM) Analysis

SEM analysis reveals the surface morphology of the pure TiO_2, 1 % Ag doped TiO_2 and 2 % Ag doped TiO_2 nanoparticles. Images obtained from SEM show that pure TiO_2 nanoparticles have more spherical shape as compared to 1 % Ag doped TiO_2 and 2 % Ag doped TiO_2 nanoparticles. Following order of nanoparticles shows degree of spherical shape.

$$TiO_2 > 1\ \%\ Ag - TiO_2 > 2\ \%\ Ag - TiO_2$$

Figures 4.4 - 4.9 show surface morphology of pure and doped nanoparticles at resolution of X 10,000 and X 50,000. These figures depict that Ag metal is not uniformly distributed on the surface of nanoparticles due to which irregularity increases as doping of metal increases.

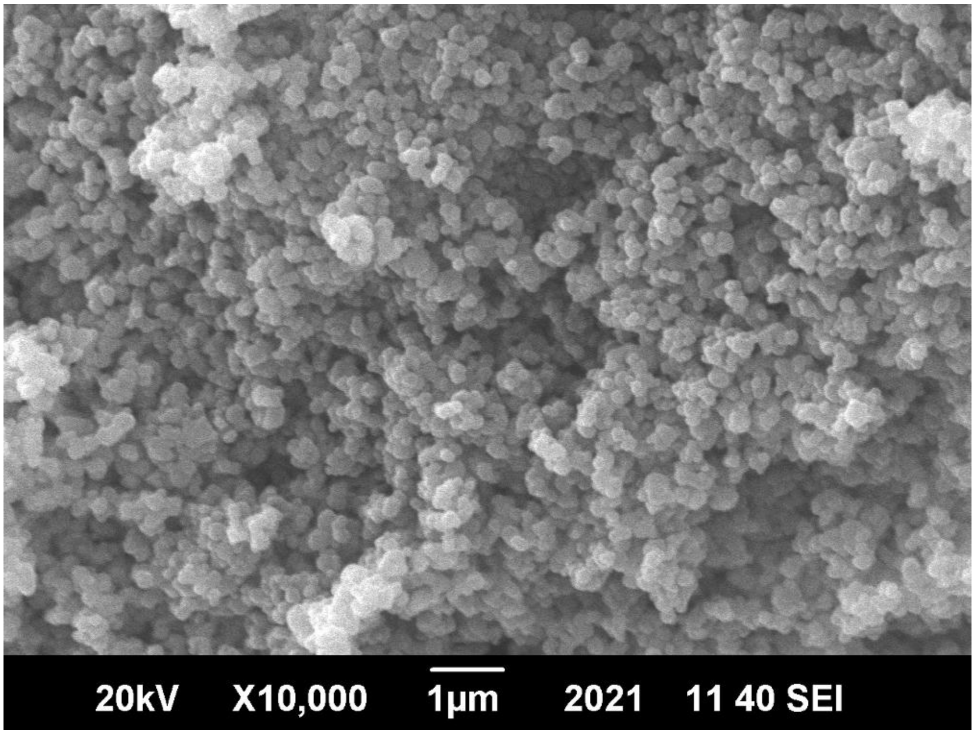

Figure 4.4: SEM Image of Pure TiO_2 Nanoparticles at X 10, 000

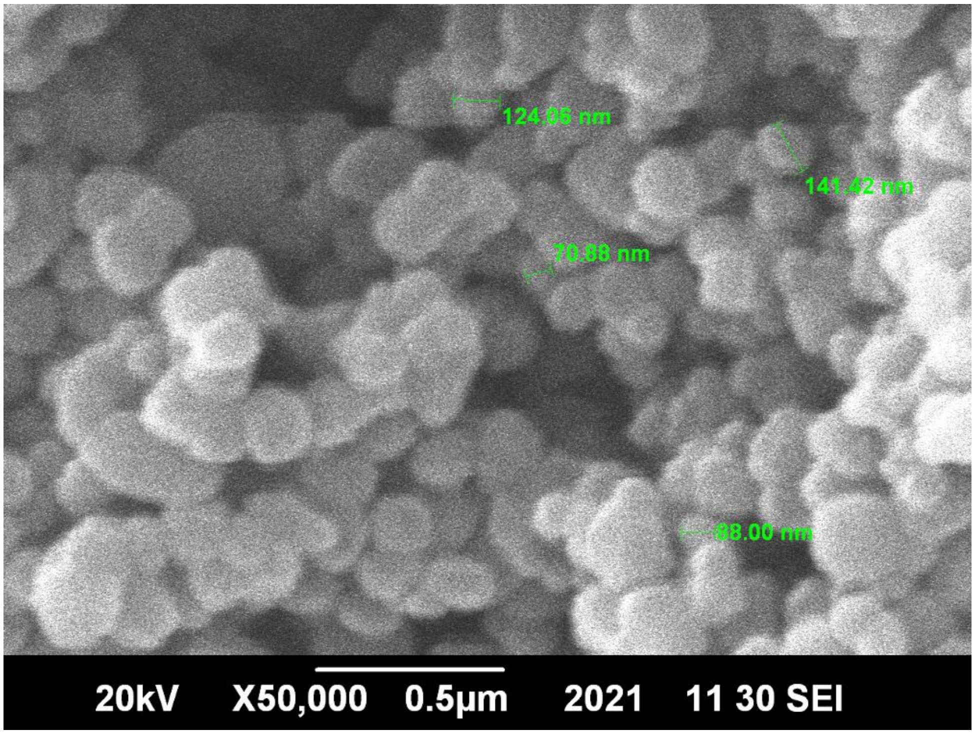

Figure 4.5: SEM Image of Pure TiO_2 Nanoparticles at X 50, 000

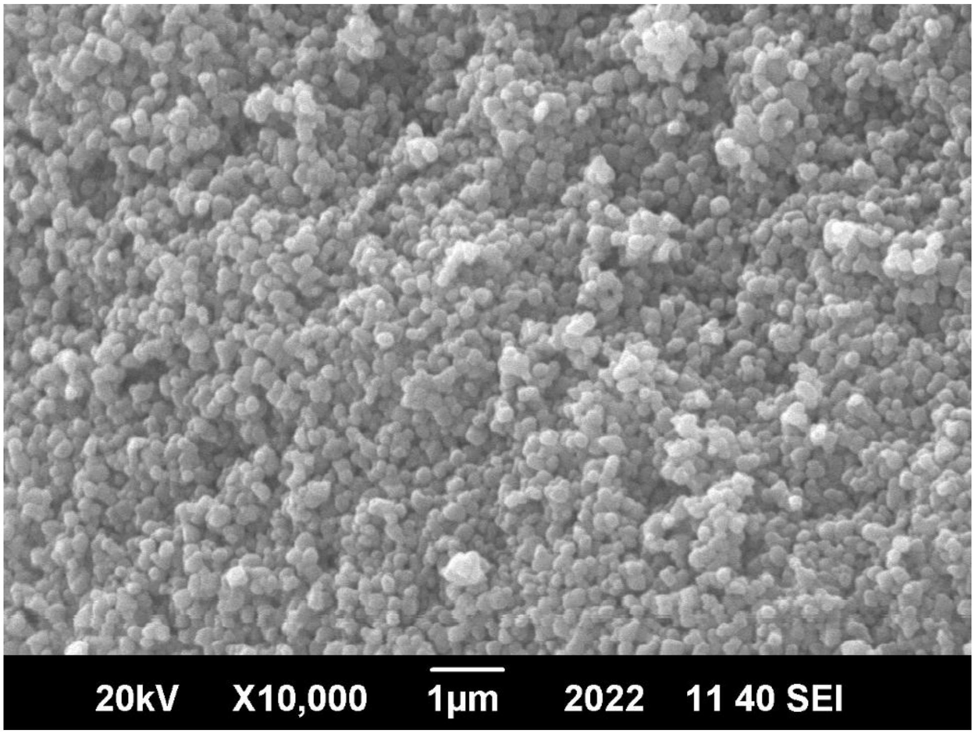

Figure 4.6: SEM Image of 1 % Ag - TiO_2 Nanoparticles at X 10, 000

