SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES FOR THE TREATMENT OF WATER FROM BACTERIAL INFECTION

Olkeba Geresu Beji

A Thesis submitted to the department of Applied Physics

School of Applied Natural Science

Presented in Partial Fulfillment of the requirement for the Degree of Master's in Material Physics

Office of Graduate Studies

Adama Science and Technology University

August, 2021

Adama, Ethiopia

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Advisor: Solomon Tiruneh Dibaba (PhD)

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Approval Sheet

I, the advisor of the thesis entitled **"Synthesis and characterization of zinc oxide nanoparticles for the treatment of water from bacterial infection"** and developed by Olkeba Geresu hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Solomon Tiruneh (PhD)	_____	_____
Advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Olkeba Geresu have read and evaluated the thesis entitled **"Synthesis and characterization of zinc oxide nanoparticles for the treatment of water from bacterial infection"** and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Material physics.

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Finally, approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

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Declaration

I hereby declare that this Master Thesis entitled **"Synthesis and characterization of zinc oxide nanoparticles for the treatment of water from bacterial infection"** is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

Olkeba Geresu	_____	_____
Name of student	signature	Date

Recommendation

I, the advisor of this thesis, hereby certify that I have read the revised version of the thesis entitled **"Synthesis and characterization of zinc oxide nanoparticles for the treatment of water from bacterial infection"** prepared under my guidance by Olkeba Geresu submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Material Physics

Therefore, I recommend the submission of revised version of the thesis to the department following the applicable procedures.

Solomon Tiruneh (PhD)	_____	_____
Advisor	Signature	Date

ACKNOWLEDGMENT

My first thankfulness goes to the Almighty God for giving me health and strength throughout this work. The research conducted during my work would not have been possible without the support of many people. First and foremost my gratitude finds my advisor Dr. Solomon Tiruneh for his excellent guidance, assistance, timely advice and contribution of valuable suggestions during my thesis work.

I accord my thanks to Dr.Abebe Belay for providing me zinc acetate dihydrate chemical during my lab work. And also I would like to acknowledge the advanced physics and biology laboratory of ASTU for allowing me to use essential materials of this research work.

I have no words to express my gratitude thanks to Mr.Lata Guta and Eticha Abdisa for their assisted me on the isolation of bacteria and the microbial laboratory activity in biology laboratory of ASTU.

My heart-felt word of thanks also goes to my friends Gurmu, and Azeb for their friendly co-operation, support and advice in various ways.

I would like to express my gratefulness thanks to applied physics department staff Dr. Mesfin Asfaw, former head department of applied physics, for his willingness and support me in different way, and to Dr. Megersa Wedajo, Dr. Alemu Kebede and Dr. Tewodros Yirgashewa, too for feed me immeasurable knowledge in the corresponding courses.

I acknowledge and appreciate my beloved classmates and friends all those who contributed in one way or the other to the fulfillment of this research

Also my special thanks to my uncle Mr. Bayisa Sorsa and my friend Mr.Habshalo Roba for their invaluable financial support and moral supports towards my research work.

Finally, my immeasurable thanks go to my wife Birhane Reta and my family for their invaluable financial support, love and encouragements during my studies.

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List of Abbreviations

AC	Alternating current
Al_2O_3	Aluminum oxide
ARC	Antireflective coatings
AFM	Atomic force microscope
Astu T_1	Adama science and Technology University tank 1
Astu T_2	Adama science and Technology University tank2
$BaTiO_3$	Barium titanate
CVD	Chemical vapor deposition
Ceo_2	Cerium oxide
CO_2	Carbon dioxide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DLE	Deep level emission band
E.coli	Escherichia coli
eV	Electron volt
FDA	Food and Drug Administration
FE	Free exciton
FTIR	Fourier transform infrared ray spectroscope
Fe_2O_3	Iron (III) oxide
GaN	Gallium nitride
GPa	Gigapascal
GRAS	Generally recognized as safe
HCL	Hydrochloric acid

H_2O_2	Hydrogen peroxide
MgO	Magnesium oxide
NNI	National Nanotechnology Initiative
$Na_2Zn\ (OH)_4$	Sodium Zinc Hydroxide
NPS	Nanoparticles
$PbTiO_3$	Lead Titanate
Pb (Zr,,Ti)O_3	Lead zirconate titanate
PL	Photoluminescence
PVDF	Polyvinylidene fluoride
ROS	Reactive oxygen species
SEM	Scanning Electron Microscope
SnO_2	Tin oxide
TCO	Transparent conductive oxides
TiO_2	Titanium dioxide
UV-vis	Ultraviolet-visible
XRD	X Ray Diffraction
ZnO NPs	Zinc oxide nanoparticles
ZrO_2	Zirconium oxide
$ZnCl_2$	Zinc chloride

ABSTRACT

Without water survival is impossible. But water is getting polluted with different pollutants such as bacteria. Various methods has been tested for removing organic contaminants, among which nanomaterials has got significant attention due to their unique size tuneable properties that make this suitable for antibacterial applications. In these work, zinc oxide nanoparticles were synthesized through the sol gel method for removal of bacteria from west water. The obtained nanoparticles were characterized by x-ray diffraction (XRD), Scanning electron microscope (SEM), photoluminescence (PL), UV-Visible Spectroscopy and Fourier transform infrared ray spectroscope (FTIR). Then, the average crystallite sizes of 26.18 nm and 27.29 nm were obtained for the ZnO nps annealed for 90 min and 2 hrs respectively. The band gap energy of ZnO NPs determined using UV-vis absorption data were found to be 3.27 ev and 3.25 ev for 90 minute and 2 hour time of annealing at temperature 500℃ *respectively which increases as the size of ZnO nps decreases. The FTIR peaks showed relatives peaks at 433 cm^{-1} and 557 $cm^{-1}$for synthesized ZnO nps which have 26.18 nm and 27.29 nm crystallite size respectively which identifies the absorption peaks of ZnO nps. Evaluation of the antimicrobial activity of ZnO nanoparticles against E.coli isolated from the collected wastewater was done. Results indicate that ZnO nanoparticles could potentially be strong antimicrobial reagents to reduce the infection of water from bacteria. For the synthesized 26.18 nm ZnO nps the measured zone of inhibition was* 12.07 ± 0.85 mm, 10.57±0.70 mm, 8.17 ± 0.95 mm and 7.37 ± 0.550 mm at concentration of 100 µg/ml, 50µg/ml, 25 µg/ml and 12.5 µg/ml respectively and for 27.29 nm ZnO nps the11.73 ± 0.38 mm, 10.33 ± 0.47 mm, 7.8 ± 0.87 mm, 7.2 ± 0.20 mm at concentration of 100 µg/ml, 50 µg/ml, and 25 µg/ml 12.5 µg/ml respectively. *Overall, both the synthesized ZnO nps show the antibacterial activity and the antibacterial activity increased as the concentration of the synthesized zinc oxide nanoparticles increases.*

***Key** words: Antibacterial activity, Sol- gel method, Zinc Oxide, E.coli*

1. INTRODUCTION

1.1 Back ground of the study

Nanoscience, nanotechnology, nanomaterials or nanoparticles are only a few of the new nano-containing terms that occur frequently in scientific reports, in popular books as well as in newspapers and that have become familiar to a wide public, even of non-experts. Nanotechnology is the science of the small; the very small. It is the use and manipulation of matter at a tiny scale. At this size, atoms and molecules work differently, and provide a variety of surprising and interesting uses. Nanotechnology and Nanoscience studies have emerged rapidly during the past years in a broad range of product domains. It provides opportunities for the development of materials, including those for medical applications, where conventional techniques may reach their limits. Nanotechnology should not be viewed as a single technique that only affects specific areas. Although often referred to as the 'tiny science', nanotechnology does not simply mean very small structures and products. Nanoscale features are often incorporated into bulk materials and large surfaces(Pal et al., 2011).

Nanotechnology is compromised as a group of novel technologies capable of designing, producing, characterizing and controlling structures, materials, devices and systems at the nanometer scale, i.e. less than 100 nanometers. This term is very transversal, being used across many fields, such as chemistry, medicine, biology, physics, materials science, environment, and engineering, amongst others. The core building blocks of nanotechnology are NPs(Muhammad et al., 2019). Nanostructure materials are a single phase or multiple polycrystalline solids with a typical average size of a few nanometers (1 nm $=10^{-9}$m). Basically the range from (1-100) nm is taken as nano-range for convention as per National Nanotechnology Initiative in the US. As the particle size decreases to some extent, a large number of constituting atoms can be found around the surface of the particles, which makes the particles highly reactive with prominent physical properties, hence, manipulation and control of the material properties via mechanistic means is needed(S. Sharma et al., 2014). The structures or materials at the nanometer scale can be classified as: 0D (zero dimension) if all three spatial dimensions are in the nanometric range, i.e.

nanoparticles or clusters; 1D (one dimension) if two dimensions are in the nanometric range, like nanotubes, nanorods and nanowires; or 2D (two dimensions) if only one spatial dimension is nanometric, such as in thin films or nanosheets. 3D (three dimensional) materials implies that the 0D, 1D and 2D elements are in close contact forming interfaces (Nunes et al., n.d.).They are widely used in a number of processes that include material science, agriculture, and food industry, cosmetic, medical, and diagnostic applications. Nano size inorganic compounds have shown remarkable antibacterial activity at very low concentration due to their high surface area to volume ratio and unique chemical and physical features. In addition, these particles are also more stable at high temperature and pressure. Some of them are recognized as nontoxic and even contain mineral elements which are vital for human body (Siddiqi et al., 2018). Various types of nanoparticles such as metal oxides nanoparticles, polymer nanoparticles and metal nanoparticles have been reported. Metal oxide nanoparticles such as Al_2O_3, MgO, ZrO_2, CeO_2, TiO_2, ZnO, Fe_2O_3, and SnO; are the most versatile materials due their diverse properties and functionalities.

Amongst these nanoparticles, zinc oxide (ZnO), also known as zincite has attracted much attention within the scientific community as a ‘future material’ and thus an important n-type semiconducting metal oxide (Nethavhanani, 2017). The ZnO is insoluble in water and ethanol but is soluble in dilute mineral acids and is a fine powder, white or slightly yellow (Savi et al., n.d.). Zinc oxide NPs has unique physical and chemical properties, such as high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photostability, is a multifunctional material. According to unique structural and optical properties of ZnO semiconductor material, there are many potential important applications based on that material, including as an anti-reflection coating (ARC) in solar cells. Antireflective coatings (ARC) made of ZnO on top to improve the optical properties of the coating(Ramelan et al., 2017). Zinc oxide has received much more attention because ZnO having wide band gap (3.37eV), large excitation binding energy (60meV) and higher melting point (2248K)(Jayasree ALURI & and Basaveswara Rao MANDAVA, 2016). Its high band gap ensures a large breakdown field, and the thermal stability of the material allows high temperature operation. Moreover Zinc Oxide is environmental friendly and ease to synthesize(Kariper, 2015). The piezo- and pyroelectric properties of ZnO mean that it can be used as a sensor, converter, energy generator and Photocatalist in hydrogen production. Because of its hardness, rigidity and piezoelectric constant it is an important material in the ceramics industry, while its low toxicity,

biocompatibility and biodegradability make it a material of interest for biomedicine and in pro-ecological systems (Belay, 2017a).

Nowadays, ZnO NPs are predominantly used as antimicrobial agents, delivering systems vaccines and anti-cancer systems, Photocatalist, biosensors, energy generators and bio-imaging materials (Haque et al., 2020). Zinc oxide is known for its antibacterial properties from the time immemorial due to its stability and is considered as harmless substance for humans and marine life. Numerous studies have been shown that nanoparticles formulation can be used as an efficient antimicrobial agent. Because of the catalytic and antimicrobial characteristics of nanoparticles, they are considered as efficient alternatives for disinfection of water and west water system. Antibacterial features of zinc oxide nanoparticles are because of their small size and their high rate of surface to volume which lets them react closely to the microbial membrane and destroy them. The operating mechanism of the effect of zinc oxide nanoparticles on bacterial is by damaging the protein, DNA and destroying the cell wall of bacteria (Mostafaii et al., n.d.).

Several fabrication techniques are used to produce ZnO NPs such as thermal hydrolysis techniques, hydrothermal processing, sol-gel method, vapor condensation method, spray pyrolysis and thermo chemical techniques(Haque et al., 2020). In the present investigation the sol-gel method for synthesis of ZnO nanoparticles was chosen as it is simplest method, consumes less power and can be carried out in robust atmosphere and works at lower temperature when compared to other methods such as chemical vapor deposition (CVD), hydrothermal, and plasma which require high temperatures in the process of synthesis. The sol-gel method has a principle similar to the solid state reaction. The process involves heating the mixture to stoichiometric reagents to make the ions move so that the desired solid product is obtained (Watanabe et al., 2017).

The structural properties of as prepared ZnO nanoparticles was studied by using X Ray Diffraction(XRD) and the morphology of ZnO nanoparticles was examined under Scanning Electron Microscope (SEM). The transparency and absorption of synthesized ZnO nanoparticles was studied using UV visible spectrophotometer. The formation of ZnO nanoparticles was confirmed from FTIR analysis.

1.2 Statement of the problem

A high antimicrobial efficiency of ZnO-NPs was observed already before several years, although their antimicrobial mechanism is still not well known. The application of ZnO NPS for antibacterial activity using photocatalytic mechanism was widely reported. However, because of absorption peak of the NPs, the photocatalytic performance of the ZnO NPs was limited to utilization of only UV light which is difficult to harvest. Hence, in this study the direct or electrostatic interaction between ZnO-NPs and live cells of microorganism (bacteria), the cellular internalization of ZnO-NPs, and the production of active oxygen substances (oxidative stress) such as H_2O_2 in cells due to metal oxides have been suggested as the possible causes of cell membrane disruption(Nosáľ & Reinprecht, 2018). Contamination of drinking water with bacteria is a major global problem as it causes irreversible damage to humans and animals and spreads numerous epidemics and chronic diseases.

To overcome these problem nanotechnologies are the best solution. Zinc is a necessary element to our health and ZnO nano particles also have good biocompatibility to human cells(Paper & Submission, 2016). Currently, investigations on the 'sol gel' synthesis and characterization of ZnO nanoparticles for treatment of water from bacterial infections was studied because of its simplicity, economic and regarded as a good antibacterial agent because it is stable under harsh processing conditions, and is considered as a safe material for humans and animals. Compared to organic materials, inorganic materials like ZnO have greater durability, selectivity, and heat resistance.

1.3 Significance of the study

The study is so significant that it improves wastewater quality for human being, animals, plants, environment etc. This study addresses the common synthesis and characterization of zinc oxide nanoparticles by sol-gel approach for the treatment of water from bacterial infection applications. Some of the most important advantages of the synthesis of zinc oxide nanoparticles by sol–gel methods were easiness of the synthesis, low temperature of the final decomposition, control on the chemical composition and also zinc oxide nanoparticles was removes heavy metal ions, high surface to adsorption ratio and low maintained cost. Therefore as syntheses of ZnO nanoparticles can be used for treatment of water from bacterial infection using direct contact of the ZnO NPs

with bacteria and electrostatic interaction between them due to its special characteristics such as size tunable large surface area to volume ratio that enhance the interaction between the bacteria and the NPs.

1.4 Objective of the study

1.4.1 General objective of the study

The main objective of this work was to Synthesis and characterizes zinc oxide nanoparticles for the treatment of water from bacterial infection.

1.4.2 The specific objectives

The specific objectives are:

- to synthesis zinc oxide nanoparticles of low dimension by using zinc acetate dehydrate ($Zn(CH_3COO)_2.2H_2O$) as precursor.
- determining the crystal structure and particle size with X-Ray Diffraction (XRD)
- measuring the absorption and emission intensity of the Synthesized ZnO nps.
- determining the band gap of the synthesized zinc oxide nanoparticles from the UV-vis data.
- to improve the quality of drinking water to humans, animals and the environment.
- to investigate the morphological and electrical properties of ZnO nps.
- to apply a synthesized Zinc Oxide nanoparticles for antibacterial activity.

1.5 Delimitation of the study

A zinc oxide nanoparticles (ZnO NPs) was used in an increasing number of industrial products such as rubber, paint, coating, and cosmetics. In the past two decades. ZnO NPs have become one of the most popular metal oxide nanoparticles in biological applications due to their excellent biocompatibility, economic, and low toxicity. ZnO NPs have emerged a promising potential in biomedicine, especially in the fields of anticancer and antibacterial fields, which are involved with their potent ability to trigger excess reactive oxygen species (ROS) production, release zinc ions, and induce cell apoptosis. These zinc oxide nanoparticles can be synthesized

by different methods such as, hybrid electrochemical thermal method, gas condensation method, direct thermal decomposition method, spray pyrolysis method and hydrothermal processes, which involves high temperatures but in this study the zinc oxide (ZnO) nanoparticles were synthesized by sol gel method for the treatment of water from bacterial infection application only because sol gel method is low processing temperature, high pure compound, uniform nanostructure and easily shapeable.

