Preparation and Antibacterial Activity of Ag-TiO_2 Composite

By

Sangavi.K

(15PPH010)

A dissertation submitted to the

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore – 641 043

In partial fulfilment of the requirements for the Degree of

Master of Science in Physics

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CERTIFIED AS A BONAFIDE RESEARCH WORK

10/4/17
Signature of the Supervisor

10/4/17
Signature of the Head of the Department

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SANGAVI.K

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INTRODUCTION

CHAPTER- I

INTRODUCTION

1.1 INTRODUCTION:

Nanotechnology is emerging as a rapidly growing field with its application in Science and Technology for the purpose of manufacturing new materials at the nanoscale level. The word ‘Nano’ means, one-billionth of a physical quantity. One Nano meter is a unit of length equal to one billionth of a meter. The physical and the chemical properties of the materials is different in the form of nano size compare to the materials in molecular form [1]. It originated from two different from which is known as primary (natural) and secondary or artificial (synthesized compounds) which may be organic or inorganic. The Nanoparticles have different forms (helix, zigzag, belt, sphere, oval, prism, cube, helical or pillar) and can form agglomerations or be dispersed [2]. The Nanoparticles are used in different fields like medicine, pharmaceuticals, textiles, communication, and the electronics industry with the purpose of improving the quality of life.

In last 25 years, photo activation of titanium oxide have been evaluated to develop a new alternatively safe and eco-friendly approaches for disinfection. A TiO_2 nanoparticle is the most fascinating material characterized by high chemical stability, high photo reactive, non-toxicity, cheapness, broad spectrum activity and antibiosis. Titanium dioxide takes a vital role in the area of renewable energy. In dye sensitized solar sell titanium dioxide used as a transparent conductive electrode due to its wide band gap.

A TiO_2 nanoparticle have the property named as ‘Photo catalytic activity’ they are used in the separation of water, energy production, air and water purification, sterilization of surfaces, synthesis of organic compounds and in the reduction environmental pollution. Photo catalytic effect of TiO_2 has been depend on the crystalline structure, doping, surface area, surface hydroxyl group etc,.due to doping TiO_2 photo activity is strongly influenced by the presence of noble metals like Ag,Au,etc[3].

Because of its anti biotic activity the TiO_2 nanoparticles has been used as biocide agent against various micro organisms like fungi, virus and bacteria [4]. In this chapter we focused on the microbial activity of Ag doped TiO_2 Nanoparticles TiO_2 and Ag both of the elements have an antibacterial activity.

The Ag Nanoparticles are one of the most important noble metal due to their highly chemical and physical properties. Because of this properties Ag have attracted by many fields especially in electrocatalystic and antibacterial activity, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components.

1.2 NANOTECHNOLOGY:

In naturally many particles are present at nanoscale level. For example, catalysts, porous materials, certain minerals, soot particles, etc., They have unique properties particularly because of the nanoscale features. There are many different views of precisely what is included in nanotechnology. However for nanoparticles should obey some specific properties, which are followed here:

1. Small size, measured in 100s of nanometers or less
2. Unique properties such as surface to mass ratio, allowing for catalytic promotion of reactions, as well as their ability to absorb and carry other compounds, etc., because of the small size[5].
3. Control the structure and composition on the nm scale in order to control the properties.

1.3 CLASSIFICATIONS OF NANOPARTICLES:

There are various approach for classify the Nanoparticles. Commonly Nanoparticles are classified as three different forms, based on their shape. Those classifications is followed by

- One dimension

  In 1D nanofilms dimension should be lower than 100 nm. These are belongs to the thin film, layers and coating type. This type is called 1D nanoparticles because of, in this type the magnitude of one coordinate is lower than 100nm.
- Two dimension

  This types dimension also have the magnitude of two coordinates less than or equal to 100nm. Nano tubes fibber and nano wires are example for this group, carbon nanotubes.
- Three dimension

  In 3D nano structure all three coordinates magnitude is lower than or equal to 100nm. Nanoparticles, quantum dots, nanoshell, nanorings and microcapsules are some examples for this type[6].

There have another method to classify the nanoparticles. Those are

- Non intentionally made nanoparticles (primary nanoparticles)
- Intentionally made nanoparticles (secondary nanoparticles)

None intentionally made nanoparticles are materials belongs to the natural or that produced by without human intention, for examples proteins, viruses, production of nanoparticles during volcano eruption. The next one is intentionally made nanoparticles. These nanomaterials synthesised through a defined fabrication process, for example Ag, Au, TiO_2 nanoparticles [5,6].

1.4 SYNTHESIS METHODS:

There have some specified methods for synthesis nano scale particle or molecules. Those methods are classified here[7,8].

1. Sol-gel technique
2. Solvothermal synthesis
3. Mechanical Attrition
4. Chemical reduction
5. Vacuum Deposition and Vaporizations
6. Laser ablation
7. Inert gas condensation
8. Electrodeposition
9. Biosynthesis of nanoparticles

1. Sol-gel technique:

In Sol-gel technique discrete particles are integrated network precursor involved in chemical solution that mainly used for the fabrication of metal oxides hence it is a chemical technique. The precursor sol can be either deposited on the substrate to form a film or used to synthesize powders.

2. Solvothermal synthesis:

In Solovothermal synthesis process the polar solvents are involved in different condition like at temperatures above their boiling points and in the condition of under pressure at versatile low temperature. Hence the reaction does not involve in lower temperature because the solubility of reaction get significantly increases in Solovothermal condition.

3. Mechanical attrition:

Unlike many of the methods mentioned above, mechanical attrition produces its nanostructures not by cluster assembly but by the structural decomposition of coarser grained structures as a result of plastic deformation. Elemental powders of Al and β-SiC were prepared in a high energy ball mill. More recently, ceramic/ceramic nanocomposites WC-14% MgO material has been fabricated. The ball milling and rod milling techniques belong to the mechanical alloying process which has received much attention as a powerful tool for the fabrication of several advanced materials. Mechanical alloying is a unique process, which can be carried out at room temperature. The process can be performed on high energy mills, centrifugal type mill and vibratory type mill, and low energy tumbling mill. High energy mills include:

- Attrition Ball Mill
- Planetary Ball Mill
- Vibrating Ball Mill
- Low Energy Tumbling Mill
- High Energy Ball Mill

4. Chemical reduction:

Sodium borohydride, hydrazine hydrate and sodium citrate are some of the commonly used reducing agents in which the ionic salts get involved in reduction process by an appropriate medium in the presence of surfactant were involved using reducing agents are used.

5. Vacuum deposition and vaporization:

Before proceeding to the other methods, it is important to understand the terms vacuum deposition and vaporization or vacuum evaporation. In vacuum deposition process, elements, alloys or compounds are vaporized and deposited in a vacuum. The vaporization source is the one that vaporizes materials by thermal processes. The process is carried out at pressure of less than 0.1 Pa and in vacuum levels of 10 to 0.1 MPa. The substrate temperature ranges from ambient to 500°C. The saturation or equilibrium vapour pressure of a material is defined as the vapour pressure of the material in equilibrium with the solid or liquid surface.

6. Inert gas condensation:

In inert gas condensation there is an ultra-high vacuum chamber filled with helium or argon gas at typical pressure of few 100 Pascal's where different metals are evaporated in separate crucibles inside. As a result of inter atomic collisions with gas atoms in chamber, the evaporated metal atoms lose their kinetic energy and condense in the form of small crystals which accumulate on liquid nitrogen filled cold finger.

Example gold nanoparticles have been synthesized from gold wires. Synthesis using bio organisms is compatible with the green chemistry principles. Environmental friendly, nontoxic and safe reagents are mainly involved in green synthesis of nanoparticle.

7. Laser ablation:

The laser ablation laser beam is a technique that used for removing materials from a solid surface. Absorbed laser energy and evaporates mainly involves when the material is heated at low laser flux. The material is converted to plasma in case of higher flux. For example Carbon nanotubes can be produced by this method.

8. Electrodeposition:

Nanostructured materials can also be produced by electrode position. These films are mechanically strong, uniform and strong. Substantial progress has been made in nanostructured coatings applied either by DVD or CVD. Many other non-conventional processes such as hypersonic plasma particle deposition (HPPD) have been used to synthesize and deposit nanoparticles.

The significant potential of nanomaterial synthesis and their applications is virtually unexplored. They offer numerous challenges to overcome. Understanding more of synthesis would help in designing better materials. It has been shown that certain properties of nanostructured deposits such as hardness, wear resistance and electrical resistivity are strongly affected by grain size. A combination of increased hardness and wear resistance results in a superior coating performance.

9. Biosynthesis of nanoparticles:

Nanoparticles prepared by utilizing bacteria, fungi and plant extracts as reducing agents, botanical extracts, Bio reduction. These biological methods, so called green synthesis methods, are not only benign and environment friendly but also cost effective, rapid, less laborious, easily scalable to large scale and more efficient than conventional methods[9].

1.5 APPLICATION OF NANO TECHNOLOGY:

- Nanomedicines -Nano drugs, Medical devices, Tissue engineering
- Chemical and Cosmetics- Nanoscale chemicals and compounds, paints, coatings etc
- Materials- Nanoparticles, carbon nanotubes, biopolymers, points, coatings
- Food Sciences- Processing, nutracetical food, nanocapsules.
- Environment and Energy- Water and air purification filters, fuel cells, photovoltaic
- Military and Energy- Biosensors, weapons, sensory enhancement
- Electronics- Semiconductors chips, memory storage, photonica, optoelectronics
- Scientific Tools Atomic force, microscopic and scanning tunnelling microscope
- Agriculture -Atomic force, microscopic and scanning tunnelling microscope.

These are the general and common applications of nano technology in various fields, nanotechnology gives a new way for approaching the materials in research[10]. In this chapter we concentrate on Titanium dioxide and silver nanoparticles synthesis and their antibacterial activity.

1.6 TITANIUM DIOXIDE:

Titanium dioxide is a molecule which contains single titanium atom and two oxygen atoms[11]. Chemical formula for the molecule is TiO_2. Titanium dioxide is a white solid inorganic substance that is thermally stable, non-flammable, poorly soluble, and not classified as hazardous according to the United Nations' (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

Titanium is the ninth most common element in the earth's crust. Titanium dioxide is typically thought of as being chemically inert [12]. Even after half a century of research, investigation of the fundamental properties of TiO_2 crystal phases remains very important properly due to their important role to effectively utilize solar energy. Titanium dioxide exists in many polymorphs. Among them anatase, rutile, and brookite are well known minerals in nature. Nanomaterials show a higher tolerance to structural distortion than bulk materials due to their inherent lattice strain. As a result, the surface modification of TiO_2 nanoparticle appears to be more beneficial than the modification of bulk TiO_2. Fig 1.1 shows the classification of TiO_2.

