# Nanocoating of Dental Mirrors by TiO$_2$ & TiO$_2$/ZnO Nano-composite to Induce Anti-fog and Anti-microbial Properties

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A thesis submitted in partial fulfilment of the requirement for the degree of

Master of Science (MS)

In

**Healthcare Biotechnology**

By

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**July, 2019**

Annex A to NUST Letter No.0972/102/Exams/Thesis-Cert
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# DECLARATION

I, **Faisal Sheraz Shah,** declare that this research work titled "**Nanocoating of Dental Mirrors by $TiO_2$ & $TiO_2$/ZnO Nano-composite to Induce Anti-fog and Anti-microbial Properties**" is my own work. The work has not been presented elsewhere for assessment. The work here in was carried out while I was a post-graduate student at Atta-ur-Rahman School of Applied Biosciences, NUST under the supervision of Dr. Attya Bhatti. The material that has been used from other sources has been properly acknowledged / referred.

Faisal Sheraz Shah
00000204987

DEDICATED TO
BELOVED PARENTS
MY WORLD BANK OF DUA'S

# ACKNOWLEDGEMENT

**"In the name of ALLAH, the most Beneficent, the most Merciful"**

And indeed, we bestowed upon Luqman Al-Hikmah (wisdom) saying: "*Give thanks to ALLAH, and whoever gives thanks, he gives thanks for (the good of) his ownself. And whoever is unthankful, then verily, ALLAH is ALL-Rich (Free of all wants). Worthy of all praise"* (31: 12 Al-Quran).

Praise to Allah the sustainer of the universe and blessings be upon the final Prophet Muhammad (P.B.U.H) the source of guidance for humanity.

I would like to thank first and foremost my supervisor Dr. Attya Bhatti. It has been an honor to be her MS student. She has taught me, both consciously and un-consciously, how good science is done in all the time I have spent at the university. I appreciate all her contribution of time, ideas and moral support and effort to find us funding to make our research experience productive and stimulating. The joy and enthusiasm she has for research have not been less than contagious and motivational for me.

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I would close this chapter with a verse from the Holy Quran, "*And my success (in my task) can only come from Allah. In Him I trust, and unto Him I look"* (11:88 Al- Quran).

Faisal Sheraz Shah

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# LIST OF ACRONYMS

| ACRONYM | GENERIC NAME |
|---|---|
| µg | Microgram |
| nm | Nanometer |
| ml | Milliliter |
| mg | Milligram |
| mM | Millimolar |
| rpm | Rate Per Minute |
| DI water | De Ionized Water |
| CFU | Colony Forming Unit |
| $TiO_2$ | Titanium Dioxide |
| ZnO | Zinc Oxide |
| TTIP | Titanium Isopropoxide |
| SEM | Scanning Electron Microscope |
| XRD | X-Ray Diffraction |
| EDX | Energy Dispersive X-Ray |
| MRSA | Multiple Resistance Staphylococcus Aureus |
| LBL | Layer by Layer |

| | |
|---|---|
| UV | Ultra Violet |
| kV | Kilo Volt |
| CRT | Cathode Ray Tube |
| HCL | Hydrochloric Acid |
| CB | Conduction Band |
| VB | Valence Band |
| CVD | Chemical Vapor Deposition |
| PVD | Physical Vapor Deposition |

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# ABSTRACT

The number of oral infections is expanding day by day because of unhygienic environment and poor sterilization of instruments. Also, the repeated sterilization decreases the quality of the instrument, that thusly expanding the health cost facilities. Presently, there are no dental mirrors with antifogging property along with being antimicrobial in the meantime. With the end goal to beat such issues some vital advances are fundamental. In this way, we guess that if infections and microbes can be purified utilizing strong oxidizing forces of photocatalysts, dental and therapeutic instruments shouldn't be exposed to excessive heat. The reasons for this examination are to shape the anatase $TiO_2$ photocatalytic thin films on glass slide and dental mirror utilizing hydrolysis of titanium alkoxide, and to look at hydrophilicity, the level of oxidizing power, and the pellucidity of the anatase $TiO_2$ photocatalytic thin films. In this study, $TiO_2$ and $TiO_2$/ZnO nanocomposite has been synthesized via sol gel technique and an endeavor is made to gander at antifog and antimicrobial properties under Ultra-Violet light by using $TiO_2$ nanoparticles and its composite with ZnO nanoparticles. $TiO_2$ if utilized as a part of blend with ZnO as $TiO_2$-ZnO nanocomposite will come about into upgrade consolidated properties with the likelihood of funding the new properties of the composite also. This composite in association with this examination will be used to do covering of dental mirrors and other instruments which will add numerous profitable characteristics to them.

# Chapter1

# INTRODUCTION

As day by day the number of oral infections is expanding because of unhygienic environment and poor sterilization of instruments. Also, the repeated sterilization decreases the quality of the instrument, that thusly expanding the health cost facilities. With the end goal to beat such issues some vital advances are fundamental. Photocatalysts have as of late been utilized for applications essentially dentistry, pharmaceutics and are portrayed by two characteristics: superhydrophilicity (Fujishima & Honda, 1972) and robust oxidation (Wang *et al*., 1997). Photocatalysts have the ability to keep fogging free mirrors and windows, and also while day and artificial light illuminated on particular photocatalyst, numerous sorts of inorganic and natural compounds can also be decayed. Anatase $TiO_2$ is a standout amongst most agent photocatalysts. Commonly there are two procedures to shape Nanoparticles and thin films of anatase $TiO_2$ such as dry procedures, like, sputtering(Dumitriu *et al*., 2000; Takagi, Makimoto, Hiraiwa, & Negishi, 2001) and CVD (Battiston, Gerbasi, Porchia, & Marigo, 1994), or by wet procedures, for example, dip coating (Y. Takahashi & Matsuoka, 1988; Yoko, Yuasa, Kamiya, & Sakka, 1991), sol-gel(Tsuzuki, Murakami, Kani, Kawakami, & Torii, 1990), spray coating(M. Takahashi, Mita, Toyuki, & Kume, 1989) and spin coating methods(Anast, Jamting, Bell, & Ben-Nissan, 1994). Although wet-processing tasks are moderately simple and no specific hardware is required, while arrangement of Titania's thin film uniformly on exceptional molded substrate surfaces is knotty. One major drawback of dental mirrors is their fogging due to patient breathing during examination. Also, water sprayed from the hand piece and disseminated particles during drilling and condensing the restorative materials just as salivation in oral cavity contaminate the reflective mirror surface and interfere with the vision of dentist(Funakoshi & Nonami, 2007). Impaired vision due to mirror contamination and interference from debris, mist and sprays has always been problematic for dentists. During years, many attempts have been made to overcome this issue such as manufacturing of mirrors with air and water spray in the rim of the mirror surface that continuously siphon the water across the reflective surface, spontaneously heated mouth mirrors that prevent accumulation of water or fogging of mirrors, and mirrors with a rotor and centrifugal force that expel water or other materials on the reflective surface.

Another problem is the need for repeated autoclave sterilization of instruments. High temperature and pressure of autoclave can corrode the metals and decrease the quality of dental instruments. The metal frame of the mirror is damaged by repeated sterilizations and decreases the quality of mirror(Smith, Bagg, Hurrell, & McHugh, 2007). Fogging is a major obstacle for dental therapeutic instruments mainly dental mirror, as it obstructs the clear image of diagnosis and also contaminate the surface. Therefore, after the use of instruments, disinfection is necessary. Fogging can be overcome by practicing nanotechnology through lodging suitable semiconductor photocatalyst on the mirror's surface(Funakoshi & Nonami, 2007). By repeated sterilization of dental therapeutic instruments, metal frame of the mirror could be disintegrated and also thin film of photocatalyst could be peeled off by the repeating exercise of sterilization or by use of disinfecting agents. because of such mentioned reasons, commercially no such fog free dental mirror has been executed. It is accounted for that the different sorts of infection and microscopic organisms, for example, Escherichia coli(Ireland, Klostermann, Rice, & Clark, 1993), Pseudomonas aeruginosa (Amezaga-Madrid, Nevarez-Moorillon, Orrantia-

Borunda, & Miki-Yoshida, 2002), and methicillin-resistant Staphylococcus aureus (MRSA) (Amezaga-Madrid et al., 2002) can be killed by strong oxidizing impact of photocatalyst by irradiating light on surface of photocatalyst. In this way, we guess that if infections and microbes can be purified utilizing photocatalyst oxidizing power, dental and therapeutic materials shouldn't be exposed to excess heat means. The goals behind this assessment are to shape thin films of titania on glass slide and dental mirror making effective use of titanium alkoxide hydrolysis, and to take a gander at the hydrophilicity, the degree of oxidizing power, and the pellucidity of photocatalytic thin films of anatase $TiO_2$.

Titania is an extensively used material with multiple functions as a result of its physical and chemical properties, splendid firmness similar to its non-toxic quality, phenomenal openness and inexpensiveness. Financially they can be enriched in paints, coatings, hues and sunscreens(Wakefield, Green, Lipscomb, & Flutter, 2004; Zallen & Moret, 2006). $TiO_2$, due to its potent photocatalytic action, just as its potential to disrupt synthetic compounds similar to super hydrophilic, antibacterial activity, and usage related to energy, was able to promptly attract research interests. The UV photon is capable of stimulating the photocatalyst, leading to the excitation from the valence band (VB) to the conduction band (CB) of an electron. Though, one of the serious concerns that leads to reduction in suitability of photocatalysis procedure is recombination of photo-generated electrons and hole pairs. $TiO_2$ is favored with positive outcomes in hydrogen production, photovoltaic, photocatalyst, lithium-particle batteries modules and power modules because of excellent surface action and appropriate electronic band structure (Liu & Aydil, 2009). Apparently, on a nanoscale level, applications of nanoparticles mostly were based on the structure and morphology of that nanoparticle. Due to increase in the quantum confinement impacts, in the nanoscale, the moving patterns of electron and hole could be different, and the electronic band structure could be shifted. The colossal surface-to-volume extent of nanomaterial can surprisingly manufacture the reaction sites at surface and can even modify the movement of surface particles reactant.

Explicit surfaces with high vitality should in like manner end up firm and enhance their reactant properties in the nanoscale level. Eventually, on the establishment of appropriate nano-estimate structures with all around engineered amalgamation, geometry, and crystallography can moreover update the execution of TiO2 semiconductor-based systems and gadgets (Roduner, 2006). In procuring the preeminent properties of TiO2 material, manipulating the shape, structure and size of the colloidal precursor is the foundation step. TiO2 powders have been picked up either by precipitation from titanium salts solution or alkoxides or decisively from minerals that bear titanium. The most usual strategies were relied upon the hydrolysis of acidic Ti (1V) salts solutions (Chowdhury & Viraraghavan, 2009). In any case, these Titania powders have ordinarily required in the properties like compatible (uniform) shape and size, furthermore non-agglomerated state needed for quantitative examinations of colloidal wonders (Chen, Lee, Yeng, & Chiu, 2003). Behindhand, bundles of philosophies were accounted for titania nanoparticles formation, through managed nucleation and development forms in dilute Ti (IV) solutions. Some of them are sol-gel methodology and aqueous technique, which are having bit of leeway of low reaction temperature. It has been revealed that the emergent nanostructure of titanium dioxide incorporated by hydrolyzing titanium alkoxide, unambiguously impacts the characteristics of the end powder and crystallization lead (Banfield & Veblen, 1992). In this report, we have endeavored to plan anatase and rutile $TiO_2$ by moderately simple sol-gel technique and an attempt is made to look at the antifogging and antimicrobial properties under Ultra-Violet light by using $TiO_2$ nanoparticles and its composite with ZnO nanoparticles.

$TiO_2$ if utilized as a part of blend with ZnO as $TiO_2$-ZnO nanocomposite will come about into upgraded consolidated properties with the likelihood of finding new properties of the composite also. This composite in connection to this investigation will be utilized to do coating of dental mirrors and dental instruments which will add numerous valuable qualities to them.

## 1.1. Objective of Work:

This study sought to assess the efficacy and antibacterial property of dental mirrors coated with $TiO_2$ nanoparticles and $TiO_2$-ZnO nanocomposite.

