ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESIZED ZnO NANOPARTICLES

Dissertation submitted to the University of Kerala for the partialfulfilment of the requirements for the degree of

MASTER OF SCIENCE

In

BOTANY

ABSTRACT

This project work focuses on antibacterial property of green synthesized Zno nanoparticle. ZnO finds a wide variety of applications and is one of the most studied wide band gaps (~3.2eV at room temperature) material. ZnO exhibits interesting electrical, optical and chemical properties. Here structural and optical property of the as-synthesized nanoparticle were characterized by XRD and UV-Visible spectroscopy X-Ray Diffraction technique was used to identify the crystalline structure, phase purity and crystallite size of the as-prepared Zno nanostructures. It was observed that ZnO nano powders are highly crystalline and single phase with hexagonal structure. UV-Visible absorption studies reveal the Optical characterisation of the as-prepared ZnO nanostructures were conducted using JASCO V760 Spectrophotometer. Direct bandgap of the ZnO nanostructures is obtained by this study. Bandgap of the samples were found to be 3.18eV. Antibacterial studies showed that green synthesised grown ZnO nanostructures can be used effectively for the destruction of human pathogens Kpneumonia and Shigella

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 Nanoscience

Nanoscience is an emerging area of science which involves the study of materials on an ultra- small scale and the novel properties that these materials demonstrate. The structures with a dimension in a range of 1-100 nanometers are called nanoparticles. One nanometer is defined to a billionth of meter that is 10^{-9}meter. Nanoscience is cross disciplinary, meaning scientists from a range of fields including chemistry, physics, biology, medicine, computing, material science and engineering are studying it and using it to better understand our world. The nanoworld of nanoscience provides scientists with a rich set of materials useful for probing the fundamental nature of matter. Nanoscience has useful application in our everyday life ranging from consumer goods to medicines. Nano dimension have some special features like large ratio of surface area to volume, large fraction of surface atoms to bulk. The smallness of size makes many barriers transparent. The size depends properties like absorption of light of a certain frequency due to theonset quantum effect at nanometer scale gave the nanoparticle (NPs) the name Quantum Dots.

1.2 Nanotechnology

Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is that the control of matter on the molecular level in scales smaller than 1 micrometer, normally 1-100 nanometers, and therefore the fabrication of devices within that size range. The term nanotechnology was first defined by Norio Taniguchi of Tokyo science university in 1974 paper as “nanotechnology mainly comprises processing of separation, consolidation and deformation of materials by one atom or by one molecule”. Nanotechnology is often seen as an extension of existing sciences into nanoscale, or as a recasting of existing sciences employing a newer, more modern term. Two main approaches employed in nanotechnology arebottom-up approach and top-down approach. Within the “bottom-up” approach, materials and devices are built from molecular components which assemble themselves chemically by principle of molecular recognition. Within the “top down” approach, nano-objects are constructed from larger entities without atomic-level control. The impetus for nanotechnology come from a renewed interest in colloidal science, joined with a new generation of analytical tools like atomic force microscope (AFM), and also scanning tunneling microscope (STM). Combined with refined processes like electron beam lithography and molecular beam epitaxy, these instruments allow deliberate manipulation of nanostructures, and led to observation of novel phenomena.

1.3 Historical aspects of nanotechnology

Concept of nanotechnology was first mentioned in 1867 by James Clerk Maxwell. When he proposed as an experiment, a tiny entry known as Maxwell’s Demon which can handle individual molecule. In 1914 Richard Adolf Zsigmondy use nanometer for the first time to characterize the particle size. He determined it as 1/1000000 of millimeters. In 1959 a well- known physicist Richard Feynman a Nobel laureate first introduced the idea of nanotechnology. In 1960 he published his article titled “There is plenty of room at the bottom” which discussed idea of nanomaterials. He explored possibility of manipulating materials at scale of individual atom and molecule for finding the increasing ability to examine and control matter at nanoscale. In 1980’s Eric Drexler introduced the concept of nanotechnology to engineering through concept of molecule manufacturing. He introduced nanotechnology as a scientific field that mainly revolve around molecular manufacturing and the vision is called molecular nanotechnology. By applying molecular manufacturing to industries, he declared that coal can be changed into diamond and computer chips can be made from sand. In early 1980’s nanoscience and nanotechnology got boosted with two major developments. The invention of scanning tunneling microscope and the birth of cluster science. These two developments led to the discovery of fullerenes in 1985 and the structural assignment of carbon nanotubes. The 1990's Huffman Kretschmar discovered how to synthesis and purify bulk quantity of fullerenes.

1.4 Nanoparticles

Nanoparticles have dimension between 1 - 100nanometers. They show considerably different properties compared with their bulk counterparts; size reduction is the major reason behind this. When size of particle is too small to be comparable to wavelength of the electron, quantum confinement effect is taken into consider. Quantum confinement means to confine the motion of randomly moving electron to restrict its motion in particular energy levels and reflects the atomic realm of materials. The decrease in confining dimension will makes energy level discrete and this increases band gap. Since band gap and wavelength are inversely related to each other the wave length increases with decease in size [1]. Surface area to volume ratio has a significant effect on the properties of a material. Material made up of nanoparticles have larger surface area when compared to same volume of material made up of larger particles. The surface area to volume ratio increases as radius of the sphere decreases and vice-versa. It also means that when a given volume of materials is made up of smaller particles, surface area of material increases. As particle size decreases, a larger proportion of particles are found at surface of the material. This makes nanoparticles more chemically reactive. As chemical reaction occurs between particles that are on the surface, given mass of nanomaterial will be much more reactive than the same mass of material made up of larger particles. This means that materials that are inert in their bulk form are reactive when produced in their nanoparticle form [2].

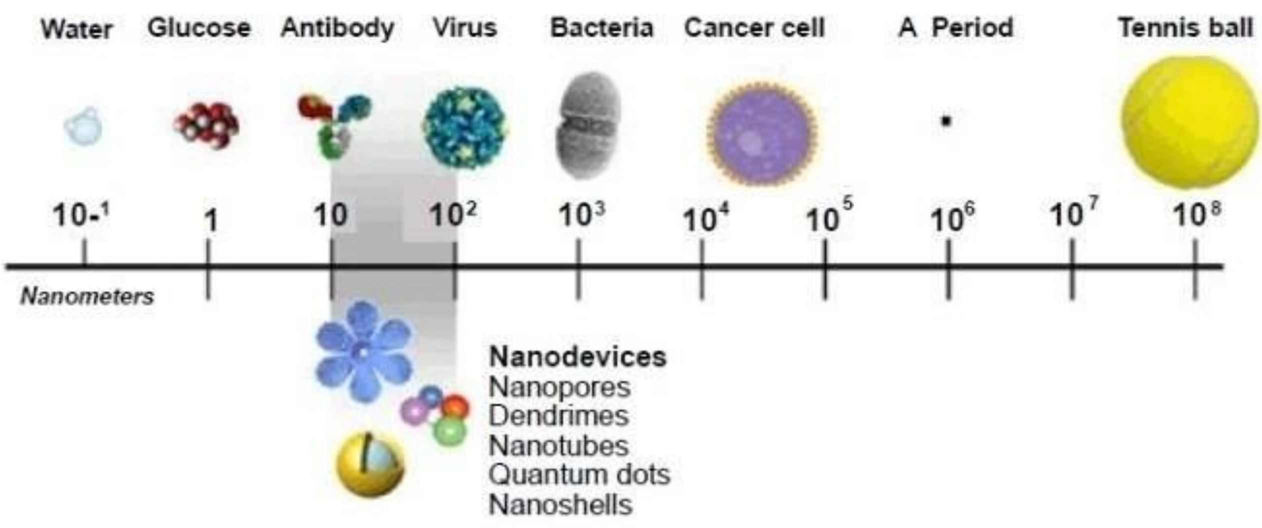


Figure1.1 Size comparison of nanoparticles [3].

