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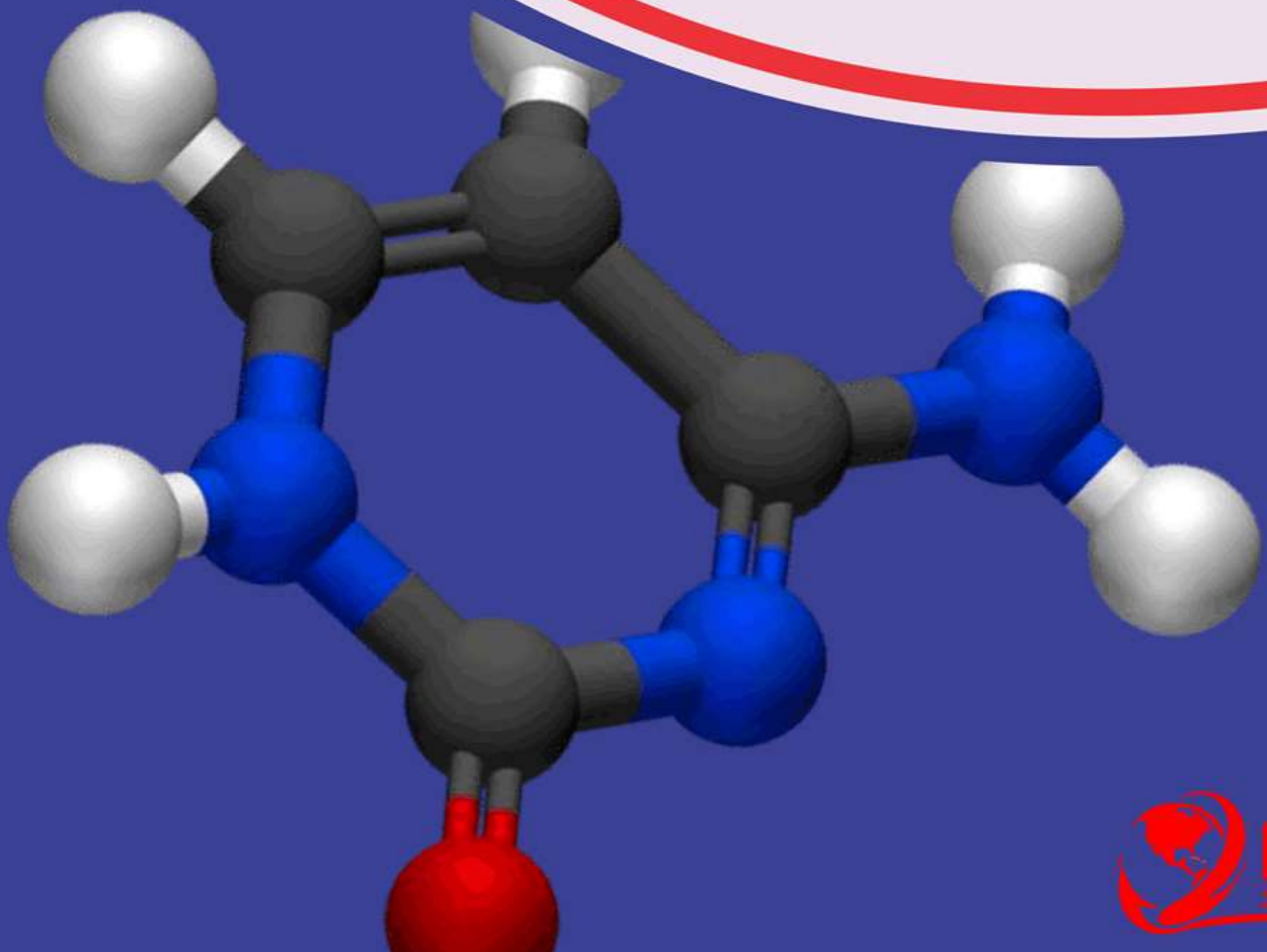

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Synthetization and Characterization of Zinc Oxide Nanoparticles by X- Ray Diffractometry (XRD), Fourier Transforms, Infra-Red Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and Antibacterial Activity Test

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Abstract

Purpose: Nanomaterials with their derivable potentials offer wide obtain ability and have recently aroused much attention for biomedical applications. Nowadays, nanomaterials-based colorimetric sensing is a quickly emerging field of sensing applications. Nanomaterials are considered as the main component of colorimetric determination of hydrogen peroxide to replace the natural enzyme-based sensors because of some associated intrinsic drawbacks. Considering the advantageous properties of ionic liquid (IL) for various applications, significant attention has been made to the use of ionic liquid stabilized metal NPs which may serve as a regulator to enhance the catalytic performance of the metal nanoparticles in the different IL reaction medium.