- Synthesis, characterization and evaluation of anti-fogging property of $TiO_2$ & $TiO_2$/ZnO nanocomposite.
- Evaluation of antimicrobial activity of Titania nanoparticles & $TiO_2$/ZnO nanocomposite.

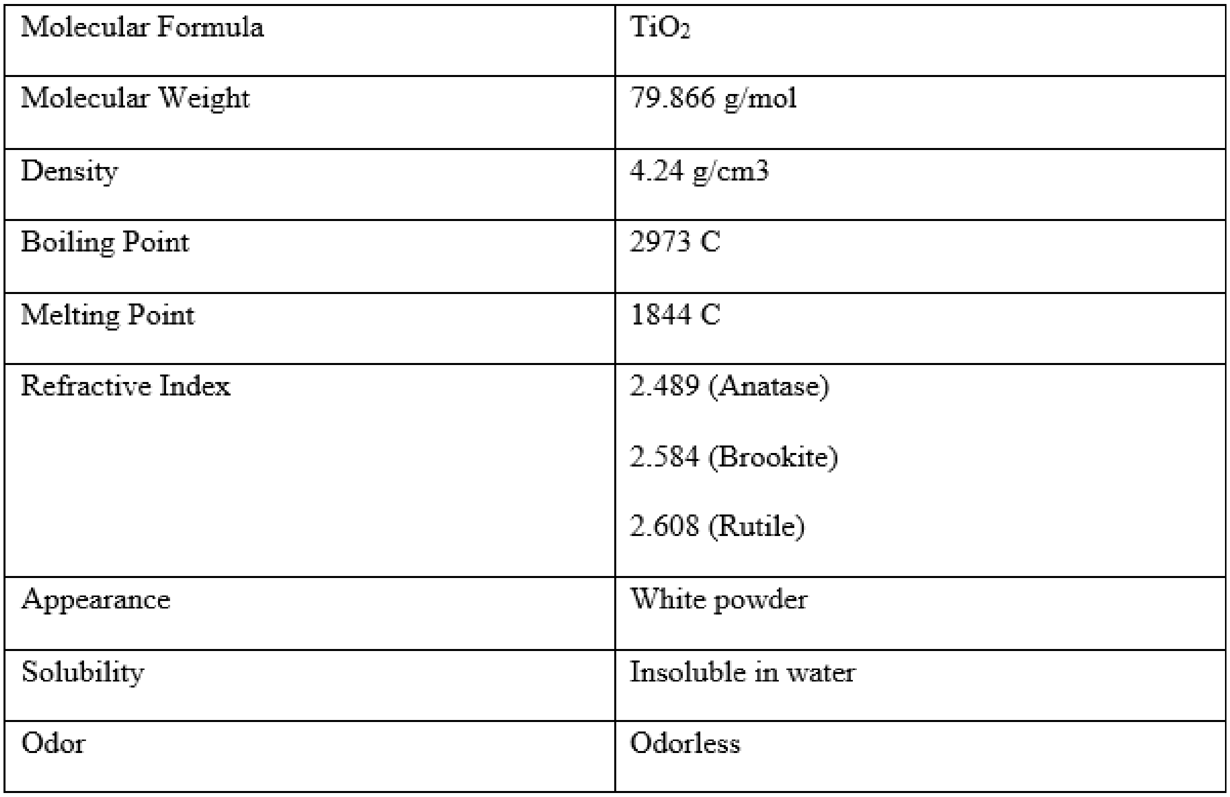
# Chapter 2

# LITREATURE REVIEW

Different articles have been considered on $TiO_2$ nanoparticles and spoke to here in this area. The concise talk of $TiO_2$ nanoparticles on their different crystal framework, integrating procedures and reaction mechanism were additionally given. This substance contains subtleties of photocatalysis procedure and variables such as quantity of catalyst, temperature, pH, time and concentration influencing photocatalysis.

## 2.1. Characteristics of $TiO_2$:

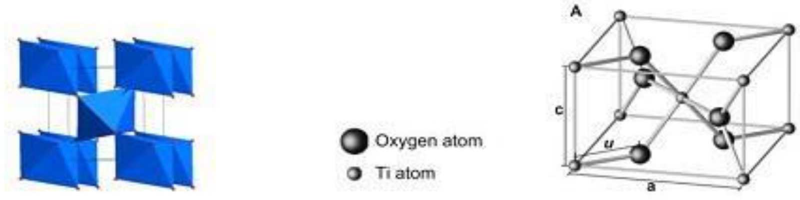
Table 2.1- Titania ($TiO_2$) peculiarities (Chen et al., 2003)



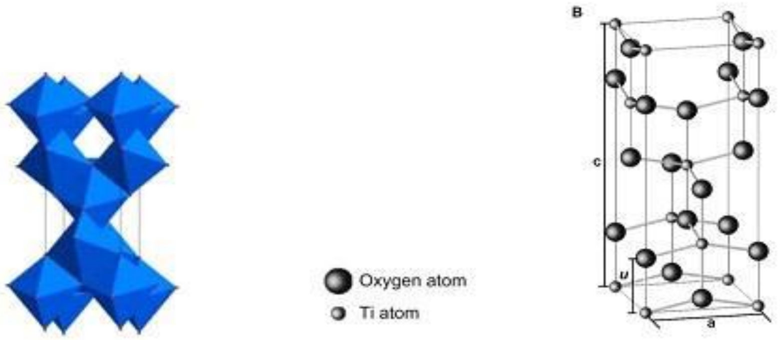
| | |
|---|---|
| Molecular Formula | $TiO_2$ |
| Molecular Weight | 79.866 g/mol |
| Density | 4.24 g/cm3 |
| Boiling Point | 2973 C |
| Melting Point | 1844 C |
| Refractive Index | 2.489 (Anatase)<br>2.584 (Brookite)<br>2.608 (Rutile) |
| Appearance | White powder |
| Solubility | Insoluble in water |
| Odor | Odorless |

## 2.2 $TiO_2$ Crystal lattice Structure:

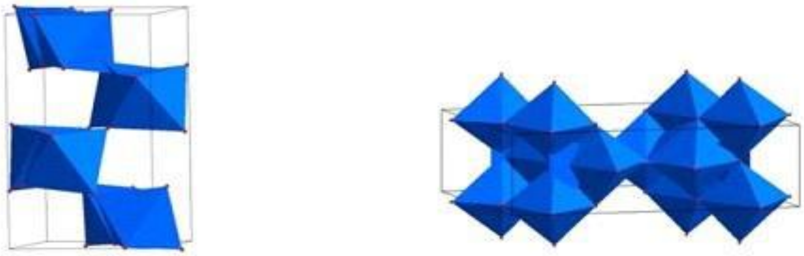
In 1992, (Banfield & Veblen, 1992) contemplated different crystal lattice structure of titania. Typically, TiO2(B), Anatase, Rutile & Brookite are four different sorts of polymorphic stages of Titania. Additionally, couple of unstable stages of Titania also exist, for instance, TiO2 (hollandite, columbite, baddeleyite, ramsdellite) have been observed. Each phase has its own properties, so along these lines, each phase requires particular condition and to procure particular stage and give particular kind of execution.



[A] Rutile



[B] Anatase



[C] Brookite

[D] $TiO_2$ (B)

**Figure 2.1:** Structure of different phases of $TiO_2$ (Banfield & Veblen, 1992)

Roundly, metastable stages of Titania are Anatase, brookite and $TiO_2$ (B) and amongst them, rutile is the steadiest phase and under high pressure these anatase, brookite and $TiO_2$ (B) transforms into rutile phase. This kind of state sustainability liaison too exists in $TiO_2$ nanomaterial production. Most of the nanomaterial commonly acquired anatase phase. Anatase phase normally exists in moderately low temperature condition. $TiO_2$ nanostructures acquired rutile phase under annealing or high temperature deposition. Solution based frameworks commonly yield $TiO_2$ (B) and Brookite polymorphic state. The other kind of metastable states can shape by using specific types of precursors under specific conditions. Figure 2.1 diagrammatically demonstrates four different configurations of Titania by utilizing major structure hinder as Ti−O octahedrons.

Polymorphic states of Titania have diverse nanostructures which dependably display distinctive development practices and favored morphologies just as shows diverse symmetry. $TiO_2$ rutile phase demonstrates tetragonal geometry (Figure 2.1 [A]). Generally, the morphology of Rutile phase is only obvious through engineered $TiO_2$ nonpowered. The anatase stage additionally indicates tetragonal geometry yet has fundamentally longer c-axis contrasted with a-axis; Figure 2.1 [B]). Anatase stage $TiO_2$ demonstrates trunked octahedron morphology in its nanostructures similar to rutile stage with same most minimal energy surfaces. Brookite demonstrates orthorhombic geometry structure and has a huge unit cell comprising of eight Titania groups (Figure 2.1 [C]). This expansive unit cell likewise displays in $TiO_2$ (B) with similarly more open gem structure than other polymorphic stages (Figure 2.1 [D]). $TiO_2$ (B) is monoclinic having an especially long a-axis (1.216 nm). Though, both brookite and $TiO_2$(B) stages are less routinely gotten in engineered Titania nanostructures.

(Gong, Selloni, Batzill, & Diebold, 2006) inferred that the initial move toward sensible exploratory plan for getting ready $TiO_2$ nanoparticles with all around indicated measurement, shape, crystallinity and stage, which is the essential significance for achieving wanted usefulness and execution is understanding the structure of the crystal.

## 2.3. Synthesis Procedure of $TiO_2$:

There are namely two well defined routes that are generally use to prepare $TiO_2$ in different form like powder, thin films and crystallite from, namely by (Carp, Huisman, & Reller, 2004)

1. Synthesis by solution route

2. Synthesis by gas phase

### 2.3.1 Synthesis by Solution Route

Generally thin films of $TiO_2$ are prepared by this route because of its convenience and ease. Additionally, homogeneity and stoichiometry can also be controlled, the amalgamation of composite materials just as complex arrangement. Negative marks of this course lie in its staggering expense, substantial preparing time just as nearness of contaminations, for example, carbon. Routinely used synthesis by solution route procedures are given below,

a) Sol gel way

b) Synthesis by hydrothermal mean

c) By Solvothermal route

d) By precipitation way

### 2.3.2. Synthesis by Gas route:

The claim to fame by this channel is framing coatings to adjust distinctive characteristics, for example, erosion and wear obstruction, optical, thermal and electrical properties of different substrate. By infiltration route, they can be utilized to form composite materials. Generally utilized procedures of gas phase are:

1) Chemical Vapor Deposition Method
2) Physical Vapor Deposition Method
3) Spray Pyrolysis Deposition Method

## 2.4. Synthesis by Sol gel:

(Hench & West, 1990) inferred that, sol gel strategy is a standout amongst utmost broadly utilized for incorporating $TiO_2$ nanoparticles because of different points of interest, for example, particles high homogeneity, low handling temperature, blend conditions, dependability and flexibility of preparing. In sol-gel approach, firstly, mix two different solution, one is the precursor of the required nanoparticle and the other is hydrolysis solution. In this approach, hydrolysis solution is supplemented drop by drop in solution containing precursor and kept at room temperature under constant stirring in order to tune size of particle ($TiO_2$) and also hydrolysis rate. It is frequently affirmed that utmost brilliant feature of sol gel method is that size of particle can be tuned, generally by polymerization and condensation of metal organics to acquire gel. Titanium Isopropoxide, titanium ethylhexoxide and titanium butaoxide are most common precursors used for the preparing Titania nanoparticles using Ti as a source. TiCl4 is also use by some of the researchers as a Ti source for synthesis of Titania.

(Brinker & Scherer, 2013) was concentrated on a standout amongst utmost generally utilized precursors, metal alkoxides, having an organic ligand connected to a metal or metalloid molecule. Higher reactivity of metal alkoxides with water make them attractive precursor molecule. A sol can be portrayed as colloidal suspension having particles in range of 1 to 1000nm in fluid, and is upheld by little range powers like van der Waals and other surface charge forces. At particular pH, mechanically gel can be molded by mixing discrete particles that hinder precipitation. Also, by hydrolysis and condensation a 3-dimensional shape can be organized.

(Jagadale et al., 2008) characterize various channels which can be perused to attain sol gel structure are exhibited in Figure 2.3. Xerogel is attain by evaporation when gel is dehydrated under favorable condition. The resultant xerogel volume is 5 times fewer than mass of original gel. Drying gel in autoclave, another final product aerogel is obtained.