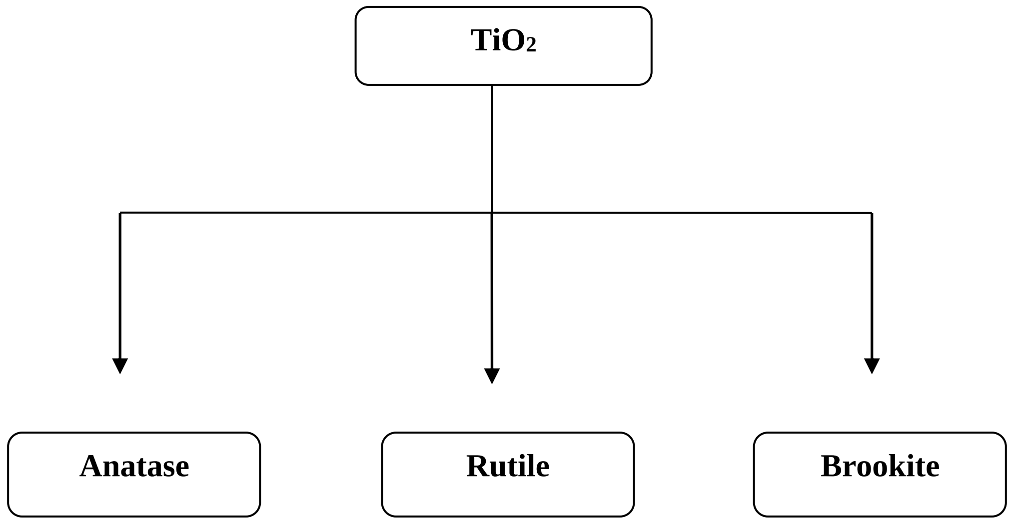


Fig. 1.1 Classification of TiO_2 nanoparticles

1.6.1 ANATASE:

Anatase form of TiO_2 has tetragonal structure but the distortion of the TiO_6octahedron is slightly larger for the anatase phase. Muscat et al[13]. Found that the anatase phase is more stable than the rutile at 0 K, but the energy difference between these two phases is small (~2 to 10 kJ/mol). Compare to all other polymorphs phase anatase is recommended for the application of photocatalytic applications due to its high electron mobility, lower density and dielectric constant. Fig 1.2 shoes of anatase phase.

Due to higher Fermi level, lower capacity to absorb oxygen and higher degree of hydroxylation in the anatase phase its photo reactivity is higher. Selloni reported that the reactivity of (001) facets is greater than that of (101) facets in an anatase crystal[14].

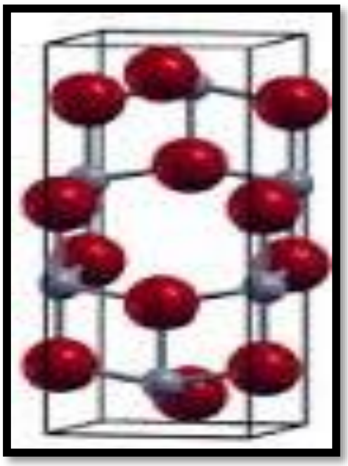


Fig.1.2 Anatase phase of TiO_2 nanoparticles

1.6.2 RUTILE:

Rutile TiO_2 has a tetragonal structure and contains 6 atoms per unit cell. The TiO_6 octahedron is slightly distorted. The rutile phase is stable at most temperatures and pressures up to 60 kbar, where TiO_2 becomes the thermodynamically favourable phase. Zhang et al. found that anatase and brookite structures transformed to the rutile phase after reaching a certain particle size, with the rutile phase becoming more stable than anatase for particle sizes greater than 14 nm[15]. Fig 1.3 for Rutile phase of TiO_2

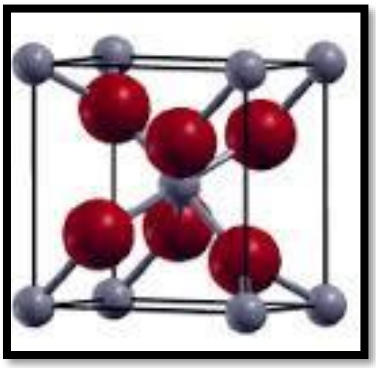


Fig. 1.3 Rutile phase of TiO_2

Once the rutile phase formed, it grew much faster than the anatase. The activity of the rutile phase as a photocatalyst is generally very poor. However, Sclafani et al concluded that the rutile phase can be active or inactive, depending on its preparation conditions.

1.6.3 BROOKITE

Brookite TiO_2 belongs to the orthorhombic crystal system. Its unit cell is composed of 8 formula units of TiO_2 and is formed by edge-sharing TiO_6 octahedral. It is more complicated, has a larger cell volume and is also the least dense of the 3 forms and is not often used for experimental investigations.Fig 1.4 for the phase of Brookite. Some of the properties of Anatase, Rutile, Brookite forms of TiO_2 are tabulated below [16]. Table 1.1 shows the comparision of TiO_2properties.

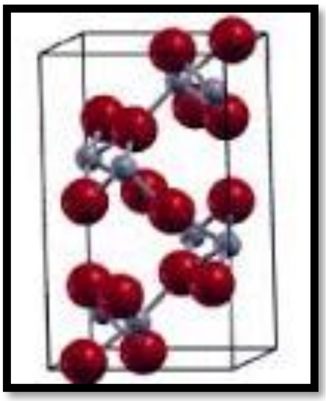


Fig.1.4Brookite phase of TiO_2nanoparticles

Properties	Anatase	Rutile	Brookite
Crystal structure	Tetragonal	Tetragonal	Orthorhombic
Lattice constant	a = 3.784 c = 9.515	a = 4.5936 c =2.9587	a = 9.184 b = 5.447 c = 5.154
Space group	I41/amd	P42/mnm	Pbca
Molecule	2	2	4
Volume/ molecule	34.061	31.2160	32.172
Density	3.79	4.13	3.99
Ti–O bond length	1.937(4) 1.965(2)	1.949 (4) 1.980 (2)	1.87–2.04
O–Ti–O bond angle	77.7° 92.6°	81.2° 90.0°	77.0°–105°
Optical band gap	~3.4 ev	~3.0 eV	~3.3 eV

Table 1.1 comparison of Anatase, Rutile, Brookite properties.

1.7 DOPING:

Doping of TiO_2 has been an important approach in band gap engineering to change the optical response of semiconductor photo catalysts. The main objective of doping is to induce a bath chromic shift, i.e., a decrease of the band gap or introduction of intra-band gap states, which results in the absorption of more visible light. Doping may lead to photocatalystic systems that exhibit enhanced efficiency. It is desirable to maintain the integrity of the crystal structure of the photocatalystic while changing its electronic structure by doping.

For modifying the photocatalystic effect doping is the one of the main process to increase the efficiency. Metals, non-metals are added as a doping material and noble metals like Pt, Ag, Au, Pd, Ni, Rh and Cu. Compare to all other doping materials, noble metals are very effective at enhancing photo catalysis by TiO_2.

Because the Fermi levels of these noble metals are lower than that of TiO_2, photo excited electrons can be transferred from the conduction band of TiO_2 to metal particles deposited on the surface of TiO_2, while photo generated holes in the valence band remain on TiO_2.

This greatly reduces the possibility of electron-hole recombination, resulting in efficient separation and higher photocatalystic activity. Numerous studies have found that the properties of these kinds of composites depend strongly on the size of the metal particle, dispersion and composition. When the size of the metal particles is less than 2.0 nm, the composites display exceptional catalytic behaviour[17]. It has been suggested that too high a concentration of metal particles reduces photon absorption by TiO_2 and allows the metal particles to become electron-hole recombination centres, resulting in lower efficiency.

TiO_2 thin films possess deodorizing, antibacterial and self-cleaning function under ultraviolet light. However, its disadvantage is that the band-gap energy of TiO_2 is ~ 3·2 eV, therefore, UV illumination is necessary to photoactivate this semiconductor. The bacterial activity of TiO_2 nanoparticles is directly related to the Ultraviolet absorption and the formation of various reactive species such as superoxide and hydroxyl radicals. The TiO_2 has inherent drawback as an efficient biocide agent due to large bandgap energy and fast recombination rate of photo generated electron hole pair. Photocatalystic decomposition of TiO_2 induced only in the range of UV.

To improve photocatalystic performance of TiO_2 various approaches can be developed [18]. For example doped the noble metals give the good result in photo catalytic performance. This method improves the range of absorption due to decrease the band gap energy of TiO_2. Silver or silver ions have long been known to be excellent materials for breeding spectrum antibacterial agents.

According to various studies, it is believed that Ag reacts with proteins by combing the –SH groups of enzymes, which leads to the inactivation of the proteins the antibacterial mechanism of Ag+ on bacteria. Ag+ makes DNA molecules to loose their replication abilities. Silver doped materials are chemically durable and release Ag+ for a long time. This excellent antibacterial activity is not restricted by UV illumination.

1.7.1 SILVER NANOPARTICLES:

The use of Ag Nanoparticles is rapidly increasing in current century because of their unique optical, electrical, and thermal properties and is incorporated into industrial application of electronics, catalysis, and photonics. In recent years, with higher integrated density of electronic components, there are growing demands for the thickness or the width of printed electronic circuits due to considering the space between these circuits. Therefore, the synthesis of Ag Nanoparticles becomes an important issue in the electronic industry.

Nanoparticles properties depend on the aspect ratio, crystal size, crystalline density and morphology. Narrow sized and uniformly distributed Nanoparticles possess higher chemical and physical properties due to their higher aspect ratio[18]. Ag Nanoparticles possess very high aspect ratio regardless of their synthesis process which determines surface related properties such as solubility and stability. High aspect ratio of Ag Nanoparticles is essential for different application e.g. catalysis, microbial resistance etc. One of the widely studied properties of Ag Nanoparticles is Surface Plasmon Resonance which is also found when aspect ratio is high.

High ratio of surface area to volume ratio of Ag Nanoparticles exhibits microbial resistance and develops resistant strains. Today researcher's main concerns are optical, catalytic and microbial properties. Ag Nanoparticles are synthesised by various methods, like Physical Approach, Biological Approach, Chemical Approach, Photo chemical Approach.

Since ancient times among various antimicrobial agents, silver has been most extensively studied and used to fight against infections and prevent spoilage. Silver nanoparticles are among the most widely commercialized engineered nanomaterials, because of their antimicrobial properties[19]. They are already commonly used in medical devices, household products and industry.

The bactericidal activity of silver nanoparticle against the pathogenic, multidrug-resistant (MDR) as well as multidrug-susceptible strains of bacteria was studied by many scientists, and it was proved that the silver nanoparticles are the powerful weapons against the MDR bacteria such as Pseudomonas aeruginosa, ampicillin-resistant Escherichia coli, erythromycin-resistant Streptococcus pyogenes, methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Staphylococcus aureus (VRSA).

The broad spectrum of silver nanoparticles includes microorganisms in general, as Gram-positive and Gram-negative bacteria, filamentous fungi, yeasts and viruses. Its most striking property is to have a large surface area. The antifungal activity of silver nanoparticles was also reported by several researchers [20].

According Marambio Jones &Hoek in submicromolar concentrations, silver ions are internalized and react with thiol groups of cellular proteins that lead to uncoupling of ATP synthesis from respiration, loss of proton motive force and interference with phosphate efflux system. At levels of milli-molar silver nanoparticle induce the detachment of the cell wall membrane from the cytoplasm, with the possible release of intracellular content, DNA condensation and loss of replicative capacity. Free radicals produce oxidative stress in reactive oxygen species (ROS) resulting in membrane damage and DNA. Finally, silver nanoparticle increase cell membrane permeability and subsequently penetrates into cells[21].

1.7.2 APPLICATION OF SILVER NANOPARTICLES:

Ag nanoparticles are used in various fields. Some uses of Ag nanoparticles are followed by,

1. Catalysis: The selective oxidation of alcohols, alkanes and alkenesFor the synthesis of industrially interesting products including water splitting degradation of organic pollutants.
2. Biology and Medicine: Ag is a highly antimicrobial material used in water purification, wound care, medical devices and drug delivery.
3. Optics: Optoelectronic devices, active waveguides in optical devices (amplifiers)
4. Electronics: electronically conductive adhesives (ECAs).