Methodology: The peroxidase-like activity of IL coated metal NPs (IL-MNPs) have been considered for the catalytic oxidation reaction of chromogenic substrate 3,3,5,5- tetramethylbenzidine (TMB) in the presence of H2O2 at an estimated wavelength of 652 nm.

Results: The synthesized metal nanoparticles (Ag) were produced using a chemical reduction method. Various characterization techniques like FTIR, UV-Visible spread Reflectance Spectroscopy [UV-VIS DRS], were employed, which verified the structure, nano-size and successful combination of metal dopant ion into the samples. The molecular structure of ionic liquid with varying cations was produced and confirmed by 1H-NMR spectroscopy. The ionic liquid was coated on metal nanoparticles to enhance their conductivity.

Unique Contribution to Theory, Practice and Policy: Optimized reaction conditions like pH, temperature and catalyst dosage affect catalytic activity and color sensing properties. The coating of [Min] Ac on Ag achieved low detection limits and colorimetric detection of [Pyr] based Ag.

Keywords: *Nanoparticles, Nano Materials, Synthetization, Zinc Oxide*

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INTRODUCTION

Nanomaterials

Nanomaterials are the basis of nanotechnology and nanoscience. The field of study and development known as nanostructure science and nanotechnology is vast and cross-disciplinary, and it has experienced rapid global growth in recent years. The range and functionality that may be usable, as well as how materials and things are manufactured could all be revolutionized by it. It has already had a large commercial influence, and it will undoubtedly continue to do so in the future. At least one dimension of Nano scale materials is fewer than 100 nanometers. Nanomaterials are of special interest because they exhibit novel optical, electrical, magnetic, and other capabilities at this scale. In sectors like electronics, health, and other areas, these emergent traits have the potential to have significant effects. The nanoparticles' surface to volume ratio is substantially higher than it is in their commercial form, which could make them more chemically reactive [1]. A momentous group of semiconductors called transition metal oxides find uses in a variety of fields, including catalysis, electrical and magnetic fields, water purification, medication delivery, and UV detectors. There are several different kinds of transition metal oxide, such as tin oxide (SnO2), copper oxide (CuO), titanium dioxide (TiO2), and zinc oxide (ZnO). Because of their numerous qualities, including biocompatibility and cost-effectiveness etc. Zinc oxide nanoparticles stand out among transition metal oxides [2].

Zinc Oxide

Rarely found in nature, zinc oxide or ZnO is an inorganic substance also known as zincite. Nevertheless, synthetically made ZnO accounts for the majority of its economic utilization. Usually, it takes the form of a white powder that is essentially insoluble in water [3]. Because of the existence of manganese impurities, it is typically red or orange in color [4]. Following iron oxide, zinc oxide is the second most prevalent metal [5]. Zinc oxide is sometimes referred to as a member of the group 11 -VI semiconductors, whose valency is on the frontier between covalent and ionic semiconductor [6] whereas zinc belongs to the second group and oxygen belongs at ambient temperature, zinc oxide is a crystalline semiconductor with a large and direct band gap (3.37eV) [7]. The n-type doping of the ZnO semiconductor is caused by oxygen emptiness [4]. Additionally, zinc oxide has a high exciton binding energy of 60 meV [6]. Zinc oxide occurs as mineral zincite or as white powder commonly known as zinc white. Zinc oxide, an amphoteric oxide, can function as either an acid or a base [4]. Hexagonal wurtzite, cubic zinc blender, and the rarely examined cubic salt are three crystal forms of Zinc oxide. The hexagonal wurtzite is most common and stable at ambient conditions [9] . Due to its coordination number of 4, the ZnO is Sp3 hybridized. In a standard tetrahedron, the corners of each anion are encircled by four more cations, and vice versa. The lattice constant of the ZnO hexagonal unit cell is a=3.2500 Å and c=5.2060 Å [10]. Zinc nano structural can be divided into one – dimensional (1D) includes nano needless, nano rings, nano tubes etc. Two – dimensional (2D) includes nano sheets, nano plates etc. while flowers, snowflakes etc. exhibit 3D morphology of Zinc oxide [11]. Each morphology exhibits distinct physical and chemical characteristics, and each has a different use [7].