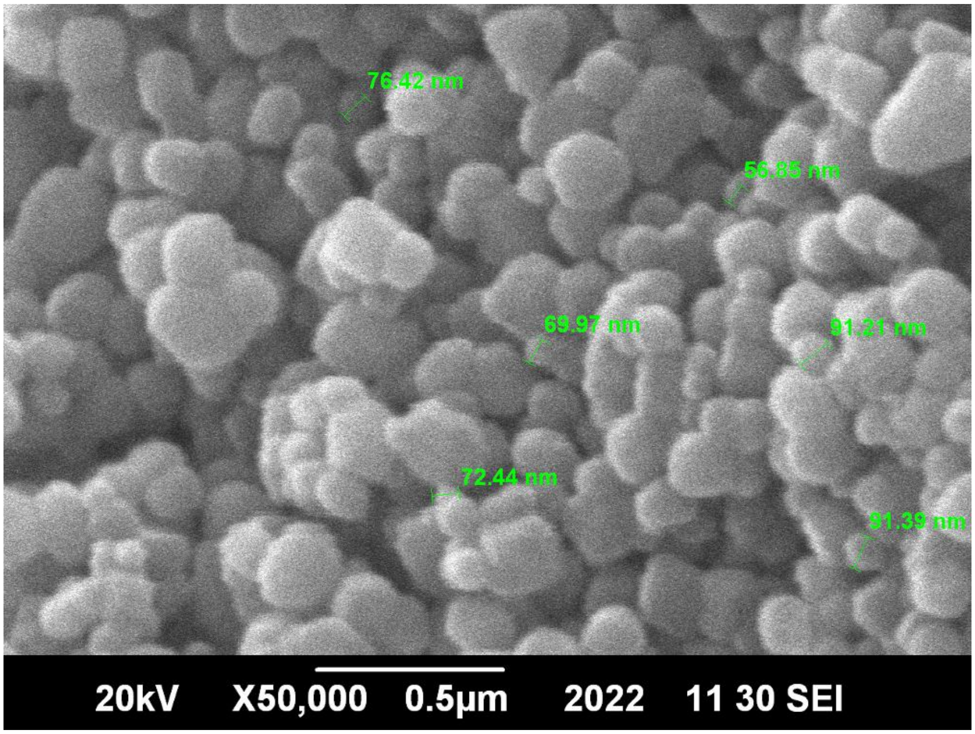

Figure 4.7: SEM Image of 1 % Ag - TiO_2 Nanoparticles at X 50, 000

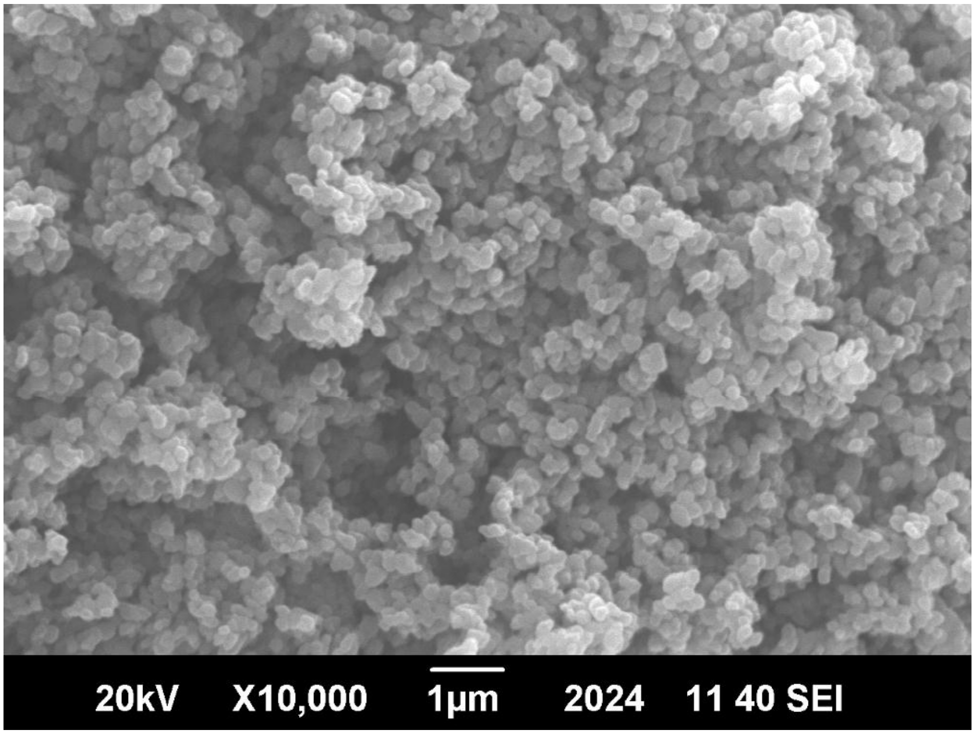

Figure 4.8: SEM Image of 2 % Ag - TiO_2 Nanoparticles at X 10, 000

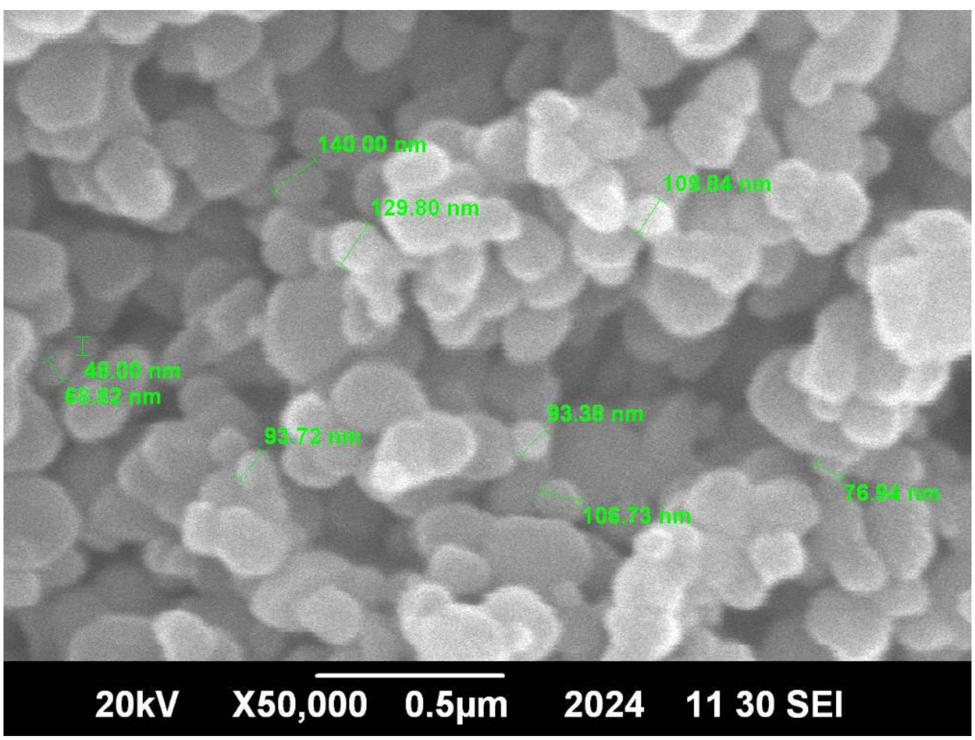

Figure 4.9: SEM Image of 2 % Ag - TiO_2 Nanoparticles at X 50, 000

4.1.3 Energy Dispersive Spectroscopy (EDS) Analysis

EDS examines elemental composition of pure TiO_2, 1 % Ag - TiO_2 and 2 % Ag - TiO_2 nanoparticles. Figures 4.10 - 4.12 and Table 4.2 shows relative elemental mass composition of each category of nanoparticles (TiO_2, 1 % Ag - TiO_2 and 2 % Ag - TiO_2). This also confirms that nanoparticles contain silver and titania only while no alien element or impurity is introduced in the synthesis process.

Table 4.2: EDS Results of Nanoparticles

Sr. No.	Nanoparticles	Relative Elemental Mass Ratios		
		Ti	O	Ag
1.	Pure TiO_2	59.75	40.25	0
2.	1 % Ag - TiO_2	49.74	49.06	1.19
3.	2 % Ag - TiO_2	49.90	47.77	2.33