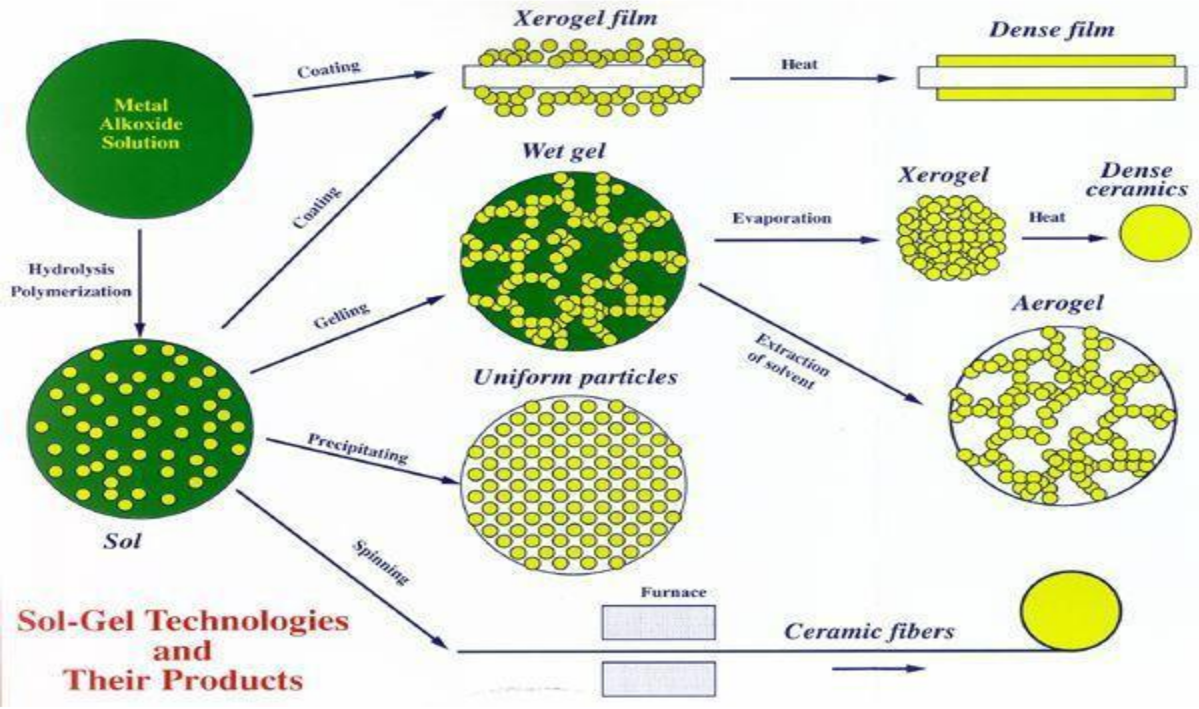


**Figure 2.2:** Sol-gel process (Lee & Yang, 2005)

However, the mass of an aerogel is quite similar to the mass of original gel because of scarcity of capillary pressure during drying.

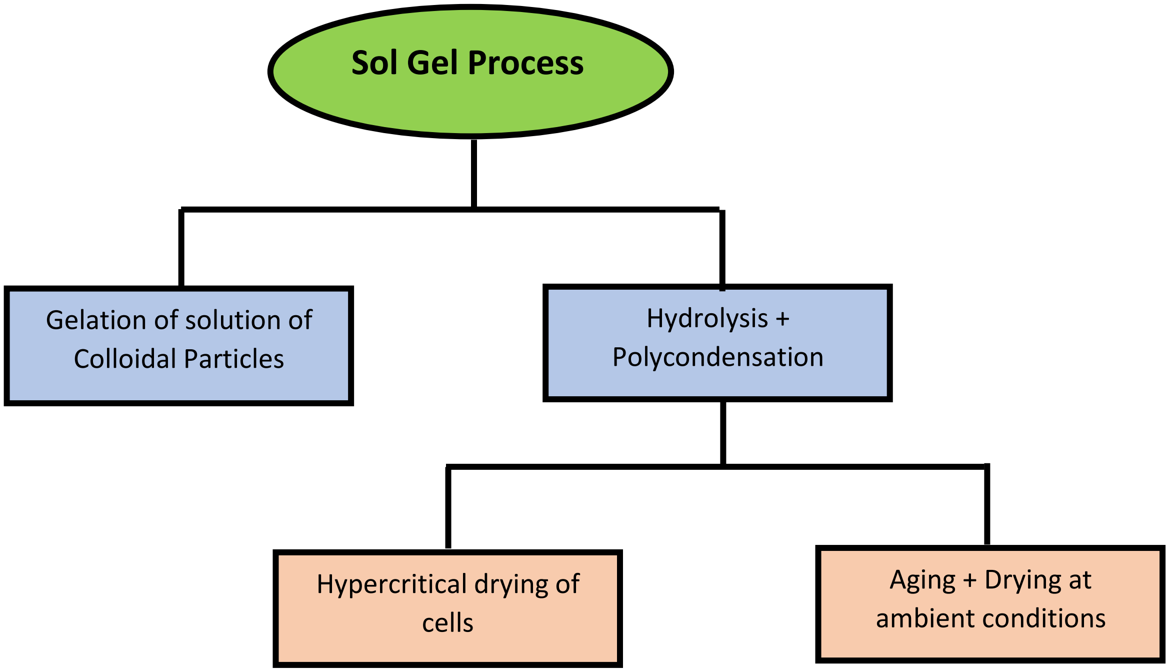


**Figure 2.3:** Sol-gel routes

This pictorial representation shows the basic two routes of gel formation. The gel formed by the route of Hydrolysis and Condensation; their formation heavily relies upon the reaction kinetics. The steps such as ageing, drying, stabilization and purification acquired by the techniques, all depend on the structure of the gel.

### 2.4.1. Reaction Mechanism:

(Lee & Yang, 2005) depicts that inorganic polymerization is the basis of sol-gel technique. This technique comprises of various gaits starting from hydrolysis to polycondensation and then dehydrating the material and finally calcination to obtain the desired structure. First step involves the precursor's hydrolysis either in the presence of alcohol or water.

$Ti(OR)4 + 4H_2O$ $Ti(OH)_4 + 4ROH$ (hydrolysis)

Where R = any alkyl group

(Oskam, Nellore, Penn, & Searson, 2003) discloses that acid or base along with water or alcohol also facilitate in hydrolysis of precursor solution. Typically, the process of sol-gel initiates and advance by the titanium alkoxide hydrolysis step and this hydrolysis is catalyzed by an acid and then succeed by condensation. Slow hydrolysis rate, less water content and higher quantity of Titanium alkoxide solution in process chamber is normally endorsed for the propagation and production of Ti-O-Ti chain. Anyhow, by foundation of Ti-O-Ti chains, close symmetry of three-dimensional polymeric skeletons is acquired.

(Behnajady, Eskandarloo, Modirshahla, & Shokri, 2011) interpreted that higher quantity of Ti-OH leads to minute installation of 3D polymorphic skeleton that resulted in a loosely organized first order particle. Ti-O-Ti chains are advanced due to excessive amount of water.

$Ti(OH)_4$ $TiO_2 + 2H_2O$ (condensation)

Organic precursor dissipation requires calcination at higher temperature. Titania nanoparticles synthesis by sol-gel route has been depicted in a flow chart in figure 2.4.

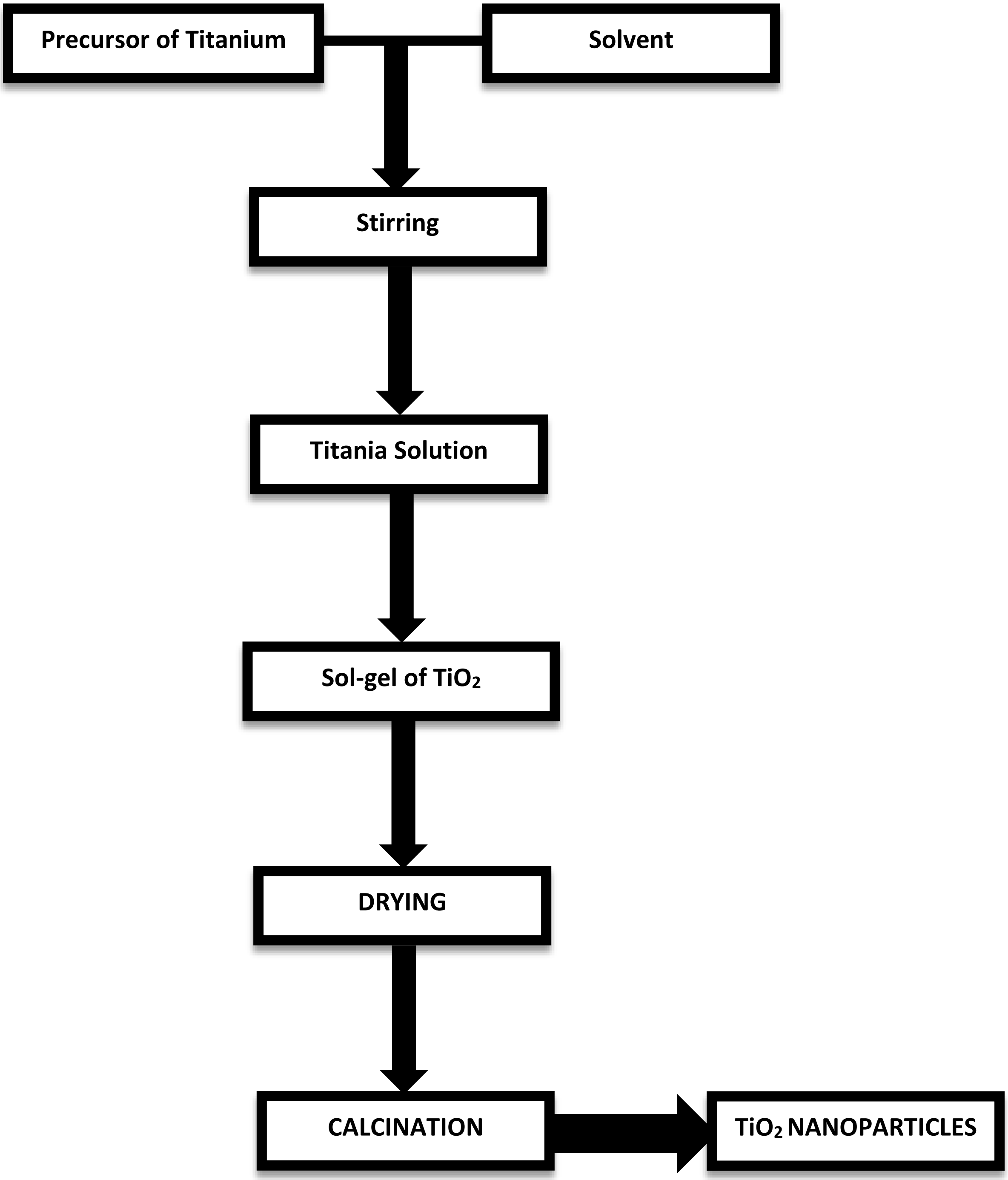


**Figure 2.4:** Flow Sheet representation of $TiO_2$ nanoparticles

Some of the factors such as pH, temperature and the impurities present in solution highly influence size of the particle and by governing these parameters, the size of the particle can be tuned.

(Tumma, Nagaraju, & Reddy, 2009) clinched that pure anatase state nanoparticles are commonly synthesized by utilizing moderate acids like acetic acid and triethanolamine from Titanium isopropoxide as Ti source. Stronger acids often form $TiO_2$ nanoparticles of Brookite phase. Titania nanoparticles in Brookite phase has been inclined by thermolysis of $TiCl_4$ in aqueous solution of HCl. Ti:Cl concentration ratio strongly determine the composition of reaction product. Titanium tetra isopropoxide in nitric acid yield $TiO_2$ nanoparticles of pure rutile phase. Also, TiCl4 or TiCl3 in Hydrochloric solution yield rutile phase $TiO_2$ nanoparticles.