Properties of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles have acquire much more importance in the recent years due to their attractive and outstanding properties such as chemical [12], mechanical and thermal stability, high potency [13], high electrochemical coupling coefficient, broad range of Ultraviolet

radiation absorption, transparency for visible light, increased selectivity [14], large exciton binding energy [15],high electrical, optical properties [16], low toxicity [17], high electron mobility and, strong room temperature luminescence [3], high photosensitivity [18], biocompatibility [19], biodegradability, high solubility [20] , inexpensive and earth abundant compound [21]. It has been shown that zinc oxide, both in bulk form and as well as nanoparticles, has biodegradability. ZnO have photocatalytic and photo-oxidizing ability against chemical and biological species [22]. Due to its similar band gap to tin dioxide (TiO2), zinc oxide has been suggested as a probable photo catalyst. However, zinc oxide has absorbed a large fragment of the solar spectrum. The effects of zinc oxide as a photocatalytic agent on the photodegradation of water contaminants are an important application [23]. Higher breakdown voltage, the capacity to withstand huge electric fields, reduced electronic noise, high temperature and high-power operation are advantages of ZnO having a large band gap [9]. In the drug industry, ZnO NPs are used in soaps, toothpaste, dental inlays, and powders [13].

Applications of Zinc oxide nanoparticles

Zinc oxide is broadly used as an ingredient in a variety of substances including ceramics, glass, rubber, cement, paints, sealants, adhesives, food, batteries etc. [9]. ZnO nanoparticles are primarily used in rubber industry in order to increase the performance of high polymers in terms of their toughness, intensity, antiaging, and other functions [24]. Because of its flexibility, zinc oxide is frequently used in sunscreens and cosmetics due to its exceptional UV absorbing property. Typically, zinc oxide is employed as an inorganic physical blocker. **Due** to their native capacity to filter both UVA and UVB rays, zinc oxide is particularly helpful in sunscreens [25]. Zinc oxide is widely used to treat a variety of skin conditions in products such as antiseptic ointments, antidandruff shampoos etc. One of the main aspects of Zinc oxide nanoparticles (Zn NPs) is zinc oxide (ZnO) tap accessible in the market and employed as a gauze by athletes to avoid damage of soft tissues [26].

Zinc is one of the most important structural components of the human body, and a cofactor for more than 300 enzymes [14] without which many enzymes become inactive while the other two members mercury and cadmium of the same element group and having the same electronic configuration are noxious. Zinc is involved in body metabolism and plays an important role in protein synthesis. Due to small particle size, they are efficiently absorbed by the body. Therefore, nano zinc is usually used as a food additive. Zinc oxide has been listed as “Generally Recognized as safe (GRAS) by the US Food and Drug Administration (FDA) due to its non – toxic properties [24]. ZnO demonstrated potential uses in the poultry and livestock sectors, particularly as an addition to feed and an ingredient in animal diets. Many studies have been conducted to confirm the probability of using zinc oxide nanoparticles as dietary supplements to enhance growth performance [27].

Recent research suggested that ZnO might be a potential coating material for dental and orthopedic implants because of its antibacterial properties. Additionally, the Dox-ZnO nano complex, a composite form of zinc oxide and the anticancer medication doxorubicin, has been hailed as a promising cancer treatment appliance [26]. Due to their unique physical and chemical properties, zinc oxide nanoparticles, one of the most significant metal oxide nanoparticles, are effectively used in a variety of different sectors. It is considered to be a viable material in electronic application, optoelectronic application, laser technology [5], agriculture [7], power generators, photo printing electrophotography, etc. [11]. Due to its similarity to the features of GaN, zinc oxide is a viable candidate for optoelectronic applications in the short wavelength range (green, blue, UV), information storage and sensors. ZnO nanoparticles are

an excellent candidate for a number of applications such as bio sensor, gas sensor, solar cells, and photo detector [28]. The development and productivity of food crops may be enhanced by zinc oxide nanoparticles (NPs). Different zinc oxide nanoparticle concentrations were applied to peanut seeds. In order to improve seed germination, seedling vigor, and plant growth, zinc oxide nanoscale treatment (25 nm mean particle size) at 1000 ppm concentration was applied. These zinc oxide nanoparticles also demonstrated effectiveness in boosting stem and root growth in peanuts [3].