1.5 Classification of nanoparticles

Nano particles can be made of one or more numbers of atoms or molecules that can exhibit a wide range of properties based on the size dependence. Mainly nanoparticles can be classified based on their dimensionality, morphology, composition, uniformity and agglomerations.

Classifications based on dimension:

According to the size nanoparticles are classified into zero dimensional, one dimensional, two dimensional and three dimensional:

a) Zero-dimensional (0D)

The particles which have nano-dimension in all the three directions are called zero dimensional materials. Nanoparticles can be amorphous or crystalline. They can be single crystalline or polycrystalline. Nanoparticles are composed of single or multi-chemical elements. It exhibits various shapes and forms. Nano particles can exist individually or incorporated in a matrix. They can be metallic, ceramic or polymeric.

b) One-dimensional (1D)

In these types of nanomaterials one of the dimensions will be outside the nanometer range. Examples of 1D materials are nanotubes, nanorods, and nanowire etc. They can be amorphous or crystalline, single crystalline or polycrystalline. Nanoparticles can be chemically pure or impure. They can be metallic, ceramic, or polymeric [4].

c) Two-dimensional (2D)

In this of nanomaterials two dimensions are outside the nanometer range. This type of materials exhibit plate-like shapes. Examples of two-dimensional nanomaterials are nanofilms, nanolayers, and nano coatings etc. They can be amorphous or crystalline and made up of various compositions. It is deposited on a substrate. They can be metallic, ceramic, or polymeric.

d) Three-dimensional (3D)

All dimensions of these type of nanomaterials are outside the nanometer range. These include bulk materials composed of individual blocks which are in nanometer scale(1-100nm). They have large surface area too. They possess a nanocrystalline structure. For 3D nanomaterial the electrons are completely delocalized.

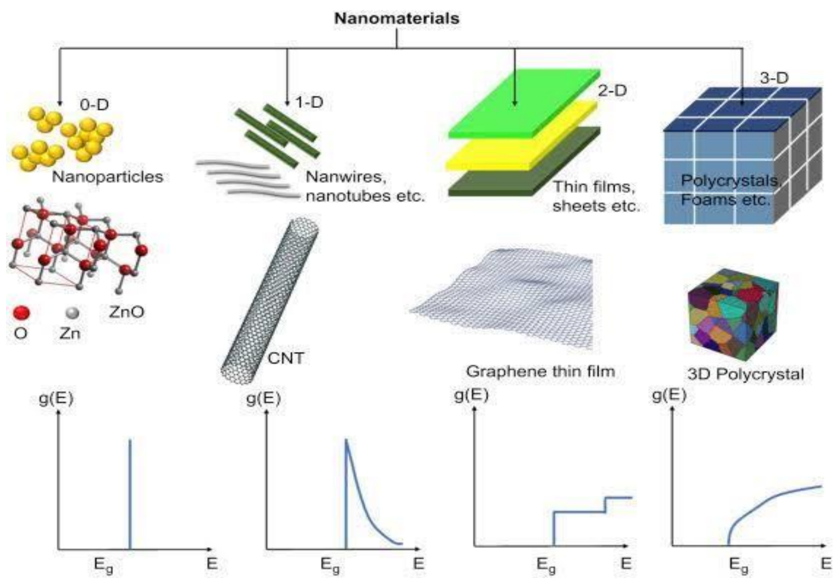


Figure 1.2 Nanoparticles and density of states [5].

1.6 <u>Important properties of nanoparticles</u>

Comparing with the bulk materials, nanoparticles have several numbers of properties. Nanoparticles are unique because of their large surface area and this property dominates over the bulk materials. That is why they are widely used in the research and technology. Some of the common properties of nanoparticles are electrical, mechanical, physical, magnetic, thermal and optical.

a) <u>Electrical properties</u>

The dielectric properties, electrical transport and hall effect for nanomaterials differ from those for micron sized materials due to increased interfacial atoms or ions and sinking of large amounts of defects at or near the grain boundaries. The electrical resistivity of nanocrystalline metals is higher than that in both coarse grained polycrystalline metals and alloys. If crystal size is smaller than electron mean free path, grain boundary scattering dominates and hence electrical resistivity as well as the temperature coefficient is increased. The magnitude of electrical resistivity and hence the conductivity in composites can be changed by altering the size of the electrical conducting component [6].

b) **Mechanical properties**

Mechanical properties of nanoparticles have novel applications in many fields such as surface engineering and nanomanufacturing. Different mechanical parameters such as elastic modulus, hardness, stress and strain adhesion and friction can be surveyed to know the exact mechanical nature of nanoparticles. Besides these parameters surface coating, coagulation and lubrication also aid to mechanical properties of bulk material. Elastic constants of nanocrystalline have been reduced considerably compared to those of bulk materials. This is due comparatively higher inter atomic spacing in the boundary regions. The strength of nanocrystalline materials increases considerably than that of coarse-grained materials. Hardness also increases with decreasing grain size in the conventional coarse grain materials. It is referred to as Hall Petch effect [7].

c) **Physical properties**

Nanoparticles have a comparatively smaller melting point or phase transition temperatures and have significantly reduced lattice constant because of the high fraction of surface atom in the total amount of atoms and high surface area. Many barriers will appear due to the smallness of the size. Gold particles, coming to the nanometer range appear red in color. Absorption of solar radiation is high in nano range because the particles are too small [8].

d) **Magnetic property**

The nanometer sized particles are super paramagnetic, a property resulting from their tiny size- only a few nanometers- a fraction of the width of human hair. Super paramagnetic nanoparticles are not magnetic when located in zero magnetic field. But they rapidly become magnetized when an external field is applied. When we return to zero magnetic field they suddenly change to a non- magnetized state. The magnetic properties of nanoparticles are used for the drug delivery, therapeutic treatment, contrast agents for MRI imaging, bio separation and in-vitro diagnostics etc. [9]

e) Thermal property

Comparing to the fluids in solid form, the metal nanoparticles have higher thermal conductivity. For example, the oxides such as alumina have thermal conductivity higher than that of water. So, fluid containing suspended solid particles are expected to display notable enhanced thermal conductivity compared to those of conventional heat transfer fluid [10].

f) Optical property

Optical activity in a nanomaterial can be obtained through several different mechanisms, depending on the nanomaterial size, composition and arrangement. Optical properties of nanoparticles are independent to some extent. Noble metal nanoparticles have size dependent optical properties and a strong UV visible extinction band spectrum which is not seen in the bulk materials. When a metal absorbs light of resonant wavelength it will cause electron cloud to vibrate. This process normally happens at the surface of the particle and therefore they are called surface plasmon resonance. Oscillations of electron clouds are the plasmons. The incident photon frequency of the excitation band is constant with respect to the collective excitation of the conduction electron. Then it is called localized surface plasmon resonance. When the size of nanosphere is less than the wavelength of incident radiation then a resonance occurs and it will generate the surface plasmon resonance. During this time the frequency of surface plasmon is equal to the frequency of radiation due to constructive interference. Localized surface plasmon resonance spectrum depends upon the size, shape, interatomic spacing of the nanoparticle, the dielectric properties and its local environmental conditions includes the substrate, adsorption andsolvent.

g) Antibacterial properties

Disinfection using organic compounds has a lot of disadvantages including toxicity to the system and the evolution of the bacteria to develop antibacterial resistance. Hence, the development of inorganic substitutes such as metal oxide nanoparticles became of great importance. In recent years, various techniques have been proposed on the pathway of nanoparticle – biomolecule interaction. Doping is a widely used method for improving the antimicrobial properties of nanoparticles. It is speculated that future research would be focused upon developing new dopant materials and methods to incorporate them into suitable nanoparticles. Also, the process of development of these antibacterial surfaces and their activities should have no negative impact on the environment. N A Smirnov et al demonstrated the antibacterial properties of silicon nanoparticles, prepared by laser ablation, in several liquids on Gram-positive and Gram-negative bacteria. The presence of singlet and other oxygen species on the sample surface led to the damage of bacterial membranes, thereby destroying them effectively [11]. Mohamed et alconducted research where gold nanoparticles along with laser exposure were used as a local antibacterial approach to inhibit the growth of C. pseudotuberculosis [12].