2 LITERATURE REVIEW

2.1 Historical aspects and growth of Nanoscience and nanotechnology

The term “Nano” comes from the Greek word “dwarf” which generally elaborates the particle of size roughly in the range of 1 to 100 nanometers. The American physicist and Nobel Prize laureate Richard Feynman introduce the concept of nanotechnology in 1959. During the annual meeting of the American Physical Society, Feynman presented a lecture entitled “There’s Plenty of Room at the Bottom” at the California Institute of Technology (Caltech) suggesting the possibility of manipulating things at atomic level. In this lecture, Feynman made the hypothesis “Why can’t we write the entire 24 volumes of the Encyclopedia Britannica on the head of a pin?”, and described a vision of using machines to construct smaller machines and down to the molecular level. This new idea demonstrated that Feynman’s hypotheses have been proven correct, and for these reasons, he is considered the father of modern nanotechnology. After fifteen years, Norio Taniguchi, a Japanese scientist was the first to use and define the term “nanotechnology” in 1974 as: “nanotechnology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule”(Bayda et al., 2020).He speculated on the possibility and potential of Nanosized material. The field of nanotechnology is one of the most active research areas in modern material science. Over the last few decades, the syntheses of novel metal NPs have been the subject of many applied researches due to their unique properties. “Nano” is used to indicate 1 billionth of a meter (or) 10^{-9} (Girish, 2018).

Nanotechnology can be understood as a technology of design, fabrication and applications of nanostructures and nanomaterials, as well as fundamental understanding of physical properties and phenomena of nanomaterials and nanostructures(Ahmed et al., 2015). Nanotechnology is one of the most promising technologies of the 21^{st} century. It is the ability to convert the nanoscience theory to useful applications by observing, measuring, manipulating, assembling, controlling and manufacturing matter at the nanometer scale. The National Nanotechnology Initiative (NNI) in the United States define Nanotechnology as “a science, engineering, and technology conducted at the nanoscale (1 to 100 nm), where unique phenomena enable novel

applications in a wide range of fields, from chemistry, physics and biology, to medicine, engineering and electronics"(Bayda et al., 2020).

Nanoscience is a convergence of physics, materials science and biology, which deal with manipulation of materials at atomic and molecular scales; while nanotechnology is the ability to observe measure, manipulate, assemble, control, and manufacture matter at the nanometer scale. There are some reports available, which provided the history of nanoscience and technology, but no report is available which summarize the nanoscience and technology from the beginning to that era with progressive events. Therefore, it is of the utmost requirements to summarize main events in nanoscience and technology to completely understand their development in this field(Bayda et al., 2020).

Nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from their bulk counterparts. It deals with the study of the objects and systems in which at least one of its dimensions is less than 100 nm. Nanoscience offers an exciting possibility to study a state of matter, which is intermediate between bulk and isolated atoms or molecules, as well as the effect of spatial confinement on electron behavior.

Nanotechnology is defined as the design, characterization, production and application of structures, devices and systems by controlling size and shape at the nanometer scale. At the nano-region, the boundaries between traditional disciplines such as physics, chemistry, biology, materials science and engineering disappear and a synergistic interdependence emerges(The & Nanoscience, n.d.). There are four main types of NPs which includes organic NPs, inorganic NPs, semi-conductor NPs and noble metallic NPs. Organic NPs includes carbon based NMs like fullerenes. Magnetic NPs are included in the category of inorganic NPs. Oxides of metals like zinc and titanium are the examples of semi-conductor NPs while noble metallic NPs includes silver and gold etc. The most studied type of NPs among these is the metallic NPs, the studies on which can be dated back to 19^{th} century when Michael Faraday synthesized gold colloid solution in 1850s. In 1857, Michael Faraday studied the preparation and properties of colloidal suspensions of "Ruby" gold. Their unique optical and electronic properties make them some of the most interesting nanoparticles. Faraday demonstrated how gold nanoparticles produce

different-colored solutions under certain lighting conditions(Bayda et al., 2020). Nanoscience and nanotechnologies are widely seen as having huge potential to bring benefits in areas as diverse as drug development, water decontamination, information and communication technologies, and the production of stronger, lighter materials. They are attracting rapidly increasing investments from governments and from businesses in many parts of the world(Dowling et al., 2004).

2.2 Nanoparticles/Nanomaterials

Nanotechnology is one of the great advancements in various industries that manipulate materials on the atomic or molecular scales. Resulting materials have new characteristics and functions with very small sizes; these smaller materials are referred to as nanomaterials or nanoparticles (NPs) and defined as particles that are less than 100 nm in at least one dimension(Musee et al., 2015). Nanomaterials have attracted great attention for their unique, superior, and dispensable properties that can be distinguished from conventional macroscopic materials. Their discrete property arises especially from their higher surface to volume ratios and increased percentage of atoms at the grain boundaries. They represent an important class of materials in the development of novel devices that will enable applications in many areas, such as the physical, biological, biomedical and pharmaceutical area(*Effect of zinc oxide and silver nanoparticles on intestinal*, 2013).

Many kinds of NPs, including metal NPs, oxide NPs, semiconductor NPs, and even composite NPs, have been widely used in electrochemical sensors and biosensors. Metallic NPs are very interesting, because of the fact that these particles show size-dependent characteristics of the material. Especially in the nanometer scale, these size dependent properties are observable. Metal NPs with at least one dimension approximately 1-100 nm have received considerable attention in both scientific and technological areas due to their unique and unusual physico-chemical properties compared with that of bulk materials. This means the NPs have change in color when their size differs, whereas bulk materials do not have change in color in even if their size varies.

2.3 ZnO nanoparticles

Zinc oxide nanoparticles are nanoparticles of zinc oxide (ZnO) that have diameters less than 100 nanometers. Zinc oxide is a direct gap semiconductor with a considerable fraction of ionic bonding. Many particular features of ZnO are determined by the fact that, among the elements of the sixth group, the ionization energy of oxygen is the highest, which leads to a strongest interaction between the Zn3*d*- and O2*p*-orbitals(Rodnyi & Khodyuk, 2011). They have a large surface area relative to their size and high catalytic activity. ZnO is a wide-band gap semiconductor with an energy gap of 3.37 eV at room temperature. It usually appears as a white powder and is nearly insoluble in water. The powder is widely used as an additive for numerous materials and products including plastics, ceramics, glass, cement, rubber (e.g. car tyres), lubricants, paints, ointments, adhesives, sealants, pigments, foods (source of Zn nutrient), batteries, ferrites, fire retardants, etc. ZnO is present in the Earth crust as a mineral zincite; however, most ZnO used commercially is produced synthetically. ZnO is nontoxic and is compatible with human skin making it a suitable additive for textiles and surfaces that come in contact with human body. The increase in surface area of nanoscale ZnO compared to bulk has the potential to improve the efficiency of the material function (Barnali Ashe, 2011).

Zinc oxide nanoparticles (ZnO NPs), as one of the most important metal oxide nanoparticles, are popularly employed in various fields due to their peculiar physical and chemical properties. ZnO NPs are firstly applied in the rubber industry as they can provide wear proof of the rubber composite; improve performance of high polymer in their toughness and intensity and antiaging, and other functions. Because of the strong UV absorption properties of ZnO, they are increasingly used in personal care products, such as cosmetics and sunscreen. In addition, ZnO NPs have superior antibacterial, antimicrobial, and excellent UV-blocking properties. Therefore, in the textile industry, the finished fabrics by adding ZnO NPs exhibited the attractive functions of ultraviolet and visible light resistance, antibacteria, and deodorant. Apart from the applications mentioned above, zinc oxide can also be used in other branches of industry, including concrete production, photocatalysis, electronics, electro technology industries, and so on(Jiang et al., 2018).

ZnO is a key technological material and finds use in a large gamut of applications like interconnects in nanoelectronics, piezoelectric devices, optoelectronics, chemical sensors and

AFM tips. ZnO has three key advantages. First, it is a semiconductor, with a direct wide band gap of 3.37 eV and a large excitation binding energy (60 meV). Second, because of its non-central symmetry (wurtzite structure), ZnO is highly piezoelectric, which is a key property in building electromechanically coupled sensors and transducers. Finally, ZnO is a bio-safe and biocompatible material, and can be used for biomedical applications(Desai & Haque, 2007).

It is generally known that zinc as an essential trace element extensively exists in all body tissues, including the brain, muscle, bone, skin, and so on. As the main component of various enzyme systems, zinc takes part in body's metabolism and plays crucial roles in proteins and nucleic acid synthesis, hematopoiesis, and neurogenesis. Nano-ZnO, with small particle size, makes zinc more easily to be absorbed by the body. Thus, nano-ZnO is commonly used as a food additive. Moreover, ZnO is graded as a "GRAS" (generally recognized as safe) substance by the US Food and Drug Administration (FDA). With these properties, ZnO NPs have received more attention in biomedical applications. Compared with other metal oxide NPs, ZnO NPs with the comparatively inexpensive and relatively less toxic property exhibit excellent biomedical applications, such as anticancer, drug delivery, antibacterial, and diabetes treatment; anti-inflammation; wound healing; and bioimaging(Jiang et al., 2018).

Zinc oxide (ZnO) is an n-type semiconductor material with promising catalytic, electronic, and optical properties. In the abovementioned fields, it possesses great potential because of its high electron mobility, high thermal conductivity, wide band gap (3.37 eV), and large exciton-binding energy (60 meV) and exhibits UV absorption in the range 200–350 nm and emission in the near UV and visible range from 500 to 600 nm and piezoelectricity. In addition, mono crystalline ZnO structures show a superior fatigue resistance, high isoelectric point and charge transfer properties, biocompatibility, and low impact on the environment at the end of life cycle (Valentina et al., 2014).

ZnO nanomaterials are easy to fabricate, and raw materials and precursors are easily available. The combination of semiconducting and piezoelectric properties has been addressed as the 'piezotronic effect, whereas the combination of optical and piezoelectric properties gives rise to the so-called piezo-phototronic effect. Recently, ZnO nanostructures have been widely studied as gas and mechanical strain sensors, thanks to the high electromechanical coupling, as well as

energy nano generators responding to mechanical deformation. A reasonable power density has been generated from ZnO-based piezoelectric harvesting devices (Valentina et al., 2014).

2.4 Crystal structure and properties of ZnO nanoparticle

2.4.1 Crystal structure

ZnO is an amphoteric oxide which is either a covalent or an ionic semiconductor(Nalumaga, 2017). Zinc oxide crystallizes in three forms as shown in figure 1: hexagonal wurtzite, cubic zinc blende, and the rarely observed cubic rocksalt(M. Science & Panwar, 2009). In these structures, each anion is surrounded by four cations in tetrahedral coordination, and vice-versa having sp3 covalent bonding. These compounds also have ionic nature which tends to enhance the band gap beyond the limit of covalent bonding. However, the large difference of the electro negativity of the two constituents of ZnO (Oxygen = 3.44 and Zinc = 1.65) is responsible for the strong ionic bonding between them. The ZnO is a wide direct band gap semiconductor whose ionicity lies at the boundary of ionic and covalent semiconductors(*Chapter-1 Introduction*, n.d.).

The wurtzite structure is most stable and thus most common at ambient conditions. The zincblende form can be stabilized by growing ZnO on substrates with cubic lattice structure. In both cases, the zinc and oxide are tetrahedral. The rocksalt NaCl-type structure is only observed at relatively high pressures ~10 GPa. During this phase transition, there is reduction in volume by 17% .The hexagonal and zincblende ZnO lattices have no inversion symmetry (reflection of a crystal relatively any given point does not transform it into itself). This and other lattice symmetry properties result in piezoelectricity of the hexagonal and zincblende ZnO, and in pyroelectricity of hexagonal ZnO. The wurtzite structure has hexagonal unit cell arrangement in 3 dimension with two lattice parameters *a* and *c* in the ratio of *c/ a* = $\sqrt{8}/3$ =1.633, with a density equal to 5.605 g cm-3 (for ideal wurtzite structure).

The wurtzite structure deviates from the ideal arrangement in a real ZnO crystal by changing the c/a ratio. The lattice parameter ratio deviation from ideal one may be due to free charge, impurities, stress and temperature. Since the c/a ratio also correlates with the difference of the

electronegativities of the two constituents, and also components with the greatest differences show largest departure from the ideal c/a ratio.

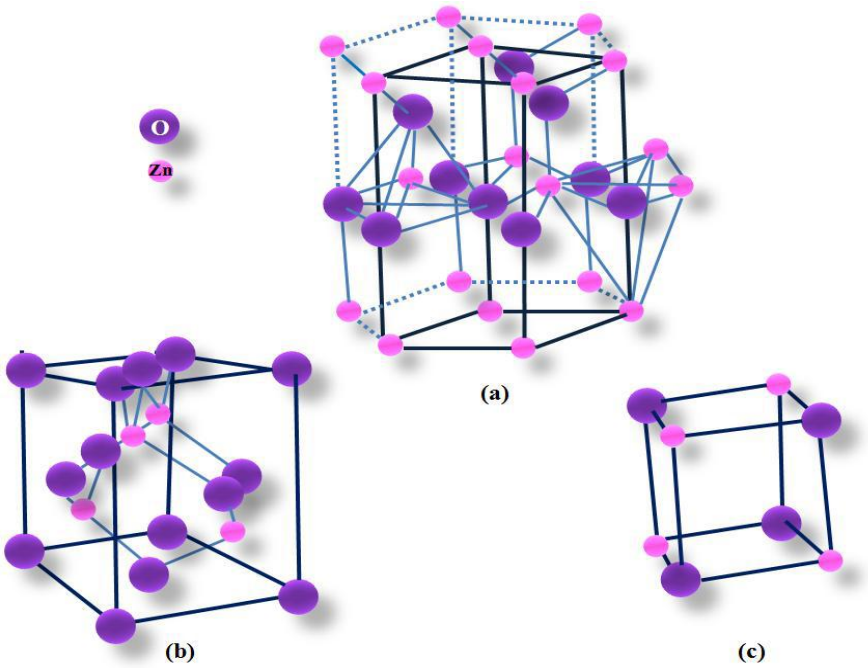


Figure 1: Crystal structure of ZnO (a) wurtzite (b) zinc blende and (c) rocksalt.

2.4.2 Electrical Properties

It is very much essential to realize the electrical properties of ZnO nanostructures for different applications especially in electronics. But it is difficult to measure the electrical properties of ZnO that is why lot of variation in the reported results has been found(Hussain, n.d.). The fundamental study of the electrical properties of ZnO nanostructures is crucial for developing their future applications in Nano electronics. ZnO has a quite large band gap of 3.3 eV at room temperature. The advantages of a large band gap include higher values of breakdown voltages, sustaining large electric fields, high-temperature and high-power operations. ZnO has n-type character, in the absence of doping. Non stoichiometry is usually the origin of n-type character. Due to defects such as oxygen vacancies and zinc interstitials, ZnO nanowires are reportedly show n-type semiconductor behavior(Paul, n.d.).

2.4.3 Chemical Properties

ZnO has the following chemical properties(Paul, n.d.):

- ZnO occurs as the mineral zincite or as white powder known as zinc white. It is usually orange or red in color due to manganese impurity.
- Crystalline zinc oxide is thermochromic, which changes from white to yellow colour when heated and reverting to white colour on cooling. This change in colour is caused by a very small loss of oxygen at high temperatures.
- Zinc oxide is amphoteric, that is it reacts with both acids and alkalis. With acid it reacts to form familiar compound such as zinc sulfate. With alkali it forms zincates.

$$ZnO + 2HCl \longrightarrow ZnCl_2 + H_2O$$

$$ZnO + 2NaOH + H_2O \longrightarrow Na_2 (Zn (OH)_4)$$

- ZnO decomposes to form zinc vapor and oxygen at about 1975 Å ◦C, indicating its considerable stability. Heating with carbon converts ZnO into Zn, which is more volatile(Paul, n.d.).

$$ZnO + C \longrightarrow Zn + CO$$

2.4.4 Piezoelectric properties

Piezoelectricity is the capability of certain materials to generate an alternating current (AC) voltage when subjected to mechanical stress or vibration, or to vibrate when subjected to an AC voltage, or both. Piezoelectric materials are broadly into two category (i) natural and (ii) man-made materials. Natural piezoelectric materials are Berlinite (structurally identical to quartz), cane sugar, quartz, Rochelle salt, topaz, tourmaline, sodium potassium tartarate and bone. Man-made piezoelectric materials are Ceramics, sintered form of finely ground powdered mixture made of ferroelectrics of oxygen-octahedral types are lead zirconate titanate [$Pb(Zr, Ti)O_3$], Lead titanate ($PbTiO_3$), Barium titanate ($BaTiO_3$), Zinc oxide (ZnO) and polymer is polyvinylidene fluoride (PVDF)(Ramar & Balasubramanian, 2018).

ZnO is a promising piezoelectric material, because it shows an effective accumulation of charges when mechanical stress is applied on it. Since ZnO has both semiconducting and piezoelectric properties, therefore it has enormous potential in energy harvesting applications. The polarity present in the ZnO crystal is because of its tetrahedral structure in which oppositely charged ions produce positively charged zinc and negatively charged oxygen polar surfaces, that creates conventional dipole moment and spontaneous polarization along the c-axis. Therefore one can say that piezoelectricity initiates from the polarization of the tetrahedrally coordinated unit. In principle the piezoelectric effect changes an applied mechanical stress into an electrical voltage or vice versa and it is not similar to ferroelectricity. There are two possible ways to determine piezoelectric properties. One is called direct piezoelectric effect and the other is called converse or indirect piezoelectric effect. In the direct piezoelectric effect force is applied on the material in order to create strain in it, which generates charges on the material's surface due to electrical polarization that results output voltage signal. Vice versa in converse piezoelectric effect an external voltage is applied in order to generate strain in the material. Polarity in ZnO is also critical because it has substantial influenced on different properties like growth direction, etching, piezoelectricity and defect generation. Moreover due to the absence of center of symmetry in the wurtzite crystal structure of ZnO, it exhibits pyroelectric behavior as well besides being piezoelectric(Hussain, n.d.).