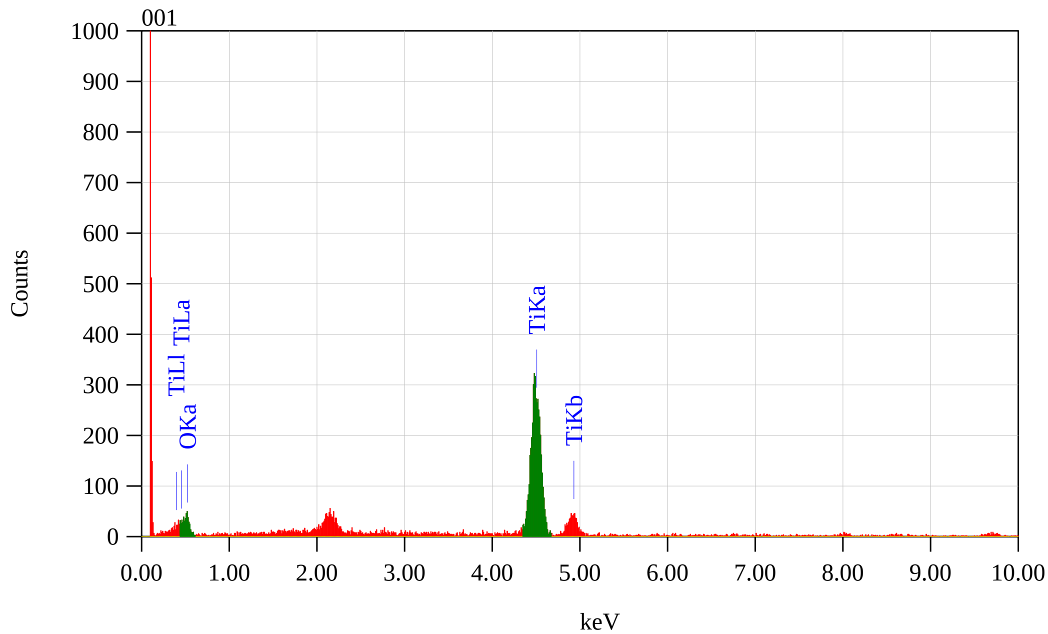

Figure 4.10: EDS Image of Pure TiO_2 Nanoparticles

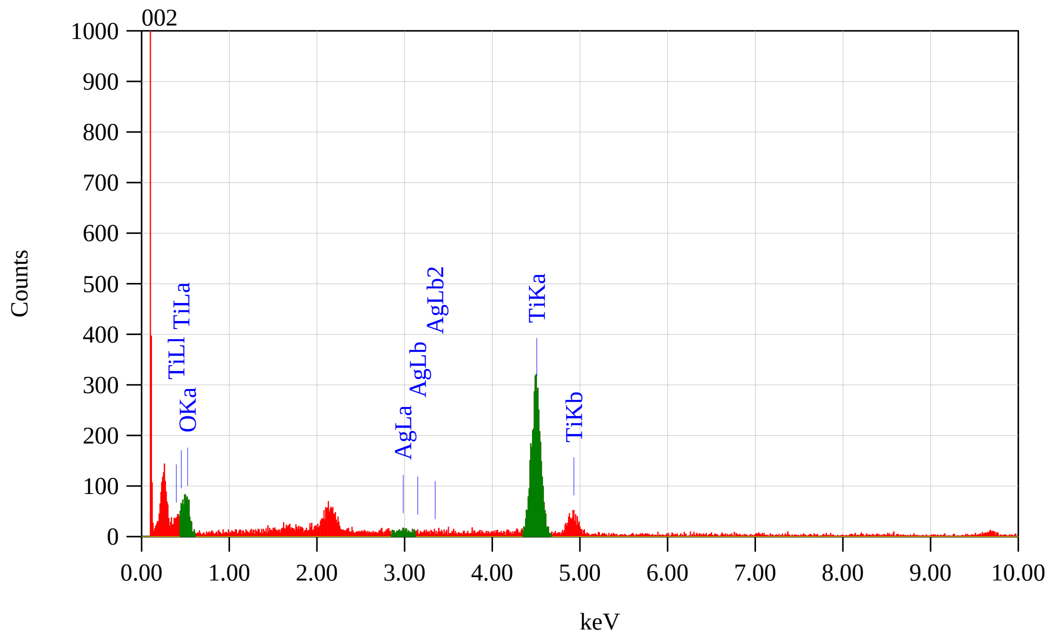

Figure 4.11: EDS Image of 1 % Ag - TiO_2 Nanoparticles

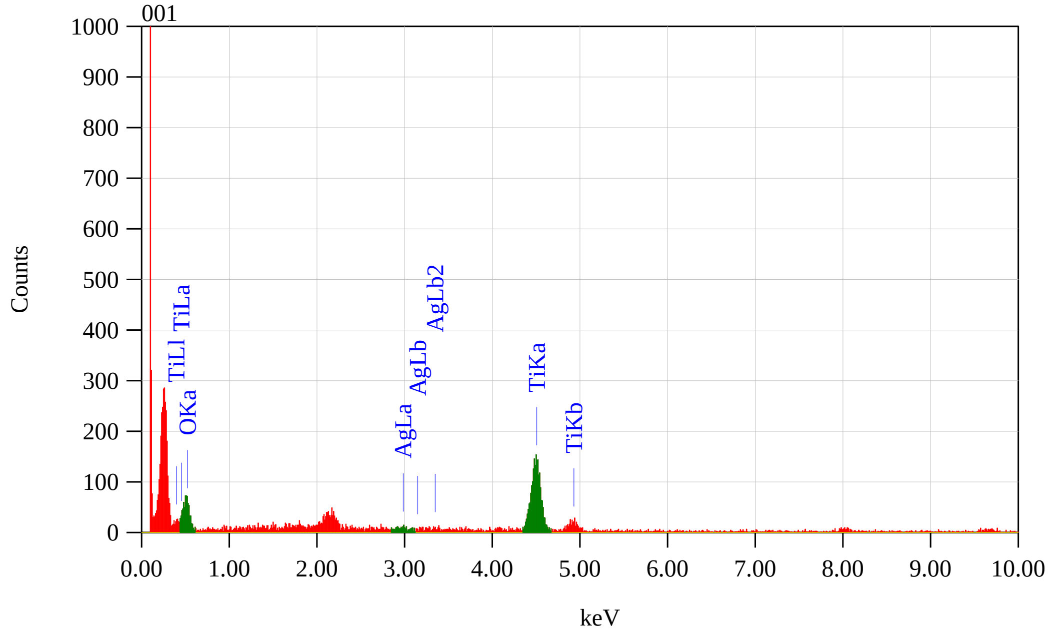

Figure 4.12: EDS Image of 2 % Ag - TiO_2 Nanoparticles

4.2 Microbial Disinfection Studies

4.2.1 Experiment with *Pseudomonas aeruginosa*

2 % Ag - TiO_2 embedded ABS film is more effective against *Pseudomonas aeruginosa* as compared to pure ABS, TiO_2 and 1 % Ag - TiO_2 embedded ABS films. Figure 4.13 shows that only 25 % *Pseudomonas aeruginosa* can survive on 2 % Ag - TiO_2 embedded ABS film after 1 hour of spray while complete disinfection results after 2 hours of exposure with 2 % Ag - TiO_2 embedded ABS film. In addition, it is also revealed that Ag doping of TiO_2 increases photocatalytic ability of TiO_2 nanoparticles.