(Loryuenyong, Angamnuaysiri, Sukcharoenpong, & Suwannasri, 2012) evaluated the $TiO_2$ nanoparticle synthesis by sol-gel method, using precursor Titanium isopropoxide and isopropanol as a solvent. After the complete reaction of 2-3 hours the engineered $TiO_2$ nanoparticles were then calcined at 500C. It was disclosed that the anatase to rutile stage change, crystallite development, and pore crumbling happened with an expansion in calcinations temperature. Isopropanol as a solvent was used to inhibit the phase transformation from anatase to rutile, and also by controlling the hydrolysis rate the phase transformation could also be controlled. It was also observed that by using isopropanol as a solvent, an improvement in the photocatalytic activity of $TiO_2$ was observed due to thermal stability of anatase phase in contrast to other solvents like ethanol.

## 2.5 Titanium dioxide antimicrobials ($TiO_2$):

The intensity of Titania nanoparticles was re-found by (Fujishima & Honda, 1972) which has been referred to since 1921 as a photocatalyst. Before that titania was only known as a physical self-cleaning agent and was applied on windows, glasses and tiles (Zaleska, 2008). Hypothetically, the mechanism of photocatalysis is the generation of actuated species at the illuminated surface of semiconductor, having higher energy of photons in conduction band instead of valence band. Titania only activated in UV light as it has a band gap range in between 3-3.2ev (Puzenat, 2009). Therefore, Titania nanoparticles show antimicrobial activity when irradiated with UV light. This averts the microbe's adherence, or inactivates microorganisms that hold fast to a surface because of generation of reactive oxygen species. Anyhow, reactive oxygen species half-life remains for a small period of time, and they likely exist just in the locale close to the impetus surface since they can be promptly smothered in watery situations because of a high recombination rate. Utilizing a photoelectron-synergist framework with an outside potential predisposition can smother the charge recombination (Nie et al., 2014). In term of environmental contamination, Titania nanoparticles are widely utilized in air purification and water disinfection (Sunada, Watanabe, & Hashimoto, 2003). (Matsunaga, Tomoda, Nakajima, & Wake, 1985) was the first one who used titania as disinfecting agent. Titania nanoparticles are widely studied because of strong oxidizing power and stability both in powder form and in thin film form (Armelao et al., 2007). Considerably, titania surfaces are assured as promising material in future prescription, since it isn't noxious and does not cause ecological contamination. Out of these anatase exhibit strong oxidizing power because of greater hole trapping rate and higher adsorptive capability (Visai et al., 2011).

## 2.6. Titanium Dioxide as a Photocatalyst:

(Carp et al., 2004) optimal photocatalyst, expressed underlying qualities:

1) Chemically and biologically inert

2) Stable photo catalytically

3) Ease in synthesis and usage

4) Effectively enacted by daylight

5) Capable to adequate catalyze reactions

6) Low cost

7) Safe for humans and environment

$TiO_2$ is only a semiconductor that meets the above highlighted criteria. But lack of absorption in the visible region is a main impediment in utilizing $TiO_2$ nanoparticles.

(Kubota et al., 2001) further explained that $TiO_2$ nanoparticles has the ability to degrade pollutants like fungi, viruses, bacteria and organic compounds into innocuous product like water, carbon di oxide due to generation of reactive oxygen species. This chaotic response of Titania catalyst can be applied in certain applications to avert astringent loss in some cases like in paints.

(Cong & Xu, 2011) elaborated that in view of thermodynamics, phase anatase and rutile have the capability of boot up photocatalytic oxidation due to concurrence in valence band. However, studies reveal that anatase phase is more potent photocatalyst as compared to other two forms. A conceivable clarification could be because of the lifetime of the charge transporters which has been appeared to be longer for anatase when contrasted with that of rutile. Fewer studies show that anatase and rutile phase mixture could be an active photocatalyst in contrast to solo anatase phase. The combination of these two phases may give the synergistic effect.

In contrast to photocatalytic importance of titania, $TiO_2$ is utilized in many other purposes like gas sensors, biomaterials (hemocompatibility with the human body), antimicrobial activity.

# Chapter 3

# METHADOLOGY

## 3.1 Titania nanoparticles synthesis:

Titania nanoparticles were synthesized in the laboratory by (Jalali & Mozammel, 2017) using Titanium tetraisopropoxide as a precursor. Initial 6ml of 2-propanol and 6ml of HCL were blended in 85 ml of distilled water and afterwards 5.2 ml of TTIP was added in a mixture. Mixture was allowed to stirred for 2-3 hours at 60C. Solution color changed from white to transparent solution. Solution kept at rest for 12 hours and then dried at 100C. Resulting solution was further refined by calcination for 1 hour in 500C for yielding crystalline nature nanoparticles.

### 3.1.1 $TiO_2$/ZnO nanocomposite synthesis:

$TiO_2$/ZnO nanocomposite was synthesized by preparing 0.2M solution that was made by weighing 0.57g of Zinc acetate dihydrate and 13.57ml of de-ionized water. The solution was stirred for 30 minutes at 80C. Then 0.5g of Titania powder was mixed in 20ml of de-ionized water and added in Zinc Oxide solution above and allowed to magnetically stirred for further 30 minutes and then allowed the solution to cool at room temperature. 0.8 molar solution of Sodium hydroxide was also prepared separately by adding 0.24g of NaOH in 7.66ml of distilled water and allowed the solution to stirred for 20 minutes at 80C and afterwards cool down at room temperature. This prepared Sodium Hydroxide solution was then added dropwise in the primary solution under vigorous stirring for 5 hours at room temperature. After 5 hours of vigorous stirring precipitates of $TiO_2$/ZnO formed that were aged for 24 hours, following filtration and washing for several times utilizing distilled water. Before calcination the resulting solution was dried at 100C for 24 hours and then calcined for 2 hours at 500C in Muffle furnace to obtain crystalline nanocomposite and then grind into powder.

### 3.1.2 Dip Coating:

Dip coating is well defied commercially available and applied method among different wet thin film deposition procedures. In 1939 Jenaer Glaswerk was the first one who win the patent based on dip coating process (Geffcken & Berger, 1939). Fundamentally, the procedure might be distinguished into three significant specialized steps:

1) **Immersion & Dwelling**: Object is submerged into solution of suitable precursor at a consistent rate pursued by particular stay time for adequate association time of substrate and coating solution.

**2**) **Deposition:** In this time, substrate is pulled upward with a uniform speed to form a thin film of precursor solution. Surplus fluid will deplete from surface.

**3) Evaporation:** In this, solvent starts to evaporate from liquid, framing thin film of precursor, which can be further smoothed by warmed drying. For burning other residual organics and impurities the coating might be exposed to further heating treatment.

## 3.2 Titania Nanoparticle Characterization:

### 3.2.1 Scanning Electron Microscope analysis:

This is a technique in which the light emitted electrons are focused on surface of specimen. These electrons interact with the sample surface and photons or electrons are knocked off from material’s surface in result. These knocked off electrons are then focused on the detector. The detector’s output then modulates the brightness of cathode ray tube (CRT). For every point where the electron beams are focused and interact, it is plotted on consequent point on CRT and material’s image is produced(Jerosch & Reichelt, 1997).

The electron-surface interaction results in the emission of X-rays, secondary electrons and backscattered electrons(Oatley, 1982). Common SEM mode for detection is via secondary electrons and such electrons are discharged from close to surface of the sample. So, a pronounced and clear image of sample is obtained. It can reveal sample detail even less than 1nm in size. Also, elastic scattering of incident electrons also takes place and release back scattered electrons. They emerge from deeper locations as compared to secondary electrons. So, their resolution is comparatively low. X-rays also emit from the atoms when an inner shell electron knocks off from its shell [97].

SEM as it has easy sample preparation and we can figure our sample’s morphology, chemistry, crystallography and orientation of planes. Magnification of SEM can be controlled from 10 to 500,000 times.

SEM analysis was conducted by Scanning Electron Microscope (voltage:10-20 kV), spot size of 20-40nm, and working distance of 10mm. Images were recorded in Secondary Electron mode at low and high magnifications.

### 3.2.2 X-Ray Diffraction:

This technique is performed to examine the crystalline phase of the material. It is a non-destructive technique and it provides fingerprints of Bragg’s reflections of crystalline materials(Stanjek & Häusler, 2004). X-ray beam diffractometer comprise of 3 essential elements. A cathode tube, x-ray detector and sample holder. X-rays are produced by heating filament element which accelerates electrons towards a target which collide with target material with electrons. Crystal is composed of layers and planes. So, x-ray which has wavelength having similar to these planes is reflected that that angle of incidence is equal to angle of reflection. “Diffraction” takes place and it can be described as by Bragg’s Law:

$$\mathbf{2d\sin\theta = n\lambda}$$

When Bragg’s law is satisfied, constructive interference takes place and “Bragg’s reflections” will be picked up by the detector. These reflections positions tell us about inter-layer spacing. X-ray diffraction tells us about the phase, crystallinity and sample purity. By this technique one can also determine lattice mismatch, dislocations and unit cell dimensions.

### 3.2.3 Contact Angle Measurement:

It is a technique used to determine the contact angle of a droplet on a surface. A water drop is dropped on a sample surface and then is measured through different means. Here we use Drop Shape Analyzer DSA-25 shown in figure 4.5 present in SCME, NUST

**Figure 3.1:** Drop Size Analyzer DSA-25

Drop shape analysis is a picture study strategy for characterizing the contact point from the shadow picture of sessile drop. A water droplet is allowed to drop onto a sample. Make sure the drop is sessile. a drop picture is recorded with the assistance of a camera and shifted towards drop shape investigation programming. A blueprint acknowledgment is basically dependent on grey scale examination of image. A geometrical model advising the shape of drop is fitted to contour in the subsequent stage.

### 3.2.4 Antifogging Test:

Effectively developed thin films of titania coated on glass substrate was examined by showering water on glass substrate surface against the uncoated one for anti-fogging analysis. The glass substrate which was coated with titania did not show any water droplets and fog on it due to Titania's hydrophilic nature, whereas glass substrate which was not coated show water droplets on it. Hence, Titania hydrophilic property was clearly observed.

## 3.3 Collection of Bacterial strains:

Four (4) microbial organisms were obtained from Virology lab ASAB, Nust Islamabad. These microorganisms include Gram Negative (*Pseudomonas aeruginosa, Klebsiella pneumonia, E. coli*) & Gram Positive (Methicillin-resistant Staphylococcus aureus) bacteria. Specimen were streaked on Nutrient Agar and allowed for overnight incubation at 37C.

### 3.3.1 Preparation of Nanoparticle Concentration:

Various concentration of Titania nanoparticles was prepared for their antimicrobial activity. Weight of nanoparticles was measured and added in propanol following continuous vortexing at high resolution, accompanied with sonication of 1 hour before use. The nanoparticles concentration prepared were 0.25mg/mL, 0.5mg/mL, 075mg/ml, 1.0mg/mL.

### 3.3.2 Preparation of Mc Farland Standard:

For comparing the turbity of bacterial cultures 0.5 Mc Farland was used in disk diffusion assay. 0.05mL of 1.17% barium chloride dehydrates (BaCL2.H2O) was mixed with 9.95mL of 1% of sulphuric acid. Spectrophotometer was used to examine the absorbance and was found to be 0.9 for prepared standard. The mixture was sealed in test tube and stored in dark.

### 3.3.3 Antibacterial Disk Diffusion Assay:

First of all, nutrient agar plates were prepared, for this nutrient agar was dissolved in distilled water and autoclaved, it was then poured inside laminar flow hood on petri plates. The bacterial sample was brought out from -80ºC and placed in ice packed box to thwart samples from thawing; it was then streaked onto nutrient agar plates. Plates that were streaked were incubated for 16 hours, in order to enable bacterial colonies to multiply. 0.9% of normal saline was made by mixing 0.9g of sodium chloride in 100mL of distilled water and autoclaved before use. 5mL of normal saline was added in sterile test tube in the laminar flow hood. Using sterile loop microbial colonies were picked from 16 hours cultured plates and released into the normal saline till the turbidity was compared to 0.5

Mc Farland Standard. 300µl of microbial inoculum in normal saline was pipette out and dropped on to the nutrient agar plate. The sterilized spreader was then used to spread the bacterial inoculum evenly throughout the plate. Sterile filter paper discs made from Whatman filter paper were located on inoculated plates with the help of sterile syringe. Different concentration of Titania nanoparticles (0.25, 0.5, 0.75, 1.0, mg/mL) was added and absorbed by filter paper discs. As a negative control, deionized water was used. Plates were incubated overnight at 37ºC.