Zinc Oxide Nanoparticles and Their Antibacterial Properties

Zinc oxide has exceptional performance and cutting – edge uses, however zinc oxide, a material of enormous interest, provides striking advantages in biomedical and therapeutic settings. Nanomedicine has garnered significant interest in order to achieve medication delivery criteria, reduce antibiotic concentration, and manage drug-resistant harmful bacteria [26]. Due to the emergence of microbial resistance to metal ions, antibiotics, and the creation of resistant strains, zinc nanoparticles have recently become to a potential antibacterial agents due to their increased surface area to volume ratio [29].Due to its great effectiveness against antibiotic-resistant strains and minimal toxicity to mammalian cells, ZnO seems to be one of the most promising compounds for clinical translation [17].However, ZnO are generally used as antimicrobial agents, delivering system, vaccines , bio- imaging [30], gene transfer, biological labeling, antiviral, and medical implant coatings [22].

ZnO has a remarkable effect on cancer cells. The size, shape and concentration of zinc oxide significantly affect its anticancer properties. Numerous studies have demonstrated the notable anticancer properties of the zinc oxide nanoparticles [22]. The human body can more easily absorb zinc oxide nanoparticles because of their comparatively small particle size. This makes them a viable inorganic substance. This contributes to its safety. Zinc oxide is therefore frequently employed as an anticancer, antidiabetic, and anti-inflammatory material [31].

Table 1: Physical Properties of Zinc Oxide (8)

Properties	**Measured value**
Crystal structure	Hexagonal, Wurtzite
Molecular weight	Zn:65.38,0:16 and Zno:81.38
Lattice constant	a=3.246 Å, c = 5.207Å
Density	5.67 g/cm3 or 4.21 x 1019 ZnO molecules/mm3
Melting point	Tm = 2250 K
Heat of fusion	4470 Cal/mole

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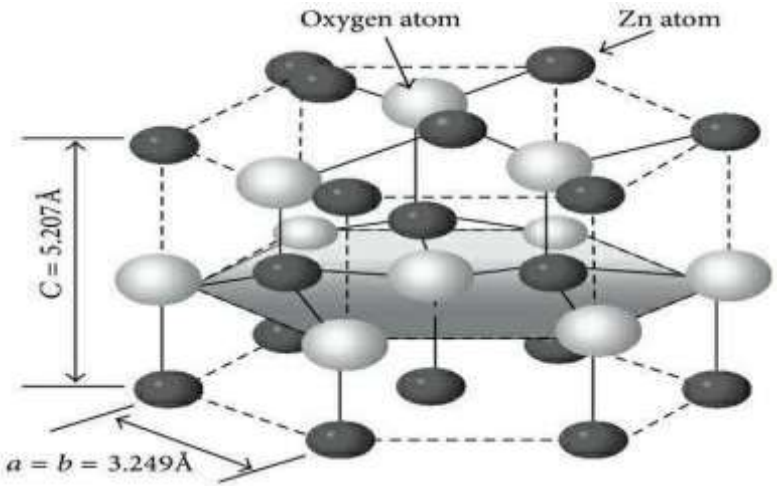

Figure 1: Schematically Wurtzite Crystal Structure of Zinc Oxide (7)