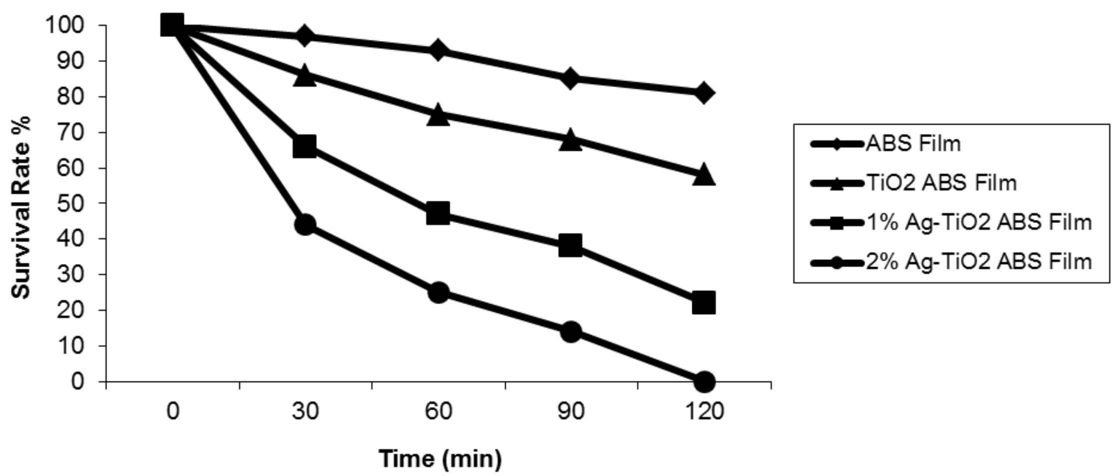

Figure 4. 13: Survival Rate of *Pseudomonas aeruginosa*

Results show that 2 % Ag - TiO_2 nanoparticles embedded ABS films are effective for the disinfection of *Pseudomonas aeruginosa.* So, coating of 2 % Ag - TiO_2 nanoparticles was decided on computer keyboard buttons. Following order shows survival rate of *Pseudomonas aeruginosa* on different categories of TiO_2 nanoparticles embedded ABS films:

ABS Film > TiO_2 ABS Film > 1 % Ag - TiO_2 ABS Film > 2 % Ag - TiO_2 ABS Film

4.2.2 Experiment with General Aero-flora

Table 4.3 shows frequencies of keystrokes recorded on a computer keyboard for the period of one month. This also shows colonies of general aero-flora in terms of Colony Forming Units (CFU) on each button which were observed after a period of one week. Additionally, this table also provides information regarding grouping of letters.