1.8 APPLICATIONS OF TITANIUM DIOXIDE:

Photo catalysis refers to the chemical reaction that occurs when light strikes a chemical compound that is light sensitive, such as titanium oxide. When light strikes titanium dioxide, the base compound in Titanium dioxide, a chemical reaction will be repeated in the immediate region and cause the breakdown of organic toxins, odours, and more. This reaction has many valuable results, several are listed below.

Deodorization applications:

Titanium dioxide doesn't cover up smells, like conventional air freshners, it actually attacks the root of the smell by causing the breakdown of the origin of the odor (ammonia, aldehyde gas [smoke], etc.,

Water purifying applications:

Titanium dioxide causes detrimental organic matter such as organic chlorine compounds, tetrachlorethylene, trihalomethane and other harmful substances to be broken down.

Environmental improvement applications:

Titanium dioxide also actively removes environmental pollution substances, such as NO_2 emitted by exhaust gas etc. from the atmosphere. SO_2, a detrimental organic matter present in the atmosphere, are also broken down.

Antibacterial applications:

Titanium dioxide has a more helpful role in our environmental purification due to its nontoxicity, photo induced super-hydrophobicity and antifogging effect. These properties have been applied in removing bacteria and harmful organic materials from water and air, as well as in self-cleaning or self sterilizing surfaces for places such as medical centres. Bacteria such as e-coli, yellow staphylococcus, fungi etc. are broken down by TiO_2 (the main ingredient in Titanium dioxide) nanoparticles[21].

1.9 OBJECTIVE OF WORK:

The aim of the present work is,

- To synthesis Silver doped Titanium dioxide nanoparticle
- To characterize the synthesized nanoparticles by SEM, XRD, Raman analysis.
- To studies the antibacterial activity of the synthesized nanoparticles.

REVIEW OF LITERATURE

CHAPTER-II

REVIEW OF LITRATURE

2.1 INTRODUCTION:

A literature review about previous research work and the discussion published on silver doped Titanium dioxide [Ag-TiO_2] nanoparticles and its antimicrobial activity. Literature review on silver doped Titanium dioxide nano particles synthesis by sol-gel method and other methods have been reported here.

2.2 LITERATURE OF REVIEW:

E.Albert et al., [2016]presented a report on antibacterial activity of silver nanoparticles doped in titanium coating by sol-gel method. Titanium layer structure and other physical properties are characterised by Transmission and electron microscopy. Ag content were examined by Rutherford backscattering spectrometry. Antibacterial activity of Ag-TiO_2 was studied by colony forming and agar diffusion method under dark and light illumination. The TiO_2 show antibacterial activity and the Ag content increase its behaviour.By the result Ag-TiO_2 nanoparticles show antibacterial property [22].

AnnaZielinska-Jurek et al., [2015]worked on Ag-Pt modified TiO_2nanocomposites synthesised by sol-gel process. Bimetallic modified TiO_2shows better photocatalytic activity under visible light irradiation, better than monometallic nanoparticles.The samples were characterized by X-ray diffraction [XRD] and scanning transmission electron microscopy [STEM], DRS, measurement corroborate that the most activated photo catalyst under visible light contain fine small Ag nano particles deposited on self synthesised TiO_2 surface. The activity of silver nanoparticles was studied with respect to various bacteria's. By the result of this work, less than 5nm size silver nanoparticles are recommended for the inhibition oh pathogenic microorganism growth [23].

Xiangyu Zhang et al., [2015]one dimensional Ag doped anatase TiO_2 nanowire array prepared by magnetron sputtering with hydrothermal process. The silver titanium layer was deposited by sputtering and the concentration of Ag nanoparticles was controlled by technology. Then by hydrothermal growth silver doped TiO_2 nanowires were prepared and characterized by X-ray diffraction [XRD], transmission electron microscopy [TEM]. This nanowire had excellent antibacterial properties against E-coli. By their work Ag doped TiO_2 nanoparticles have the antibacterial property was confirmed [24].

Rafeala S. Andre et al., [2015]silver doped Titanium nanoparticle was synthesised by using microwave-assisted hydrothermal method and characterised using by X-ray diffraction [XRD], and UV-vis spectra, FEG-SEM micrograph, Raman spectroscopy. The tests were conformed presence of Ag and Ti nanoparticles. Antibacterial property against plank tonic and bio film forming cells of MRSA and Candida spp of Ag-TiO_2 nanoparticles were confirmed by this work. They suggested the possibility of Ag-TiO_2 nanoparticle for medical and industrial application[25].

Thomas Verdier et al., [2014]studied inactivation of Escherichia coli bacteria by photocatalysis involving TiO_2 nanoparticles alone or in transparent coatings [varnishes] and investigates different parameters that significantly influence the antibacterial activity. The antibacterial activity of TiO_2 was evaluated through two types of experiments under UV irradiation: in slurry with physiological water [stirred suspension]; and in a drop deposited on a glass plate. The results confirmed the difference in antibacterial activity between simple drop-deposited inoculums and inoculums spread under a plastic film, which increased the probability of contact between TiO_2 and bacteria [forced contact]. In addition, the major effect of the nature of the suspension on the photocatalytic disinfection ability was highlighted. Experiments were also carried out at the surface of transparent coatings formulated using nanoparticles of TiO_2. The results showed significant antibacterial activities after 2 h and 4 h and suggested that improving the formulation would increase efficiency [26]**.**

Zoe VinethQuiñones-Jurado et al., [2014] confirmed that the surface plasmon resonance of Ag nanoparticles plays an important role in relation to the nanoparticles size and consequently with the antibacterial effect of the nanocomposites. They observed that under visible light the reactivity of TiO_2 cannot be amplified when it is supporting Ag nanoparticles that have an inactive photocatalytically surface. The results confirmed that the antimicrobial effectiveness of nanocomposites based on Ag nanoparticles supported-TiO_2 is closely associated to the contact surface area and to the electronic performance of the noble metal. The results confirmed that the antimicrobial effectiveness of Ag-supported TiO2 is achieved principally due to a favoured exposure and amount Ag nanoparticles, without a photocatalytic effect of TiO_2 and a surface plasmon resonance effect of Ag nanoparticles that can be considered. An extended Ag nanoparticles surface reduced the catalytic activity due to electronic confinement barriers. However, higher surface-to-volume ratio of Ag nanoparticles ensured more surface area to act by direct contact against bacteria, changed the permeability of the cellular membrane [27].

M. Harikishore et al., [2014]prepared nanocrystalline pure TiO_2 and 5 mol% silver doped TiO_2 [Ag-TiO_2] powders by sol-gel route. The prepared powders were characterized by X-ray diffraction [XRD] and UV-Visible spectroscopy [UV-Vis] for their phase composition and optical properties, respectively. The antibacterial property of TiO_2 and Ag-TiO_2 was assessed by spread plate method against Escherichia coli as test bacteria. The photocatalytic activity of Ag-TiO_2 was studied by measuring degradation of Methylene blue [MB] under UV Irradiation and the results were compared with pure TiO_2. Further, the effect of temperature and dye concentration on degradation efficiency of pure TiO_2 was studied. XRD results confirmed the presence of anatase phase in both the samples and the average crystallite size of both samples were found in the order of 6-15 nm. The antibacterial activity of pure TiO_2 and Ag-TiO_2 is assessed by spread plate method against 105 cells/mL of Escherichia coli bacteria after an incubation period of 24 h. Bacterial colonies were not observed in Ag-TiO2 treated plates whereas, numerous bacterial colonies were observed in TiO_2 treated plates. Photocatalytic activity of both TiO_2 and Ag-TiO_2 samples was examined by decomposition of MB for 5 h under UV irradiation. It was found that the photocatalytic degradation depends on the catalyst crystallinity and dye concentration [28].

D.Nithyadevi et al., [2014]synthesisedAg doped TiO_2 coreshellnanoparticlesby reverse micelle process within the size 10-50nm. HRTEM, X-ray diffraction [XRD], scanning electron microscopy [SEM] were used to characterised the nano particles through tests. By the test presence of Ag and titanium nanoparticle and their size were conformed. By disc diffusion method nanoparticles antibacterial properties were studied. Outcome of this work showed Ag doped TiO_2 have high antibacterial property compared to pure TiO_2 nanoparticles against S.aureus [29].

Kiran Gupta et al., [2013]structural and optical properties and comparative photocatalytic activity of TiO_2 and Ag-doped TiO_2 nanoparticles against different bacterial strains under visible-light irradiation. The TiO_2 and Ag-doped TiO_2photocatalysts were synthesized by acid catalyzed sol–gel technique and characterized by X-ray diffraction [XRD], transmission electron microscopy [TEM], UV–visible spectroscopy and photoluminescence [PL]. The decreased band-gap energy of Ag-doped TiO_2 nanoparticles in comparison to TiO_2 nanoparticles was investigated by UV–visible spectroscopy. The antimicrobial activity of TiO_2 and Ag-doped TiO_2 nanoparticles [3% and 7%] was investigated against both gram positive [Staphylococcus aureus] and gram negative [Pseudomonas aeruginosa, Escherichia coli] bacteria. The photocatalysis efficiency of TiO_2 and Ag-doped TiO_2 was tested by the percentage viability reduction of bacterial colonies under visible-light irradiation. The viability of P. aeruginosa was reduced to zero at 40 mg/30 mL culture of Ag-doped TiO_2 [7%] while S. aureus and E. coli showed zero viability at 60 mg/30 mL culture. In the case of 3% doping all bacterial culture were killed at 80 mg/30 mL culture. The pure TiO_2 [crude and annealed] nanoparticles showed poor photocatalytic activity, while doping of silver ions improves the efficiency under visible-light irradiation [30].

Shenglin Mei et al., [2013]utilized a dual process encompassing anodization and silver plasma immersion ion implantation [Ag PIII] is to produce titania nanotubes [TiO_2-NTs] containing Ag at different sites and depths. The concentration and depth of the incorporated Ag can be tailored readily by changing the PIII parameters. The Ag-embedded TiO_2-Nanotubes which retain the nanotubular morphology are capable of sterilizing oral pathogens as opposed to pure Ti plates and pristine TiO2-NTs. Biological assays indicate that the in vitro and in vivo biocompatibility of the sample plasma-implanted at a lower voltage of 0.5 kV [NT-Ag-0.5] is significantly compromised due to the large amount of surface Ag [31].

P.Rameshet al., [2012]synthesised silver and Titanium dioxidenanoperticles by sonochemical and colloidal method. Synthesized nanoparticles were characterised by various analysis test like X-ray diffraction [XRD], Scanning electron microscopy [SEM] and Transmission electron microscopy [TEM]. The TEM images haveconfirmed that both Ag & TiO_2Nanpparticless are regular spheres with the sizes in the range of 20-50 nm. The X-ray diffraction analysis of Ag-Nanoparticless showed that the facecentered cubic and TiO_2 Nanoparticles have bodycentered crystalline nature. In addition, antimicrobial activities against Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, and Klebsiella pneumonia were studied.

Even though both materials Ag and TiO_2 Nanoparticles have antimicrobial activities, when compared between them, the Ag Nanoparticles have much higher antimicrobial properties than TiO_2 nanoparticles. Their results suggested that Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems [32].