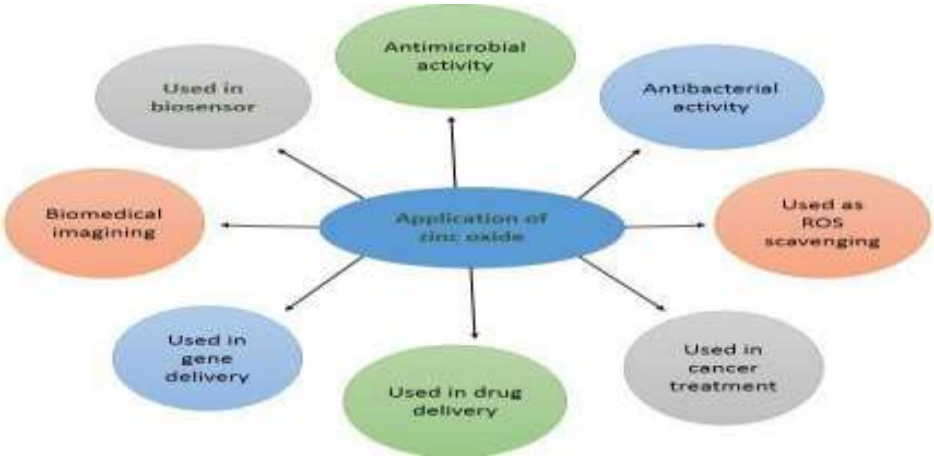

Figure 2: Applications of Zinc Oxide

Hyperglycemia is a condition associated with insulin. It has been found that zinc plays a significant role in the synthesis, storage, and secretion of insulin [22]. Zinc oxide can be regarded as of the most frequent drugs due to its remarkable inter disciplinary history and its safe applications [26]. ZnO nanorods having a diameter of 50 nm or less showed superior antibacterial potency over ZnO nanorods of larger dimensions [21]. Numerous studies have examined a wide range of bacterial species, and zinc nanoparticles have demonstrated antibacterial effectiveness over these with various morphology. Both on a nano scale and on a micro scale, zinc oxide is now being examined as an antibacterial agent. When the particle size of zinc oxide was decreased to nano range, then to nano size, it showed substantial anti-bacterial activity. Zinc oxide has been shown to have bactericidal processes when it comes into contact with bacteria on their surface or in their core [32]. Moreover, size, shape, concentration in the culture media, crystallinity, and type of bacteria (Gram – positive or Gram – negative

bacteria) affects the bactericidal activity of ZnO nanoparticles [21]. Zinc oxide nanoparticles show antimicrobial properties which make it a potential agent for various applications.

Researchers have successfully incorporated zinc oxide, an antimicrobial agent, into textiles, cellulose fiber to inhibit microbial growth. For this reason, zinc oxide is widely accepted as beneficial as an antibacterial agent and due to its durability and selectivity, it is considered a safe material for humans [33].

The following summarizes the mechanism of action of Zinc oxide nanoparticles: cell membrane disruption, bending to proteins and DNA, production of reactive oxygen species (ROS), disruption to the bacterial DNA amplification processes, and changes in the expression of a variety of genes. When interacting with bacteria, zinc oxide nanoparticles cause the generation of reactive oxygen species (ROS). The Zinc oxide nanoparticles have an impact on the respiratory chain and inhibit various enzymes. As a result, singlet oxygen, hydroxyl radicals, hydrogen peroxide, superoxide anion and other ROS are formed and accumulate. ROS can harm the proteins and DNA inside the bacteria cell [34]. The consequences of antibacterial activity of Zinc oxide nanoparticles is due to high sensitivity of the lipid bilayer of bacteria to the reactive oxygen species generated by these zinc oxide nanoparticles [35]. Furthermore, Zinc oxides nanoparticles cause cytoplasmic shrinkage and disturbance of cell walls leading to cytoplasmic leakage [22].

Due to its versatility in fabrication and wide range of uses, ZnO can therefore act as a magical material. As a result, the majority of researchers have used ZnO that is readily available commercially, while some have manufactured it using other methods. However, numerous research have demonstrated that particle homogeneity, shape, and size had a significant impact on the characteristic and performance of Zinc oxide. . Zinc oxide antibacterial activity was significantly influenced by the particle morphology. Therefore, the most fundamental requirement for biological usage is control over the particle morphology, size, and homogeneity [26].

LITERATURE REVIEW

Synthesis of Zinc Oxide Nanoparticles

Radzimska *et.al.* Conducted a study on the most significant ZnO preparation techniques, broken down into metallurgical and chemical approaches, are presented in this work. Chemical approaches for obtaining zinc oxide included the mechanochemical process, controlled precipitation, sol-gel method, solvothermal and hydrothermal method, method using emulsion and microemulsion environment, and other techniques. This review's next section described the ZnO modification techniques. The major description concerned the alteration using polymer matrices and inorganic (metal oxides) and organic (carboxylic acid) components. Lastly, we outline potential uses in a number of industries, including rubber, pharmaceuticals, cosmetics, textiles, electronics, electrotechnology, and photocatalysis**.**

Akhtar *et al.* used controlled precipitation method to synthesized uniform ZnO NPs by using aqueous solution of zinc nitrate, ethylene glycol and ammonia as a precursor, and also examined the effect of different synthesis parameters; i e. temperature, composition of reactant mixture and pH on particles morphologies. SEM images of the prepared ZnO particles show that they are mostly composed of irregular morphologies in the form of discrete particles or embedded material in a gelatinous matrix. Through XRD, the crystalline nature of prepared ZnO NPs were identified. The composition and purity of synthesized ZnO were determined through FT-IR. Thermal stability was confirmed by TG/DTA [26].