2.4.5 Optical properties

Optical characterization is made by the diffuse reflectance (R) measurements(Dakhsi et al., 2011).Optical properties and processes in ZnO as well as its refractive index were extensively studied many decades ago. The renewed interest in ZnO is fuelled and fanned by its prospects in optoelectronics applications owing to its direct wide band gap of 3.37 eV at room temperature with large exciton energy of 60 meV and efficient radiative recombination. The strong exciton binding energy, which is much larger than that of GaN (25 meV), and the thermal energy at room temperature (25 meV) can ensure an efficient exciton emission at room temperature under low excitation energy. As a consequence, ZnO is recognized as a promising photonic material in the blue UV region (Tang et al., 2014).

2.4.6 Mechanical properties

ZnO is a relatively very soft material with approximate hardness just 4.5. Its elastic constants are relatively smaller than those of other III-V semiconductors, e.g. GaN. The high heat capacity and high heat conductivity, low values of thermal expansion and high melting points are some of the characteristics of ZnO. ZnO has been proposed to be a more promising UV emitting phosphor than GaN because of its larger exciton binding energy (60 meV). Among the semiconductors bonded tetrahedrally, it's found that ZnO has the highest piezoelectric tensor(Paul, n.d.).

Since ZnO has been extensively used in developing piezoelectric nanodevices, therefore it is very important to have good understanding of mechanical stability and reliability of ZnO nanostructures. Because if a piezoelectric device is fabricated and it is not working properly, then it is hard to understand that whether it is due to the failure of the grown nanostructures or the failure of something else. So in such a case the mechanical characterization of nanostructures plays an important role. These include parameters like hardness, stiffness, toughness, yield strength, piezoelectric constant; Young's and bulk module, and adhesion to the substrate. It is important to point out that if the diameter and length of the nanostructure varies then it also affects the mechanical properties. Therefore in order to have efficient and reliable piezoelectric devices, the characterization of its mechanical properties is the backbone. That is why mechanical properties of different materials have been extensively studied(Hussain, n.d.).

2.5 Synthesis methods of zinc oxide nanoparticles

Nanoparticles are known as the fundamental building blocks of nanotechnology, a study dedicated to materials sized from 1 to 100 nm. As a result, the synthesis of many nanoparticles has become an important component in nanoscale science and engineering. Amongst them, zinc oxide (ZnO) nanoparticles are of great interest due to several favorable properties including good transparency, high electron mobility, wide band gap, and room- temperature luminescence. A wide range of nanoparticles in the form of colloids, clusters, powders, tubes, rods, wires, thin films, etc. can be prepared by a variety of methods (Nethavhanani, 2017).

There are two general types of strategies used in synthesis of nanomaterials–the bottom-up and top-down approach as shown in figure 2. The first one uses atoms and molecules to create nanostructures which can obtain by chemical synthesis, biological methods or controlled deposition and growth. Conversely, the top-down approach refers to slicing or cutting of bulk material to get nano sized particles. Both methods are used to prepare nanostructures on a large, industrial scale. It is obvious that each has its advantages and disadvantages: for example top-down techniques can cause serious damage to the crystal structure of nanomaterials, which directly affects their physicochemical properties. In addition to knowing the synthesis methods, an important issue as well as a challenge is finding a way to control the shape and size of nanostructures. These parameters influence the optical properties of NPs, so it is important to select the most appropriate synthesis method(Król et al., 2017).

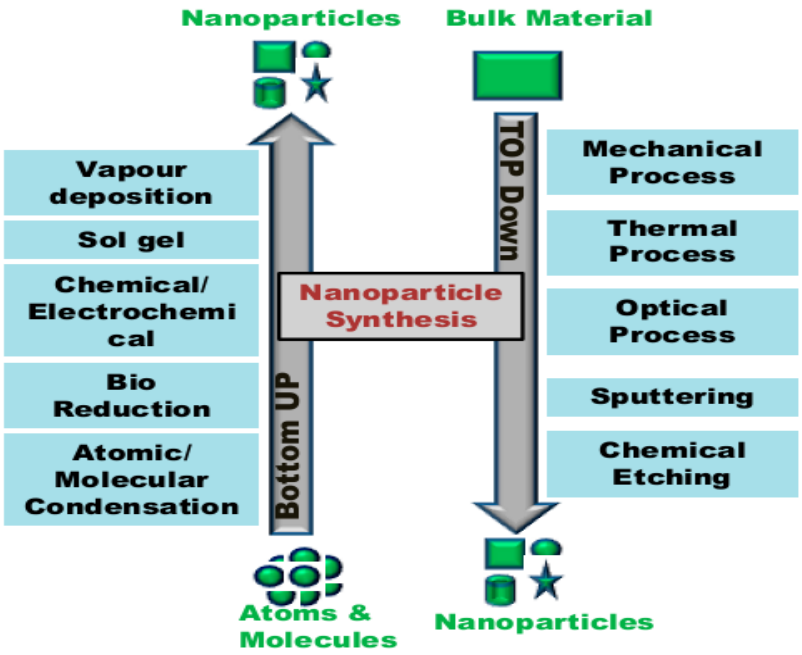


Figure 2: The concept of top down and bottom up technology: different methods for nanoparticle synthesis(Bayda et al., 2020).

Zinc oxide has been a focus of attention in a broad range of research fields, and therefore a growing number of synthesis methods have been developed. Metallic nanoparticles should have a specified size, and their shape and aggregation level should be as small as possible. Since the synthesis route determines the latter properties of the material, of the preparation method is a

very important issue in the design of ZnO NPs for a specific application. In the past several years, various approaches, including hydrothermal method, solvent-based ultrasonic irradiation, micro emulsion, sol-gel synthesis or green methods such as plant extracts/bacteria strains have been used to prepare zinc oxide nanocomposites with different morphologies. All of them can be divided into three main groups: physical, chemical and biological methods(Król et al., 2017).

2.5.1 Physical methods

Among the physical synthesis of zinc nanomaterials, we can distinguish such methods as physical vapor deposition, arc plasma method, thermal evaporation, ultrasonic irradiation or laser ablation. Physical vapor deposition (PVD) has been used to produce ZnO nanowires networks with a very good repeatability. This process consists of the evaporation of the precursor (metal oxide), the subsequent transport and the final condensation onto the substrate. The growth is catalyzed by metal nanoparticles. It is a relatively simple process and the deposition is controlled by temperature, pressure and carrier gas flow(Jimenez-Cadena et al., 2010). ZnO NPs can be synthesized by laser ablation of zinc metal (bulk material) in a solution this method was have several benefits such as technical simplicity and chemical pureness of the yield. The mechanism of laser ablation depends on physical properties of metals and environment medium. Therefore, the ablation of metals is an intricate subject .Ablation of metal target commences with the sorption of laser beam energy. When the laser beam interacts with the metal target, the heat can generate and the photoionization of the metal target can occur. After that, metal nanoparticles will be released from the metal plate as the different phase that depends on the absorbed energy and plasma plume expands(Reza Sadrolhosseini et al., 2019). The synthesis efficiency and the characteristics of produced nano zinc particles depend upon many process parameters, including ablation time, fluence and wavelength of the laser. The laser ablation conditions may lead to narrow distribution of size, shape and physical parameters of nanoparticles.

Another physical method is thermal evaporation. It is a process in which condensed or powdered source material is vaporized at elevating temperature and then the resultant vapor phase condenses under certain conditions such as temperature, pressure, atmosphere or substrate to form the desired product. Thermal evaporation is realized by thermal heating (usually resistive) of an evaporated material placed in an evaporation source. It is carried out in a vacuum at pressure $P < 10^{-5}$ torr. The thermal evaporation is the gentlest PVD method with evaporated

particle energies ~1500 K (0.12 eV). This method is the simplest PVD method and needs comparatively low power consumption. They reviewed various growth morphologies such as nanorods, nanobelts, nanocombs and nanorings. The thermal evaporation method used there was found to have some advantages - it is simple, low cost, and catalyst-free. Besides, it can be used for fabrication of ZnO nanotubes on an industrial scale for photocatalytic degradation, because of their high photocatalytic activity.

2.5.2 Biological methods

Nanoparticles have been produced with physical and chemical methods for a long time, but recently the development of green synthesis seems to be an approach generating much interest synthesis biological systems is a promising alternative and provides many advantages in comparison to traditional chemical methods. This type of nanoparticle preparation is more eco-friendly, allows the researchers to control the synthesis of NP size and shape and does not use toxic and expensive organic solvents. Biological methods require considering several process parameters such as selection of the organisms that are the most suitable (with regard to their enzyme activities and biochemical pathways) and optimal conditions for cell growth or enzyme activity (medium, temperature, pH, and buffer). Optimization of these factors makes it possible to control the morphology of ZnO nanoparticle. Among biological methods, we can distinguish synthesis with bacteria, yeast, fungi and plant extracts. In the case of biosynthesis with plants, it is supposed that they are able to reduce metal ions by flavonoids, terpenoids and polysaccharides and through reduction, such plants can produce nanoparticles. Furthermore, some plants are known for their heavy metal accumulation and the ability to detoxify such substances. There are many mechanisms of metal tolerance in plants including cell wall binding, chelation of metals and active transport of metal ions into the vacuole. This property has attracted the attention of researchers and can be used in biosynthesis of metal and metal oxide nanoparticle - it is possible to use Zn hyper accumulating plants as a source of precursor to form zinc oxide NPs(Król et al., 2017).

2.5.3 Chemical methods

Chemical methods are classified according to the physical state, i.e. solid phase, liquid phase (so-called wet chemical methods) or vapor phase applied for the synthesis of zinc nanocomposites.

The wet chemical methods are the ones most commonly used, and they can be a valuable alternative to gas phase synthesis, with known commercial applications. Water-based chemical methods offer numerous advantages such as being environmentally friendly, using cheap and easy to handle reagents as well as uncomplicated equipment, and they simultaneously require only low energy input. Moreover, they make the easy tailoring of synthesis parameters throughout the whole process possible, which is helpful in gaining control of the composition, shape and size of the resulting nanomaterials. Among the wet-chemical methods we can distinguish micro emulsion, sol-gel synthesis, precipitation, hydrothermal and solvothermal method(Król et al., 2017).

2.5.3.1 Sol-gel method

The sol-gel technique is one of the attractive and versatile wet chemical methods. The sol-gel process involves the transition of a solution system from a liquid "sol" (mostly colloidal) into a solid "gel" phase(Sajjadi, 2005). The sol-gel approach appears to be one of the most promising methods to prepare ZnO nanoparticles. Sol-gel method involves the formation of solid particles in a liquid called sol followed by the crosslinking of the ‘sol’ to form a continuous network of particles (enclosed with liquid) called a gel. The most commonly used precursors are the metal alkoxides, which are compounds whereby a metal is bonded to one or more alkyl groups through an intermediate oxygen atom. Other precursors that serve as sol forming constituents include metal organic compounds, salts of inorganic acids and salts of organic acids. The importance of utilizing sol-gel technique is that this method produces best thermal stability ratio, best solution resistance, higher mechanical stability and the probability to the transform simulation(Azeez et al., 2020).

A typical sol-gel process, as the one shown in Figure 3, generally involves different stages, namely, hydrolysis of the precursor/s, condensation and polymerization process that establishes metal–OH–metal or metal–O–metal bridges between metallic atoms of the precursor materials, growth of particles, and agglomeration of particles. These stages are followed by the formation of networks that extend throughout the liquid medium, resulting in the formation and congealing of the gel. A number of ZnO nanoparticles, nanorods or nanotubes have been synthesized using the sol gel method. For the Growth of ZnO with high crystallinity, a sufficient amount of OH^- ions are necessary(KHAN et al., 2013). The synthesizing ZnO nanoparticles via the sol gel

method using zinc acetate dihydrate (Zn $(CH_3COO)_2.2H_2O$) as a precursor, ethanol (CH_2COOH) as solvent, and sodium hydroxide (NaOH) and distilled water (H_2O) as solvent medium were reported(Nethavhanani, 2017).

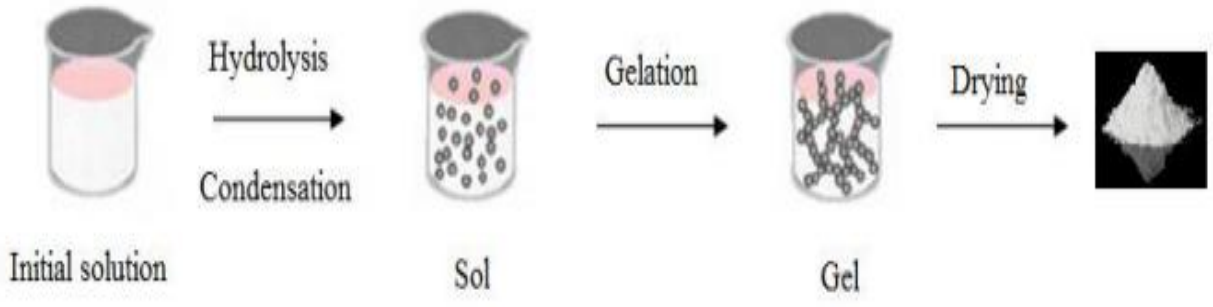


Figure 3 : The schematic diagram of a sol gel process for nanoparticles (Nethavhanani, 2017).

The advantages of the sol-gel method (Nethavhanani, 2017):

I. Sol gel formation is a relatively low temperature process.
II. Uses less energy consumption thus less pollution too.
III. Sintering at low temperature possible.
IV. Allows the fine control of the product's chemical composition.
V. Can create very fine powders of high purity

2.6 Characterization techniques of ZnO nanoparticles

In order to apply ZnO NPs for any of applications characterization should be hold after synthesis of the material. Several physical techniques have been developed to characterize the size, morphology, crystalline structure, temperature effects and other remarkable properties of ZnO NPs. The most commonly used characterization techniques are X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), UV-Visible Spectroscopy, Fluorescence Spectroscopy and FT-IR Spectroscopy. The detail information about these techniques is explained as follows.

2.6.1 X-ray diffraction (XRD)

The crystalline structure of nanoparticles can be confirmed by using X-ray diffraction analysis. The lattice parameters and crystalline grain size are predicted by using the Scherrer equation(Thakral et al., 2021). From the XRD pattern analysis, we determined peak intensity, position and full-width at half maximum (FWHM) data(Singh, 2020). X-rays diffraction measurement has been performed with a diffractometer (X´ pert PANalytical) equipped with Cu KαI (λ=1.5406 nm)(Zaman, n.d.). X-ray is an energetic electromagnetic radiation appearing at the region between gamma-rays and ultraviolet, which is comparable with the size of an atom. When X-rays interact with a crystalline substance (phase), one gets a diffraction pattern. The reflection of incident monochromatic X-ray from successive planes of crystal lattices when the difference between the planes of complete number n of wavelengths leads to famous Bragg's law. In 1912, W. L. Bragg recognized a predictable relationship among several factors(M. O. F. Science et al., n.d.).

1. The distance between similar atomic planes in a mineral (the interatomic spacing) which we call the d-spacing and measure in angstroms.
2. The angle of diffraction which we call the theta angle and measure in degrees. For practical reasons the diffractometer measures an angle twice that of the theta angle. Not surprisingly, we call the measured angle '2-theta'.
3. The wavelength of the incident X-radiation, symbolized by the Greek letter lambda and, in our case, equal to 1.54 angstroms.

The lattice spacing is calculated from Bragg's formula equation below:

$$n\lambda = 2dsin\theta \qquad 2.1$$

Where, d is lattice spacing and θ is angle of incidence, λ is wavelength and n is diffraction order.

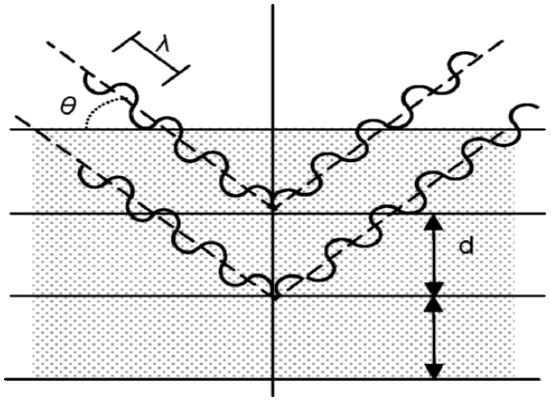


Figure 4 : Condition for constructive interference(Spectroscopy, n.d.).

In a typical XRD pattern, the diffracted intensities are plotted versus the detector angle 2θ. Each peak is then assigned a label indicating the spacing of a crystal plane. Bragg's law states the condition for sharp diffraction peaks arising from crystals which are perfectly ordered. Actual diffraction peaks have a finite width resulting from imperfections, either the irradiation source or the sample. A useful phenomenon is that as crystallite dimensions enter the nanoscale the peaks broaden with decreasing crystal size. It is known that the widths of the diffraction peaks allow the determination of crystallite size(Spectroscopy, n.d.).

Furthermore, the average crystallite size D is estimated according to the Debye–Scherer formula(Vijayaprasath, Murugan, Hayakawa, et al., 2016).

$$D = \frac{k\lambda}{\beta \cos\theta} \qquad 2.2$$

Where D is the crystal size, λ is the wavelength of the x-ray radiation (λ= 0.15406nm) for CuK, K refers to Scherrer's constant usually taken as 0.89, β is full width at half maximum (FWHM) of diffraction Peak and θ refers to Bragg diffraction angle.