1.7 Synthesis methods of nanoparticles

The method of preparation of nanoparticles can be classified mainly into two procedures.

a) Top-down approach

By this method, the nanosized particles are obtained by the method of cutting or slicing the bulk material. Top-down synthesis techniques are extension of those that have been used for producing micron sized particles. This approach is inherently simpler and depend either on removal or division of bulk material or on the miniaturization of bulk fabrication processes to produce the desired structure with appropriate properties. The failure regarding this approach is the surface imperfection. For example, nanowires made by lithography are not smooth and may contain lot of impurities and structural defects on its surface. Examples of this techniques are high-energy wet ball milling, electron beam lithography, gas phase condensation, aerosolsprays,etc..

b) Bottom-up approach

Here an assembly of smaller scale particles combined together to form a material which have dimension greater than from its beginning. It can be atom or molecule or cluster by cluster. Materials are built from molecular components which assemble themselves chemically by the principles of molecular recognition. The bottom-up has more advantages than the top-down approach because it has better chance of producing nanostructure with less defect better homogeneous chemical composition and good short and long ordering. Sol-gel synthesis, colloidal precipitation, hydrothermal synthesis, template assisted sol-gel synthesis, organometallic chemical route, reverse-micelle route, electro-deposition, etc. are some of the well-known bottom-up techniques for the preparation of nanoparticles.

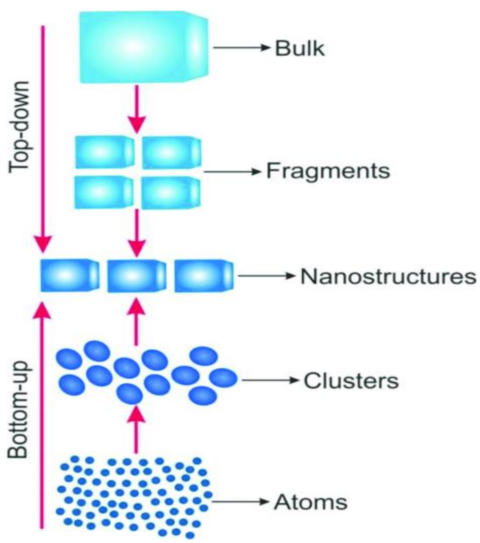


Figure 1.3 Illustration of top- down and bottom- up approach [13].

- **Green Synthesis**

The development of reliable, sustainable, and eco-friendly synthesis procedures is required to prevent the production of unwanted or harmful by-products. To achieve this goal, green synthesis using ideal solvent systems and natural resources (such as organic systems) is required. Biosynthesis is a method of synthesizing nanoparticles using micro-organisms (Bacteria, fungi etc.), plant parts (like roots, leaves, stems, seeds, fruits) and algae, their extracts are rich in phytochemicals which acts as both reducing and stabilization agent. Among the available green methods, the utilization of plant extracts is a rather simple and easy process for the synthesis of metal or metal oxide nanoparticles relative to bacteria and/or fungi mediated synthesis, these products are known together as biogenic nanoparticles. This approach is environment friendly, cost-effective, biocompatible und safe. The formation of nanoparticles consists of two important steps: first is the nucleation followed by particle growth in the second step and requires reducing and stabilizing organic molecules, The reaction parameters such as solvent, temperature, pressure, and pH conditions all play role in green synthesis methodologies based on biological precursors (acidic, basic, or neutral), Plant biodiversity has been widely considered for the synthesis of metal/metal oxide nanoparticles due to the availability of effective phytochemicals in various plant extracts, particularly in leaves such as ketones, aldehydes, flavones, amides, terpenoids, carboxylic acids, phenols, and ascorbic acids which are capable of reducing metal salts into metal nanoparticles, such nanomaterials find applications in biomedical diagnostics, antimicrobials catalysis, molecular sensing, optical imaging, and labelling of biological systems.

1.8 Zinc Oxide

ZnO oxide is an important member of the II-VI semiconductor group, it is an inorganic substance that exists in the form of a white solid. US FDA has recognized ZnO as GRAS (Generally recognized as safe) metal oxide. The immense semiconducting properties of ZnO nanoparticles like high catalytic activity, anti-inflammatory, wound healing and UV filtering properties are due to its wide bandgap (3.37 eV) and high exciton binding energy (60 meV). Among all the metal oxides, ZnO nanoparticles have been in the interest of researchers for the past few years due to their exceptional electronic, optical, magnetic, mechanical and chemical properties that are remarkably different from their bulk counterpart. They are also inexpensive to produce, safe and can be prepared easily. Also, ZnO is a multifunctional material with unique physical and chemical properties like high chemical stability, high electrochemical coupling coefficient, a broad range of radiation absorption, paramagnetic nature and high photostability. Thus, making it preferable for use in photovoltaic, photocatalytic, cosmetics, electrochemical, light-emitting diode and antibacterial applications. Even though ZnO nanoparticles can be toxic to humans, biosynthesized nanoparticles appear safer. Among all known materials, ZnO has a variety of particle structures, ZnO can arise in one-dimensional structures like needles, nanorods, helixes, ribbons, tubes, wires, combs and belt, in two-dimensional structures like nanosheets and nanoplates and three-dimensional structures contain flower, snowflakes, coniferous, urchin-like, dandelion etc. Zinc Oxide mainly crystallizes in two forms, hexagonal wurtzite and cubic zine blende. ZnO synthesized by plant extracts mostly crystallizes in hexagonal wurtzite structures, which are most stable at ambient conditions and are thus the most common, it is a hexagonal lattice and it is characterized by interconnecting two sublattices of Zn" and O, such that each Zn' ion is surrounded by tetrahedra of O' ions and vice-versa. This tetrahedral coordination gives rise to a polar symmetry along the hexagonal axis and is responsible for several properties of ZnO, including piezoelectricity and spontaneous polarization and is an important factor in crystal growth, etching and defect generation.

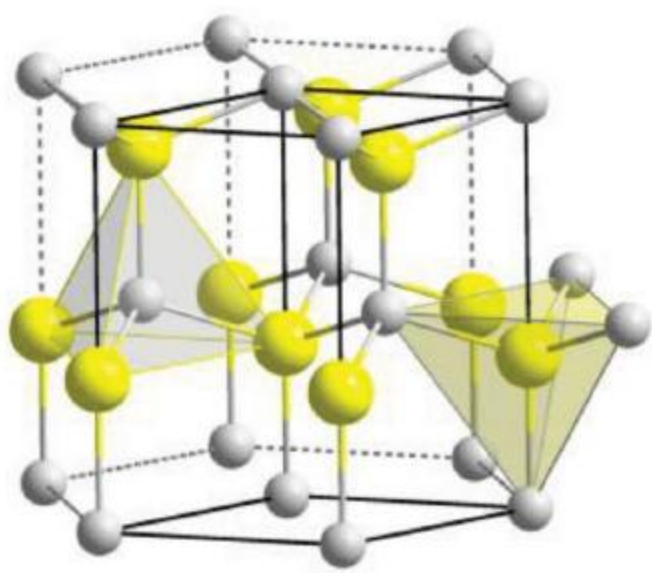


Figure 1.2 The hexagonal wurtzite structure of ZnO[14}.