### 3.3.4 Time Kill Assay:

Time kill cure data analysis was done to assess Titania nanoparticles antibacterial activity and $TiO_2$/ZnO nanocomposite and evaluates microbial reduction in vitro after exposed to $TiO_2$ and $TiO_2$/ZnO nanocomposite. Time kill analysis estimates the difference un microbial population inside a predefined examining period in vitro after exposed to an antibacterial test specimen. In this test, test specimen either nanoparticles or their dilution comes in contact against known populace of microbes for a predetermined time frame and also predetermined temperature. at the objective inspecting time, test specimen is then neutralized and enduring organisms are then listed.

In this study the bacterial culture was prepared and incubated for 24 hours then afterwards it was diluted until 0.5 McFarland standard was obtained. Then 50ul of inoculum was added to fresh liquid broth along with 30ul active ingredients. There were different concentrations of tested nanoparticles to determine the response of nanoparticles and nanocomposite at various concentrations. Microplate reader was used to read the plate at 620nm after regular intervals for 12 hours.

# Chapter 4

# RESULTS

The information including crystal phase, band gap energy, active surface area, surface morphology and purity are of significant importance to assess photocatalytic behavior of synthesized Titania nanoparticles. The characterization techniques such as SEM-EDX and XRD were employed to know surface morphology, crystallite size and other properties of $TiO_2$ nanoparticles. Furthermore, Time Kill Assay and Disc Diffusion Assay were done to analyze the antimicrobial properties of the synthesized nanoparticles.

## 4.1 Characterization of Titania Nanoparticles:

### 4.1.1 Shape and Size Analysis through SEM:

Scanning Electron Microscopy analysis of Titania nanoparticles revealed them to be homogenous in their shape. The morphological analysis of $TiO_2$ are depicted in picture, where a little agglomeration of the particles can be seen due to increase in aging time for SEM analysis. The synthesized nanoparticles were found to be have a spherical morphology.

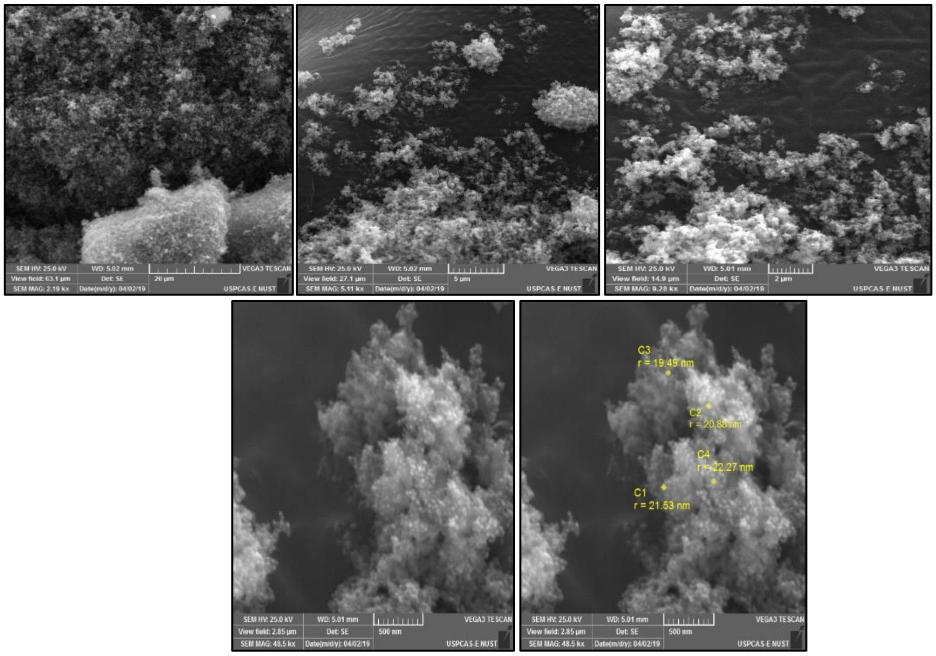


**Figure 4.1:** Images showing the morphological analysis of $TiO_2$ nanoparticles at various magnifications. At higher magnification it can be seen that $TiO_2$ nanoparticles are spherical in shape.

The morphological analysis of $TiO_2$ coated thin films was also performed by Scanning Electron Microscope which shows that our glass substrate was uniformly coated with Titania. Also, it can be seen that Titania nanoparticles coated on the substrate had spherical configuration and the mean size of that coated nanoparticles was 20-40nm in range.

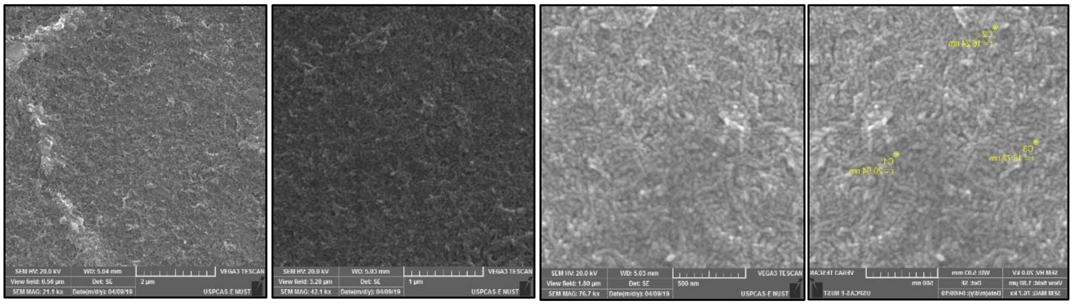


**Figure 4.2:** Images showing the morphological analysis of $TiO_2$ coated thin films on glass substrate at various magnification. Higher magnification shows the shape and average size of Titania nanoparticles.

### 4.1.2 Energy Dispersive X-ray Spectroscopy:

EDS was performed to validate the presence of desired nanoparticles in the test sample. In order to confirm the presence of Titania nanoparticles in the sample Energy Dispersive X-ray Spectroscopy was performed which revealed that only Titania nanoparticles are present in the sample. As carbon tape was used to fix the nanoparticles on the stub so, a small peak of Carbon was also detected.

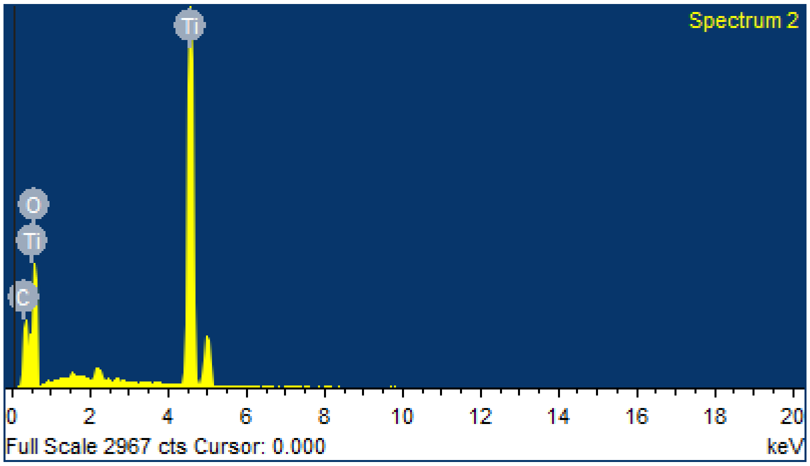


**Figure 4.3:** Picture depicting the elemental analysis of $TiO_2$ nanoparticles emitting Titanium and Oxygen peaks. Also, carbon peak was observed in the sample. This analysis confirms the presence of Titania in a sample.

**Table 4.1:** Elements and their respective weight and atomic percentages in $TiO_2$ sample

| Element | Weight% | Atomic% |
|---|---|---|
| C K | 19.68 | 29.71 |
| O K | 52.84 | 59.89 |
| Ti K | 27.48 | 10.40 |
| Totals | 100.00 | |

The elemental analysis of $TiO_2$ coated thin films was also examined by Energy Dispersive Spectroscopy which confirm that the slide was coated with Titania. Peaks of some other elements were also observed but that peaks were of glass slide on which $TiO_2$ was coated.

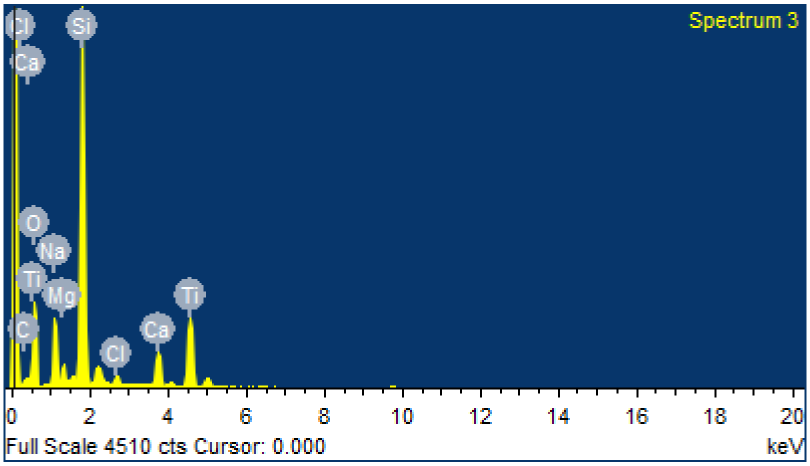


**Figure 4.4:** Picture depicting the elemental analysis of $TiO_2$ coated thin films on glass substrate by EDS machine. Titanium and Oxygen peaks were observed in the sample along with peaks of some other elements. As the coating was very fine and thin and was prepared on glass slide, so other peaks were of glass slide.

**Table 4.2:** Elements and their respective weight and atomic percentages in $TiO_2$ sample

| Element | Weight% | Atomic% |
|---|---|---|
| O K | 43.69 | 59.76 |
| Na K | 8.13 | 7.74 |
| Mg K | 1.75 | 1.58 |
| Si K | 28.53 | 22.23 |
| Cl K | 0.87 | 0.54 |
| Ca K | 4.24 | 2.32 |
| Ti K | 12.79 | 5.84 |
| Totals | 100.00 | |

### 4.1.3. X-Ray Diffraction Analysis (XRD):

Structural analysis was done to determine the phase, crystallinity and planes of prepared $TiO_2$. Pure Titania and nanocomposites prepared were analyzed to determine the phase of Titania obtained. It was seen that “anatase” phase was observed in all the samples.

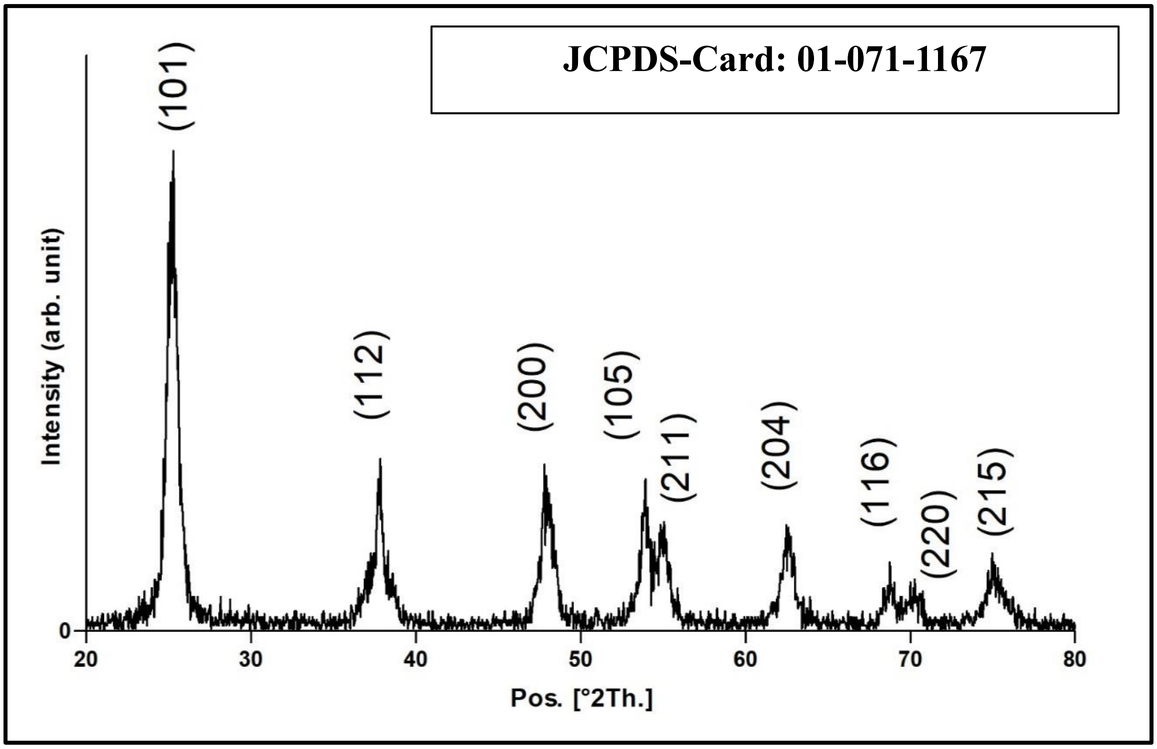


**Figure 4.5:** XRD pattern of $TiO_2$ observed over scale of 20-80

All the peaks obtained through XRD were indexed and they were found to be matching with that of Tetragonal anatase phase of $TiO_2$ nanoparticles. The diffraction peaks observed at 2θ= 25.271, 36.877, 37.698, 38.509, 47.980, 53.761, 54.992, 62.010, 62.572, 68.593, 70.198, 73.858, 74.897, 75.932, 80.505, 82.027, 82.529, and 83.034 were matching the JCPDS card no. 01-071-1167. It confirms that no other extra peak was omitted and the prepared Titania nanoparticles were pure and in anatase phase.