The lattice constants 'a' and 'c' and the spacing 'd_{hkl}' for wurtzite structure of ZnO have been calculated according to equation (3)(Belay, 2017b).

$$a = \sqrt{\frac{1}{3}} \frac{\lambda}{\sin \theta_{100}} \quad 2.3$$

$$c = \frac{\lambda}{\sin \theta_{002}} \quad 2.4$$

Where, $\lambda = 1.54\text{Å}$ is the wavelength of the X-ray radiation used, d is the interplanar distance, θ_{100} and θ_{002} are the angles of the diffraction peaks (1 0 0) and (0 0 2) respectively.

The bond lengths are calculated using the relation below(Vijayaprasath, Murugan, Asaithambi, et al., 2016)

$$L = \sqrt{\frac{a^2}{3} + \left(\frac{1}{2} - u\right)^2 c^2} \quad 2.5$$

Where,a and c are the lattice parameters and u is a positional parameter. The positional parameter u can be calculated by the relation,

$$u = \frac{a^2}{3c^2} + 0.25 \quad 2.6$$

The volume (V) of the unit cell for hexagonal system is calculated from the equation

$$V = 0.866 \times a^2 \times c \quad 2.7$$

Dislocation density(δ), which represents a number of defect states present in the sample. Mathematically, dislocations are a type of topological defect and are determined using Equation(8)(Raj et al., 2016), where D is the particle size

$$\delta = \frac{1}{D^2} \quad 2.8$$

Lattice strain is a measure of the distribution of lattice constants arising from crystal imperfections, such as lattice dislocation. There are other sources of strain, which are the grain boundary triple junction, coherency stresses, contact or sinter stresses, stacking faults, etc(Bindu & Thomas, 2014). The strain-induced broadening in powders due to crystal imperfection and distortion was calculated using the formula,

$$\varepsilon = \frac{\beta_{hkl}}{4\tan\theta} \quad 2.9$$

The total broadening of the diffraction peak is due to the sample and the instrument(Al-saadi et al., 2014). The total peak broadening is represented by the sum of the contributions of crystallite size and strain present in the material and can be written as

$$\beta_{hkl} = \frac{k\lambda}{D\cos\theta} + 4\varepsilon\tan\theta \quad 2.10$$

2.6.2 UV-Visible Spectroscopy

UV–vis spectroscopy was used to characterize the optical absorption properties of ZnO(Kumar et al., 2013). Ultraviolet-visible (UV-vis) spectroscopy is widely utilized to quantitatively characterize organic and inorganic nanosized molecules. A sample is irradiated with electromagnetic waves in the ultraviolet and visible ranges and the absorbed light is analyzed through the resulting spectrum(Spectroscopy, n.d.). It is relatively facile and low-cost characterization method that is often used for the study of nanoscale materials. It measures the intensity of light reflected from a sample and compares it to the intensity of light reflected from a reference material. NPs have optical properties that are sensitive to size, shape, concentration, agglomeration state and refractive index near the NP surface, which makes UV-Vis spectroscopy an important tool to identify, characterize and investigate these materials, and evaluate the stability of NP colloidal solutions.

Different sized materials can be characterized, ranging from transition metal ions and small molecular weight organic molecules, whose diameters can be several Ångstroms, to polymers, supramolecular assemblies, nano-particles and bulk materials. Size dependant properties can also be observed in a UV-visible spectrum, particularly in the nano and atomic scales. These include peak broadening and shifts in the absorption wavelength. Many electronic properties, such as the band gap of a material, can also be determined by this technique. The band gap energy (E_g) of ZnO NPs was also calculated using equation (11)(Zinc & Nanoparticles, 2020).

$$E_g = \frac{(hc)}{\lambda} = \frac{1240}{\lambda} eV \qquad 2.11$$

Where h is the Planck's constant $6.626 \times 10^{-34} Js$, c is the light velocity $3 \times 10^8 \frac{m}{s}$ and λ is the peak wavelength (nm)

The band energy of the ZnO nps can be also estimated by the following relation(Bindu & Thomasa, 2017).

$$ahv = \beta\left(hv - E_g\right)^n \qquad 2.12$$

where β is a constant and $E_g$is the optical band gap of the material, α absorption coefficient, n is a number which characterizes the nature of electronic transition between valance band and conduction band, which may have values 1/2, 2, 3/2, and 3 corresponding to the allowed direct, allowed indirect, forbidden direct, and forbidden indirect transitions, respectively. It is well known that direct transition across the band gap is feasible between the valence and the conduction band edges in "k space". In the transition process, the total energy and momentum of the electron– photon system must be conserved. It is known that ZnO is a direct band gap semiconductor, then from the above equation, it is clear that plot of $(\alpha h\nu)^2$ vs. hν was indicate a divergence at an energy value E_g, where the transition takes place. The band gap value depends on the nature of the transition (i.e., the n value) determined.

2.6.3 Scanning Electron Microscope

The scanning electron microscope (SEM) is one of the most versatile instruments available for the examination and analysis of the microstructure morphology and chemical composition characterizations(Zhou et al., 2007).The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample.

In most applications, data are collected over a selected area of the surface of the sample, and a 2-dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques. Accelerated electrons in an SEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons (that produce SEM images), backscattered electrons (BSE), diffracted backscattered electrons (EBSD that are used to determine crystal structures and orientations of minerals), photons (characteristic X-rays that are used for elemental analysis and continuum X-rays), visible light (Cathodoluminescence), and heat.

Secondary electrons and backscattered electrons are commonly used for imaging samples: secondary electrons are most valuable for showing morphology and topography on samples and backscattered electrons are most valuable for illustrating contrasts in composition in multiphase samples (i.e. for rapid phase discrimination SEM analysis is considered to be "non-destructive"; that is, x-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyze the same materials repeatedly.

The SEM is routinely used to generate high-resolution images of shapes of objects (SEI) and to show spatial variations in chemical compositions: 1) acquiring elemental maps or spot chemical analyses using EDS, 2) discrimination of phases based on mean atomic number (commonly related to relative density) using BSE, and 3) compositional maps based on differences in trace element "activators" (typically transition metal and Rare Earth elements) using CL.

The SEM is also widely used to identify phases based on qualitative chemical analysis and/or crystalline structure. Precise measurement of very small features and objects down to 50 nm in size is also accomplished using the SEM(*Synthesis and characterization of zinc oxide nanoparticles by sol-gel process Synthesis and Characterization of Zinc Oxide Nanoparticles by Sol-Gel Process*, 2012). The scanning electron microscope works as same principle as an optical microscope, but it measures the electrons scattered from the sample rather than photon. In scanning electron microscopy (SEM) an electron beam is focused into a small probe and is rastered across the surface of a specimen.

Electron microscope works on the basic ideas of optical microscope, but uses electrons instead of light. "Lens" used here are not made of optical materials (glass), but electrical field. In scanning electron microscopy (SEM) an electron beam is focused into a small probe and is rastered across the surface of a specimen. Several interactions with the sample result in the emission of electrons or photons which occur as the electrons penetrate the surface. The emitted electrons or photons can be collected with an appropriate detector to yield valuable information about the material. It is a best suited method to for observing the shape of the sample. The beam diameter decides the resolution of the resulting image.

2.6.4 Photoluminescence (PL)

PL spectroscopy concerns monitoring the light emitted from atoms or molecules after they have absorbed photons. PL spectroscopy is suitable for the characterization of both organic and inorganic materials of virtually any size, and the samples can be in solid, liquid, or gaseous forms. Electromagnetic radiation in the UV and visible ranges is utilized in PL spectroscopy. The sample's PL emission properties are characterized by four parameters: intensity, emission wavelength, bandwidth of the emission peak, and the emission stability. The PL properties of a material can change in different ambient environments, or in the presence of other molecules. Many nanotechnology-enabled sensors are based on monitoring such changes. Furthermore, as dimensions are reduced to the nanoscale, PL emission properties can change, in particular a size dependent shift in the emission wavelength can be observed. Additionally, because the released photon corresponds to the energy difference between the states PL spectroscopy can be utilized

to study material properties such as band gap, recombination mechanisms, and impurity levels(Spectroscopy, n.d.).

Photoluminescence properties of zinc oxide and nano-substance were considered by(Article, 2020).Zinc oxide gives the photoluminescence emission from the UV and visible ranges depending upon preparation shape, routes, deep level, size and surface defects. Zinc oxide permits the organization of photoluminescence properties to create as white light. Furthermore, the efficient transfer of energy in zinc oxide to the carbon nanotubes makes it suitable not just for energy gathering applications, but also as photo-detectors, biosensors and thermal imaging at low temperatures. Furthermore, it was shown that zinc oxide NP embodiment from (metal oxide matrix) creates changes in the (PL) response causing the inactivation of (surface defects)(Hamad & Azeez, 2020).

2.6.5 Fourier Transform Infra-Red (FTIR)

Each chemical bond, in solids or molecules, has specific (quantized) vibrational modes. These modes, acting as oscillators, can absorb photons of energy corresponding to the oscillator transition. The absorption spectrum is thus “a picture” of the chemical bonds present in the sample, provided that they are IR active, i.e. having a dipole moment, capable of absorbing the electromagnetic radiation(Yao, 2015).

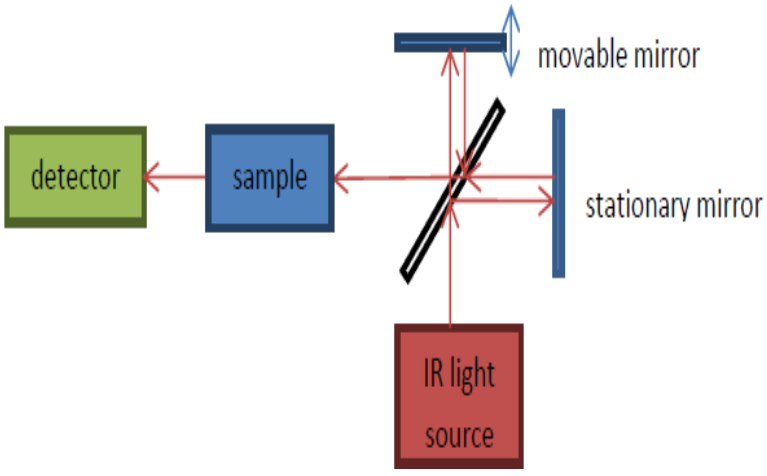


Figure 5: Schematic diagram of the operational principle of the Michelson interferometer.

IR absorption spectroscopy can be performed using either a monochromatic source or a broad spectrum IR source. Before the incident light arrives at the sample, it is divided by a beam splitter into two light beams and reflected by a stationary and a movable mirror, respectively. The two reflected light beams then recombine and undergo interference which is normally called the Michelson interferometer. The recombined light illuminates the studied samples, and the transmitted or deflected light is detected. The transmittance spectrum can be obtained from the collected signal and the interferogram is treated by the Fourier transform. The absorption spectrum can also be converted from the transmittance spectrum using programs integrated in the FTIR instrument.

Fourier transform infrared spectroscopy (FTIR) is a technique based on the measurement of the absorption of electromagnetic radiation with wavelengths within the mid-infrared region (4000–400 cm^{-1})(Mourdikoudis & Pallares, 2018). FTIR and Raman spectroscopes are used for vibrational information of nanoparticles. They provide information on the fingerprint regions of chemical bonds in molecules(Mustapha et al., 2020).

2.7 Application of ZnO nanoparticles

ZnO has different chemical and physical properties. It can be used in numerous fields. Zinc oxide is important in a wide range of applications, from medicine to agriculture, from paints to chemicals and from tires to ceramics (Azeez et al., 2020). ZnO NPs have attracted intensive research efforts for their unique properties and versatile applications in transparent electronics, ultraviolet (UV) light emitters, piezoelectric devices, chemical sensors, and spin electronics. ZnO is nontoxic; it can be used as photocatalytic degradation materials of environmental pollutants. Bulk and thin films of ZnO have demonstrated high sensitivity for many toxic gases. ZnO is currently listed as a “generally recognized as safe (GRAS)” material by the Food and Drug Administration and also used as food additive. ZnO nanostructures exhibit high catalytic efficiency, as well as strong adsorption ability, and are more frequently used in the manufacture of sunscreens. ZnO nanoparticles of size less than 100nm have high efficiency in scattering light and induce cosmetically desired whitening to the skin. It has also been used in foot care, ointments, and over-the-counter topical products(Naveed Ul Haq et al., 2017).

Most preferentially, among different metal oxide nanoparticles, zinc oxide (ZnO) nanoparticles have their own importance due to their vast area of applications, for example, gas sensor, biosensor, cosmetics, storage, optical devices, window materials for displays, solar cells, and drug-delivery (Sabir et al., 2014). ZnO is also a biocompatible material having high Isoelectric Point (IEP) of about 9.5 which makes it suitable for absorption of proteins with low IEPs where the protein immobilization is primarily driven by electrostatic interaction(Senthilkumar et al., 2021). The ZnO-NPs were tested as an additive for modification of resins used for wood and wood-based composites surface treatment with the aim to achieve antimicrobial properties of their surfaces and to enhance their protection against weathering. It shows excellent antibacterial activity of such treated wood surfaces, especially against the Gram-negative bacteria (*E. coli*), and also significant improvement of their mechanical resistance. (BUŞILĂ, 2018).

2.7.1 Application of zinc oxide nanoparticles for antibacterial treatment

Most of the bacteria and pathogenic fungi are harmful for environment, agriculture, and living organisms. The antibacterial character of ZnO NPs against pathogenic fungi and bacteria is due to change in the cell permeability when the plasma membrane of bacterial cell comes in contact with ZnO NPs. This is due to the reason that ZnO NPs move to the cytoplasm and affect the normal functioning of cell resulting in the formation of zone of inhibition against the microbes(R. Sharma et al., 2020).

Further, ZnO NPs damage the cell membrane which results in the death of bacteria. This can be explained by the mechanism that oxygen species are released on the surface of NPs that react with hydrogen to produce hydrogen peroxide. The generated hydrogen peroxide either stops the growth of bacteria or kills the bacteria. The bacterial cell membrane disruption takes place by ZnO NPs, due to formation of superoxide and hydroxyl radicals. The zone of inhibition is directly proportional to the antibacterial activity of NPs, but inversely proportional to the size of ZnO NPs. Hence, as the size of NPs decreases, higher is the zone of inhibition and greater is the antibacterial action. The formation of hydrogen peroxide is related to the size and surface area of synthesized NPs. Smaller the ZnO NPs and larger the surface zone per unit area, greater is the formation of oxygen species and higher is the formation of hydrogen peroxide. The antibacterial

activity has also been found to depend upon the shape of nanoparticles, type of synthesis and concentration of thee ZnO NPs.

Generally the size of the nanoparticles may have a greater impact on their activity, probably because of a greater accumulation of the nanoparticles inside the cell membrane and cytoplasm(Jones et al., 2008).Also by increasing ZnO NP concentrations in discs, the zone of growth inhibition has also been significantly improved due to the effective diffusion of ZnO nanoparticles in the nutrient broth medium(Rajeswari, 2020).

There various mechanisms for the antimicrobial activity of ZnO nanoparticles (Fig. 6). These are as follows:

2.7.1.1 Reactive Oxygen Species (ROS)

The antimicrobial activity of the ZnO nanoparticles involves the release of oxygen species from the surface of ZnO which cause fatal damage to microorganisms. Several studies indicated ROS formation as the main mechanism responsible for ZnO-NPs antibacterial activity(Sirelkhatim et al., 2015). ROS are known to cause oxidative stress by damaging DNA, cell membranes and cellular proteins. The rupture of the cell wall is due to the surface activity of ZnO which causes the decomposition of the cell wall and subsequently the cell membrane, the leakage of cell contents, and eventually cell death. The mechanism was presented by Padmavathy and Vijayaraghavan as follows (Dimapilis et al., 2018):

ZnO is activated by UV and visible light to form electron-hole pairs:

$$ZnO + hv \longrightarrow e^{-} + h^{+}$$

The electron-hole pairs split water molecules from ZnO suspension into •OH and H^{+}:

$$h^{+} + H_2O \longrightarrow \bullet OH + H^{+}$$

O_2 molecules yield superoxide anion:

$$e^{-} + O_2 \longrightarrow {}^{\bullet}O^{-}_{2}$$

Superoxide anion reacts with H^{+} to generate HO_2• radicals:

$$\bullet O_2 + H^+ \longrightarrow HO_2^\bullet$$

$HO_2^\bullet$ interferes with electrons generating hydrogen peroxide anions which react with H^+ to produce hydrogen peroxide molecules:

$$HO_2^\bullet + H^+ + e^- \longrightarrow H_2O_2$$

The hydrogen peroxide can penetrate the cell membrane and kill the bacteria.