1.8 Properties of Zinc Oxide nanoparticles

ZnO nanoparticles have tremendous physical properties. As the dimension of the semiconductor material is reduced to a nanometre or even smaller scale, some of their physical properties undergo changes known as the "quantum size effects". Quantum confinement, for example, increases the bandgap energy of quasi-one-dimensional (QID) ZnO, as demonstrated by photoluminescence. The physical properties of ZnO nanoparticles include magnetic, electrical, mechanical and photoluminescence.

i. Mechanical properties

Bai er al, [15] used TEM to classify the bending modulus of ZnO nanobelts based on an electric field induced resonant excitation. A unique TEM sample holder was made to apply an oscillating electric field between a ZnO nanobelt and a fixed electrode in this process The nanobelt was shaken by this electric field, and resonant oscillation was achieved by tuning the driving frequency, The bending modulus was measured using the conventional elasticity theory, As a nano resonator and nano cantilever, the ZnO nanobelt proves to be a promising material, As compared to a traditional microtechnology-fabricated cantilever, it has a higher sensitivity. Hughes et al. [16] stated that they were able to manipulate the ZnO nanobelt to the desired length and location. This demonstrates the possibility of using it as an atomic force microscopy (AFM) cantilever.

ii. Electrical properties

Individual ZnO nanorods and nanowires have been subjected to electrical transport measurements. Following various techniques, solitary ZnO nanowires were configured as a field-effect transistor (FET), ZnO nanowires have been documented to exhibit n-type semiconductor activity due to inhabitant defects such as oxygen vacancies and zinc interstitials. Chu and Li [17] used electrochemical deposition to study the growth and electrical properties of doped ZnO. They discovered that the doping ions affected the electrical properties of ZnO, only pure ZnO exhibits resistive switching behaviour in this study, demonstrating that defects in ZnO play a key role in inducing resistive switching behaviour.

iii. Electrochemical properties

The electrochemical properties of nanostructured material are analysed by cyclic voltammetry and galvanostatic cycling. El-Shaarawy et al. [18] analysed that the electrochemical study of different ZnO samples showed specific capacitance ranging from 77 to 330 Fg(⅒) depending on the morphological structure and the best capacitance was found for the nanowire structure with good cycling capability, EIS studies also confirmed that nanowire ZnO is reputable in supercapacitor application due to its minor charge transfer resistance (Ra) compared with that of other morphologies, Sabu et al. [19] observed that ZnO/graphene nanocomposite exhibits improved cycling stability compared to bare ZnO because of the buffering, confining, and conducting effects of the incorporated graphene.

iv Photoluminescence properties

Rauwel et al, [20] investigated the photoluminescence properties of ZnO and carbon materials and found that ZnO emits photoluminescence in the UV and visible region depending on the synthesis routes, size, shape, deep level, and surface defects, for example, ZnO, allows the control of the photoluminescence properties to create white light. Furthermore, the effective energy transfer from ZnO to carbon nanostructures makes them ideal candidates for biosensors, photodetectors, and low-temperature thermal imaging, in addition to energy harvesting applications. They have also shown that the passivation of surface defects causes differences in the PL response when ZnO nanoparticles are embedded in a metal oxide matrix. ZnO has been found to emit a variety of coloured emissions, including orange, blue, green and red.

v. Magnetic properties

Garcia er al. [21] showed that even in the absence of magnetic ions, the absorption of certain organic molecules onto ZnO nanoparticles modifies its electronic structure giving rise to a ferromagnetic like behaviour at room temperature.

1.9 Applications of Zinc Oxide nanoparticles

Because of its diverse properties zinc oxide nanoparticles has a wide range of applications ranging from pharmaceuticals to agriculture, tyres to ceramics and from paints to chemicals.

i) Medicinal applications

ZnO nanoparticles are widely used in the productions of various kinds of medicines due to their antimicrobial, disinfecting and drying properties, formerly it was used as an orally administered medicine for epilepsy and later for diarrhoea but presently it is applied in the form of ointments and creams, it was also found to accelerate wound healing and is therefore used in dermatological substances against inflammation and itching. It has a peeling effect at higher concentrations, ZnO is chiefly used as a component of dental pastes and temporary fillings in dentistry, it is also used in various nutritional products and diet supplements which provides dietary zinc. ZnO nanoparticles are widely used in the productions of various kinds of medicines due to their antimicrobial, disinfecting and drying properties, formerly it was used as an orally administered medicine for epilepsy and later for diarrhoea but presently it is applied in the form of ointments and creams, it was also found to accelerate wound healing and is therefore, used in dermatological substances against inflammation and itching, it has a peeling effect at higher concentrations. ZnO is chiefly used as a component of dental pastes and temporary fillings in dentistry, it is also used in various nutritional products and diet supplements which provide dietary zinc.

ii) Cosmetics

ZnO nanoparticles were widely used in sun creams due to their ability to absorb UV-A und UV-B radiation, to solve the problem of an insufficient white layer and to produce a new medium that is more transparent, less adhesive and easy applicability, a new cream formula containing, a combination of ZnO and TiO_2 was created, it is an extremely good medium in sun creams since they do not irritate the skin and are easily absorbed into the skin.

iii) Textile Industry

ZnO coatings are more air-permeable and efficient as UV blockers compared with their bulk counterparts and are, therefore a boon for textile applications. Thus, zine oxide nanostructures have become very popular as UV- protective textile coatings. Ates et al, [22] found that zine oxide nanowires grown on cotton fabric exhibits self-cleaning, super hydrophobicity and UV blocking properties. Poznan University of Technology and the Textile Institute in Lodz researched the use of ZnO in polyester fibers and the results showed the product's favorable dispersive/morphological and adsorption properties. On analyzing the microstructure and properties of unmodified textile products and those modified with zinc oxide showed that the modified product could protect against UV radiation and bacteria.

iv) Electronics and Electro technological industries

The wide bandgap and high bond energy of ZnO makes it useful in photoelectronic and electronic equipment, in devices emitting a surface acoustic wave, in field emitters, sensors, UV lasers, and solar cells. ZnO is used in FED (Field emission display) equipment, such as televisions as it exhibits the phenomenon of luminescence chiefly photoluminescence. It can be used for the production of light-permeable electrodes in solar batteries and also as transparent electrodes in photovoltaic and electroluminescent equipment. ZnO nanoparticles are also used in gas sensors to detect CO and CO2 (in mines and alarm equipment) but can also be used for the detection of other gases (H3, SF, C.H10, C H, OH), The production of varistors is another important application of ZnO in electronics. They are used to protect high-voltage lines, and in electrical equipment protecting against atmospheric and network voltage surges and as lightning protectors.

v) Agricultural uses

ZnO nanoparticles have the potential to enhance the yield and growth of food crops. When treated with different concentrations of zine oxide nanoparticles, seed germination, seedling vigor and plant growth were enhanced. It was also proved to be effective stem and root growth when ZnO was applied at different concentrations in Bacillus subtilis, Streptococcus pneumonia, Pseudomonas aeruginosa und E.coli cultures Paula and Ban found a sharp increase in enzymatic activity with maxima at a ZnO concentration. Thus, they investigated the role of ZnO nanoparticles in the field of Biotechnology.

vi) Miscellaneous applications

Zine oxide has uses in criminology for mechanical fingerprint analysis, it is also used as a constituent in cigarette filters as it selectively removes certain components from cigarette smoke, It also removes

Sulphur and its components from various liquids and gases particularly industrial waste gases. ZnO and its derivatives are also used as an additive to car lubricating oils, reducing consumption and oxygen corrosion, ZnO is used as a source of Zinc, which is an essential nutrient and so it is added to many food products including breakfast cereals, ZnO and its derivatives are also used in the process of producing and packing meat products and vegetable products due to their chemical and antifungal properties, It is also used in the production of typographical and offset inks, Zinc oxide reacts with silicates to produce zinc silicates, which are water- and fire-resistant materials used as binders in paints. These fire-resistant and adhesive substances are used in the construction industry for the binding of cement.