The XRD patterns of the $TiO_2$/ZnO nanocomposite were also observed and matched with the peaks of synthesized ZnO to confirm their presence in the sample. All the diffraction peaks of $TiO_2$ are mentioned above, while the diffraction peaks of ZnO in composite after calcination were $2\vartheta$ = 31.73, 34.36, 36.21, 47.47, 56.53, 62.75, 66.29, 67.85, 68.99, 72.43 and $76.85^{o}$ matched with the wurtzite phase ZnO having JCPDS card No. 01-080-0074. The diffraction planes obtained through XRD of $TiO_2$/ZnO identified both $TiO_2$ and ZnO in the composite and there was no impurity in the sample.

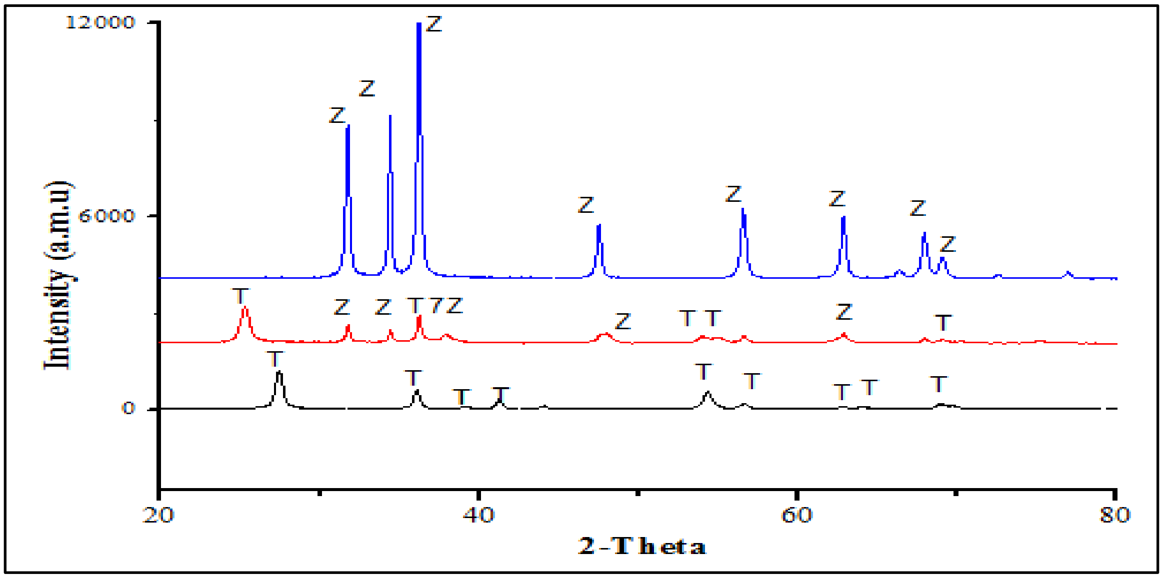
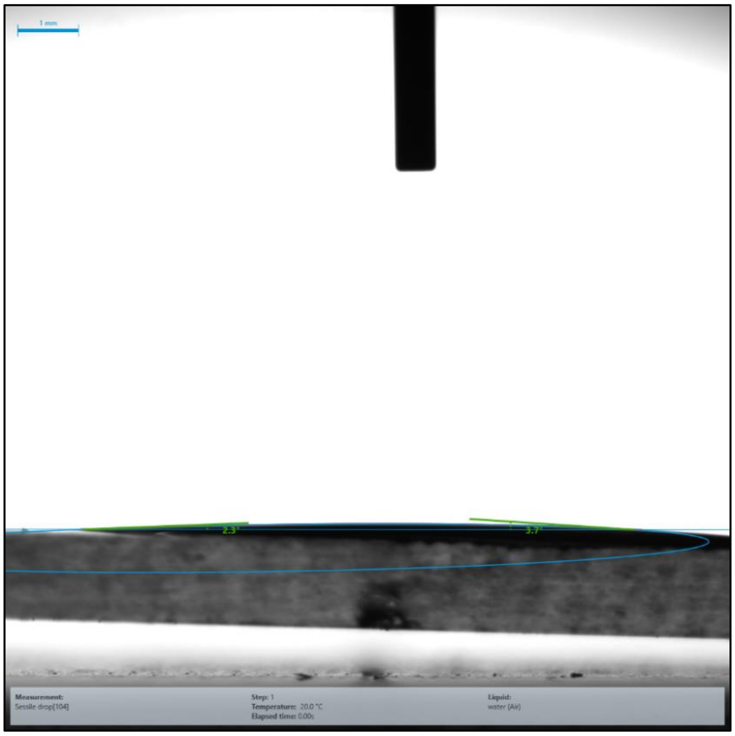


Figure 4.6: XRD patterns of $TiO_2$, T7Z and ZnO showing that the composite had peaks that matched with that of ZnO and accepted patterns of $TiO_2$. The intensity of the ZnO peaks decreased and that of $TiO_2$ increased with the change in weight percentage of $TiO_2$ in composite.

### 4.1.4 Contact Angle Test:

The contact angle measurement of the thin films prepared was done by contact angle goniometer. This was done to evaluate the effect of surface roughness on the contact angle. DI water was used as a solvent to measure the contact angles over thin films.

It was examined that after coating the slide with Titania nanoparticles, the contact angle of thin film was decreasing upto 3 due to decrease in surface roughness and the angle shift towards hydrophilic nature.



Measurement: Sessile drop[104]
Step: 1
Temperature: 20.0 °C
Elapsed time: 0.00s
Liquid: water (Air)

Figure 4.7: Contact Angle of $TiO_2$ coated slide was measured by goniometer. It can be seen that the slide coated with titania had the contact angle of 3, which confirms the super hydrophilic nature of $TiO_2$ nanoparticles.

The contact angle of $TiO_2$/ZnO nanocomposite was also evaluated by contact angle goniometer. De-ionized water was used as a solvent to examine the contact angle of composite. The contact angle of $TiO_2$/ZnO nanocomposite was approximately 23. This also shows the hydrophilic nature of $TiO_2$/ZnO nanocomposite.

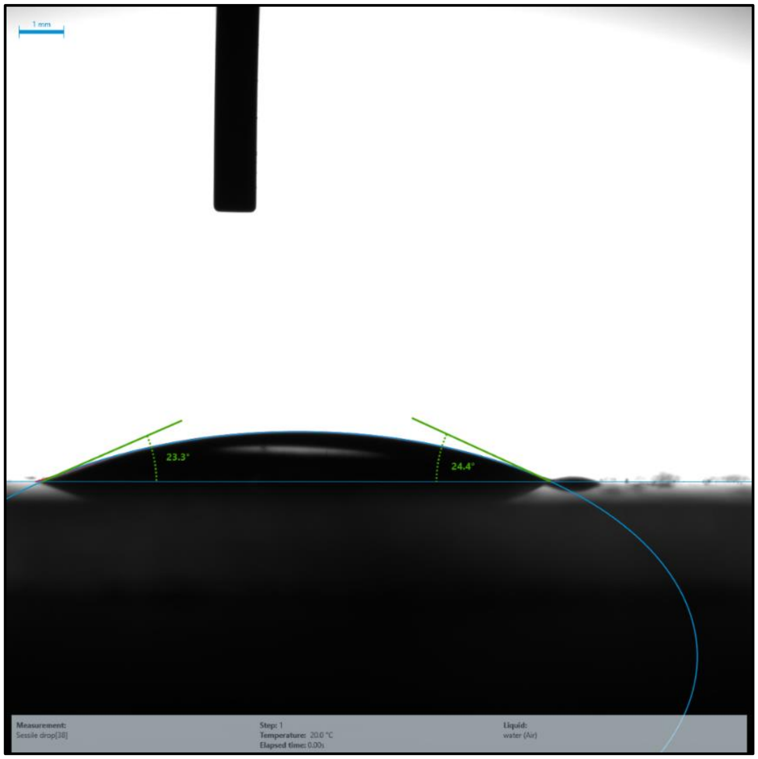


Figure 4.8: Picture depicting the contact angle of $TiO_2$/ZnO nanocomposite measured via goniometer. It reveals the hydrophilic nature of the composite and show the contact angle at nearly 23.

### 4.1.5 Antifogging Test:

$TiO_2$ coated slides were assessed for their hydrophilic properties. In order to examine the antifogging property of Titania, water was sprayed on the coated slide and along with the normal slide. The thin films of Titania shows the hydrophilic properties as no fog was formed on the coated slide as compared to that of control slide. Water droplets moved towards periphery on the coated slide which shows the hydrophilic nature of the Titania.

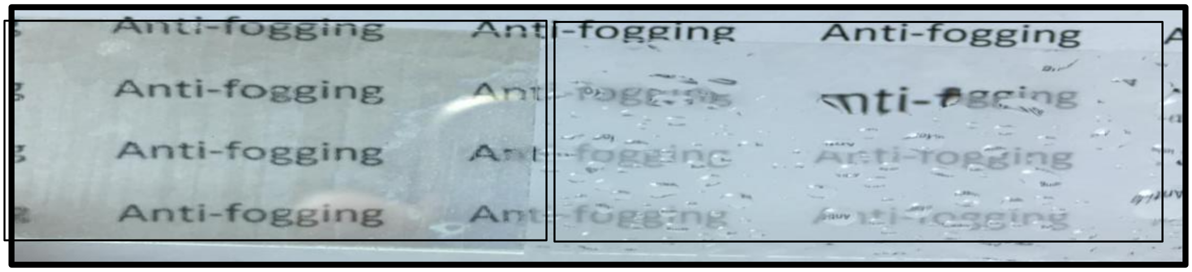


**Figure 4.9:** $TiO_2$ coated and uncoated normal slide was compared and test for antifogging property. It can be seen that no fog was observed on $TiO_2$ coated slide while the uncoated slide showed fog.

Similarly, $TiO_2$/ZnO nanocomposite coated slide was examined for its antifogging property. As, $TiO_2$/ZnO nanocomposite hydrophilic nature confirmed by contact angle test, $TiO_2$/ZnO nanocomposite also exhibited antifogging property. In the case of $TiO_2$/ZnO nanocomposite when water was sprayed on it, the water droplets moved towards periphery in the form of sheet due to nanocomposite hydrophilic nature.