Mohsen Behpour et al., [2012]in theirresearch work, photocatalyst titanium dioxide was doped with silver and modified by polyethylene glycol by sol gel method and the samples were characterized by X-ray diffraction [XRD] and scanning electron microscopy [SEM]. The purpose of the present study was to evaluate the photocatalytic bactericidal effects of prepared nanocomposite on human pathogenic bacteria under visible light irradiation. The Ag doped TiO_2 nanoparticles, modified by PEG, were successfully prepared by a simple sol gel dip coating method as antimicrobial materials. The antimicrobial susceptibility was tested using bacteria S. aureus, Salmonella paratyphi, Shigelladysanteriae and pathogenic fungi Candida albicans. The obtained results showed that bioactivity of differed depending on microbial strain, Ag content and presence of visible light and silver doped Titanium composted materials may contain the advantages of both materials: silver has a higher antimicrobial activity, and TiO_2 can last longer, and able to be controlled by illumination [33].

A.A.Hebeish et al., [2012] presented a report on, TiO_2 and Ag-TiO_2 nanowires antimicrobial activity. TiO_2 nanowires were prepared by the hydrothermal method and Ag doped TiO_2nano particles was prepared by photo reducing method. The prepared nanomaterials were characterised using x-ray diffraction [XRD], scanning electron microscopy [SEM] and Transmission electron microscopy [TEM]. And nanomaterials are confirmed by the above tests. Cotton fabrics where coated with TiO_2 and Ag- TiO_2 nano wires. The coated cotton materials shows good antibacterial activity against gram positive bacteria, gram negative bacteria and fungi. Based on the results of this study Ag-TiO_2nano particles were recommended for possible medical and industrial applications [4].

Tessy M. Lopez Goerne et al., [2012]synthesised Ag doped TiO_2 by sol gel method. Nanoparticles were characterised by FTIR, UV-Vis and XPS, TEM microscopy, EDS, also BET and DRX analysis were carried out. Outcome of this work is Amorphous materials were apparently obtained. Ag-TiO_2 nanoparticles were tested against several Gramnegative and Gram-positive bacteria including enterophatogenic Escherichia coli and highly resistant strains such as methicillin-resistant Staphylococcus aureus and they showed sensibility in most of cases. The results of this study demonstrated that nanostructured sol-gel TiO_2-Ag have a bactericide effect including highly pathogenic bacteria such as EPEC and MRSA even more than conventional bactericides with the advantage of suitability for repeated use with potential to surface application [34].

Kim, Soo-Hwan et al., [2011]reported The antibacterial activities of silver nanoparticles [Ag-NPs] were studied with respect to Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli by observing the bacterial cells treated or not with Ag-Nanoparticles by field emission scanning electron microscope [FE-SEM] as well as measuring the growth curves, formation of bactericidal reactive oxygen species [ROS], protein leakage, and lactate dehydrogenase activity involved in the respiratory chain. Bacterial cells were treated with Ag-NPs powder, and the growth rates were investigated under varying concentrations of Ag-NPs, incubation times, incubation temperatures, and pHs. By their result, to determine the lowest concentration that completely inhibited visible growth, the minimum inhibitory concentration [MIC] was used. 100 and 150 µg/ml Ag-NPs powder were used, growth was inhibited; however, when 50 µg/ml Ag-NPs was used, growth was only slightly inhibited. The antibacterial activities of the Ag-NPs against the Gram-positive S. aureus and Gramnegative E. coli were almost identical [35].

Altangerelamarjargal et al.,[2011]reported Ag-TiO_2nano composites synthesis via ethylene glycol medium and studied their antibacterial properties. The salient features of this method include simple operation, large scale production and one medium [solvent] to produce two different nanoparticles.Clusters of Ag and TiO_2 nanoparticles with an average size of 20 – 30 nm and narrow size distribution are formed after annealing at 400 and 500 ^{o}C. Samples were characterized by field emission scanning electron microscopy [FE-SEM], transmission electron microscopy [TEM], X-ray diffraction [XRD] and X-ray photoelectron spectroscopy [XPS]. And their antibacterial properties were evaluated using E. coli as model organism under visible light irradiation. The obtained AgTiO_2nanocomposites indicated that they have good crystallinity, high purity and excellent antibacterial properties [36].

BinyuYu et al., [2011]in this study TiO_2 and Ag–TiO_2 composite nanofilms were successfully prepared on silicon wafer via the sol–gel method by the spin-coating technique. The as-prepared composite Ag–TiO_2 and TiO_2 films with different silver content were characterized by scanning electron microscopy [SEM], atomic force microscopy [AFM], x-ray diffraction [XRD] and x-ray photoelectron spectroscopy [XPS] to determine the topologies, microstructures and chemical compositions, respectively. The antimicrobial effect of the synthesized nanofilms was carried out against gram-negative bacteria [Escherichia coli ATCC 29425] by using an 8 W UV lamp with a constant relative intensity of 0.6 mW cm^{-2} and in the dark respectively. The synthesized Ag–TiO_2thinfilms showed enhanced bactericidal activities compared to the neat TiO_2nanofilm both in the dark and under UV illumination. Silver ion release kinetics combined with an antimicrobial efficacy test and the cytotoxicity of the composite films. Such mesoporousTiO_2 substrate structures with silver loading could have promising applications as antibacterial materials for biomedical use and in the water treatment field [37].

Yue Lin et al., [2011]worked on mono dispersed Ag-TiO_2 core shell nanoparticles synthesized in solution via colloid-seeded deposition process. And the samples were characterised by X-ray diffraction [XRD] and scanning electron microscopy[SEM] and UV-vis spectra. Inductively coupled plasma atomic emission spectrometry [ICP-AES] showed low amount of Ag ion leaching from the Ag-TiO_2 core shell nanoparticles. Ag ion released from the Ag-TiO_2 core shell nanoparticles by ICP-AES detection showed this structure could control release of Ag ion. The antibacterial properties of above core shell nanoparticles against E. coli by inhibition testing indicate that this material acquires excellent antibacterial effects. The antibacterial capability of the core shell nanoparticles was attributed to its structural feature of Ag nanoparticles [38].

Lixiangmai et al., [2009]studied TiO_2 doped with Ag nanoparticles deposited on titanium plates by sol-gel method. The anti microbial properties where studied as a function of annealed temperature using E-Coli as a benchmark microorganism. When the annealed temperature increased Ag nano particles deposited on TiO_2 film, were of metallic nature and could grow to larger. The nanoparticles were characterised using x-ray diffraction [XRD], scanning electron microscopy [SEM] and Transmission electron microscopy [TEM]. Nanoparticles size was confirmed by the above tests. The outcome of the work is Ag- TiO_2 nano particles have positive antibacterial activity. And the results indicated that smaller size of Ag nanoparticles shows better antibacterial activity [39].

S-Q SUN et al., [2007]reported A liquid phase deposition method has been devised for the deposition of Ag–TiO_2 thin films on ceramic tiles with glazed surface at a low temperature. The Ag–TiO2 thin films obtained were well adhered, homogenous and coloured by interference of reflected light. The films were characterized by X-ray diffraction and scanning electron microscopy [SEM]. From these analyses, it was found that silver ions were trapped in TiO_2 matrix and their reduction could be achieved at 600°C annealing temperature. The antibacterial activity against S. aureus and E. coli has been studied applying the so called antibacterial-drop test. The Ag–TiO_2 thin films exhibited a high antibacterial activity. In the present study Ag–TiO_2 thin films show high antibacterial activity eliminating the E. coli and S. aureus. One reason is that the silver ion is released from the Ag–TiO_2 thin films in a certain extent. The antibacterial functionality of Ag–TiO_2 thin films was not compromised even after aging in a weathering chamber [40].

Renjis T. Tom et al., [2003]reported a one-step route for the synthesis of Ag-TiO_2, particles in nanometer dimensions, with controllable shell thickness. This scalable procedure leads to stable and freely dispersible particles and bulk nanocomposite materials have been made this way. The procedure leads to particles of various morphologies, with a crystalline core in the size range of 30-60 nm diameters and an amorphous shell of∼3 nm thickness in a typical synthesis. The material has been characterized with microscopic, diffraction and spectroscopic techniques**.** Absorption spectroscopy, electron microscopy, X-ray diffraction, infrared spectroscopy, cyclic voltammetry, XPS, and TG were used to reveal various properties of the material. Nonlinear optical measurements indicate that these materials are strong optical limiters with a high laser damage threshold [41].

METHODOLOGY

CHAPTER-III

METHODOLOGY

3.1. INTRODUCTION:

This chapter describes the experimental techniques used in the synthesis of silver doped titanium nanoparticles which is used for the antibacterial agent. Solvothermal method is used here to synthesis the nanocompound. The various analysis techniques such as X-ray Diffraction, Raman spectroscopy and Scanning Electron Microscope methods are used for conformation and study the crystal structure, phase, vibrational modes and morphology.

3.2. METHOD OF PREPARATION:

Various methods can be handled to prepare nanoparticles like, Sol-gel method, Solvothermal method, Microwave method, Hydrothermal method etc., Here Solvothermal method is adapted to synthesis Ag-TiO_2 nanoparticles. Solvothermal method is a common scientific method and it is almost identical to the hydrothermal method. Solvothermal synthesis is a method for preparing a variety of materials such as metals, semiconductors, ceramics, and polymers. The temperature can be elevated much higher than that in hydrothermal method.

Solvothermal method is defined as "a chemical reaction in a closed system in the presence of a solvent (aqueous and non-aqueous solution) at a temperature higher than that of the boiling point of such a solvent". The selected temperature depends on the required reactions for obtaining the target-material through the involved process. This method is normally has better control than hydrothermal methods of the size and shape distributions and the crystallinity of the TiO_2 nanoparticles [42]. The Solvothermal method has been found to be a versatile method for the synthesis of a variety of nanoparticles with narrow size distribution and dispersity. The Solvothermal method has been employed to synthesize TiO_2 nanoparticles and nanorods with/without the aid of surfactants.

3.3EXPERIMENTAL DETAILS:

MATERIALS USED:

- Titanium isopropoxide 97% ($C_{12}H_{28}O_4Ti$)
- Silver nitrate 99.9% ($AgNO_3$)
- Sodium borohydrate ($NaBH_4$)
- Acetic acid(CH_3COOH)
- Hydrochloric acid (HCl)
- Double distilled water(H_2O)

3.4 METHOD OF PREPARATION:

STEP1: Double distilled water, Hydrochloric acid, acetic acid; Titanium isopropoxide and Silver nitrate were taken in the volume ratio

STEP2: Double distilled water, Hydrochloric acid, Hydrochloric acid, Sodium borohydrate and acetic acid are mixed together by using magnetic stirrer to make homogeneous.

STEP3: 3mM of silver nitrate solution were added dropto the homogeneous solutiontill get the yellow colour solution.Obtain solution is shown in fig.3.1

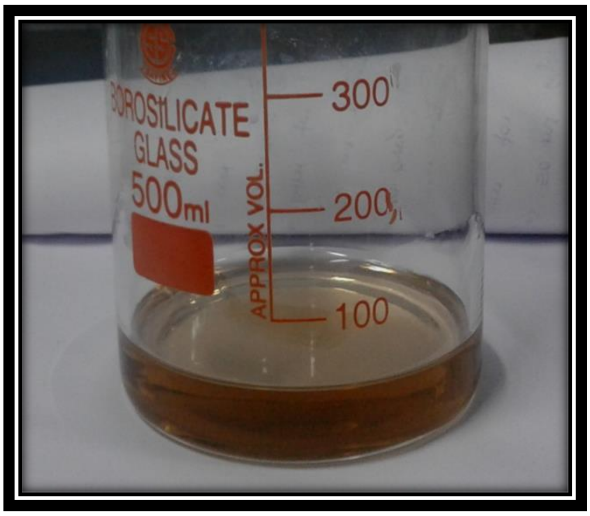


Fig.3.1 Silver Solution

STEP 5:2ml of Titanium isopropoxide were added drop wiseand kept in stirrer.