CHAPTER 2

Review Of Literature

Rasli *et al.* [23] reported the biosynthesis of zinc oxide nanoparticles fam aloe vera extract using zine nitrate as the precursor. Of the four biosynthesis parameters studied by a two-level factorial screening, the precursor concentration was the most significant parameter that affected the production of ZnO nanoparticles. The XRD analysis showed that it was in the hexagonal phase and that the average crystalline size was 0.452um.

Sangeetha et al, [24] reported the synthesis of nanostructured ZnO using both chemical and biological methods. The biological synthesis was carried out using aloe vera extract and zinc nitrate as the precursor. XRD analysis showed all samples to have a wurtzite structure. All samples exhibited strong absorption spectra with absorption peaks ranging from 358 to 375 um.

Mofid et al. [25] reported the green synthesis of ZnO nanoparticles using aloe vera with zinc acetate as the precursor. XRD analysis confirmed the hexagonal wurtzite structure of the sample with an average grain size of 17 nm. The UV-Vis spectrum exhibited a blue-shifted absorption maximum at 347 nm. The sample also showed antibacterial activity against gram-positive and gram-negative bacteria.

Primo et al. [26] reported the eco-friendly synthesis of Zno nanoparticles using green synthesis (using aloe vera) and gelatinization (using cassava starch). The XRD pattern confirmed the average crystalline size to be 43.3 nm (Zn-Aloe vera) and 44.9 nm (Zn- starch). The UV-Vis spectra showed a difference in the absorption edge of the sample using aloe vera (3.18 eV) and cassava starch (3.24 eV), Both the synthesis methods have the same removal efficiency at low Cu ion concentration however, the sample synthesized using aloe vera showed higher removal efficiency on increasing the absorbate concentration. Thus, Zn0 nanoparticles can be used as an effective and environmental-friendly metal trace absorber in wastewater

Ali et al. [27| reported the synthesis of ZnO nanoparticles through a biogenic approach, exploiting the reducing and capping potential of aloe vera leaf extract, The XRD analysis confirmed the average size of the sample to be 15 nm. The UV-Vis spectra showed a characteristic peak at 375 nm. The sample also exhibited significant antibacterial activity against clinical bacterial isolates. The study elucidated an environmentally benign and cost-effective method for applications as nano antibiotics or drug carriers.

Varghese et al. [28] reported the green synthesis of zine oxide nanoparticles using aloe vera extract. XRD analysis confirmed the hexagonal zine oxide structure with an average particle size of 22.18 nm. The blue-shifted UV-Vis absorption peak at 340 nm confirmed the presence of ZnO in the nanoscale. The antibacterial study of the sample showed sensitivity to both Gram-positive and Gram-negative bacteria.

Chaudhary et al. [29] studied the antimicrobial activity of zine oxide nanoparticles synthesized from aloe vera extract against pathogenic bacteria and fungi. UV-Vis spectra showed maximum absorbance at 240 nm and after 24 hrs the intensity of the peaks increased. XRD pattern showed two peaks at 33.2542 and 59.0524 with Miller indices (100), (111) respectively indicating the face-centred cubic symmetry of the sample. The ZnO nanoparticles had an antibacterial effect against E coli and an antifungal effect against A. Niger however in combination with antibiotics, they showed lesser effect as compared to the antibiotic ic.

Parthasarathy et al. [30] reported the biosynthesis of Zno nanoparticles using the leaf extracts of aloe vera. The XRD pattern confirmed the mean crystalline size to be 10.35 nm, 11.66 nm, 18.75 nm and 30.16 nm. The UV analysis showed a maximum absorption peak at 436 nm. The synthesized nanoparticles showed better activity against S. aureus and S. typhi than other organisms.

Matinise et al. [31] reported the green synthesis of ZnO nanoparticles using Moringa Oleifera extract as an effective chelating agent. The XRD and EDS studies showed that annealing at 500 C in the air is required for the synthesis of the pure wurtzite Zno phase. The absorbance profile of the UV spectra starts decreasing at 350 nm approx. with an inflexion point about 380 nm. The electrochemical analysis proved the sample to have high electrochemical activity and therefore has potential electrochemical applications.

Hu et al. [32] reported the eco-friendly synthesis ol Zno nanoparticles using leaf extracts of Cucurbita pepo. The UV absorption study showed a large range of absorption value with a strong absorption peak at 352 nm. The XRD pattern showed strong diffraction peaks corresponding to hexagonal wurtzite structure. The experimental results confirmed the sample to be an important substitute for osteoporotic and bone tissue formation

Vijayakumar et al. [33] reported the biosynthesis of zinc oxide nanoparticles using Atlantia monophyla leaf extract. XRD spectrum showed sharp and narrow diffraction peaks indicating the sample to be pure and crystalline in nature with an average particle size of 33.01 am. The UV-Vis absorption studies showed a wide range absorption value and strong absorption peak ut 352 nm. The bacterial and fungal destruction was better for Zno nanoparticles than reported for plant extracts and standard drugs.

Elumalai et al. [34] described the synthesis of zinc oxide nanoparticles using leaf extract of Azadirachta indica. The XRD pattern confirmed the average crystalline size to be 18 nm. The UV-Vis absorption spectrum showed a characteristic absorption peak at 370 nm and the bund gap energy of ZnO was found to be 3.3 eV. The antimicrobial activity of the sample increased due to the increase of H:Oz concentration on the surface of Zno. Moreover, green synthesized Zno nanoparticles were more potent than bare ZnO.

Selim et al. [35] reported the synthesis of Zinc oxide nanoparticles using the extract of the aerial parts of

Deverra tortuosa. XRD analysis estimated the crystalline size to be 15.41 nm. The UV-Vis spectrum showed a characteristic peak at 374 nm and the direct band gap calculated was 3.32 ev, Both the aqueous extract of D. tortuosa and the synthesized Zno nanoparticles showed an attractive selective cytotoxic activity against two tested cancer lines offering safer and cheaper alternatives to conventional therapy tools.

Sivakumar et al. [36] reported the green synthesis of zinc oxide nanoparticles using the aqueous extract of green tea leaves. The UV-Vis spectrum confirmed the synthesized sample by exhibiting a highly blue shifted absorption maxima occurring around 325 nm. XRD analysis confirmed the hexagonal wurtzite structure of the ZnO nanoparticles and the average particle size was found to be 16 nm. The synthesized nanoparticles showed better and comparable antimicrobial activities with respect to the activities of synthetic drugs.