2.7.1.2 Release of Zn^{2+}

Another possible mechanism for the ZnO antibacterial activity is the release of Zn^{2+} ions which can damage the cell membrane and penetrate the intracellular contents. On the physicochemical characterization and antibacterial tests of ZnO nanoparticles in five media, it was suggested that toxicity of nano-ZnO against Escherichia coli was attributed mainly to the released Zn^{2+} ions as stated in reference(Dimapilis et al., 2018). Similarly, the toxicity of ZnO nanoparticles against Saccharomyces cerevisiae could result from the solubility of the Zn^{2+} ions in the microorganism-containing medium according to reference(Alamdari et al., 2020).Microorganisms are protected by a cell membrane covering them and through this membrane sufficient nutrient are transported. Gram-negative bacteria have a triple thin layer of peptidoglycan compared with Gram-positive bacteria and show less toleration to nanoparticle interaction with the cell membrane. A Gram-negative bacterium (such as E. coli) has a layer of peptidoglycan in its cell wall where porins are present in the outer layer. Porins are a family of proteins that exist in the outer peptidoglycan layer that helps passive diffusion of NPs inside the cell. ZnO NPs prefer to dissolve in the aqueous medium containing bacteria and release Zn^{+2}ions (because of free energy). The interaction between zinc ions and the cell wall results in a change in surface tension which leads to the membrane depolarization at the point of contact. As a result, the bacterial membrane shows abnormal textures such as membrane rupture or membrane blebs(Alamdari et al., 2020).

One of the main proposed antibacterial mechanisms for ZnO-NPs is release of zinc ions in medium containing ZnO-NPs and bacteria. The released Zn^{+2} have significant effect in the active transport inhibition as well as in the amino acid metabolism and enzyme system disruption. Several studies have believed that the leaked Zn^{+2}into growth media responsible for ZnO Nanotoxicity and the dissolution of ZnO-NPs into Zn^{+2} were found as size dependent.

Additionally, Zn^{+2} Release would be limited by an inherent ZnO property, mentioned earlier ZnO stability in water. The insolubility of ZnO impedes the distribution of zinc ions into the medium and thus limits this antimicrobial effect unless ZnO capped or stabilized(Sirelkhatim et al., 2015). Furthermore, it is reported that small-sized nanoparticles can penetrate the bacterial membrane easily and spherically shaped ZnO NPs release Zn^{+2} ions more effectively than rod-shaped ions(Alamdari et al., 2020).

2.7.1.3 Direct contact of nanoparticles with cell membrane

Further suggestions for the bactericide achievement are the inhibition of energy metabolism, once NPs have internalized bacteria. ZnO-NPs are bactericidal and thus disrupt membrane causing membrane dysfunction, resulting in their internalization into the bacteria. According to Heinlein et al cell damage does not necessarily result from the entry of the metal oxide particles into the cell. More importantly is the contact between the bacterial cell and particle which causes changes in microenvironment within the contact area of the organism and particle. After contact with ZnO nanoparticles, bacterial cell walls were damaged and disorganized. The abrasive ZnO caused increased membrane permeability leading to subsequent cellular internalization of the nanoparticles(Dimapilis et al., 2018).There is a creation of electrostatic forces when E. coli treated with ZnO and this electrostatic interaction between NPs and bacteria cell surface as a cause of growth inhibition, and that the total bacterial charge is negative, because of the excessive formation of separated carboxyl groups.

Thus, the cell surface is negatively charged, interestingly, ZnO-NPs contain a positive charge in a water suspension such reverse charges enhance the total effect by creating electrostatic forces, which serve as a powerful bond between NPs and bacterial surface. As a result, the cell membrane is damaged. Additionally, the interaction between E. coli and ZnO-NPs yields cell wall disorganization followed by internalization of NPs into the cells(Sirelkhatim et al., 2015).

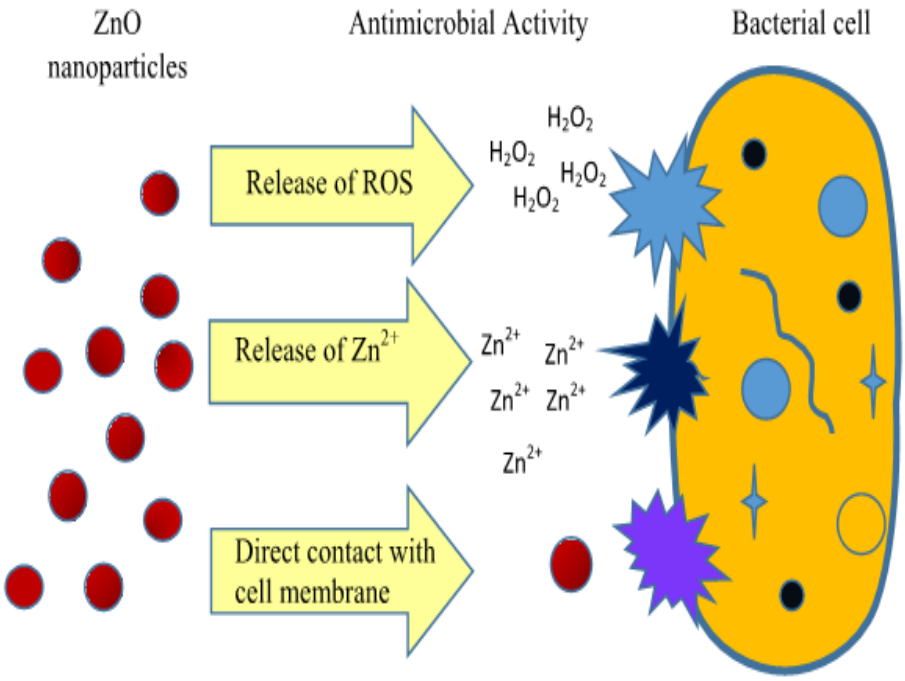


Figure 6: Zinc oxide nanoparticles disinfection mechanism (Dimapilis et al., 2018)

2.8 Gram negative bacteria

Gram-negative bacteria lose the crystal violet stain (and take the color of the red counterstain) in Gram's method of staining. Gram-negative bacteria have a cytoplasmic membrane, a thin peptidoglycan layer, and an outer membrane containing lipopolysaccharide. Gram negative bacteria appear a pale reddish or pink color when observed under a light microscope following Gram staining. But Gram positive are purple colour following Gram staining. This is because the structure of their cell wall is unable to retain the crystal violet stains so are colored only by the safranin counterstain. There is a space between the cytoplasmic membrane and the outer membrane called the periplasmic space or periplasm. This is characteristic of bacteria that have a cell wall composed of a thin layer of a particular substance (called peptidoglycan).

The genus Escherichia is a Gram-negative, non-spore forming, facultative anaerobic, rod-shaped bacteria from the family Enterobacteriaceae. Escherichia coli (abbreviated as E. coli) are bacteria found in the environment, foods, and intestines of people and animals. E. coli are a large and diverse group of bacteria(Zinnah M. A. et al., 2007). E.coli have a cell envelope structure typical

of Gram-negative cells, and thus, posses an outer membrane with a lipopolysaccharide component that is distinct from the cytoplasmic membrane(Beneduce et al., 2003). The gram negative bacteria have rods in shape, non-spore forming, with peritrichous flagella. But gram positive have cocci, bacilli, or branching filaments shape.

3 MATERIALS AND METHODS

3.1 Chemicals and solvents

The chemicals used for the synthesis of zinc oxide nanoparticles for the application of treatment of water from bacterial infection are zinc acetate dihydrate Zn $(CH_3COO)_2.2H_2O$ Assay 99.0%, sodium hydroxide (NaOH), ethanol absolute (C_2H_5OH) assay 99.8%, double distilled water, dimethyl sulfoxide (DMSO), Peptone bacteriological, MacConkey agar the crystal violet, iodine, alcohol and safranin were used. All the chemicals are analytical grade and do not undergo further purification.

The study of ZnO in its nanoscale requires accurate and well understood techniques. This section gives a detailed description on the synthesis and characterization of ZnO nanoparticles for water purification and also, a brief description of the techniques that have been utilized in this study as well as their respective sample preparations.

3.2 Instruments and Apparatus

The apparatus used for synthesis and characterized ZnO NPs are beaker, measuring cylinder, magnetic stirrer with hot plate, thermometer, Whatman filter paper, glass rod, spatula, analytical balance (FA2104) China with accuracy 0.0001g, and sample holders, crucible, puddle, Heating oven (DHG-9055) china, Box type resistance furnace (BK-5-12GJ) china, double beam UV/VIS spectrophotometer, XPERTPRO X-ray diffractometer, Fourier transform infrared spectroscopy, fluorescence spectroscopy instrument Cary eclipse, Bacteriological Incubator, Electric Heated vertical steam sterilizer (LX-B751) china, Petri dishes, cotton swab, stain less steel cork borer, micro slides and 1cm quartz cuvette.

3.3 Experimental procedure

Zinc Oxide nanostructure was synthesized by using sol-gel method. In order to prepare a sol at first, 20 gm of Zinc Acetate Dehydrate (Zn $(CH_3COO)_2.2H_2O$) were mixed into 150 ml distilled water and stirred for 20 min at 35 °C to produce a zinc acetate solution. Again, 80 gm NaOH powders was weighted, mixed into 80 ml water and stirred for around 20 min at 35 °C for

producing NaOH solution. After mixed both solutions, the titration reaction was performed by the addition of 100 ml ethanol into the drop-wise manner accompanied by vigorous stirring. The stirring was continued for around 4 hrs to complete the reaction for obtained a gel-like product. The gel was aged for around 12 hrs. Then the gel was dried at 80 °C cover night and calcined in an oven at 500 °C for 90 min. Then, the samples were grinded using mortar and pestle. Finally, ZnO nanoparticles powder was prepared. However, the overall chemical reaction for the prepared of ZnO nanoparticles by using NaOH can be expressed as:

$$Zn\,(CH_3COO)_2.2H_2O + 2NaOH \longrightarrow ZnO + 2NaCH_3COO + H_2O$$

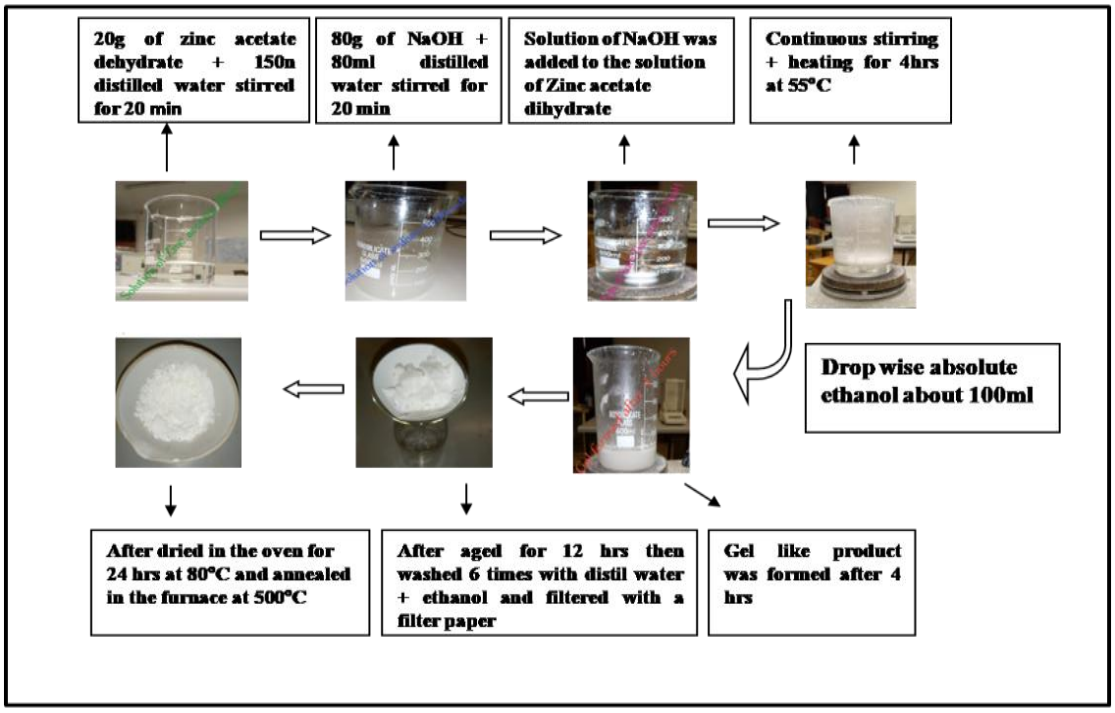


Figure 7 experimental procedures to synthesize zinc oxide nanoparticles

3.4 Wastewater collection

Three glass bottles were sterilized in the autoclaves. The unfiltered and untreated wastewater samples were collected from three different places in the sterilized glass bottles: the first two were collected from Adama science and technology university wastewater center and the left one were collected from ganda oda in Adama city. The collected wastewater samples were labeled as

Astu T_1, Astu T_2 and SGO. The sample was transferred to the laboratory immediately and stored at 4 °C to avoid any physical-chemical changes in the wastewater.100µl from each wastewater sample was used for isolating the bacteria through serial dilution and agar plate culture technique.

3.5 Bacteria Isolation From wastewater

To active the microorganism present in wastewater 4gm of peptone bacteriological was dissolved in 200ml of distilled water. Then100µl from each wastewater sample was taken and serial solution was done then the solution of peptone bacteriological added to it. After 24 hrs 12.5 gm of MacConkey agar were dissolved in 250 ml of distilled water. The activated serial dilution of wastewaters were transferred to the prepared MacConkey agar medium on Petri dishes and incubated at 37°C for 24h. Then one loop from each colony (multiculture bacteria) was transferred another MacConkey agar medium through a sterile glass rod (pure culture) at 37°C for 24 h. The growth was then visualized by performing standards Gram stain method. One loop from each pure culture bacteria were taken on the micro slides. Then the crystal violet, Iodine, Alcohol and safranin was added on the bacterial on the micro slides. All the dried slides were seen under microscopes at 100x magnification. Finally the pure cultured bacteria were taken into the broth media and incubated at 37°C for 48 hrs to antibacterial test.

3.6 Antibacterial analysis of ZnO NPs

An antibacterial analysis of ZnO Nanoparticles with bacteria isolated from wastewaters was determined by using the agar disc diffused method. Microbial strains were growth in nutrient broth aerobically at 37°C for 24 h until the turbidity of bacterial suspensions was achieved to 1.5 x108 CFU mL^{-1} by comparison with the 0.5 McFarland standards. 0.01 mL of each sub cultured bacteria organisms were spreaded using sterilized cotton swab on 20 mL of sterilized molten and cooled MHA media. Subsequently, agar wells of 6 mm diameter was impregnated on different plates with sterilized stainless steel cork borer and labeled properly. A different concentration of (100, 50, 25 and 12.5µg/ml) of solutions was added into well using micropipette. The plates' containing the microbes and synthesized ZnO nanoparticles was incubated at 37°C for 24 h in the case of bacteria to measuring the zone of inhibition. After incubation, the plates were examined for clear zone around the discs or wells. The diameter of such zones of inhibition was

measured using ruler and mean value for each concentration was recorded and expressed in millimeters and finally were compared with the standard drug (Ceftriaxone).

3.7 Methods of characterization

3.7.1 XRD characterization

The X-ray diffraction (XRD) pattern for ZnO nanoparticles was analyzed using XPERTPRO X-ray diffractometer by generating Cu-Kα radiation (λ= 0.15406nm). It is used to determine the crystalline phase of the synthesized nanoparticles. An X-ray generator operates at a voltage of 40kV and applied current of 30mA at room temperature. Intensities were measured at room temperature for angle range of $2\theta = 10° \leq 2\theta \leq 80$.

3.7.2 Scanning electron microscope characterization

The morphological feature of synthesized ZnO NPs were determined by using Scanning Electron Microscopy (SEM) (INSTRUMENT JCM-6000Plus), using electron beam energy of 10 KV and 15 KV. The synthesized ZnO NP powder is mounted on a sample holder followed by coating with a conductive metal. Then, the sample was scanned with focused fine beam of electrons. The surface characteristics of the sample were obtained from the secondary electrons emitted from the sample surface.

3.7.3 UV- Vis spectroscopy characterization

The UV-Vis absorption peaks of ZnO NPs were recorded with UV-Vis spectroscopy. UV-Vis Spectrophotometer with double monochromator using 1cm fused quartz cuvette in the wavelength region of 200 - 800 nm at room temperature. The ZnO NPs was dissolved in DMSO and its absorbance measured using 1cm quartz cuvette. The ASCII files of the measured spectra were collected by computer which interfaced with the instrument. Finally, the absorption spectra of ZnO NPs were analyzed using origin 8 software.

3.7.4 Photoluminescence spectroscopy

The emission spectra of ZnO NPs was measured with fluorescence spectroscopy instrument Cary eclipse (Instrument Serial Number MY18490002) with excitation width slit of 10 nm in the wavelength range of 350-800 nm at room temperature and excitation wave length of 320 nm. The samples was dissolved in DMSO and stirred until it dissolved. The emission spectrum of the sample was measured by 1 cm quartz cuvette. The wave length and intensity of the obtained sample were collected by computer which interfaced with the instrument. The emission spectra of synthesized ZnO NPs were analyzed using origin 8 software.

3.7.5 FT-IR spectroscopy characterization

The vibrational phonon and the quality of ZnO NPs were also characterized by Fourier transform infrared spectroscopy (FTIR) (PE IR SUBTECH SPECTRUM) in the wave number region of 4000-400 cm-1. The ZnO NPs spectra measurements were performed by putting the powder ZnO NPs on KBr pellet. The ASCII files of the measured spectra were collected by computer which interfaced with the instrument.

4 RESULTS AND DISCUSSIONS

4.1 X-Ray Diffraction Analysis

The XRD patterns of ZnO NPs synthesized by the sol-gel method annealing temperature at 500 °C for different time are showed in Fig.8. Both XRD diffraction peaks of ZnO powders are in a good agreement with wurtzite structure (hexagonal phase, space group P63mc) as reported in (JCPDS card no. 36-1451) with lattice parameters a=b= 3.253 Å and c= 5.21Å for ZnO nps calcinated for 90 minutes and a=b= 3.250 Å and c= 5.21Å for ZnO nps calcinated for 2 hrs. XRD pattern ZnO shows eleven peaks at 2θ = 31.76°, 34.45°, 36.27°, 47.56°, 56.27°, 56.62°, 56.93°, 62.89°, 63.18°, 66.40° and 67.88° and were respectively indexed to the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) planes. The samples (101) peak is the most intense peak that shows the plane is a preferred growth plane.