Orozco *et al*., conducted a systematic review on Antibiotic-resistant bacteria are becoming a rising problem when it comes to serious infections, particularly in nosocomial settings. Because of its great ability to develop resistance to bactericides and antibiotics, the ESKAPE group of multi-resistant bacteria is particularly significant. Since metal-based nanomaterials have been shown to harm bacterial cell macromolecules, they present an appealing alternative for combating them. Nevertheless, the accumulation of NPs in the environment raises concerns about bacteria becoming resistant to them and their detrimental consequences**.**

Hasnidawani *et al.* employed the sol – gel method to synthesized ZnO NPs by using zinc acetate dihydrate (Zn $(CH_3COO)_2.2H_2O$) as an initial reagent and ethanol (CH_3COOH) was used as solvent. Sodium hydroxide (NaOH) and deionized water was used as a solvent medium. The synthesized solid was employed for structural and morphological analysis using XRD, EDX and FESEM.XRD analysis to exhibit spectra of mainly oxygen and zinc peaks that confirmed the crystallinity of synthesized ZnO samples. Rod shape morphology was detected in FESEM. The purity of the product was further confirmed by EDX analysis. Through the sol – gel method, the crystal size of ZnO NPs was in the range of 81.28 nm to 84.98 nm [36].

Ghorbani *et al.* used a direct precipitation method to prepare ZnO NPs using zinc nitrate as an initial reagent and KOH as a precipitating agent. In this method the aqueous solution of KOH and zinc nitrate were prepared with distilled water. At room temperature, the KOH solution was gently added on zinc nitrate solution under effective stirring. The synthesized product was centrifuged at 5000 rpm for 20 min and then washed thrice with deionized water and finally washed with absolute alcohol. The product obtained was subjected to calculation at 500°C in the air atmosphere for 3 hours. The continuation of ZnO NPs was determined by UV – Vi's spectroscopy. ZnO NPs showed particle size around 30 ± 15 nm as observed by DLS. TEM micrographs show that the prepared ZnO samples were at nano size [37].

Kumar *et al.* used polymer precursors to synthesized ZnO NPs using (Zn $(NO_3)_2$) and poly vinyl chloride (PVC). Since they were all of analytical grade, they didn't need to be further purified. All the stock and working solutions were prepared in double – distilled water. The resultant ZnO NPs were interrogated by XRD, FT-IR, SEM, TEM. The hexagonal wurtzite structure of zinc oxide NPs was confirmed from XRD pattern. SEM images of ZnO NPs revealed that the particles in the sample are well defined, uniformly distributed, spherical in shape and having an average size 35- 40 nm. TEM images show irregular shape of ZnO NPs with average size of 35 to 40 nm. The chemical groups present in the synthesized ZnO NPs were identified by FT- IR analysis [38].

Krishnaraj *et al.* selected a hydrothermal method for the preparation of ZnO NPs using zinc nitrate hexahydrate (Zn $(NO_3)_2.6H_2O$) and NaOH as an initial reagent. The temperature required for this method was 300 °C. The FT-IR spectrum of ZnO NPs were observed in the region of 400_600 cm^{-1}. Through FT-IR, the functional groups of the synthesized ZnO were also examined. The average crystallite size of the wurtzite hexagonal phase of ZnO was 32 ± 49 nm examined by XRD results. Through SEM analysis, the flowers-like structure of the synthesized ZnO samples were observed. UV – Vi's spectroscopy helped to identify the optical properties of the particles. The peaks were observed in the 264 and 376 nm regions with energy band gaps of 4.68 and 3.536 eV. Peaks examined in the absorption spectra are because of the transition of electrons between the valence band and conduction band [39].

Wang *et al.* Synthesized ZnO NPs via solvothermal method. Zinc acetate dihydrate (Zn $(AC)_2$. $2H_2O$), ethanol (C_2H_6O), sodium hydroxide (NaOH) and methyl Orange ($C_{14}H_{14}N_3NaO_3S$,

MO) were used in the experiment. All the reagents were of analytical grade. The phase composition and crystal structure of the synthesized ZnO NPs were demonstrated through XRD technique. From the XRD pattern, the crystallinity of prepared ZnO samples was also confirmed because of absence of impurities. The elemental composition and chemical state of the synthesized ZnO NPs were determined by XPS. SEM micrographs revealed that the prepared ZnO NPs were spherical and having the diameter of 20-40 nm. Analysis through the EDX confirmed the presence of the zinc oxide NPs. The characteristic absorption spectra were obtained by UV-Vis spectroscopy. The energy gap of ZnO NPs was 2.99 eV [40].

Sharma *et al*. synthesized ZnO NPs using solution – based method using zinc chloride ($Zn(Cl)_2$) and ammonium hydroxide (NH_4OH) as precursors. The resultant ZnO NPs was characteristized by Fourier transform infra-red spectroscopy, X-ray diffraction, scanning electron microscopy and Transmission electron microscopy. XRD analysis shows that the average crystal size of ZnO NPs were in the range of 18-31 nm. SEM images show that the particles of zinc oxide NPs are somewhat rod shaped with particle size of about 95-450 nm. TEM images confirmed the average particle size 17- 50 nm. The FT-IR spectrum of ZnO NPs obtained was in the range of 450-500 nm [41].