Soto-Robles et al. [37] reported the green synthesis of zinc oxide nanoparticles using different concentrations of Hibiscus sabdariffa flower extracts. XRD analysis showed diffraction peaks corresponding to the hexagonal wurtzite crystalline phase of the sample. The size of the crystals calculated were different for different concentration, It was observed from the UV-Vis spectrum that band gap values decrease as the concentration of ions in the sample increases. The sample also exhibited good photocatalytic results comparable to the Zno nanoparticles synthesized via other extracts and other methods.

CHAPTER 3

AIM AND OBJECTIVES

3.1 X-ray diffraction spectroscopy

X-Ray Diffraction is the most commonly used techniques in determination of crystal structure of atoms.

Instrumentation:

X-ray diffractometer consists of 3 basic elements.

1.An X-ray tube

2.An X-ray holder

3.A Sample holder

X-rays are generated in a cathode ray tube by heating a filament to produce electrons towards a target by applying a voltage and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being $k\alpha$ and $k\beta$. $K\alpha$ consists of $K\alpha 1$ and $K\alpha 2$. $K\alpha 1$ has a slightly shorter wavelength and twice the intensity as $K\alpha 2$. The specific wavelengths are characteristic of the target material. Filtering by foils or crystal monochromator is required to produce monochromatic X-rays needed for diffraction. $K\alpha 1$ and $K\alpha 2$ are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single crystal diffraction. With Cu- $K\alpha$ radiation=1.5406 Å. These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-ray is recorded when the geometry of the incident x-ray impinging the sample satisfies the Bragg equation, constructive interference occurs and a peak in the intensity occurs. A detector records and processes this x-ray signal and converts the signal to a count rate which is then output to a device as aprintera monitor. The most commonly used x-ray instrument is the powder diffractometer. It has a scintillation or Geiger counter. The detector shows a range of scattering angles. Generally, it is a practice to mention scattering angle 2θ. The intensities are taken as peak heights. The d values can be calculated from the graph. A set of peaks and their heights is adequate for phaseidentification.

CHAPTER 4
MATERIALS AND METHOD

4.1 Materials used

Zinc nitrate hexahydrate (Zn (NO3)2.6H2O), sodium hydroxide (NaOH) pellets, demineralised water were used in the nanoparticle synthesis with the extracts. Aloe vera leaves were collected locally.

4.2 Preparation of the Aloe vera leaf extract

The aloe vera leaves were washed thoroughly to remove dust and impurities. The extract was prepared by adding 26 g of aloe vera in 260 ml demineralised water taken in a clean beaker. The mixture was then boiled at 80 °C for 45 minutes until the colour changes to pale yellow. Then the mixture was cooled at room temperature and the extract was separated using Whatman filter paper.

4.3 Green synthesis zno using aloe vera extract

In the green synthesis of ZnO nanoparticles, zinc nitrate and sodium hydroxide were used as precursors. For the preparation of 30 ml of 1 M zinc nitrate solution, 30 ml demineralised water was taken in a beaker and 8.9224 g zinc nitrate was added to it. The solution was then stirred for about 15 minutes in a magnetic stirrer. Then 30 ml aloe vera extract was added to it and stirred. pH of the solution was maintained at 12 by adding NaOH (5 M) drop-wise to the solution while stirring. The whole solution was stirred for about 1 hour. The 5 M solution of NaOH was prepared by dissolving 6 g of NaOH pellets in 30 ml demineralised water. The resultant solution was filtered using a filter paper. It was dried under an IR lamp and then grounded with pestle mortar into a fine powder. A resultant white powder was thus obtained.

4.4 Antibacterial Studies

The antibacterial activity of biosynthesised ZnO nanoparticles was tested by agar well diffusion method against the Gram- positive bacteria Staphylococcus aureus and the Gram-negative bacteria Shigella. Muller Hinton (MH) agar medium was used for the antibacterial assay. ZnO nanoparticles were first dissolved in 5 ml citric acid and is stirred for 45 minutes. Mueller Hinton agar medium was poured on the petri plates and are allowed to solidify. Nutrient agar and nutrient broth were used to store and subculture the bacterial pathogens. The bacterial cultures were swabbed evenly on the nutrient agar plate using a sterilized cotton bud. Four wells were made on the Mueller Hinton agar plates using a sterile cork-borer with a diameter of 6mm. The bacterial cultures were incubated for 24 hrs at 37 + 0.5 °C to make a bacterial suspension. Penicillin and citric acid were used as positive and negative control respectively. 100 µl of the biosynthesised ZnO nanoparticles were pipetted into each well and the positive control – antibiotic penicillin (10 ug/disc) was placed at the centre of the plate. The antibacterial activity of the synthesised sample was analysed by measuring the zone of inhibition in millimetres after 48 hrs of incubation.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 X-Ray Diffraction Studies

X-Ray Diffraction technique was used to identify the crystalline structure, phase purity and crystallite size of the as-prepared ZnO nanostructures using RigakuMiniflex 600 with CuKα (λ=1.5406 Å) radiation. It was observed that ZnO nano powders are highly crystalline and single phase with hexagonal structure. Fig.1 shows the obtained diffraction pattern of prepared samples.

31.70°, 34.37°, 36.23°,47.56°, 56.63°, 62.76°, 66.35°, 67.95°, 69.02°, 72.34° and 76.32° the peaks at 2ϴ of above-mentioned values corresponds to the planes 100, 002, 101, 102, 110, 103, 200, 112, 201, 004, and 202. No peaks related to impurities are observed in the spectrum.

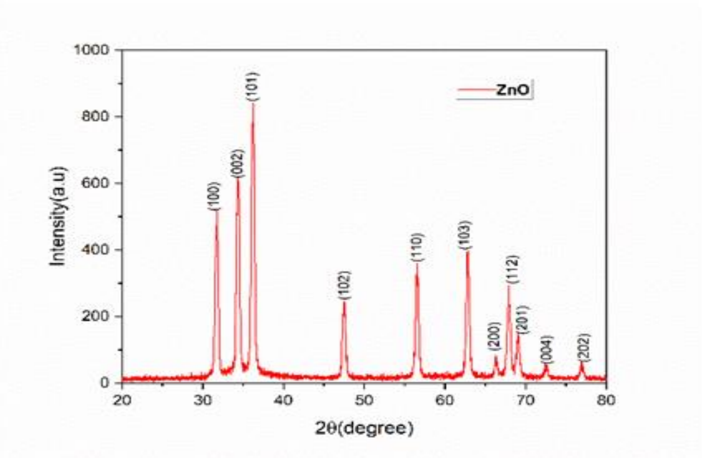


FIGURE 1. XRD spectrum of as synthesized ZnO nanostructures.

The particle size of the ZnO nanoparticles was find by using the Scherrer formula given by :

$$D = K\lambda/\beta \cos\theta \quad (1)$$

where λ is the wavelength of X-ray used ,β is the full-width half maximum and θ is the angle of diffraction. Crystallite size of ZnO nanostructures are found to be 36nm.

5.2 UV-Visible absorption studies

Optical characterisation of the as-prepared ZnO nanostructures were conducted using JASCO V760 Spectrophotometer and it is found that the prepared sample shows a strong absorption edge at 352nm.