The peak broadening in the XRD pattern clearly indicates that small nanocrystals are present in the samples. The diffraction peak intensity also markedly increases with annealing time (7010 and 7066 for ZnO calcinated for 90min and 2hrs respectively) as shown in Fig. 8. This may indicate that high time of annealing provides sufficient energy to crystallites to orient in proper equilibrium sites and causes an increase in intensity.

The average crystal sizes of ZnO NPs were calculated by Debye Scherrer formula using Eq. (2.2) based on the full width at half-maximum (FWHM) of the peaks and as shown in the Table 1. The average crystal sizes of synthesized ZnO NPs annealed for 90 minutes and 2 hours at temperature 500℃ were 26.18 nm and 27.29 nm respectively as shown in table 1. This result shows that, the average crystal sizes of ZnO NPs increased as the time of annealing increased. This serves as a powerful tool for us to control the crystallite size by choosing the suitable annealing time.

The annealing time increases the crystal size also increases, but the dislocation density and micro strain decreases as shown in table 1. Also increases of calcination time result in decrease in lattice parameter as shown in table 2. The sharp diffraction peaks of the synthesized products indicate their good crystallinity. All examined samples have their strongest line corresponding to the (1 0 1) plane.

Table 1 Crystallite size, micro strain and dislocation density of synthesized ZnO nanoparticles annealing at 500°C for 90 min and 2 hours.

Samples	Crystallite size (D) (nm)	Strain (ε) (10^{-3}) (radian)	Dislocation density (δ) (line/m^{-2}) (10^{-21})
ZnO nps-A	26.18	4.209693	1.459
ZnO nps-B	27.29	4.03558	1.343

Note: ZnO nps-A and ZnO nps-B were represents synthesised ZnO nps and annealed for 90 minute and 2 hrs respectively.

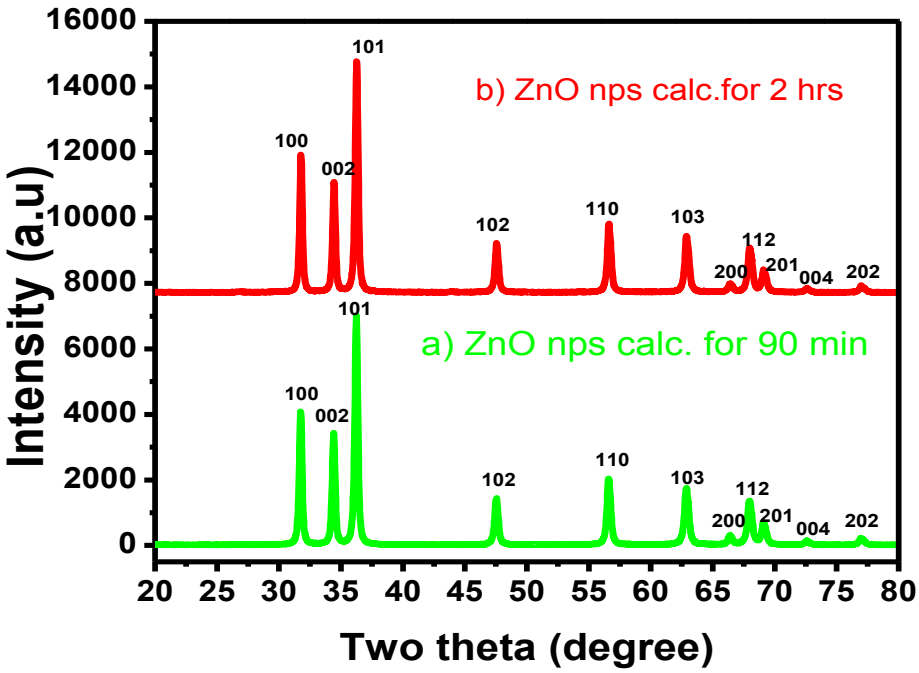


Figure 8: XRD Pattern of prepared ZnO NPs annealed at 500 ◦C for a) 2 hour0s, b) 90 minute (1 and ½ hrs).

Table 2 Here Lattice parameter, interplanar spacing, unit cell volume, positional parameter and bond length of prepared ZnO nanoparticles from the obtained XRD data of synthesized ZnO nps.

Samples	2θ	hkl	d_{khl} in nm	Lattice parameter (nm)	c/a ratio	Volume (V)(Å)^3	Positional parameter (u)	Bond length (Zn–O) (L) (Å)
ZnO nps-A	31.74	100	0.2817	a =0.3253	1.6017	47.736	0.3799	1.9171
	34.4	002	0.2605	c= 0.5210				
ZnO nps-B	31.76	100	0.2815	a=0.3250	1.6027	47.677	0.3798	1.9160
	34.4	002	0.2605	c= 0.5210				

Note: ZnO nps-A and ZnO nps-B were represents synthesized ZnO nps and annealed for 90 minute and 2 hrs respectively.

4.2 SEM analysis

Particle morphology were from scanning electron micrographs (SEM) by INSTRUMENT JCM-6000Plus at acceleration volt 10kv and 15 kV for ZnO nps synthesized by annealing for 90 minutes and 2 hrs at 500°C.The SEM image of the sample is shown in fig 9(a) and 9(b) reveals that the particles are spherical and having granular nature. Fig 9(a) shows its SEM image at higher magnification and we can see that the particles are held together because of weak physical forces.

Fig 9(b) shows the SEM images of zinc oxide under higher magnification it clearly suggests that particle separation is not good enough. It demonstrates clearly the formation of the morphology of zinc oxide nanoparticles change with the calcination time. These analyses show that high homogeneity emerged in the samples surface by increasing time of annealing. With increasing time of annealing the morphology of the particles changes to the spherical shape and nanopowders were less agglomerate. A similar result for SEM analysis was reported by ref(Xrd et al., 2016).As the particle size calculated from the XRD is in nano range we are not getting any exact information about the surface morphology of the sample from the SEM micrograph, because of the limitation of our instruments up to micro range.

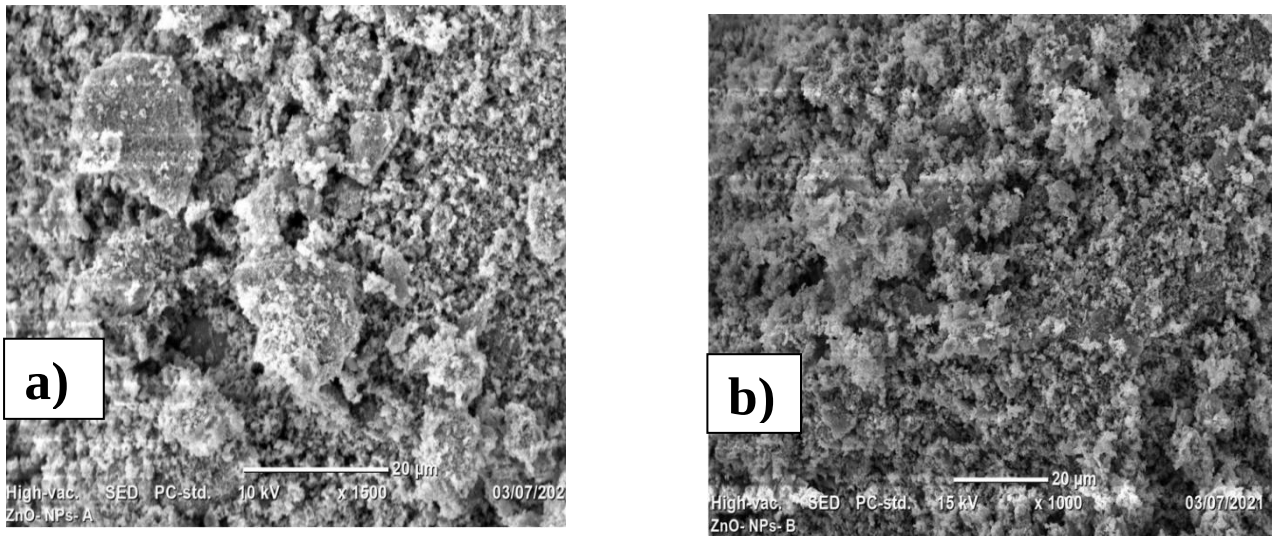


Figure 9: SEM image of ZnO nanoparticles synthesized by sol gel method annealing at 500°C for a) 90 minute b) 2 hours.

4.3 UV-Visible spectroscopy

UV-Visible spectroscopy is most widely used technique to investigate the optical properties of the particles. UV-Visible spectroscopy analysis was done in the range of 200-800 nm. Zinc oxide nanoparticles typically exhibit optical absorption around 370 nm(Manyasree et al., 2018). Fig. 10 shows UV-Vis absorption spectra of ZnO NPs at different time of annealing at 500°C. The absorption peak was observed at 379 nm and 381 nm for ZnO nps annealing at 500° for 90 minute and 2 hours respectively. The absorption peak lies much below the band gap wavelength of 388 nm of bulk ZnO(Bindu & Thomasa, 2017).

As the time of annealing increased peak absorbance wavelength shifted to red due to decreasing in quantum confinement of the particles i.e when comparing the peak absorption wavelengths of calcined samples ZnO nps annealed for 90 min and 2 hrs a slight shift towards higher number is seen by increasing the calcinations time. This shift indicates a decrease in band gap, which can be attributed to increase in particle size. The peaks are due to electronic transition from valence band to conduction band. The band gap energy of ZnO NPs calculated using Eq. (2.11) were found to be 3.27 and 3.25 for ZnO nps for 90 minute and 2 hours time of annealing at temperature 500ºC respectively. According to quantum confinement theory, the band gap of a semiconductor depends on the crystal size, and its value will increase as the crystal size

decreases(KHAN et al., 2013). The band gaps energy of ZnO NPs decreased with increasing time of annealing at constant temperature due to various particle sizes. Due to their nanodimension all the synthesized ZnO NPs exhibit blue shifted absorbance peak as compared to their bulk counterpart having absorbance peak at 386 nm at room temperature*(synthesis and characterizations of zinc oxide nanoparticles for antimicrobial applications a Thesis Submitted to Applied Physics Program Adama Science and Technology University For Partial Fulfillment of the Requirements for the Degree of Master of Scienc*, 2018).

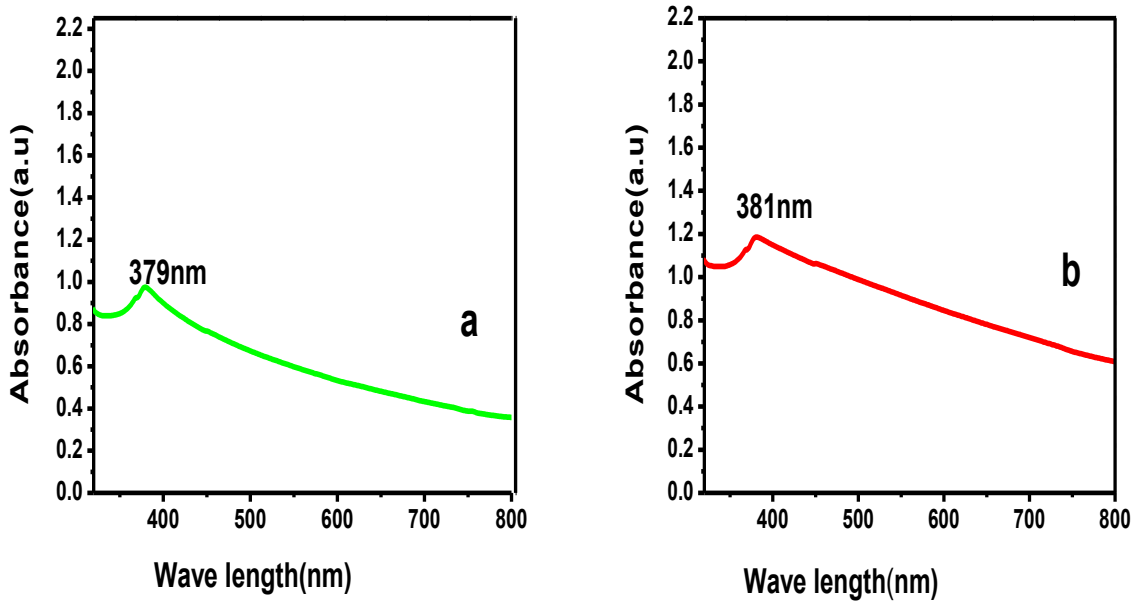


Figure 10 : UV-Vis absorption spectra of ZnO nanoparticles synthesized using sol gel method and annealed at 500 °c for a) 90 minute (1½ hrs) b) 2 hours.

The optical band gap can be also estimated using Tauc relation from equation (2.12).The estimated band gap from the plot of $(\alpha h\nu)^2$ vs. hν for ZnO nanoparticles can be seen in Fig. 11. The band gap "Eg" is determined by extrapolating the straight portion to the energy axis at $\alpha = 0$. The linear part shows that the mode of transition in these nanoparticles is direct in nature. The estimated band gap value of the ZnO nanoparticles from tauc plot relation annealed for 90 minutes was found to be 3.27 eV and 3.25 eV the one annealed for 2 hours at 500°C. The band gap value is less than that of bulk ZnO 3.37 eV this might be due to the strain arising from chemical synthesis of ZnO nanoparticle. These micro-strains highly influence the optical band gap of material.

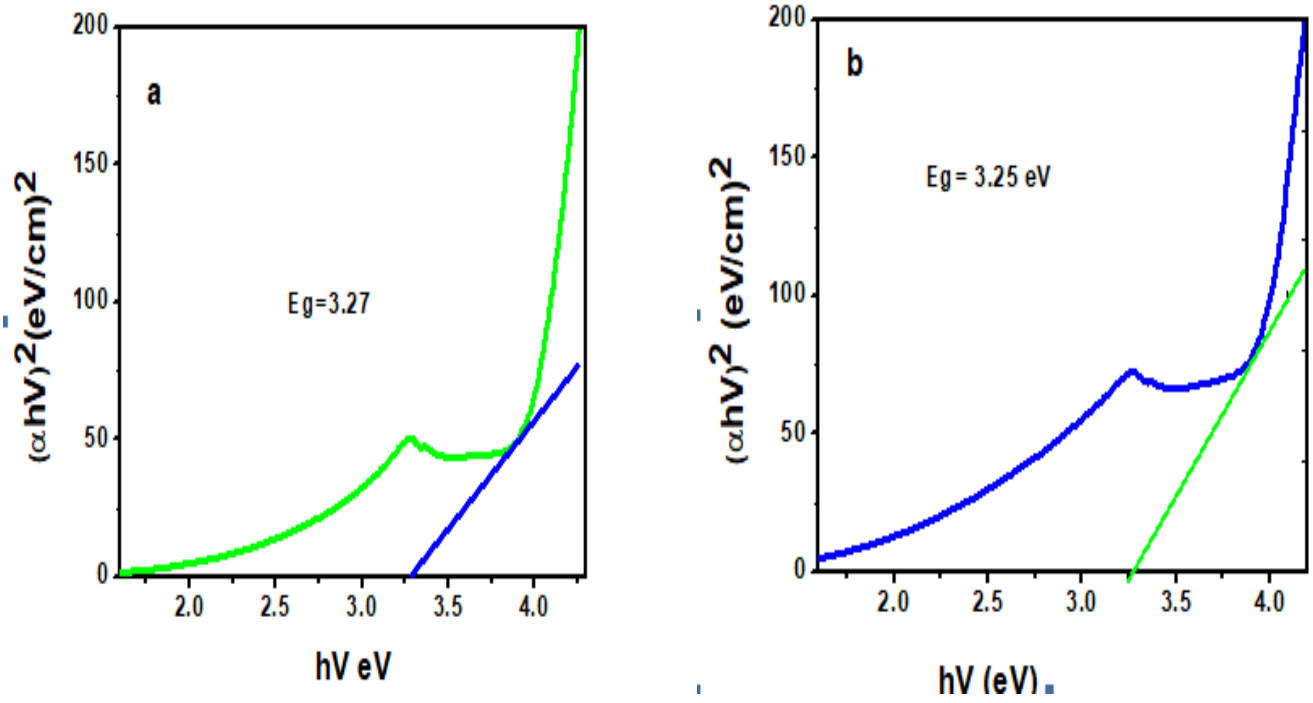


Figure 11: The Tauc plot of $(\alpha h\nu)^2$ vs. hν of ZnO nanoparticles synthesized by sol gel method and annealing at 500 °C for a). 90 minute b). 2 hours.

4.4 Photoluminescence Spectroscopy.

Photoluminescence investigation confirms the ability of nanoparticle to absorb incident energy and convert it into visible radiation(Bhagyalekshmi et al., 2018). The PL spectrum of the synthesized ZnO NPs was measured at room temperature. Figure 12 shows the emission spectra of ZnO NPs at annealing time for 90 minutes and 2 hrs at 500℃. It is well known that at room temperature the PL spectrum from ZnO typically consists of a UV emission band and a broad emission band(Tang et al., 2014). The UV emission band (300-400nm) is dominated by the free exciton (FE) emission. The broad emission band literally between 400 and 700 nm observed nearly in all samples regardless of growth conditions is called deep level emission band (DLE). The UV emission band is related to a near band-edge transition of ZnO, namely, the recombination of the free excitons. The deep level emission band has previously been attributed to several defects in the crystal structure such as O-vacancy (V_O), Zn-vacancy (V_Z).