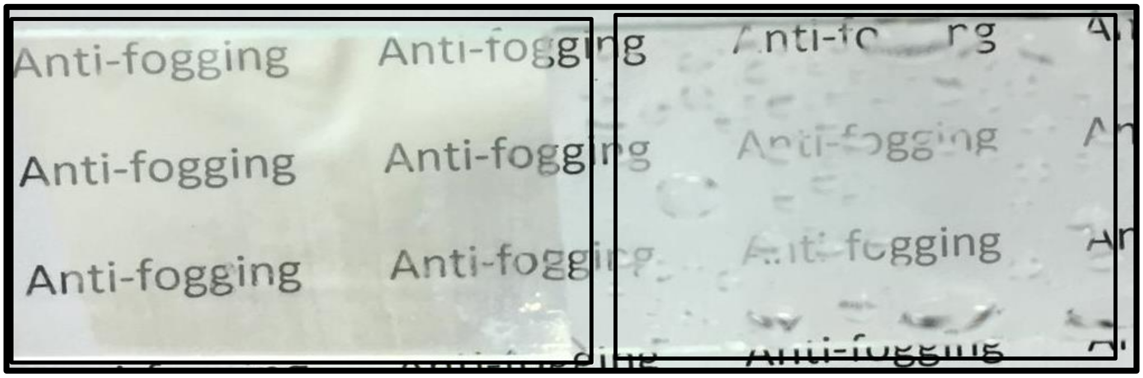


**Figure 4.10:** Image showing the antifogging property of $TiO_2$/ZnO nanocomposite. As due to composite hydrophilic nature, no fog was appeared on coated slide while on uncoated slide fog was appeared.

### 4.2 Disk Diffusion Assay:

The antibacterial properties of Titania nanoparticles and $TiO_2$/ZnO nanocomposite were tested against Pseudomonas aeruginosa, E. coli, Staphylococcus aureus, Klebsiella pneumonia and Listeria Monocytogenes as they are considered as main bacteria's that leads to nosocomial infections.

Antibacterial results of nanoparticles and nanocomposite show good antibacterial effect by inactivating bacterial growth. By increasing the concentration of titania nanoparticles and $TiO_2$/ZnO nanocomposite there was an expansion in width of inhibition zone. It was noted that titania nanoparticles and $TiO_2$/ZnO nanocomposite were active against all hospital oriented bacterial species. Graph illustrates zones of inhibition (mm) measured around nanoparticles and nanocomposite coated disks.

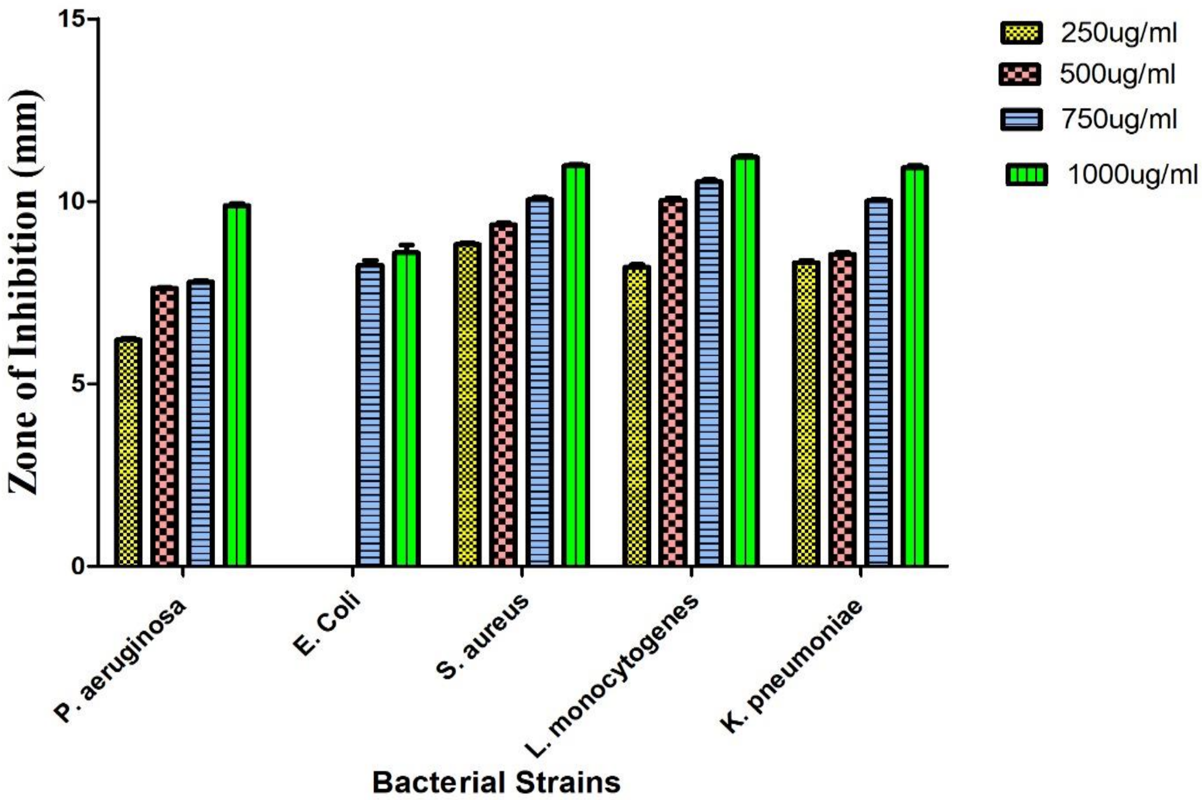


**Figure 4.11:** Disc diffusion assay for $TiO_2$ nanoparticles against (a) *Pseudomonas. aeruginosa* (b) *Escherichia. coli* (c) *Staphylococcus. aureus* (d) *Listeria. monocytogenes* (e) *Klebsiella. pneumoniae.*

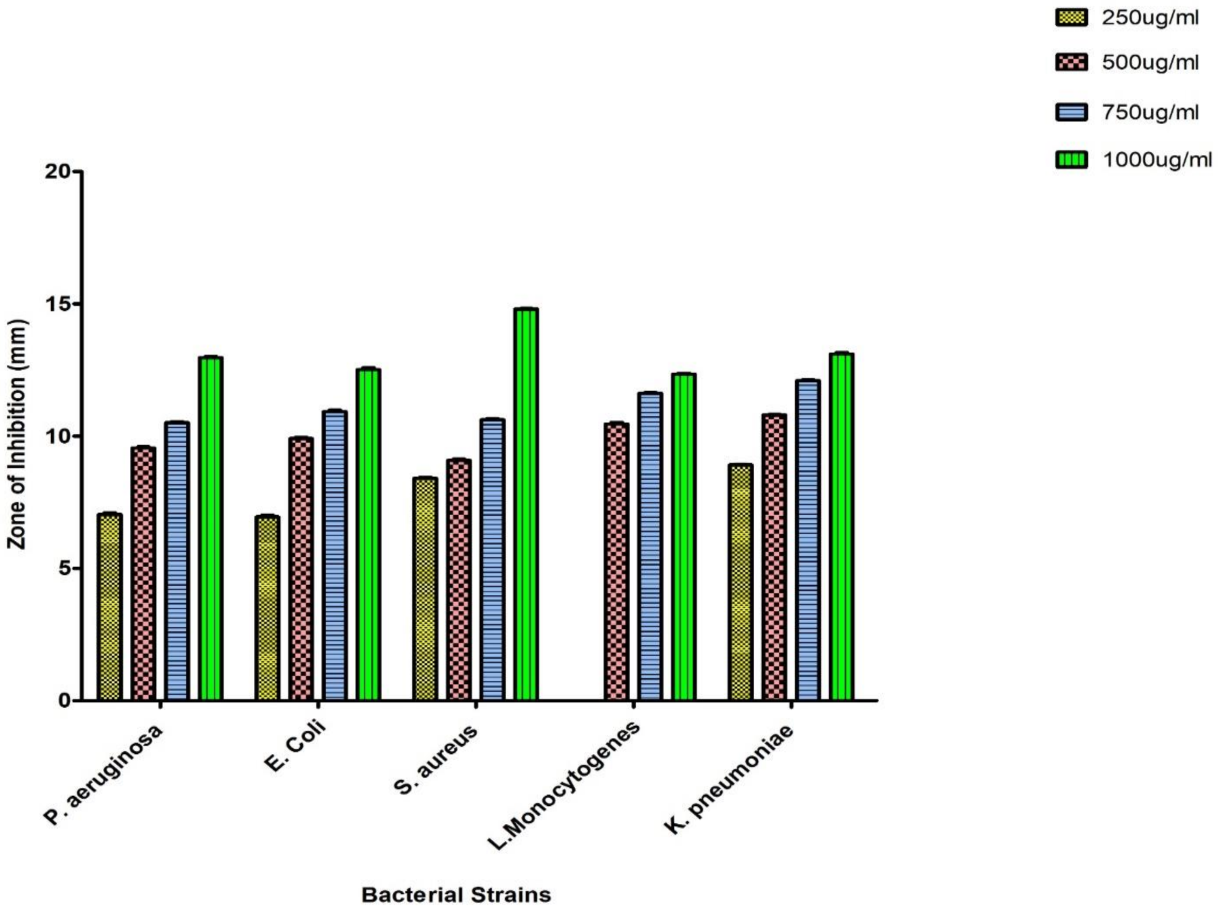


**Figure 4.12:** Disc diffusion assay for $TiO_2/ZnO$ nanocomposite against (a) *Pseudomonas. aeruginosa* (b) *Escherichia. coli* (c) *Staphylococcus. aureus* (d) *Listeria. monocytogenes* (e) *Klebsiella. pneumoniae*

Zone of inhibition around coated disks that Titania nanoparticles and $TiO_2/ZnO$ nanocomposite possess great antibacterial property and defined zone were observed. As the coating concentration increases, zone of inhibition diameter increases. Figure shows bacterial growth inhibition around different concentration of nanoparticles and nanocomposite. The maximum zone of inhibition was 1mg/l ml concentration.

### 4.2.1 Time Kill Assay:

The time kill assay was designed for 1mg/ml concentration of Titania nanoparticles. The activity was assessed for total of 10 hours. The results in the graph indicate that 1mg/ml of titania nanoparticles remain active against the bacteria for the whole duration of assay except Pseudomonas Aeruginosa which shows little resistance after 6 hours.

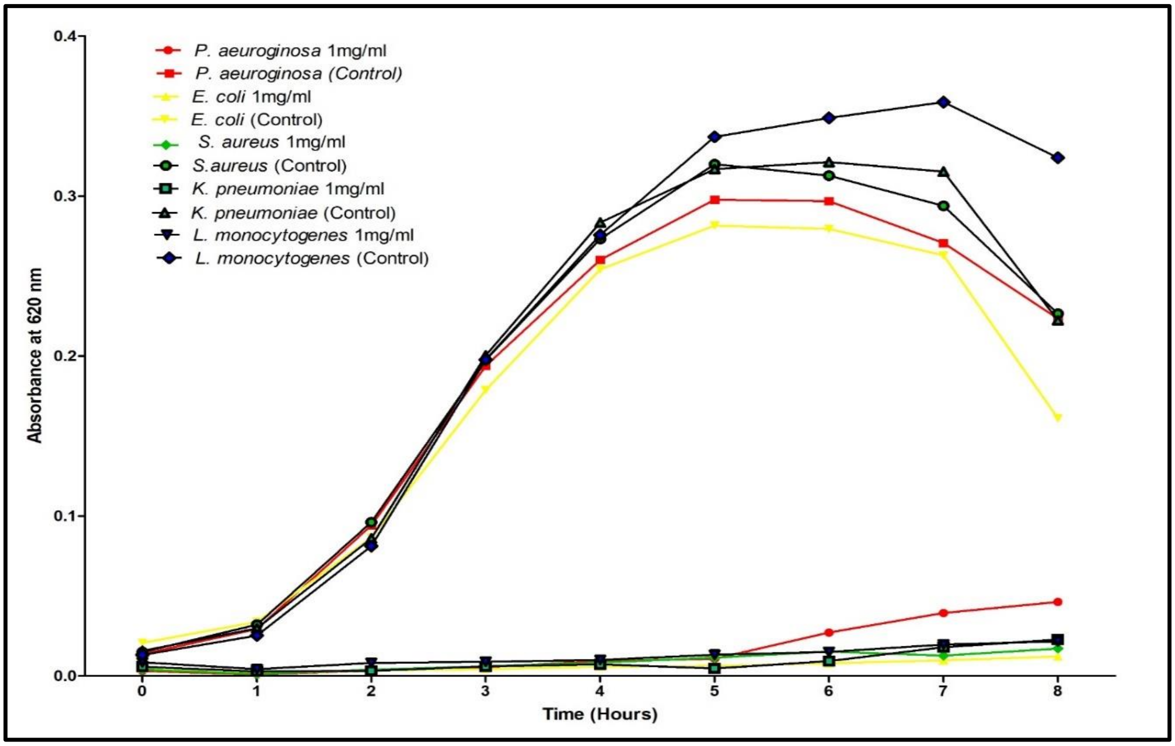


**Figure 4.13:** Graphical illustration of $TiO_2$ nanoparticles activity assessed through time kill assay against bacterial pathogens. The graphs represent activity against different bacterial strains i.e. a. *Pseudomonas aeruginosa*, b. *Escherichia coli*, c. *Staphylococcus aureus*, d. *Klebsiella pneumonia*, e. *Listeria monocytogenes.* X-axis represents the time while Y-axis exhibit absorbance at 620nm which is a representative of the bacterial population.