Drazki *et al.* used a thermal decomposition approach to prepare ZnO NPs using sodium carbonate, zinc sulfate heptahydrate, ethanol as precursor. The precursor was further calculated at 825 °C for 1hr. The properties of the resultant sample were investigated by employing X-ray diffraction, Scanning electron microscopy, Transmission electron microscopy and Energy -dispersive X-ray spectroscopy. SEM images show that all the samples were in spherical shape. XRD and TEM analysis revealed that the average crystal size of ZnO NPs were found to be 87-92 nm [42].

Kiranmayip *et al.* used the co – precipitation method to synthesize ZnO NPs. Zinc sulfate and sodium hydroxide were used as starting materials. Sodium hydroxide was added to zinc sulfate solution with constant stirring that resulted in the formation of ZnO precipitates. The structural and morphological investigation of the resultant product was carried out using various characterization techniques i. e X-ray diffraction analysis, UV-Vi's spectroscopy, FT-IR, SEM and EDX. XRD analysis shows that the average crystallite size of ZnO NPs was 35 nm. The UV-Vis spectra revealed that the prepared ZnO NPs show a broad absorption peak at 383 nm. SEM images report that the shape of ZnO NPs was spherical and well distributed. EDX confirmed the presence of the Zinc oxide NPs. The absorption peak appeared at 450 and 603 cm^{-1} was assigned to ZnO stretching vibrations [43].

Zahra *et al.* synthesized ZnO NPs through aqueous sol – gel method in acidic medium using zinc nitrate hexahydrate as starting material, aqueous isopropanol as solvent and glycerin for accomplishing the polyol system. The product formed was subjected to calculation at 500 °C, 700 °C and 900 °C. Thermal analysis of the prepared powder was carried out using Differential scanning calorimetry (DSC) and thermos gravimetric analysis (TGA). The vibration bands at 491 and 435cm^{-1} assigned to the presence of Zn-O bonds through IR spectroscopy. Field emission scanning electron microscopy (FESEM) micrographs show that the synthesized ZnO have irregular shapes and particle size is in the range of 50 to 100 nm. The results of the XRD pattern revealed that the particles were crystalline. EDX confirmed the presence of pure ZnO NPs [44].

Klink *et al.* used urea-based synthesis method to prepare ZnO NPs using urea and zinc acetate as an initial reagent. The ZnO NPs particles thus prepared were characteristized by various

techniques like Transmission electron microscopy Scanning electron microscopy, Fourier transform infra-red spectroscopy, X-ray diffraction and UV-Vi's spectroscopy. XRD data revealed the structure of ZnO NPs as hexagonal wurtzite structure. SEM micrographs showed that the synthesized ZnO NPs were spherical in shape. Through FT-IR spectra, absorption at 457cm^{-1} recognizes the peak for Zn – O stretching vibration. TEM images show agglomerated surface morphology with round hexagonal shapes and the average particle size was found to be 100, 75, 40 and 33 nm. UV – Vi's spectroscopy exhibits maximum absorption peak at 320 nm for ZnO NPs [45].

Swart *et al.* used a mixture of fuel approach in solution combustion method to prepared ZnO NPs using zinc nitrate hexahydrate, citric acid, glycine, and urea as precursors. The zinc nitrate hexahydrate was dissolved in deionized water and then stirred the solution. After stirring, the solution was kept in the furnace at 600°C to start the combustion process and thus the ZnO NPs were formed. The obtained product was characteristized by Fourier transform infra-red spectroscopy (FT-IR), X-ray diffraction (XRD), and Ultraviolet UV-Vi's spectroscopy. XRD confirmed the crystalline nature of synthesized ZnO NPs. The crystallite size was 2640 nm. The stretching mode of the vibration of ZnO at 482-455 cm^{-1} was confirmed by the FT-IR spectrum. Optical properties were determined by UV-Vi's spectroscopy. The photoluminescence spectra showed two ZnO peaks at 384 and 632 nm, respectively [46].