STEP 6: The prepared solution is kept at the temperature 90°C and stirrered vigorously using magnetic stirrer till obtain the precipitate.

STEP 7: Precipitate was collected by filtering method and collected precipitate was washed by water and ethanol several times.

STEP 8: Washed precipitate was kept in room temperature and dried at 80°C in magnetic stirrer. They obtained powder wasgrinded using agate mortar for annealing. It kept for annealing at 450°C for 3hours.

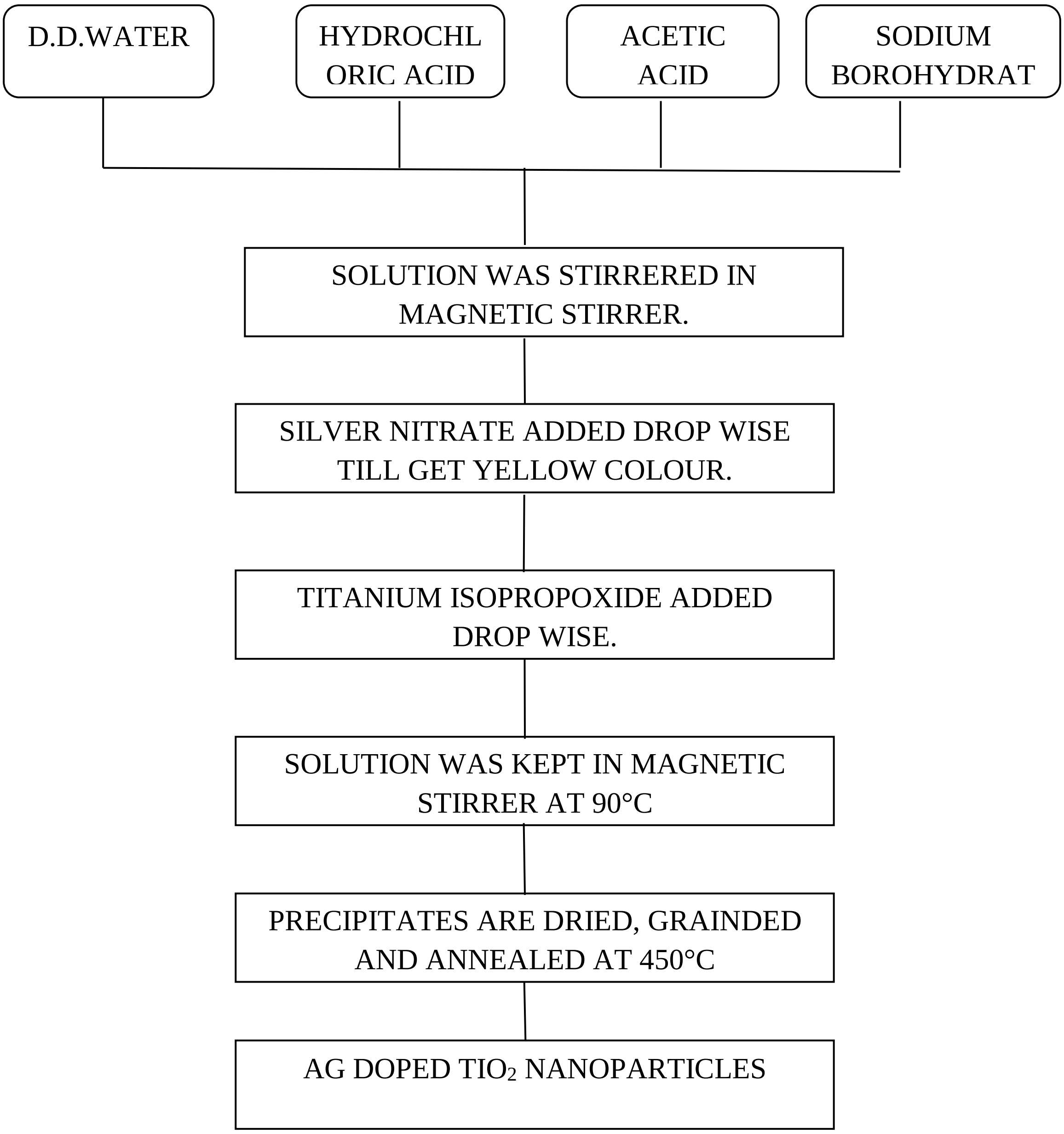


Fig.3.2 Flow chart of synthesis of Ag-TiO_2 nanoparticles

STRUCTURAL STUDIES:

Spectroscopy is the study of electromagnetic wave interaction with the matter. Spectroscopy methods based on the phenomena of emission, absorption, florescence or scattering [43]. To study the materials and their properties various spectroscopy methods are handled. For study the crystalline properties XRD spectroscopy is used. To analyse shape or nanoparticles size SEM and for examine the various vibrational modes of atom Raman spectroscopy is used.

3.5 X-RAY DIFFRACTION (XRD):

X-ray diffraction (XRD) relies on the dual wave/particle nature of X-rays to analyse fluids, to powders and crystals. This analysis technique used to study the materials, crystallinity, phase identification of a material, chemical bonding, disorders, unit cell dimensions and various other information's[44]. It is an indispensible method for analyse the materials. A primary use of the technique is the identification and characterization of compounds based on their diffraction pattern. In crystal atoms are arranged in a periodic array and thus can diffracted light. The wavelength of X- rays is similar to the distance between atoms. So the scattering of X-rays from atoms produces a diffraction pattern, which contains information about the atomic arrangement within the crystal. Amorphous materials do not have a periodic array so they do not produce a diffraction pattern. This method gives laboratories the ability to quickly analyze unknown materials and characterize them in such fields as metallurgy, mineralogy, forensic science, archaeology and the biological and pharmaceutical sciences. Identification is performed by comparison of the diffractogram to known standards or to international databases.

3.5.1. PRINCIPLE OF X-RAY:

Materials are made of atoms. Knowledge of how atoms are arranged into crystal structures and microstructures is the foundation on which we build our understanding of the synthesis, structure and properties of materials. Diffraction is the slight bending of light as it passes around the edge of an object. This principle is used in XRD to analyse the materials (crystals). When the X-ray photons fall on the material, the incident ray will be diffracted by the atoms or molecules into many specific directions. This Diffracted pattern gives the information about the material.

3.5.2. BRAGG'S LAW:

Constructive and destructive interference patterns depend on lattice spacing (d) and wavelength of radiation (i). By varying wavelength and observing diffraction patterns, information about lattice spacing is obtained. If X- ray is fall on the crystal, the incident angle is θ (Bragg angle), and λ is the wavelength of X-ray by using the Bragg's law, inter planner distance(d) of the given crystal is calculated. Fig 3.3 shows the Bragg's law pictorial representation.

Bragg's law is defined by the equation (1)

$$\mathbf{n\lambda = 2d\sin\theta} \longrightarrow (1)$$

When a parallel beam of monochromatic X-ray is incident at angle(θ), on a crystal in which the layers of ions separated(d), an intense X-ray beam is reflected when, $n\lambda=2d\sin\theta$, when 'n' is a positive integer.

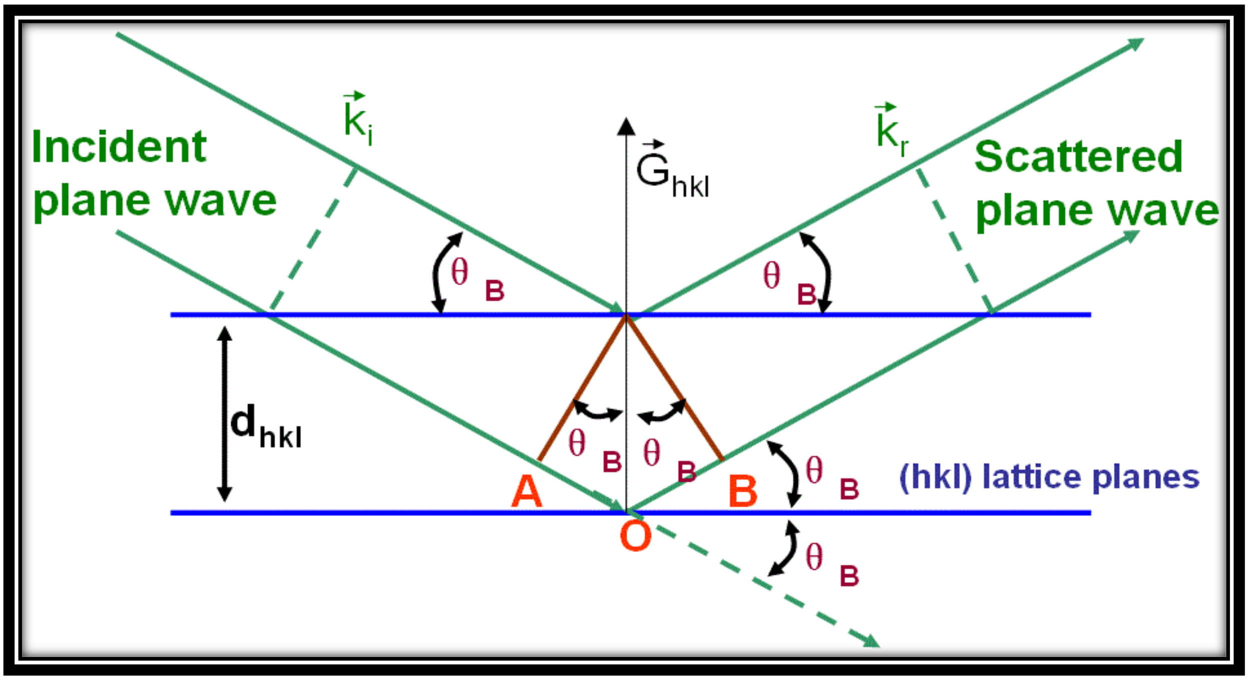


Fig.3.3 Bragg's law.

3.5.3 X-RAY POWDER DIFFRACTION:

Powder crystalline more appropriately called polycrystalline X-ray diffraction, because it can also be used for sintered samples, metal foils, coatings and films, finished parts, etc. the ideal “powder” sample contains tens of thousands of randomly oriented crystallites. Every diffraction peak is the product of X-ray scattering from an equal number of crystallites. Only a small fraction of crystallites in the specimen actually contribute to the measured diffraction pattern. Powder diffraction is mainly used for “finger print identification” of various solid materials.

Essential Parts of the Diffractometer:

- ✓ X-ray Tube: the source of X-Rays.
- ✓ Incident-beam optics: condition the X-ray beam before it hits the sample.
- ✓ The Goniometer: the platform that holds and moves the sample, optics, detector, and/or tube.
- ✓ The sample & sample holder.
- ✓ Receiving-side optics: condition the X-ray beam after it has encountered the sample.
- ✓ Detector: count the number of X-rays scattered by the sample.

When high energy electrons strike an anode in a sealed vacuum, x-rays are generated. Anodes are often made of copper, iron or molybdenum. A continuous beam of X-rays is incident on the crystal, atoms or ions are diffracting the incident ray in specific direction. The diffracted radiation is very intense in certain directions. These directions correspond to constructive interference from waves reflected from the layers of the crystal. The diffraction pattern is detected by photographic film. Fig 3.4 for the instrumentation of X-ray Powder diffraction method.