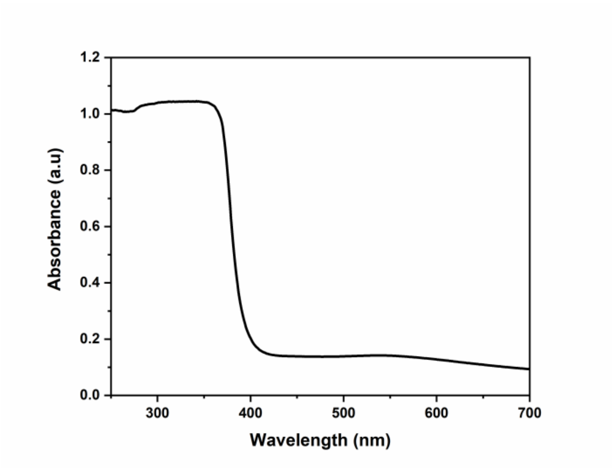


FIG 5.2

Fig.2 shows Tauc plot of the synthesized samples. Direct bandgap of the ZnO nanostructures is obtained from the Tauc's plot using the equation,

$$(\alpha h\vartheta)2 = C(h\vartheta - Eg) \quad (2)$$

where 'α', 'ϑ', 'C', and 'Eg' are the molar absorption coefficient, frequency of light, an arbitrary constant and the band gap of the nanoparticles respectively. Bandgap thus calculated is found to be 3.18 eV.

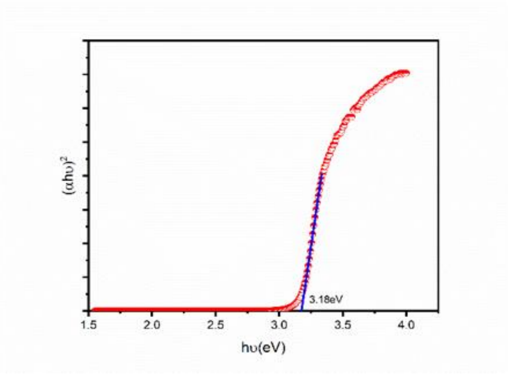
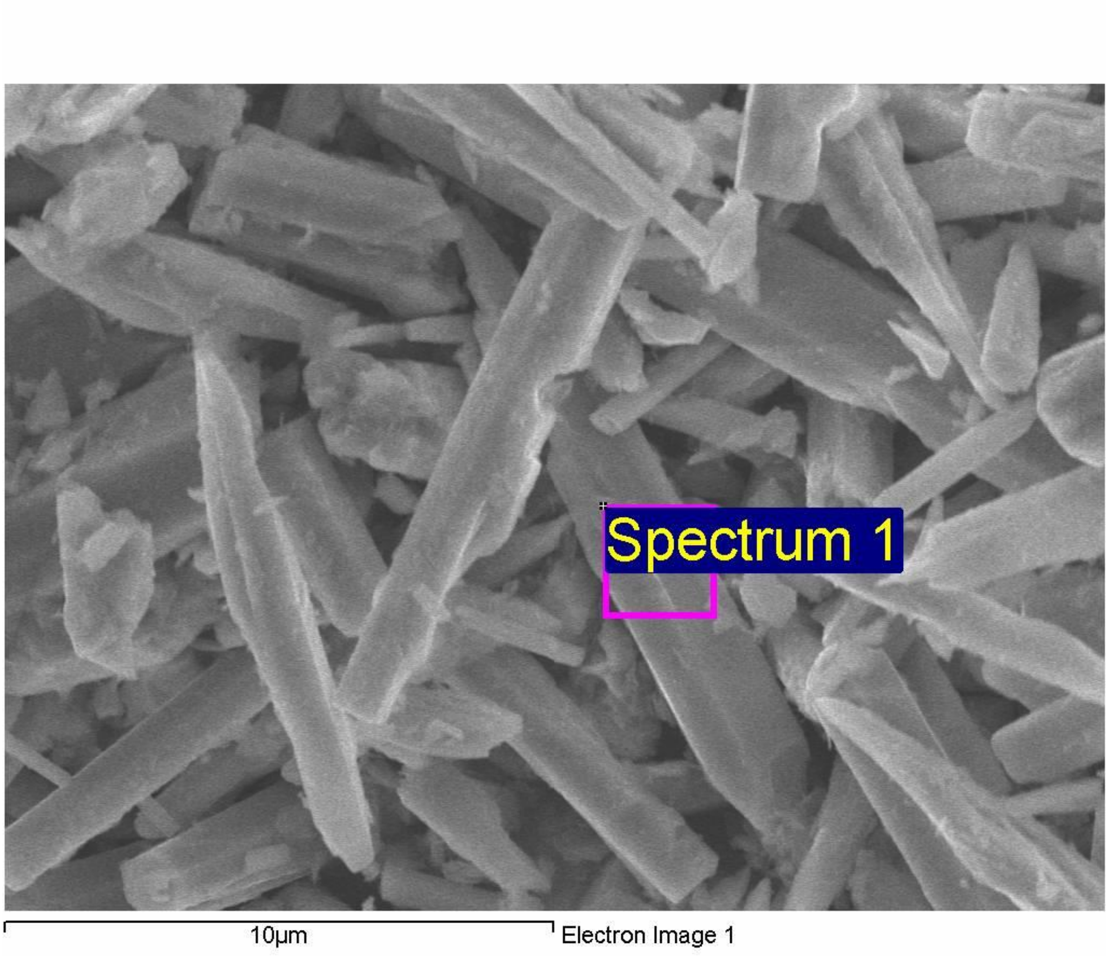


FIGURE 5.3 Tauc plot of ZnO nanostructures.

Scanning Electron Microscopy (SEM)

SEM image revealed that the prepared ZnO nanostructures follows a rod like structure with sharp edges. The rods have different thickness and width.



Antibacterial activity of ZnO nanostructures

Antibacterial activity of the hydrothermally synthesised ZnO nanoparticles was tested by agar gell diffusion method against various harmful bacteria including staphylococcus, e-coli, klebsiellapneumoniae, shigella and salmonella. Citric acid was used as the negative control in antibacterial assay. In the present study, ZnO nanoparticles were first dissolved in 5ml citric acid and is stirred for 45 minutes. Organisms such as Salmonella, Klebsillapneumoniae (K. Pneumoniae) were used as the test pathogens. Nutrient agar and nutrient broth were used to store and subculture bacterial pathogens. Muller Hinton (MH) agar medium was used for the antibacterial assay. Here the antibacterial activity of 0.5 M ZnO NPs is studied using Agar well diffusion assay. The bacterial cultures were incubated for 24 hr at 37±0.5°C to make bacterial suspension. Citric acid was taken as negative control and 100µl of bacterial suspension was uniformly spread on MH Agar plates using micropipette. The positive control- antibiotic penicillin (10µg/disc) is placed at the centre of the plate. The plates were incubated for 24 hr at 37±0.5°C. Antibacterial activity of the synthesized samples were analysed by measuring the zone of inhibition in millimetres after 48 hrs incubation. The zone of inhibition obtained for ZnO nanoparticles from the antibacterial assay is shown in the fig.5.5 and table 1.

FIGURE 5.5. Zone of inhibition of ZnO nanoparticles against (a) Shijella (b) Klebsiellapneumonia.

Sl.no	**Micro organism**	**ZnO NPs**	**+ve control**	**-ve control**
		(Diameter in mm)		
1	Staphilococcusauerus	24	23	-
2	Klebsiellapneumoniae	30	23	-
3	Shigella	29	26	-
4	Escherichia coli	22	24	-

TABLE 1. Well diffusion method zone of inhibition of ZnO nanostructures.