In this work the synthesized ZnO nps exhibited UV emission peaks at 388.93 nm for both ZnO nps annealing for 90 minutes and 2 hrs and broad peaks at 416 nm and 421 nm (violet region) for ZnO nps annealing for 90 minutes and 2 hrs at 500 ℃ respectively as shown in figure 12.

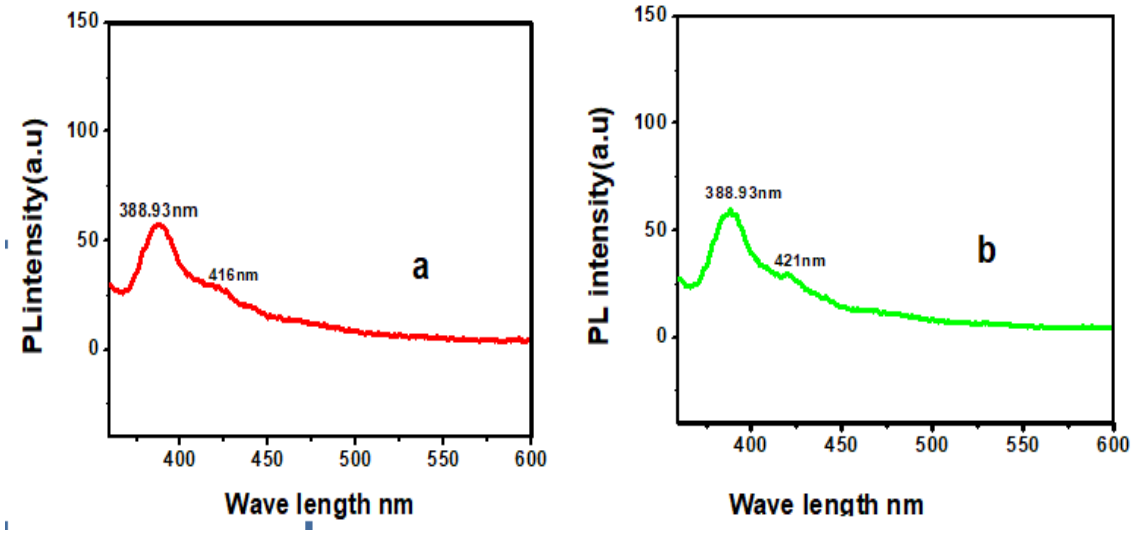


Figure 12: Emission spectra of ZnO nanoparticles synthesized and annealed at 500 °C for a) 90 min b) 2 hours.

4.5 FTIR spectroscopy

To understand the optical properties, purity, and nature of the presently studied ZnO-NPs, FT-IR spectrum was recorded at room temperature, as shown in Figure13. The FTIR spectrum of the ZnO nanoparticles synthesized by sol gel method, which was acquired in the range of 400-4000 cm^{-1}. From figure 13 the broad band observed around 3412 and 3390 cm^{-1} for the synthesized ZnO nps by annealing for 90 minutes and 2 hrs at 500°C respectively indicates the characteristics of hydroxyl (OH) group. The Presence of O-H group represents the presence of water molecules on the surface of ZnO nanoparticles which was similar to the reference(Manyasree et al., 2018). The intensity of the bands are significantly reduced at annealing for 2 hours temperature of 500°C due to removal of functional groups and the formation of pure wurtzite structure.

The FTIR spectrum, absorption at 400 cm^{-1} to 600 cm^{-1} identifies the absorption peaks of metal oxide and the presence of ZnO(Dallatu et al., 2021). In previous studies regarding ZnO NPs they were also able to observe FT-IR spectrum with the band around 400 cm^{-1}(Shamhari et al., 2018).

The pattern of absorption at 600-1000 cm^{-1} is responsible to the different modes of caused from the collision of carboxylic groups in unreacted acetate. As the size of the nanoparticles increases, the content of the carbonxylate (COO-) and hydroxyl (-OH) groups in the samples decreased(Xiong et al., 2006). The small peak observed at 2922 cm^{-1} and 2921 $cm^{-1}$for ZnO nps annealing for 90 minutes and 2 hrs respectively were due to C-H stretching vibration of alkane groups. Similarly, the band observed at 1376 and 1378 $cm^{-1}$and 1547 and 1548 $cm^{-1}$are due to symmetric and asymmetric of stretching carbonxylate respectively for both ZnO nps which attached to the ZnO nanoparticles during synthesis. Band observed at 2342 cm^{-1} and 2337 cm^{-1} are due to the existence of CO_2 molecules in the atmosphere.

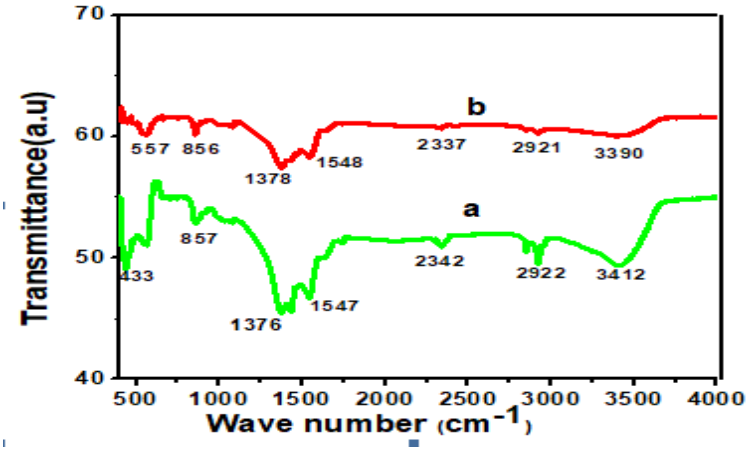


Figure 13: FT-IR spectra of synthesized ZnO NPs annealing at 500◦C for, a) 90 minute (1 and ½ hrs) b) 2 hours.

4.6 The bacteria Isolated from the collected wastewater.

Gram negative bacteria appear a pale reddish or pink color when observed under a light microscope following Gram staining; this is because the structure of their cell wall is unable to retain the crystal violet stains so are colored only by the safranin counterstain and The genus Escherichia is a Gram-negative, non-spore forming, facultative anaerobic, rod-shaped bacteria from the family Enterobacteriaceae as stated in literature review. Furthermore, colony morphology and Gram characteristics were checked by performing gram staining. All the dried slides were seen under microscopes at 100x magnification. All the bacteria were seen under the microscopes were distinguished as gram negative bacteria. Gram-negative cells have a thin

peptidoglycan layer and stain red to pink according to ref (Smith & Hussey, 2005). Figure 14 indicates the isolated Gram negative bacteria (Escherichia coli) from the collected wastewater.

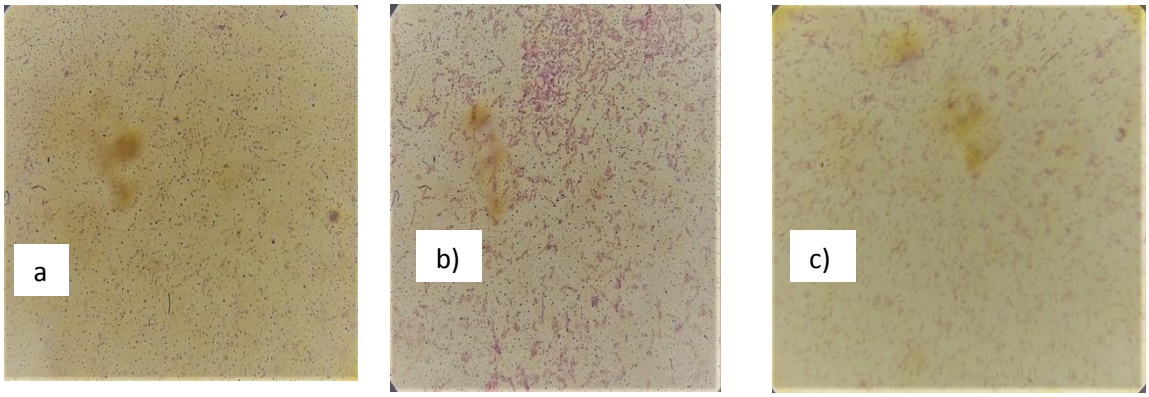


Figure 14: Representative image of isolated bacteria from (a) Astu T_1 and; (b) Astu T_2 and: (c) SGO as observed by bright field microscopy under 100 x magnifications.

4.7 Antimicrobial activity of the ZnO nanoparticles

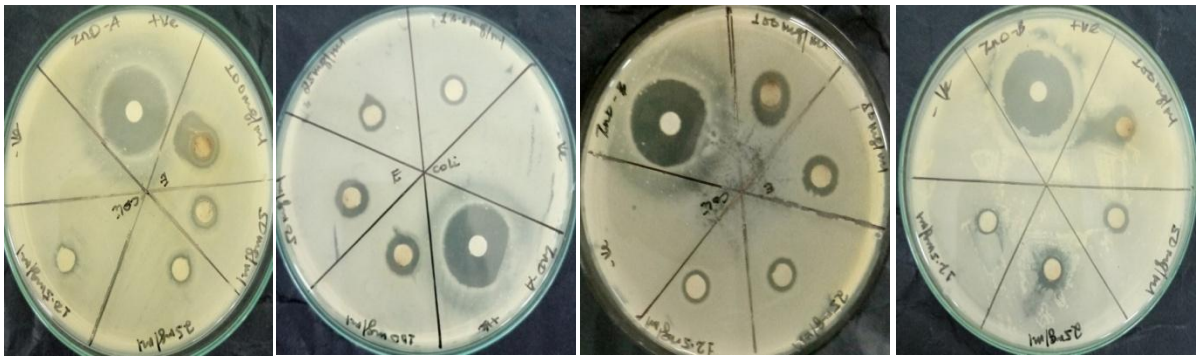
The antibacterial activity of ZnO nanoparticles ZnO NPs suspensions of different concentrations towards the isolated gram negative bacterial (Escherichia coli) from wastewater were tested by the disc diffusion agar methods. The synthesized ZnO NPs showed antimicrobial activity against the isolated Escherichia coli (E.coli) bacteria from collected wastewater was shown in figure 15.

Table 3 Zone of inhibition of E. coli at different concentrations of synthesized ZnO nanoparticles.

Samples	Conc. of the Sample (µg/ml)	Zone of inhibition (mm) Trial 1	Trial 2	Trial 3	Value = mean + SD
ZnO nps –A	100	12.4	12.7	11.1	12.07 ± 0.8
	50	10.5	11.3	9.9	10.57 ± 0.70
	25	8.2	7.2	9.1	8.17 ± 0.95
	12.5	7.1	7.0	8.0	7.37 ± 0.550
Ceftriaxone	30	26.4	25.6	21.0	24.33±2.91
ZnO nps –B	100	11.9	12.0	11.3	11.73 ± 0.38
	50	9.8	10.5	10.7	10.33 ± 0.47
	25	8.8	7.2	7.4	7.8 ± 0.87
	12.5	7.4	7.0	7.2	7.2 ± 0.20
Ceftriaxone	30	23.0	22.5	26.0	23.83±1.89

Note: ZnO nps-A and ZnO nps-B were represents synthesized ZnO nps and annealed for 90 minutes and 2 hrs respectively.

The positive control (Ceftriaxone) against on the E.coli bacteria and the zone of inhibition values was measured as shown in figure 15 and the corresponding cluster column charts are shown in figure 16. The results were given in Table 3 above. The table indicate that the synthesized zinc oxide nanoparticles (ZnO NPs) have shown a considerable antimicrobial activity on the isolated gram negative bacterial (Escherichia coli) from wastewater, the highest anti-bacterial activity of zone of inhibition values recorded 12.07 ± 0.85 for zinc oxide nanoparticles annealing at 500°Cfor 90 minute (ZnO nps-A) and 11.73 ± 38 for zinc oxide nanoparticles annealing at 500°C for 2 hours (ZnO nps-B) for similar concentration (100µg/ml). This indicates that the smaller the size of nanoparticles, the better is their antibacterial activity which was similar to ref(BUŞILĂ, 2018).



a) b) c) d)

Figure 15: Disc diffusion method of antimicrobial activity of ZnO nanoparticles on E.coli isolated from collected wastewater at different concentration (100µg/ml, 50µg/ml, 25µg/ml, and 12.5µg/ml) by ZnO nps-A fig 14a-b and ZnO nps-B fig 14c-d.

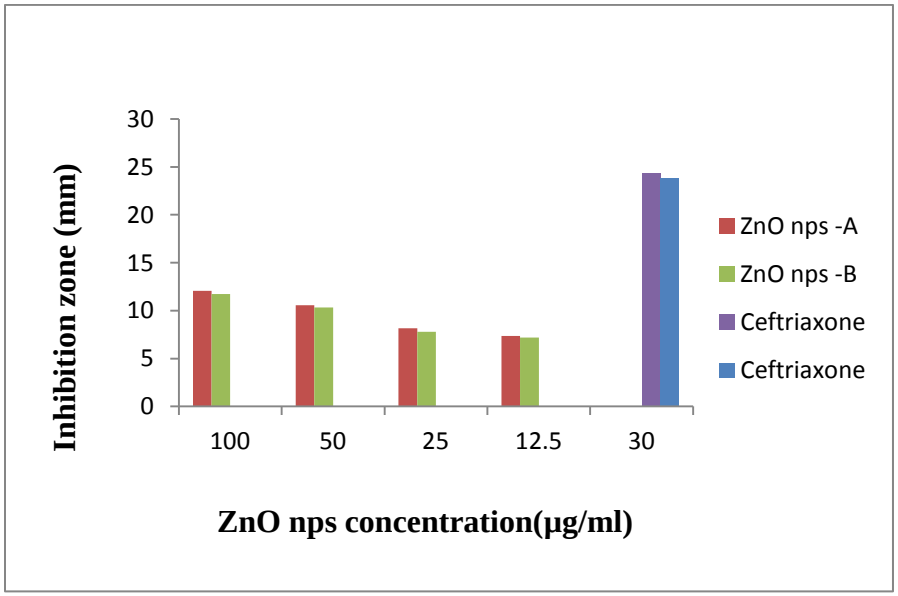


Figure 16: The bar graphs showing zone of inhibition of ZnO nps and Ceftriaxone against E.coli bacterial isolated from collected wastewater.

Figure 16 shows that Antimicrobial activities of ZnO NPs increased with increase of concentrations (12.5, 25, 50 and 100 µg/ml) and were due to the increase of H_2O_2 concentration on the surface of ZnO as indicated in the literature review. In the antimicrobial activity, initially zinc oxide nanoparticles attach to the surface of the bacterial cell membrane and then penetrate into the bacteria. After penetration, they inactivate the enzymes of the microbes, generating hydrogen peroxide. Hence bacterial cells were dead.

5. CONCLUSION

In this work, ZnO NPs has been successfully synthesized through a sol gel method using zinc acetate dihydrate as precursor for the treatment of water from bacterial infections. The synthesized zinc oxide nanoparticles were characterized using UV-Vis absorption, XRD, SEM, Photoluminescence and FTIR, which confirmed the formation of the zinc oxide nanoparticles using sol gel method. The crystallite size of the synthesized ZnO NPs was 26.18 nm for ZnO nps annealed for 90 minutes at 500 □ and 27.29nm for ZnO nps annealed for 2 hrs at 500□ which indicate that the crystal size of ZnO nps increases as the annealing time increases at constant annealing temperature. There was variation in UV-Vis absorption peaks of synthesized ZnO NPs and energy band gap of synthesized ZnO NPs was 3.27 eV and 3.25 eV for ZnO nps annealed for 90 minutes and 2 hrs respectively which indicates that the optical property of ZnO NPs influenced by calcination time.

The antimicrobial activity of synthesized ZnO nanoparticles against the isolated Escherichia coli (E.coli) bacteria from wastewater was done by different concentration 100µg/ml, 50µg/ml 25µg/ml and 12.5µg/ml. Antibacterial activity of synthesized ZnO NPs annealed for 90 minutes produces 7.37 ± 0.550, 8.17 ± 0.95, 10.57 ± 0.70 and 12.07 ± 0.85mm zone of inhibition at 12.5µg/ml, 25µg/ml, 50µg/ml and 100µg/ml concentration respectively and Antibacterial activity of synthesized ZnO NPs annealed for 2 hrs produces7.2 ± 0.20, 7.8 ± 0.87, 10.33 ± 0.47 and 11.73 ± 0.38mm zone of inhibition on the isolated Escherichia coli (E.coli) at 12.5µg/ml, 25µg/ml, 50µg/ml and 100µg/ml concentration respectively. This indicates highest concentration of synthesised ZnO nps have highest antimicrobial activity. Generally, ZnO NPs with small size and high concentration showed better antibacterial activity due to relatively high surface area to volume ration that enhances interaction between the nanoparticles and the bacteria causing its distraction.

REFERENCES

A Barzinjy, A. (2020). Structure, Synthesis and Applications of ZnO Nanoparticles: A Review. *Jordan Journal of Physics*, *13*(2), 123-135.

Abdala, E., Nur, O., & Mustafa, M. A. (2021). Photocatalytic Performance of Ca Doped ZnO Nanostructure Synthesized Via UV Incubator Shaker. *Journal of Nanoscience and Nanoengineering*, *7*(1), 1-9.