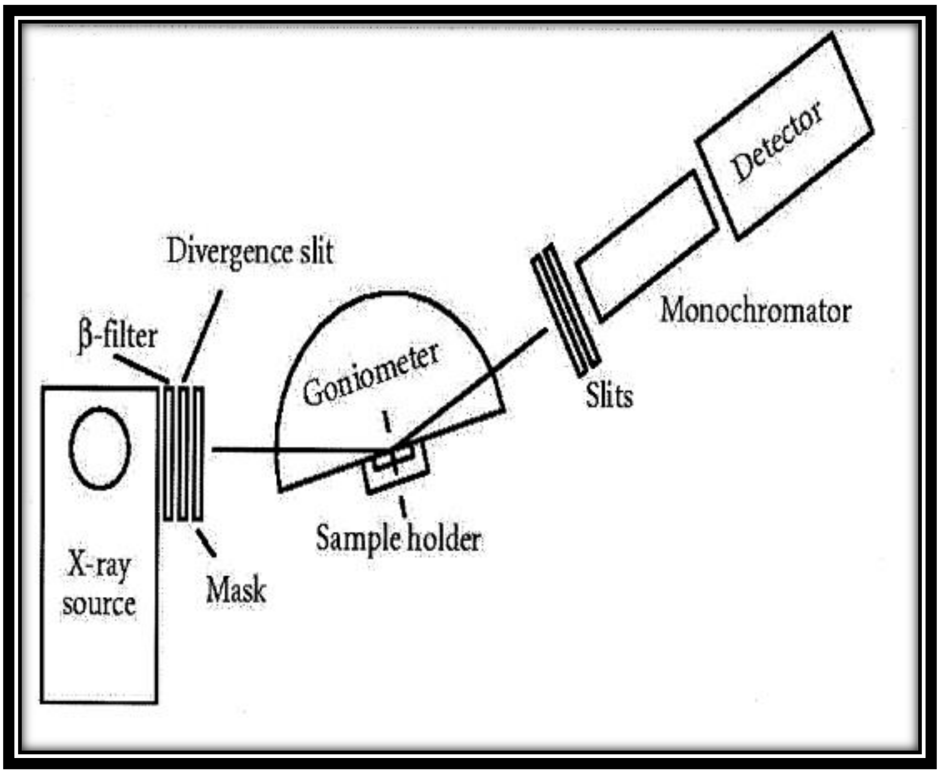


Fig.3.4 X-ray Powder diffraction method.

3.5.4 SCHERRER FORMULA:

Scherrer equation (2) is used to determine the average particle size of the crystalline material by using peak half-width (B).

$$\mathbf{t} = \frac{\boldsymbol{\kappa\lambda}}{\boldsymbol{\beta\cos\theta}} \longrightarrow (2)$$

k- Shape factor of a particle.

$\boldsymbol{\lambda}$- Wavelength of X-ray.

$\boldsymbol{\theta}$- Incident angle of X-ray.

MORPHOLOGICAL STUDIES:

3.6. SCANNING ELECTRON MICROSCOPY:

Scanning electron microscope is a scientific instrument that uses a beam of energetic electrons to examine objects on a very fine scale. In scanning electron microscopes (SEM), the effective resolution is around 1 nm. To examine the atoms or molecules required 10,000x plus magnification which was not possible using other optical microscopes. This analysis carries the following information about the material which is examined. Fig 3.5 shows the instrumentation of SEM.

Topography:

Topographic contrast enables u image the size, shape and texture of three dimensional objects. It is dependent upon the number of SEs and BSEs being emitted from different areas of the specimen as well as the trajectories they take in relation to the location of the detector.

Morphology:

The shape and size of the particles are making up the object. Direct relation between these structure and materials properties is characterised.

Composition:

Compositional contrast results from different numbers of backscattered electrons being emitted from areas of the sample differing in atomic number. The backscatter coefficient increases with increasing atomic number and so higher atomic number elements will appear brighter in the image. By using SEM compositional properties can be analysed.

Crystallographic Information:

How the atoms are arranged in the object, and the relation between these arrangements and material properties are analysed by this method.

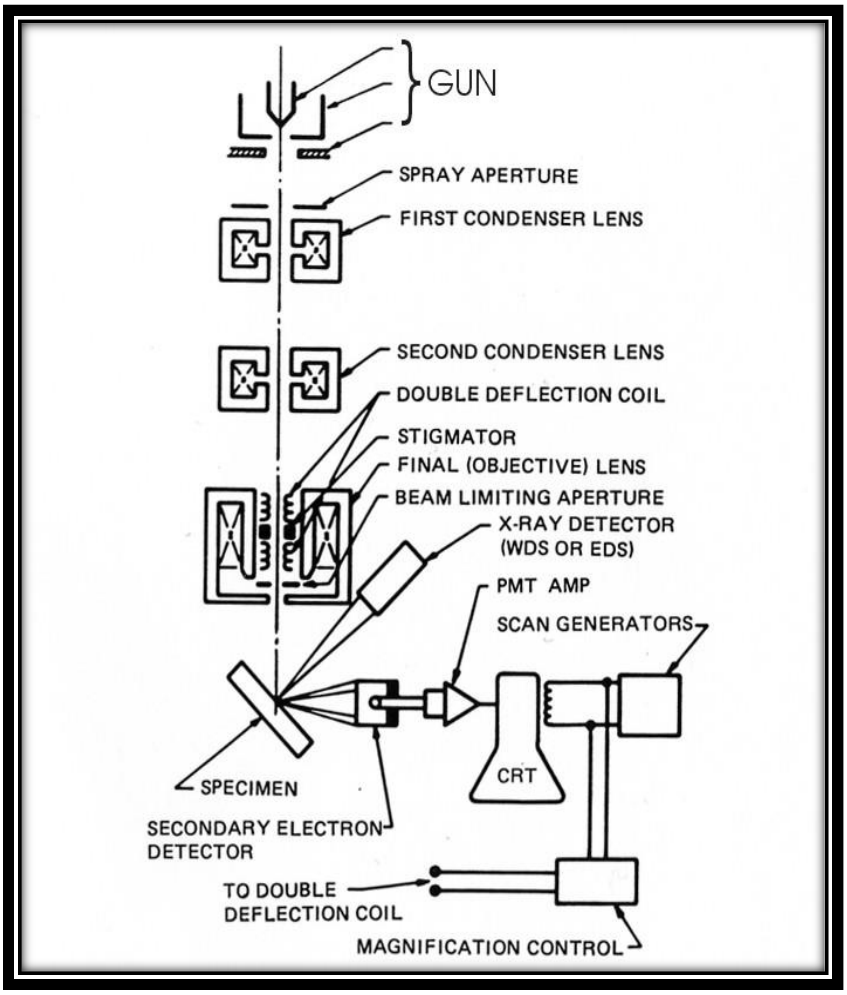


Fig. 3.5 Instrumentation model of SEM

3.6.1. WORKING PRINCIPLE OF SEM:

- The "Virtual Source" at the top represents the electron gun, producing a stream of monochromatic electrons.
- The stream is condensed by the first condenser lens (usually controlled by the "coarse probe current knob"). This lens is used to both form the beam and limit the amount of current in the beam. It works in conjunction with the condenser aperture to eliminate the high-angle electrons from the beam.
- The beam is then constricted by the condenser aperture (usually not user selectable), eliminating some high-angle electrons.
- The second condenser lens forms the electrons into a thin, tight, coherent beam and is usually controlled by the "fine probe current knob".
- A user selectable objective aperture further eliminates high-angle electrons from the beam.

- A set of coils then "scan" or "sweep" the beam in a grid fashion (like a television), dwelling on points for a period of time determined by the scan speed (usually in the microsecond range).
- The final lens, the objective, focuses the scanning beam onto the part of the specimen desired.
- When the beam strikes the sample (and dwells for a few microseconds) interactions occur inside the sample and are detected with various instruments.
- Before the beam moves to its next dwell point these instruments count the number of e- interactions and display a pixel on a CRT whose intensity is determined by this number (the more reactions the brighter the pixel).
- This process is repeated until the grid scan is finished and then repeated, the entire pattern can be scanned 30 times/sec.

3.6.2. SECONDARY ELECTRONS:

Secondary Electrons these electrons arise due to inelastic collisions between primary electrons (the beam) and loosely bound electrons of the conduction band (more probable) or tightly bound valence electrons. The energy transferred is sufficient to overcome the work function which binds them to the solid and they are ejected. The interaction is Coulombicin nature and the ejected electrons typically have ≈ 5 -10eV. 50 eVis an arbitrary cut-off below which they are said to be secondary electrons.

3.6.3. DETECTION SEQUENCE:

Secondary electrons are low energy electrons. Collect them by placing a positive voltage (100 - 300V) on the front of detector. Since this let collect a large number of the secondaries (50 -100%), to produce a “3D” type of image of the sample with a large depth of field. The type of detector used is called a scintillator/ photomultiplier tube.

OPTICAL STUDIES:

3.7 RAMAN SPECTROSCOPY:

Raman spectroscopy is a useful technique for the identification of a wide range of substances–solids, liquids and gases. It is a straightforward, non destructive technique requiring no sample preparation. This spectroscopy is a versatile method for analysis of wide range forensic sample. It is used to examine the quality as well as quantity analysis[45]. Qualitative analysis can be performed by measuring the frequency of scattering radiations. By measuring the intensity of Raman spectrum gives the quantitative information[46].

Raman spectroscopy is a spectroscopic technique based on inelastic scattering of monochromatic light, usually from a laser source. Inelastic scattering means that the frequency of photons in monochromatic light changes upon interaction with a sample. Photons of the laser light are absorbed by the sample and then reemitted.

Frequency of the reemitted photons is shifted up or down in comparison with original monochromatic frequency, which is called the Raman Effect. This shift provides information about vibrational, rotational and other low frequency transitions in molecules. This effect is based on molecular deformations in electric field E determined by molecular polarizability (α). The Raman shift does not depend upon the frequency of the incident light but it is regarded as a characteristic of the substance causing Raman Effect. For Stokes lines, $\Delta\upsilon$ is positive and for anti stokes lines $\Delta\upsilon$ is negative. Fig 3.6 shows Raman instrumentation.

3.7.1 EXPERIMENTAL SET UP:

A Raman system typically consists of four major components:

1. Excitation source (Laser).

2. Sample illumination system and light collection optics.

3. Wavelength selector (Filter or Spectrometer).

4. Detector (Photodiode array, CCD or PMT).

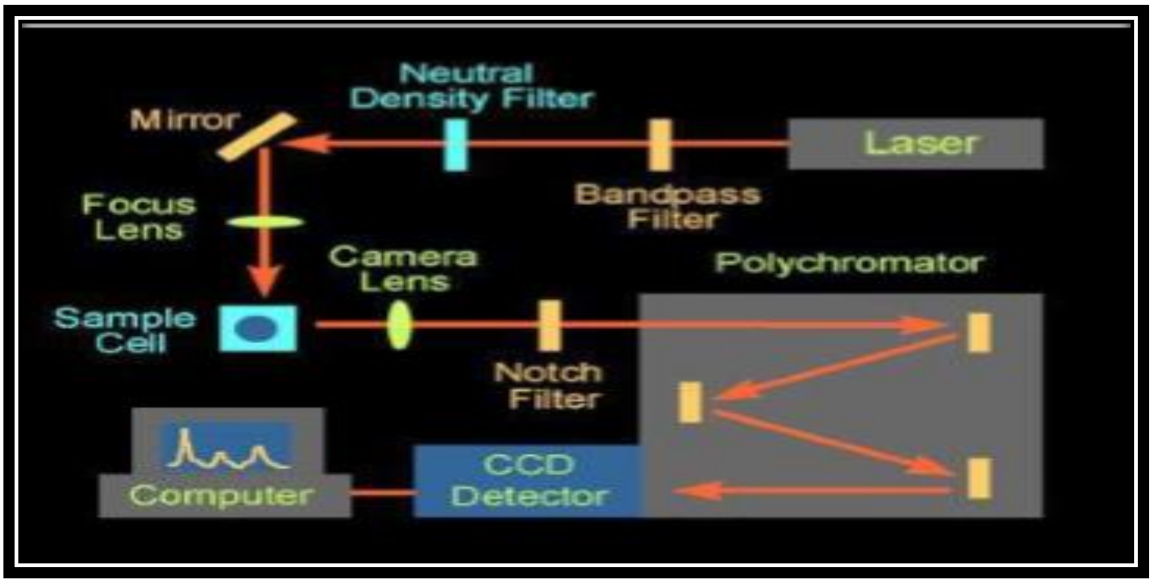


Fig. 3.6 Raman instrumentation.