CHAPTER 6

CONCLUSION

ZnO nanostructures are effectively synthesized by low-cost environmentally friendly green synthesis route from aloe vera leaf extract. Structural and optical properties of the assynthesized nanoparticles were characterized by XRD and UV-Visible spectroscopy. XRD analysis revealed that the synthesized material follows a hexagonal wurtzite nature. From UVVisible spectroscopy and corresponding Tauc plot analysis it is clear that ZnO possess a bandgap of about 3.18eV. Green synthesized ZnO nanoparticles shows better anti-bacterial activity and it is clear that ZnO possess higher antibacterial activity for Gram-negative bacteria Shigella than the Gram-negative bacteria such as Staphylococcus aureus.

From the studies it is clear that it is possible to synthesize ZnO nanoparticles without using any toxic and higher expensive chemicals. We can able to use it as a method for the preparation of ZnO for biomedical and other electronic applications.

REFERENCES

[1] J.P. Srivastava, Elements of Solid-State Physics, Fourth edition, phi Learning Pvt.Ltd.

[2] Alagarasi, Introduction to nanomaterials, (2011),424-428.

[3] Areola Adedapo Oluwasanu, Fapohunda Oluwaseua, Jimoh Adeleke, Ige Ayikunle and Ogunyele Abhibok Chris, Scientific applications and prospects of nanomaterials: A Multidisciplinary review,18(2019)946-96.1.

[4] Sumioljima, helical microtubes of graphic carbon, nature,354(1991)56.

[5]..https://www.google.com/search?q=classification+of+nanoparticles+&ei=yVOWYfakMPnYz7sPgciKmAY&ved=0ahUKEwi2rYeAgaL0AhV57HMBHQGkAmMQ4dUDCA4&uact=5&oq=classification+of+nanoparticles+&gs_lcp=Cgdnd3Mtd2l6EAMyBAgAEEMyBQgAEIAEMgUIABCABDIFCAAQgAQyBQgAEIAEMgYIABAWEB4yBggAEBYQHjIGCAAQFhAeMgYIABAWEB46BwgAEEcQsANKBAhBGABQgAVYgAVgmQ9oAXACeACAAYwBiAGMAZIBAzAuMZgBAKABAcgBCMABAQ&sclient=gws-wiz .

[6] Kendal K., Science, Atlantic publishers (1994).

[7] Dan Guo, Guoxinxie and Jianbing Luo, Mechanical properties of nanoparticles basics and applications,47(2013)013001.

[8] Y. Chan.J.M.Caruge, P.T.Snee, M.G.Bawendi,Appl.Phy.Lett.85(2004)2460-2462.

[9] www.Sepmag.eu – properties of nanoparticles.

[10] Drexler, K. E.,Nano systems, John Wiley and Sons, NY,1992.

[11] N. A. Smirnov, S. I. Kudryashov, A. A. Nastulyavichus, A. A. Rudenko, I. N. Saraeva, E. R. Tolordava, S. A. Gonchukov, Yu M. Romanova, A. A. Ionin and D. A. Zayarny, “Antibacterial properties of silicon nanoparticles”, IOP Publishing, Laser Phy. Lett. 15, (2018), 105602.

[12] Marwath M. Mohamed, Shereen A. Fouad, Hisham A. Elshoky, Gina M. Mohamed and Taher A. Salaheldin, “Antibacterial effect of gold nanoparticles against Corynebacterium pseudotuberculosis”, International Journal of Veterinary Science and Medicine, Vol. 5, (2017), Issue – 1, 23-29.

[13] Vardan Galstyan, Manohar P.Bhandari, Veronica Sberveglieri, Giorgio Sberveglieri,and Elisabetta Comini, Metal oxide Nanostructures in food Applications: Quality control and packing 6(2018)16.

[14]https://www.researchgate.net/profile/DavarBoghaei/publication/270824190/figure/fig2/AS:295128141975563@1447375440619/Colour-online-The-hexagonal-wurtzite-structure-of-ZnO-O-atoms-are-shown-as-grey.png.

[15] XD Bai , PX Gao and Z. L. Wang Applied Physics Letters 82 (2003) 4806-4808.

[16] LH William and L. W Zhong Applied Physics Letters 82 (2003) 2886-2888.

[17] D Chu and S Li New Journal of Glass and Ceramics 2 (2012) 13-16.

[18] [18] M G El - Shaarawy , M Khairy and M A Mousa Advanced Powder Technology 31 (2020) 1333-1341.

[19] KB Suresh and V Narayanan Chemical Science Transactions 2 (2013) 33-36.

[20] P Rauwel , M Salumaa , A Andres , G Augustinas and E Rauwel Journal of Nanomaterials 2016 1-12.

[21] M A Garcia , J M Merino , E Fernández Pinel , A Quesada , J de Venta , M L Rui'2 González , G R Castro , P Crespo , J Llopis , J M González - Calbet and A Hernando Nano Letters 7 (2007) 1489-1494.

[22] SA Elit and UE Husnu Thin Solid Films 520 (2012) 4658-4661.

[23] N I Rasli, H Basri and Z Harun Helion 6 (2020).

[24] G Sangeetha, S Rajeshwaria and R Venckatesh Materials Research Bulletin 46 (2011)

2560-2566.

[25] H Mofid, M S Sadjadi, M H Sadr, A Banaei and N Farhadyar Advances Nano chemistry 1.(2020) 32-35.

[26] Julia de O. Primo, C Bittencourt, S Acosta, A Sierra-Castillo, C Jean-Francois, S Jaerger,V C Teixeira, and F J Anaissi Frontiers in Chemistry 8 (2020).

[27] A Kursheed, S Dwivedi, A Ameer, S Quaiser, M S. Al-Said, A A Abdulaziz and M Javed.Journal of Colloid and Interface Science 472 (2016) 145-156.

[28] E Varghese and M George International Journal of Advance Research in Science and

Engineering 4 (2015).

[29] C Asha, K Naresh, K Ravinder and K S Raj SN Applied Sciences, A Springer Nature

Journal 1 (2019).

[30] G Parthasarathy, M Saroja and M Venkatachalam International Journal of Pharmaceutical Sciences and Research 8 (2017).

[31] N Matinise, X G Fuku, K Kaviyarasu, M Maaza Applied Surface Science 406 (2017) 339-347.

[32] H Dan, S WeiBing, Q Wei, J Jian, L XiaoLin, G XuePing, H YueFeng Journal of

Photochemistry & Photobiology, B: Biology 195 (2019) 12-16.

[33] S Vijayakumar, S Mahadevan, P Arulmozhi, S Sriram, P K Praseetha Materials Science in Semiconductor Processing 82 (2018) 39-45.

[34] K Elumalai, S Velmurugan Applied Surface Science (2015) 03.176.

[35] A S Yasser, A A Maha, I Ragab and M H M Abd El-Azim, Scientific Reports 10 (2020).

[36] S R Senthilkumar and T Sivakumar, International Journal of Pharmacy and Pharmaceutical Sciences 6 (2014) 461-465.

[37] C A Soto-Robles, PA Luque, C M Gómez-Gutièrrez, O Nava, A R Vilichis-Nestor, E

Lugo-Medina, R Ranjithkumar and A Castro-Beltrán Results in Physics 15 (2019).

[38]https://www.google.com/search?q=pictures+of+braggs+law&oq=&aqs=chrome.0.35i39l5.-1j0j1&client=ms-android-xiaomi-rev1&sourceid=chrome-mobile&ie=UTF-8#imgrc=_aVIJkbUuSdLcM .

[39] Aman Thakur, Principles, working and applications of UV spectroscopy,(2011).

[40] Brian D.Viezbicke, ShanePatel,Evaluation of the Tauc method for optical absorption

edge determination: ZnO thin film as a model system,252(2015)1700-1710.