Table 4.3: Recorded Frequencies and their Respective CFU Values

Group I			Group II		
Letter	**Frequency**	**CFU**	**Letter**	**Frequency**	**CFU**
E	2,402	133	A	2,417	10
T	1,641	127	S	2,015	4
O	1,598	1	R	1,618	3
N	1,429	1	I	1,552	1
H	813	2	L	1,152	0
M	757	2	C	777	2
D	750	15	B	543	0
P	694	0	U	512	0
V	540	3	F	479	0
G	487	0	Y	296	0
W	405	0	K	218	1
J	143	1	Z	215	0
X	91	3	--	--	--
Q	31	2	--	--	--
Total	11,781	290	**Total**	11,794	21

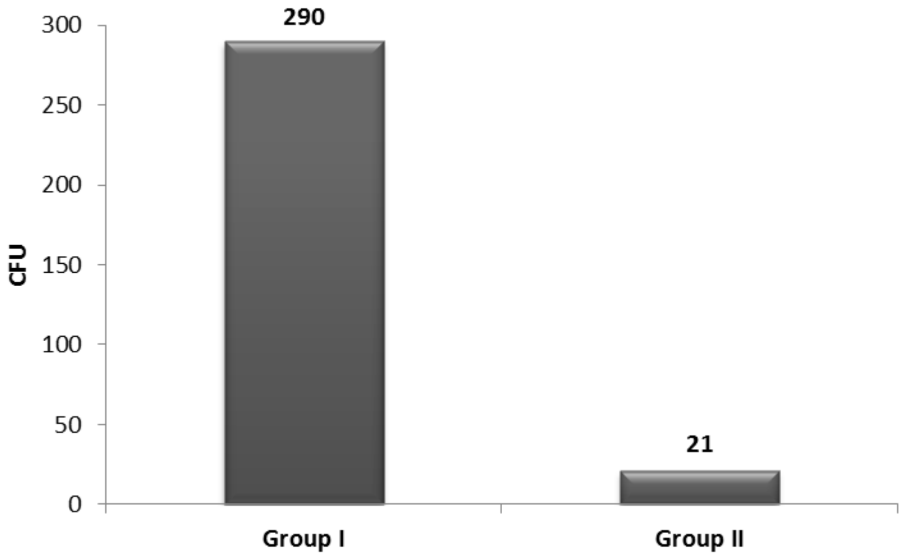

Figure 4.14: Microbial Disinfection Comparison of Group I and Group II

Figure 4.14 shows the efficiency of 2 % Ag-TiO_2 nanoparticles coated buttons (Group II) against uncoated buttons (Group I). ‘Group II’ shows 93 % effectiveness than ‘Group I’.

Two hours exposure of *Pseudomonas aeruginosa* and *Bacillus subtilis* to 1 % Ag - TiO_2 nanoparticles coated glass plates and venetian blinds result in complete disinfection of the mentioned bacterial species (Khan, 2013). This disinfection of bacterial species may be due to greater surface area to mass ratio and photocatalytic activity of TiO_2 nanoparticles.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Study reveals that 2 % Ag - TiO_2 nanoparticles show better efficiency for disinfection of *Pseudomonas aeruginosa* and general aero-flora even under room light. Catalytic activity of TiO_2 nanoparticles enhances with 2 % doping of Ag metal so self-sanitizing Acrylonitrile Butadiene Styrene (ABS) polymer can be developed after coating of 2 % Ag - TiO_2 nanoparticles.

5.2 Recommendations

Based on current research work, following recommendations are made:

- This work can be evaluated for disinfection of pathogenic microorganisms other than *Pseudomonas aeruginosa*.
- Idea can be implemented for the development of self-sterilizing keypads for cell phones, remote controls and other electronic devices.
- Field studies can be made for hospital environment.
- Research study can be extended by using different metal (Fe, Pt, Cu, etc.) doped nanomaterials.

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