In Raman instrument a sample is illuminated with a laser beam. Light from the illuminated spot is collected with a lens and sent through interference filter or spectrometer to obtain Raman spectrum of a sample. Wavelengths close to the laser line, due to elastic Rayleigh scattering, are filtered out while the rest of the collected light is dispersed onto a detector. By changing the laser light you can confirm if a peak is a true Raman peak and not a peak just associated with the wavelength of the laser light that was used. Spontaneous Raman scattering signal is very weak because most of the incident photons undergo elastic Rayleigh scattering. Therefore special measures should be taken to distinguish it from the predominant Rayleigh scattering. Instruments such as notch filters, tunable filters, laser stop apertures, double and triple spectrometric systems are used to reduce Rayleigh scattering and obtain high quality Raman spectra.

Sample is placed into the cryostat chamber where the low temperature is achieved by the use of liquid helium that cooled the cryostat. The cryostat is kept in vacuum so that laser light suffers no scattering from the particles of the air in the chamber. Before every measurement the scattered light would have to be aligned in the spectrometer so that maximum signal would hit the detector. This can be achieved by moving the sample to different positions and using lens, mirror system. multi-channel detectors like Photo Diode Arrays (PDA) or, more commonly, a Charge Coupled Devices (CCD) to detect the Raman scattered light.

RESULTS AND DISCUTION

CHAPTER IV

RESULTS AND DISCUSSION

4.1 INTRODCTION:

Silver doped TiO_2nanoparticle was synthesized by solvothermal method. The synthesizednanocompositewasanalyzed usingXRD, SEM, Raman spectroscopy. The obtained results are discussed in this chapter.

4.2 STRUCTURAL CHARACTERISATION:

4.2.1 X-RAY DIFRACTION ANALYSIS:

The X-ray diffraction analysis data obtained by powder X-ray diffractometer with CuKα radiation (k = 1.54A°).XRD is used to obtain structural information about the synthesized nanoparticles by using the principle of Bragg's law. It is used to explain the interference patternof X-raysscattered by crystals, diffraction has been developed to study the structure of all states of matter with any beam, with a wavelength similar to thedistance between the atomic or molecular structures of interest.a, b and c lattice constant and α, β and γ angles between a, b and c can be determined by XRD.

Fig 4.1 shows that the result of XRD analysis of the synthesis nanoparticles. The sharp diffracted peaks are indicated that the presence of Silver, TiO_2 particles in the prepared sample.In XRD pattern, a peak at $2\Theta = 25.46°$ matching to (110) of anatase miller indices.This characteristics peak is confirmed the presence of anatase phase. And the presence of Ag on the TiO_2 surface is confirmed by the additional peaks appearedat $2\theta \approx 38.1°$, 44.3°.

The diffracted peaks are present at the angle 2θ of 25.24°, 38.1°,44.3°, 64.54°, 77.46°, 81.76°and corresponding miller indices are, (110), (111), (200), (220), (311), (203). This pattern confirmed the presence of Ag-TiO_2 nanoparticle (JCPDS Card 04-0551) in the XRD pattern[47].

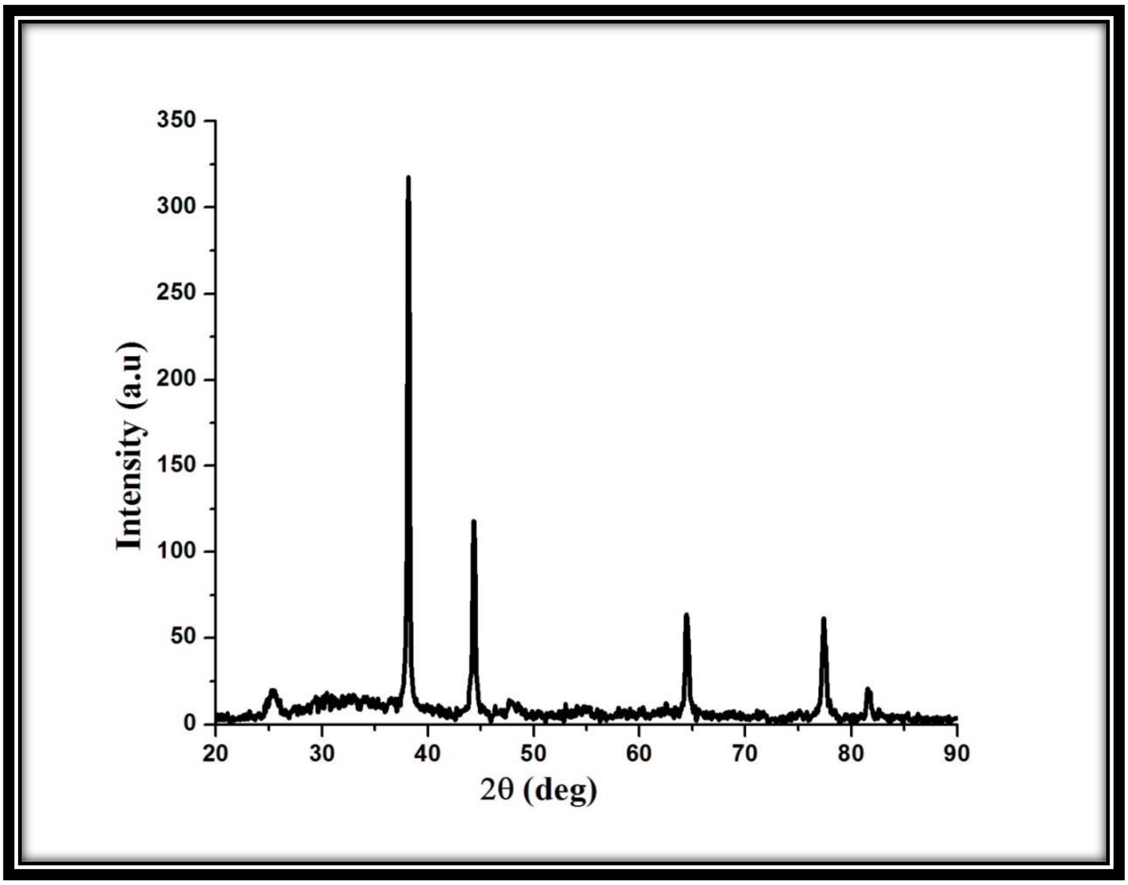


Fig 4.1 XRD analysis of Ag-TiO_2 nanoparticles

CRYSTALLINE SIZE CALCULATION:

From Scherer formula (4) the synthesized Ag doped TiO_2nano particles size can be derived, from broadening of the corresponding X-ray diffraction peaks.

$$D=\frac{k\lambda}{\beta\cos\theta} \longrightarrow (4)$$

Here,

λ- is the wavelength of x-ray radiation (λ=0.15406).

k- is the Scherrer's constant. (k=0.88).

β- is the full width at half maximum.

θ- is the diffraction angle.

The crystalline sizes of high intense peaks are calculated, by using Scherer formula 38.39nm, 29.94nm, and 24.87nm respectively.

2θ (deg)	FWHM (θ)	D=kλ / $\beta cos\theta$ (nm)	h	k	L
25.24	1.04406	7.801395	1	1	0
38.1	0.21902	38.39397	1	1	1
44.3	0.2866	29.94342	2	0	0
64.54	0.37792	24.87373	2	2	0
77.46	0.42491	23.97877	3	1	1
81.76	0.8346	12.58684	2	0	3

Tab 4.1Diameter and miller indices values for synthesised Ag-TiO_2nanoparticles.

By using Scherer formula, the nanoparticles size was calculated and tabulated to corresponding peaks values and the miller indices hkl also tabulated. The average crystalline size of synthesized nanoparticles is 22.92 nm.

4.3 OPTICAL CHARACTERISATION:

4.3.1 RAMAN ANALYSIS:

Raman spectroscopy is a form of molecular spectroscopy that involves the scattering of electromagnetic radiation by atom or molecule and this technique is a non-destructive and highly versatile technique for analysis of chemical, both organic and inorganic. It used to analyse the atoms vibrational, rotational and other low frequency modes, structural information, chemometrics analysis of molecule.Here Ag doped TiO_2 nanoparticle isanalyzed by using Raman spectroscopy.

4.3.2 RAMAN ANALYSIS OF AG DOPED TIO_2:

The result of Raman spectrum on silver doped titanium nanoparticle is shown in the fig. 4.2.The Raman peak observed at151.51 cm^{-1}, 207.10 cm^{-1},402.87 $cm^{-1}$and 616.38 cm^{-1}. 151.51 cm^{-1}, 402.87 $cm^{-1}$and 616.38 $cm^{-1}$are the high intense peak and 207.10 cm^{-1} is the low intense peak observed by Raman spectra.