Alamdari, S., Sasani Ghamsari, M., Lee, C., Han, W., Park, H. H., Tafreshi, M. J., ... & Ara, M. H. M. (2020). Preparation and characterization of zinc oxide nanoparticles using leaf extract of Sambucus ebulus. *Applied Sciences*, *10*(10), 3620.

Al-Obodi, E. E., Al-Saadi, T. M., & Sulaiman, A. F. (2018). Study of Some Physical Properties of NiyFe0. 025Mg0. 975-yO Nanoparticles Prepared by the Sol-Gel Method. *Int. J. Electrochem. Sci*, *13*, 11752-11761.

Aluri, J., Lakshmi, S. P, & Mandava, B. R. (2016). Synthesis and Characterization of ZnO Nanoparticles Using Sol-gel Process. *Sensors & Transducers*, *200*(5), 45.

Alwan, R. M, Kadhim, Q. A, Sahan, K. M, Ali, R. A., Mahdi, R. J, Kassim, N. A, & Jassim, A. N. (2015). Synthesis of zinc oxide nanoparticles via sol–gel route and their characterization. *Nanoscience and Nanotechnology*, *5*(1), 1-6.

Ashe, B. (2011). *A Detail investigation to observe the effect of zinc oxide and Silver nanoparticles in biological system* (Doctoral dissertation).

Balasubramanian, S. (2018). Development of Piezoelectric Property Materials for the Nanogenerator Production.

Bayda, S, Adeel, M., Tuccinardi, T, Cordani, M, & Rizzolio, F. (2020). The history of nanoscience and nanotechnology: from chemical–physical applications to nanomedicine. *Molecules*, *25*(1), 112. Girish, K. (2018). Neem (Azadirachta indica A. Juss) as a source for green synthesis of nanoparticles. *Asian J Pharm Clin Res*, *11*(3), 15.

Becheri, A, Dürr, M, Nostro, P. L, & Baglioni, P. (2008). Synthesis and characterization of zinc oxide nanoparticles: application to textiles as UV-absorbers. *Journal of Nanoparticle Research*, *10*(4), 679-689.

Behera, O. (2011). *Synthesis and Characterization of ZnO nanoparticles of various sizes and Applications in Biological systems* (Doctoral dissertation).

Bhagyalekshmi, G. L., Neethusha, A. P., & Rajendran, D. N. (2018, September). Synthesis, characterization and photoluminescence properties of Ce^{3+} doped ZnO nanophosphor. In *IOP Conference Series: Materials Science and Engineering* (Vol. 360, No. 1, p. 012005). IOP Publishing.

Bhushan, B, Luo, D., Schricker, S. R, Sigmund, W, & Zauscher, S. (Eds.). (2014). *Handbook of nanomaterials properties*. Springer Science & Business Media.

Bindu, P., & Thomas, S. (2014). Estimation of lattice strain in ZnO nanoparticles: X-ray peak profile analysis. *Journal of Theoretical and Applied Physics*, *8*(4), 123-134.

Bindu, P., & Thomas, S. (2017). Optical properties of ZnO nanoparticles synthesised from a polysaccharide and ZnCl2. *Acta Phys. Pol. A*, *131*(6), 1474-1478.

BUŞILĂ, M. (2018). ZnO Nanoparticles: Green Synthesis, Properties and Applications. *The Annals of "Dunarea de Jos" University of Galati. Fascicle IX, Metallurgy and Materials Science*, *41*(3), 25-29.

Conde, M. N, Dakhsi, K, Zouihri, H, Abdelouahdi, K, Laanab, L, Benaissa, M, & Jaber, B. (2011). Preparation of ZnO nanoparticles without any annealing and ripening treatment. *Journal of Materials Science and Engineering. A*, *1*(7A), 985.

Dallatu, Y. A., Shallangwa, G. A., & Africa, S. N. (2020). Synthesis and growth of spherical ZnO nanoparticles using different amount of plant extract. *Journal of Applied Sciences and Environmental Management*, *24*(12), 2147-2151.

Desai, A. V, & Haque, M. A. (2007). Mechanical properties of ZnO nanowires. *Sensors and Actuators A: Physical*, *134*(1), 169-176.

Dimapilis, E. A. S., Hsu, C. S., Mendoza, R. M. O., & Lu, M. C. (2018). Zinc oxide nanoparticles for water disinfection. *Sustainable Environment Research*, *28*(2), 47-56.

Dowling, A. (2004). Clift, R. *Groben N. et a1. Nanoscience and Nanotech. nologies: Opportunities and Uncertainties. London: The Royal Society&The Royal Academy of Engineering Report*.

Getie, S, Belay, A, Chandra Reddy, A. R, & Belay, Z. (2017). Synthesis and characterizations of zinc oxide nanoparticles for antibacterial applications. *J Nanomed Nanotechno S*, *8*(004).

Haque, M. J, Bellah, M. M., Hassan, M. R., & Rahman, S. (2020). Synthesis of ZnO nanoparticles by two different methods & comparison of their structural, antibacterial, photocatalytic and optical properties. *Nano Express*, *1*(1), 010007.

Huang, C, Notten, A, & Rasters, N. (2011). Nanoscience and technology publications and patents: a review of social science studies and search strategies. *The Journal of Technology Transfer*, *36*(2), 145-172.

Hussain, M. (2014). *Synthesis, characterization and applications of metal oxide nanostructures* (Doctoral dissertation, Linköping University Electronic Press).

Jiang, J., Pi, J., & Cai, J. (2018). The advancing of zinc oxide nanoparticles for biomedical applications. *Bioinorganic chemistry and applications*, *2018*.

Jimenez-Cadena, G., Comini, E., Ferroni, M., Vomiero, A., & Sberveglieri, G. (2010). Synthesis of different ZnO nanostructures by modified PVD process and potential use for dye-sensitized solar cells. *Materials Chemistry and Physics*, *124*(1), 694-698.

Jones, N, Ray, B., Ranjit, K. T, & Manna, A. C. (2008). Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS microbiology letters*, *279*(1), 71-76.

Kalantar-zadeh, K, & Fry, B. (2008). Characterization techniques for nanomaterials. *Nanotechnology-Enabled Sensors*, 211-281.

Kant, R., Sharma, D., Bansal, A., & Singh, R. (2020). Structural, optical and dielectric properties of Al/Mn doped ZnO nanoparticles, a comparative study. *Materials Technology*, 1-8.

Kariper, I. A. (2015). Optical and structural properties of zinc iodine thin films. *Optical Materials*, *44*, 78-83.

Kasahun, M., Yadate, A., Belay, A., Belay, Z., & Ramalingam, M. (2020). Antimicrobial activity of chemical, thermal and green route-derived zinc oxide nanoparticles: a comparative analysis. *Nano Biomed Eng*, *12*(1), 47-56.

Khan, W, Khan, Z. A, Saad, A. A, Shervani, S, Saleem, A, & Naqvi, A. H. (2013). Synthesis and characterization of Al doped ZnO nanoparticles. In *International Journal of Modern Physics: Conference Series* (Vol. 22, pp. 630-636). World Scientific Publishing Company.

Król, A, Pomastowski, P, Rafińska, K, Railean-Plugaru, V, & Buszewski, B. (2017). Zinc oxide nanoparticles: Synthesis, antiseptic activity and toxicity mechanism. *Advances in colloid and interface science*, *249*, 37-52.

Kumar, S. S., Venkateswarlu, P., Rao, V. R., & Rao, G. N. (2013). Synthesis, characterization and optical properties of zinc oxide nanoparticles. *International Nano Letters*, *3*(1), 1-6.

Manyasree, D., Kiranmayi, P., & Venkata, R. K. (2018). Characterization and antibacterial activity of ZnO nanoparticles synthesized by co-precipitation method. *International Journal of Applied Pharmaceutics*, *10*(6), 224-8.

Mideksa, K. (2018). *Synthesis And Characterizations Of Zinc Oxide Nanoparticles For Antimicrobial Applications* (Doctoral dissertation, ASTU).

Mohan, A. C., & Renjanadevi, B. (2016). Preparation of zinc oxide nanoparticles and its characterization using scanning electron microscopy (SEM) and X-ray diffraction (XRD). *Procedia Technology*, *24*, 761-766.

Morkoç, ., & Özgür, Ü. (2008). *Zinc oxide: fundamentals, materials and device technology*. John Wiley & Sons.

Mostafaii, G, Chimehi, E, Gilasi, H, & Iranshahi, L. (2017). Investigation of zinc oxide nanoparticles effects on removal of total coliform bacteria in activated sludge process effluent of municipal wastewater. *Journal of Environmental Science and Technology*, *10*, 49-55.

Mourdikoudis, S., Pallares, R. M., & Thanh, N. T. (2018). Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. *Nanoscale*, *10*(27), 12871-12934.

Muhammad, W, Haleemb, I, Ullahc, N, Khan, M. Q, & Kumar, N. S. (2019). Nanoparticle synthesis approaches at a glance. *Nanoscale Reports*, *2*, 1-10.

Musee, N, Ondiaka, M, Chimphango, A, & Aldrich, C. (2015). Modeling the fate, behaviour and toxicity of engineered nanomaterials in aquatic systems.

Mustapha, S., Ndamitso, M. M., Abdulkareem, A. S., Tijani, J. O., Shuaib, D. T., Ajala, A. O., & Mohammed, A. K. (2020). Application of TiO 2 and ZnO nanoparticles immobilized on clay in wastewater treatment: a review. *Applied Water Science*, *10*(1), 1-36.

Nalumaga, H. (2017). A Study on the Properties of ZnO. *TiO2 Nanocomposite Prepared via the Sol-gel Technique*.

Naveed Ul Haq, A., Nadhman, A., Ullah, I., Mustafa, G., Yasinzai, M., & Khan, I. (2017). Synthesis approaches of zinc oxide nanoparticles: the dilemma of ecotoxicity. *Journal of Nanomaterials*, *2017*.

Nethavhanani, T. (2017). Synthesis of zinc oxide nanoparticles by a green process and the investigation of their physical properties. Master Thesis, January, 1-228.

Nosal, E, & Reinprecht, L. (2018). Preparation and application of silver and zinc oxide nanoparticles in wood industry: The review. *Acta Facultatis Xylologiae Zvolen res Publica Slovaca*, *60*(2), 5-23.

Nunes, D., Pimentel, A., Gonçalves, A., Pereira, S., Branquinho, R., Barquinha, P, & Martins, R. (2019). Metal oxide nanostructures for sensor applications. *Semiconductor Science and Technology*, *34*(4), 043001.

Osman, D. A. M, & Mustafa, M. A. (2015). Synthesis and characterization of zinc oxide nanoparticles using zinc acetate dihydrate and sodium hydroxide. *Journal of Nanoscience and Nanoengineering*, *1*(4), 248-251.

Pal, S. L, Jana, U., Manna, P. K, Mohanta, G. P., & Manavalan, R. (2011). Nanoparticle: An overview of preparation and characterization. *Journal of applied pharmaceutical science*, *1*(6), 228-234.

Panwar, R. S, & Raina, K. K. (2009). *Preparation of modified ZnO nanoparticles by sol-gel process and their characterization* (Doctoral dissertation).

Raj, K. P., Sadaiyandi, K., Kennedy, A., & Thamizselvi, R. (2016). Structural, optical, photoluminescence and photocatalytic assessment of Sr-doped ZnO nanoparticles. *Materials Chemistry and Physics*, *183*, 24-36.

Rajeswari, M, & Agrawal, P. (2020). Rapid Water Disinfection Using ZnO Nanoparticles Synthesized from Citrus aurantifolia. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 1-8.

Ramelan, A. H, Wahyuningsih, S., Munawaroh, H, & Narayan, R. (2017, February). ZnO wide bandgap semiconductors preparation for optoelectronic devices. In *IOP Conference Series: Materials Science and Engineering* (Vol. 176, No. 1, p. 012008). IOP Publishing.

Rochman, N. T, & Akwalia, P. R. (2017, May). Fabrication and characterization of Zinc Oxide (ZnO) nanoparticle by sol-gel method. In *journal of physics: Conference series* (Vol. 853, No. 1, p. 012041). IOP Publishing.

Rodnyi, P. A, & Khodyuk, I. V. (2011). Optical and luminescence properties of zinc oxide. *Optics and Spectroscopy*, *111*(5), 776-785.

Sabir, S, Arshad, M, & Chaudhari, S. K. (2014). Zinc oxide nanoparticles for revolutionizing agriculture: synthesis and applications. *The Scientific World Journal*, *2014*.

Sadrolhosseini, A. R, Mahdi, M. A., Alizadeh, F, & Rashid, S. A. (2019). Laser ablation technique for synthesis of metal nanoparticle in liquid. *Laser Technology and its Applications*.

Sajjadi, S. P. (2005). Sol-gel process and its application in Nanotechnology. *J. Polym. Eng. Technol*, *13*, 38-41.

Savi, B. M, Rodrigues, L, Uggioni, E, & Bernardin, A. M. (2011). Synthesis of ZnO nanoparticles by Sol-Gel processing. From Chemical- Physical Application, January.

Senthilkumar, S, Dhivya, V, Sathya, M., & Rajendran, A. (2021). Synthesis and characterization of magnetite/hydroxyapatite nanoparticles for biomedical applications. *Journal of Experimental Nanoscience*, *16*(1), 160-180.

Shamhari, N. M., Wee, B. S., Chin, S. F., & Kok, K. Y. (2018). Synthesis and characterization of zinc oxide nanoparticles with small particle size distribution. *Acta Chimica Slovenica*, *65*(3), 578-585.

Sharma, R., Garg, R., & Kumari, A. (2020). A review on biogenic synthesis, applications and toxicity aspects of zinc oxide nanoparticles. *EXCLI journal*, *19*, 1325.

Sharma, S, Malik, M. A, Chandel, T, Thakur, V, & Rajaram, P. (2014, April). Growth and characterization of ZnSe nanoparticles. In *AIP Conference Proceedings* (Vol. 1591, No. 1, pp. 474-476). American Institute of Physics.

Siddiqi, K. S, ur Rahman, A, & Husen, A. (2018). Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale research letters*, *13*(1), 1-13.

Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M. & Mohamad, D. (2015). Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-micro letters*, *7*(3), 219-242.

Smith, A. C., & Hussey, M. A. (2005). Gram stain protocols. *American Society for Microbiology*, *1*, 14.

Spano, G., Goffredo, E., Beneduce, L., Tarantino, D., Dupuy, A., & Massa, S. (2003). Fate of Escherichia coli O157: H7 during the manufacture of Mozzarella cheese. *Letters in Applied Microbiology*, *36*(2), 73-76.

Sukkarieh, H. The Dramatic Element in the Second Movement of Yousef Khasho's Symphony," Al-Hussein Bin Ali": An Analytical Study.

Tang, B, Zhang, Q, Luo, Q, Wu, Z. H, & Qiu, Y. (2014). Synthesis and Optical Properties of ZnO Nanoneedles Array. *Asian Journal of Chemistry*, (18).

Thakral, F, Bhatia, G. K, Tuli, H. S, Sharma, A. K, & Sood, S. (2021). Zinc Oxide Nanoparticles: from Biosynthesis, Characterization, and Optimization to Synergistic Antibacterial Potential. *Current Pharmacology Reports*, 1-11.

Vijayaprasath, G, Murugan, R, Hayakawa, Y, & Ravi, G. (2016). Optical and magnetic studies on Gd doped ZnO nanoparticles synthesized by co-precipitation method. *Journal of Luminescence*, *178*, 375-383.

Vijayaprasath, G, Murugan, R., Asaithambi, S., Sakthivel, P., Mahalingam, T., Hayakawa, Y., & Ravi, G. (2016). Structural and magnetic behavior of Ni/Mn co-doped ZnO nanoparticles prepared by co-precipitation method. *Ceramics International*, *42*(2), 2836-2845.

Vishwakarma, A, & Singh, S. P. Synthesis of Zinc Oxide Nanoparticle by Sol-Gel Method and Study its Characterization.

Xiong, G., Pal, U., Serrano, J. G., Ucer, K. B., & Williams, R. T. (2006). Photoluminesence and FTIR study of ZnO nanoparticles: the impurity and defect perspective. *physica status solidi c*, *3*(10), 3577-3581.

Yoo, A. (2013). *Effect of zinc oxide and silver nanoparticles on intestinal bacteria* (Doctoral dissertation, University of Missouri, Columbia).

Zaman, S. (2012). *Synthesis of ZnO, CuO and their composite nanostructures for optoelectronics, sensing and catalytic applications* (Doctoral dissertation, Linköping University Electronic Press). Xu, D., Zhang, D, Zou, H, Zhu, L, Xue, M, Fang, Q, & Qiu,

S. (2016). Guidance from an in situ hot stage in TEM to synthesize magnetic metal nanoparticles from a MOF. *Chemical Communications*, *52*(69), 10513-10516.

Zhou, W., & Wang, Z. L. (Eds.). (2007). *Scanning microscopy for nanotechnology: techniques and applications*. Springer science & business media.

Zhu, Y. (2015). *ZnO nanoparticles as a luminescent down-shifting layer for solar cells* (Doctoral dissertation, INSA de Lyon).

Zinnah, M. A., Bari, M. R., Islam, M. T., Hossain, M. T, Rahman, M. T., Haque, M. H, & Islam, M. A. (2007). Characterization of Escherichia coli isolated from samples of different biological and environmental sources. *Bangladesh Journal of Veterinary Medicine*, 25-32.