On the contrary, the time kill assay of $TiO_2$/ZnO was also assessed. Time kill assay for nanocomposite was also designed in 1mg/ml concentration. The activity was assessed for 8 hours. 1mg/ml nanocomposite concentration was significant against control.

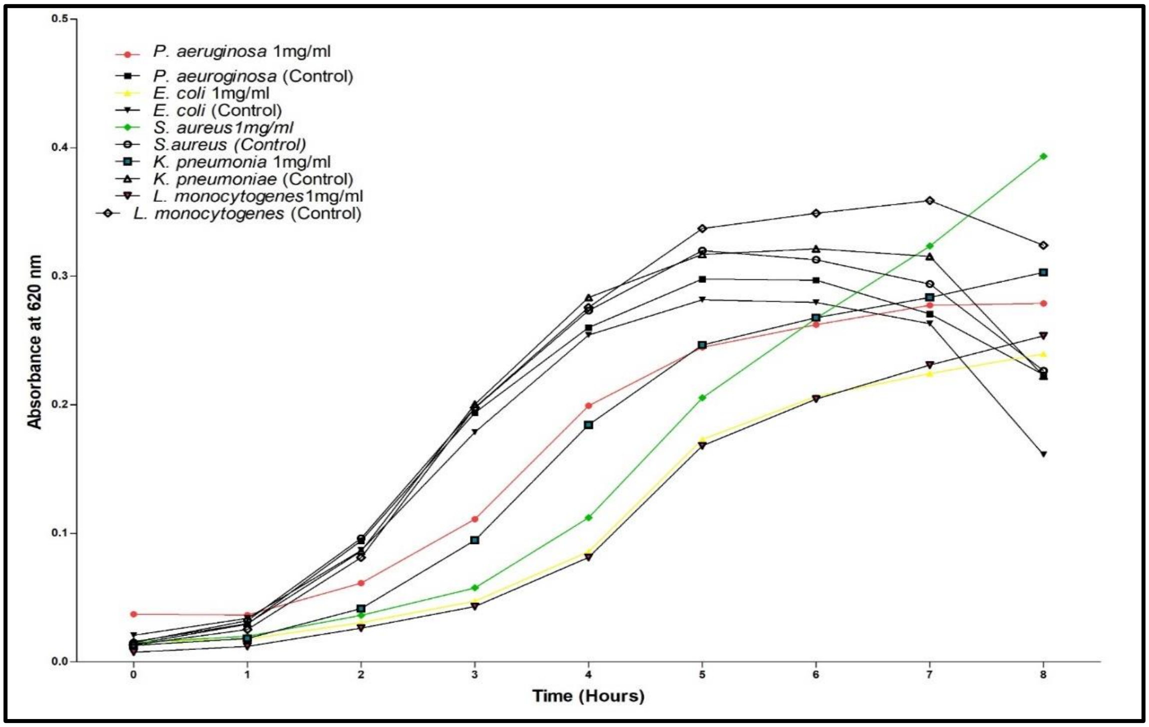


**Figure 4.14:** Graphical illustration of $TiO_2$/ZnO nanocomposite activity assessed through time kill assay against bacterial pathogens. The graphs represent activity against different bacterial strains i.e. a. *Pseudomonas aeruginosa*, b. *Escherichia coli*, c. *Staphylococcus aureus*, d. *Klebsiella pneumonia*, e. *Listeria monocytogenes.* X-axis represents the time while Y-axis exhibit absorbance at 620nm which is a representative of the bacterial population.

# Chapter 5

## DISCUSSION

Photocatalytic materials have picked up the spotlight in the field of dentistry during ongoing years due to powerful oxidizing and high hydrophilic properties(Funakoshi & Nonami, 2007). As during dental surgeries practitioners find themselves in a difficult situation such as distorted vision due to patient breathing and also the mirror contamination form debris and mist and also the transfer of microbes due to poor sterilization of the instruments where metal frame also gets damage due to repeated sterilization. This makes the whole process laborious and time consuming. So here the photocatalytic materials prove to be the blessing and disguise. Photocatalysts forestall the fogging formed on the Mirror and the glasses and also scavenge the pollutants both organic and inorganic when exposed to UV light(Funakoshi & Nonami, 2007). Due to said two attributes they are extensively used in water filtering, deodorizing and air filtering systems(Lakshmi, Renganathan, & Fujita, 1995). So far, the semiconductors that proved to be the good photocatalysts are $TiO_2$, cadmium, tungsten oxide, iron oxide and sulfide and are being used for this said purposes. Among them $TiO_2$ is biocompatible and have strong oxidizing power that caused the destruction of many microbes(Chun et al., 2007). Hence becoming significant in the field of life sciences research. $TiO_2$ is also resistant to corrosion and safe for human use, hence can be applied in dental implants, screws, orthodontic brackets and wires(Choi, Kim, Choy, Oh, & Kim, 2007). Due to Titania self-cleaning properties it is also used in water treatment purposes and bioremediation. Brookite, rutile and Anatase are the three different structures of Titania. Among them rutile and anatase are commonly utilized for industrial purposes. Rutile phase is more stable among them but the anatase have got good photocatalytic properties(Choi et al., 2007). For research purposes, Anatase phase of Titania is commonly applied. The mechanism of $TiO_2$ as vision improvement is as, when Titania is irradiated to ultra violet light, excess electrons are generated in the conduction band that causes excitation of electrons to the valence bands and as a result in valence band holes are generated, that absorbs water due to which there is decrease in contact angle, hence surface shifted towards hydrophilic, that slide the water down to the periphery and consequently vision is improved and become visible(Laskey, 1978). Generally, less than 10 liquid contact angle considered as super hydrophilic (Battiston et al., 1994).

The other property of $TiO_2$ as an antimicrobial agent is as, under moist conditions, when Titania is irradiated with light, electrons are generated and they jump towards valence bands and holes are formed. These holes react with absorbed water and create hydroxyl radicals. As excess electrons are jump to valence band more water is absorbed and the surface becomes hydrophilic more hydroxyl radicals are generated. Sufficient OH radicals enable complete mineralization of most organic materials(Prevot, Vincenti, Bianciotto, & Pramauro, 1999).

Several process can be applied for shaping Titania's thin films like spin coting, sol-gel dip coating, chemical vapor deposition. In our study, sol-gel dip coating process was applied in order to produce thin transparent layer of Titania and $TiO_2$/ZnO nanocomposite on glass substrate. Dip coating method is favorable in term of layer uniformity and conditions can be controllable. In this study, we have heated the thin films over 400C at ramp temperature for getting uniform layers. However, in this study, initially heating of dental mirror at this temperature damage them, because such high temperature damage the glue that stick the mirror to the frame and also damage the surface of the metal frame. So, our study was firstly conducted on the glass substrate. In our study lysogeny broth media was used for culturing microbes in order to assess antimicrobial efficacy of Titania nanoparticles and $TiO_2$/ZnO nanocomposite. *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* were tested against nanoparticles and nanocomposite.

In our study Sol gel synthesis method was used due to cost effectiveness and availability of the instruments. We have synthesized Titania nanoparticles and $TiO_2$/ZnO nanocomposite that include hydrolysis and condensation of the reaction. TTIP was used as a precursor and 2-propanol was used as a solvent in the reaction mixture. The reaction was stirred for 2-3 hours and then calcined at 500c for one hour.

Synthesis of particular Titania nanoparticles was confirmed by different characterization techniques that include SEM, EDS and XRD. SEM confirms the morphology of the Titania nanoparticles, EDS gave the elemental composition of the compound and XRD endorse the crystallinity of nanoparticles and also the phase confirmation of nanoparticles.

SEM analysis revealed the spherical shape of the Titania nanoparticles and composite gave irregular shape which may have occurred due to slow reaction rate which in turn affected the formation of crystals in composite. Although the morphology of composite was not clear but it was confirmed through EDS that composite had Titanium, Zinc and Oxygen as its constituent. SEM images of $TiO_2$ coated slides was also examined and their average size was calculated to be 40nm.  Presence of Titania on slides was also confirmed by EDS.

XRD was conducted to actuate nanoparticle structure and phase of synthesized nanoparticles and nanocomposite. Data was analyzed by using X-pert high score software and all the peaks of nanoparticles and nanocomposite matched with the JCPDS database. In case of Titania all the peaks were matched with JCPDS card no **01-071-1167.** in case of nanocomposite peaks other than that of Titania was also observed and upon analysis they were found to be of ZnO with JCPDS card no **01-076-0704.**

Furthermore, in our study hydrophilic property was also examined through contact angle measurement and by anti-fogging analysis. Contact angle analysis revealed that contact angle of coated slide was less as compared to uncoated slide. Lower contact angle indicated the hydrophilic nature of coated surface which is an attribute on Titania nanoparticles. Moreover, the surface can be made super hydrophilic by treating the surface with UV.

Antibacterial activity of Titania is well reported in many articles. So, this evidence led us to assess our Titania nanoparticle and its composite with zinc oxide for any possible antibacterial activity. Kirby-Bauer Disk diffusion and time kill assay was utilized to assess antimicrobial activity of synthesized nanoparticle and nanocomposite against gram positive and gram-negative bacteria(Balouiri, Sadiki, & Ibnsouda, 2016). The results indicate that upon treating Titania nanoparticles with UV, they show significant antibacterial activity. Nanocomposite also shows the antibacterial activity when checked against the same strains. The inhibition of bacterial growth shows the direct relationship with the concentration of nanoparticles. More activity was observed as the concentration increased. Time kill assay was also performed to check the efficacy of nanoparticles with respect to time. Maximum nanoparticle activity was observed at 1mg/ml in disk diffusion assay against bacterial strains. So that’s why we chose this concentration to be assessed for its time dependent antibacterial activity for total of 10 hours. Maximum antibacterial activity was observed of Titania nanoparticles.

# CONCLUSION

The nanostructures Titania and its composite with ZnO was synthesized by sol gel method. The mean crystalline size of Titania nanoparticles was 40nm. The anatase phase was confirmed by XRD analysis. Since in order to induce desired characteristics of nanoparticles and nanocomposite coating was done on glass substrate and was assessed for their antifogging and antimicrobial properties. Lower contact angle of Titania nanoparticles coated slide was examined which shows their antifogging properties. The antimicrobial property was also examined by disk diffusion assay and time kill assay which depicts that $TiO_2$ nanoparticles and $TiO_2$/ZnO nanocomposite inhibits the bacterial growth. In this manner, we presume that antifogging and antibacterial medications on glass substrate surfaces ought to be performed and best transparency would be exhibited by hydrolysis and condensation of titanium alkoxide.

## FUTURE PROSPECTS

Photocatalysis has gained the attentions in commercializing the various products by utilizing photocatalytic functions. Among numerous contenders for photocatalysis, $TiO_2$ proves to be standout among all and can be used as a main material for industrial use in future. this is because of $TiO_2$ higher stability and strong photocatalytic activity and inexpensive. Also, $TiO_2$ ensured to be safe for environment and for human. Moreover, $TiO_2$ if utilized as a part of blend with ZnO and also by varying the ratios of composite as $TiO_2$-ZnO nanocomposite will come about into upgraded consolidated properties with the likelihood of finding new properties of the composite also. This composite in connection to this investigation will be utilized to do coating of dental mirrors and dental instruments which will add numerous valuable qualities to them. Also, coat the glass substrate with anatase structure of $TiO_2$ and then transform the glass to a mirror should be recommended in future work and evaluation of the stability of coated thin films through Atomic Absorption Spectroscopy should be recommended in future studies.

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