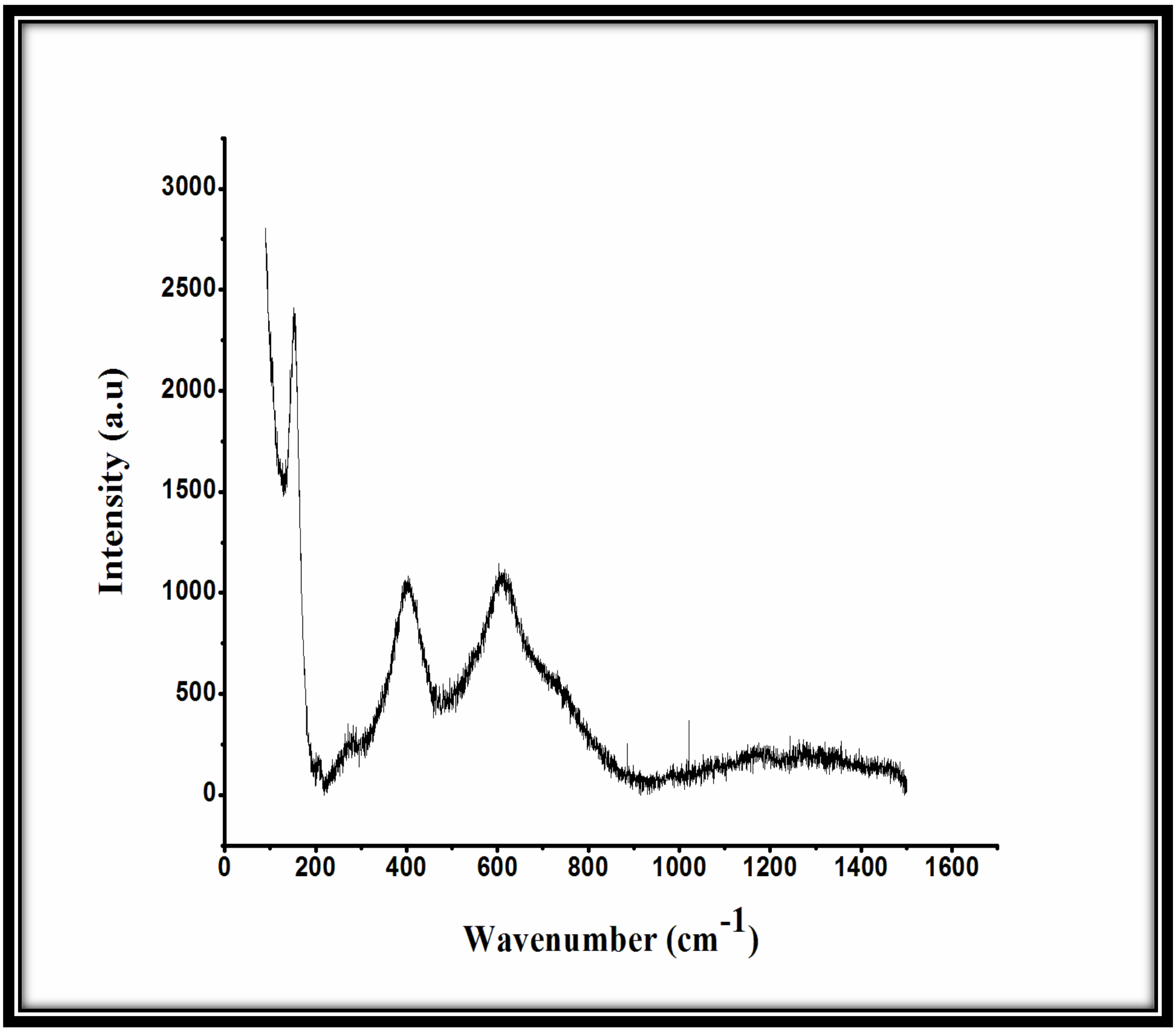


Fig. 4.2 Raman spectrum of Ag-TiO_2 nanoparticles.

Hence the Raman spectroscopy result of Ag doped TiO_2nanocompound, confirmed the presence of silver nanoparticles in the TiO_2 matrix. The shift are observed compare to pure TiO_2 nanoparticles Raman spectrum. The position of 144 cm-^{1} is shifted to 151 cm-^{1} 197cm-^{1}to 207.10 cm-^{1} and 397 cm-^{1}to 402.87 cm-^{1} respectively.These shifts are presented dueto theAgdopand. These are due to the TiO_2 nanoparticles in close vicinity to Ag nanoparticles can strongly receive the influence of the local field, there by gives the strong Raman signal[48]. The nanocomposites interface structure between Ag and TiO_2 plays an important role in Raman peak shift.

4.4 MORPHOLOGICAL ANALYSIS:

Scanning electron microscopy is used for inspecting morphological of specimens at very high magnifications using a piece of equipment called the scanning electron microscope. SEM magnifications can go to more than 300,000 X. Morphological analysis of Ag doped TiO_2 is studied by using SEM analysis.

SEM gives the information about the prepared sample's morphology information. Ag doped TiO_2 nanoparticles shape like stone structure with pours on the surface. And various size of nanoparticles are presence in the sample. The synthesis Ag-TiO_2 sample has been taken under the magnification of 14.kx. Fig 4.3 shows the SEM result of silver doped titanium nanoparticles. It consist of cluster of multiple rodes.

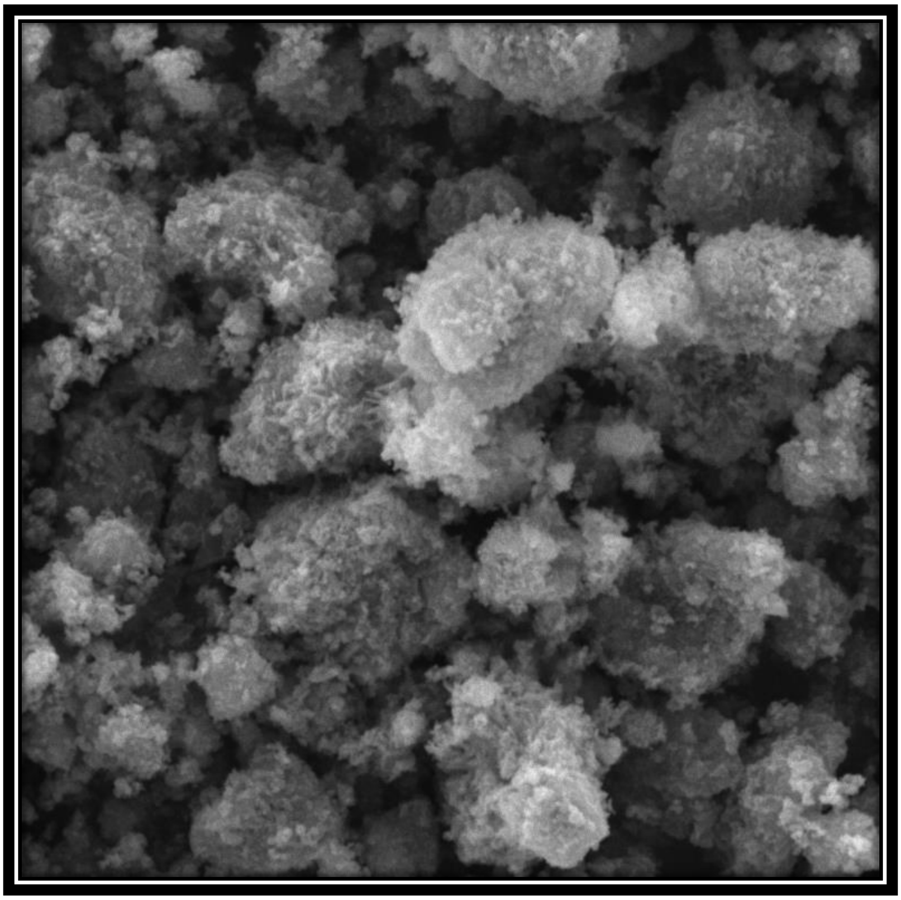
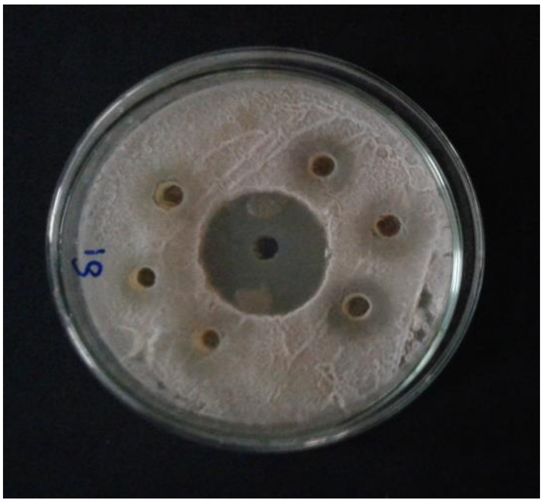


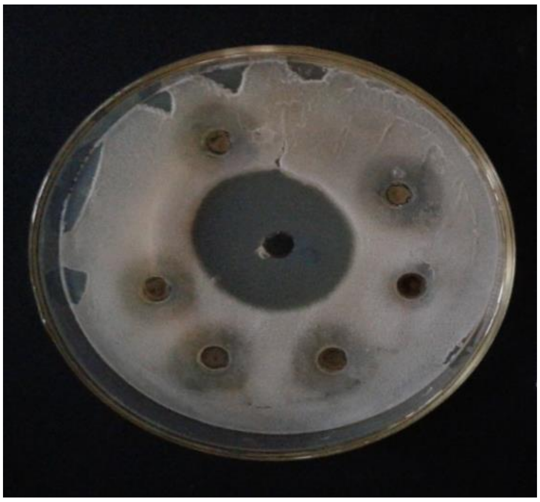
Fig. 4.3 SEM result of Ag-TiO_2nanoparticles.

4.5 ANTI-BACTERIAL ACTIVITY OF Ag-TiO_2:

The Antibacterial property of synthesized Ag-TiO_2 nanoparticles is analyzedthrough agar diffusion method of anti-microbial testing. Gram positive and gram negative bacteria's are used to study the silver doped titanium nanoparticles antimicrobial study. Those are E.coli, Staphylococcus aureus, Klebsiella pneumonia, and Bacillus subtillis. By the formation of the inhibition zone diameter, efficiency of the antibacterial activity of the nanoparticle is measured and compared with the efficiency of standard antibacterial agent.



B.Subtilis E.coli



S.AureusKlebsila Klebsiella pneumonia,

Fig.4.4 Antibacterial activity of Ag-TiO_2nanoparticles zone formation.

Zone of inhibition form around the nanocomposites are measured in mm scale and tabulated. Various concentration of Ag doped TiO_2nanocomposites dissolved in 1ml distilled water.Silver doped Titanium nanoparticles antibacterial activity compared with standard antibacterial agent (Chloramphynical).

Various concentration of dissolved silver doped titanium dioxide's antibacterial activities are shown in Fig.4.4. and the inhibition zones diameter are measured and tabulated in Table 4.2.

Concentration of Ag-TiO_2	**Zone of inhibition (mm)**			
	Klebsila	**B.subtilus**	**E.coli**	**S.aureus**
0.0005g	17	16	15	18
0.0010g	19	17	15	19
0.0015g	16	15	19	16
0.0030g	19	15	13	17
0.0050g	18	9	12	12
0.0100g	15	17	14	18
Control(mm)	33	31	34	30

Table 4.2 Ag-TiO_2nanoparticles antibacterial activity result.

The result shows 0.0010g of Ag-TiO_2 better result compare to other concentrations excluding E-coli. Its zone of inhibition is high compare to all other inhibitions range. By the result one can conclude that, the synthesized Ag-TiO_2 have an antibacterial activity against E-coli, B.Subtilis, Klebsila and S.aureus. And when the concentration level of Ag-TiO_2 decreased, antibacterial activity is improved till 0.0010g/ml.Below which 0.0010g/ml, antibacterial activity is decreased.

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

In this work, silver doped Titanium dioxide nanoparticles have been synthesised using Solvothermal synthesis method. Synthesised sample was characterised by XRD.Vibrational properties are analysed by Raman spectroscopy. SEM analysiswas used to examine the shape and morphology property of the sample. Synthesised nanocompound Antibacterial activity was examined by the Agar Diffusion Method. By the results of analysis some information's are concluded here.

- ✓ Presence Ag doped TiO_2 nanoparticle was confirmed by XRD analysis. TiO_2 present in the anatase phase. Average size of nanoparticles is 22.9 nm calculated by X-ray diffraction.
- ✓ Raman spectrum result confirmed the presence of Ag doped TiO_2nanocomposites by the Raman shift at 402.87 cm^{-1},616.38 cm^{-1} from pure TiO_2anatase phase Raman spectrum.
- ✓ The cluster of rod shape with pours on the shape of the synthesised nanoparticles with pores on the surface is confirmed by the SEM analysis.
- ✓ The antibacterial properties of synthesised nanocompound are confirmed by formation of inhibition zone using the Agar diffusion method.
- ✓ Better Inhibition zones are formed at the 0.0010g/ml ratio of nanoparticles solution.

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