Antibacterial Activity of Zinc Oxide Nanoparticles

Kiranmayip *et al*. synthesized ZnO NPs by co-precipitation method using zinc sulfate and sodium hydroxide as an initial reagent. Structural and morphological properties were analyzed by XRD, FT-IR, UV-Vis, SEM and EDX. Further, the antibacterial activity of ZnO NPs were studied against Gram- positive bacteria (Staphylococcus aureus and streptococcus mutans) and Gram- negative bacteria (Escherichia coli and Proteus vulgaris) using agar well diffusion and broth dilution method. The antibacterial activities at different concentrations 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml were measured. Results showed that degree of zone of inhibition (ZOI) at 50mg/ml was more against Gram- negative bacteria E. Coli (32±0.20 mm) and Proteus vulgaris ((30±0.45 mm) as compared to Gram- positive bacteria Staphylococcus aureus (24±0. 35mm) and Streptococcus mutans ((23±0.30mm) because of thick membranes of Gram - negative bacteria [43].

Jeyasubramanian *et al.* employed a simple precipitation method using zinc acetate and ammonium oxalate to synthesize ZnO NPs. The resultant samples were investigated by FT-IR, XRD, FESEM, and PL spectroscopy. The antibacterial activities against three bacterial strains such as Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus were reported using the broth agar method. ZnO NPs show a maximum zone of inhibition (11mm) towards Pseudomonas aeruginosa followed by Escherichia coli (9 mm). However, it shows no zone of inhibition towards Staphylococcus aureus because of the thick peptidoglycan cell membrane of Gram – positive bacteria. Amikacin was used as positive control [47].

Sharma *et al.* Prepared ZnO NPs via sol gel method using zinc acetate and oxalic acid as precursor. The precipitated obtained was thus heated at 87°C for 2hrs.The prepared solid were characterized using XRD, SEM, and UV-Vi's spectroscopy

Antibacterial testing was performed against Gram-negative bacteria (Escherichia coli) with standard (Erythromycin and Vancomycin) by disc diffusion method. Results indicate that ZnO NPs had strong antibacterial activity against Escherichia coli followed by Erythromycin, and Vancomycin respectively [48] .

Belay *et al.* used a wet chemical method to prepare ZnO NPs using zinc nitrate hexahydrate, sodium hydroxide, zinc chloride, zinc acetate dihydrate and caffeine as initial reagents. The ZnO samples were characteristized by XRD, FT-IR, SEM, and UV-Vis Spectroscopy. Antibacterial activity of ZnO NPs was determined by agar disc diffusion method for both Gram - positive (S. aureus) and Gram- negative bacteria (E.coli). Antibiotic Vancomycin was used as positive control. The result revealed that ZnO NPs exhibited good antibacterial activity than the standard antibiotics (vancomycin). Further it can also conclude that S. aureus exhibited more sensitivity towards Zn NPs as compared to E. coli bacteria [49].

Rashid *et al.* synthesized ZnO NPs via chemical method using zinc acetate heptahydrate ($ZnSO_4.7H_2O$) and sodium hydroxide (NaOH). ZnO NPs were determined by XRD, FT-IR, EDX, SEM and UV – VIS spectroscopy. Furthermore, they investigate the antibacterial activity of ZnO NPs using broth dilution method against methicillin _resistant Staphylococcus aureus (MRSA) at different concentrations (0.5, 0.25, 0.125, 0.0625, 0.3125) mg/ml. They concluded that ZnO NPs showed a maximum zone of 16± 03 mm and 17 ± 02 mm at high concentrations 0.125, 0.25 mg/ml, respectively [50].

Mena *et al.* used a thermal decomposition method to synthesize ZnO NPs using zinc carbonate and potassium hydroxide as an initial reagent. The characterization of Zinc oxide nanoparticles was examined by UV-Vi's spectrometer, thermoluminescence (TL) reader. The author further investigated the antibacterial activity of ZnO NPs against Pseudomonas aeruginosa and Escherichia coli bacterial strains by determining the zone of inhibition. They concluded that antibacterial activity of ZnO NPs is very high as in case of Pseudomonas aeruginosa which is around (9 mm) zone of inhibition [51].

Verma *et al*. synthesized ZnO NPs via precipitation method using zinc acetate and sodium hydroxide as precursors. The Prepared ZnO NPs were characterized by UV-Vis and Dynamic light scattering (DLS) spectroscopy. Antibacterial action cof ZnO NPs was tested against Gram- positive bacteria (S. aureus), and Gram- negative bacteria (E. coli) through Kirby-Bauer method. Results indicate that ZnO NPs had strong antibacterial activity against Gram - positive bacteria (S. aureus) as compared to the Gram- negative bacteria (E. coli). The antibacterial activity increases as the concentration of the zinc oxide NPS increases [52].

Hussein *et al*. Prepared ZnO NPs through thermal precipitation method using zinc nitrate hexahydrate ($Zn\ (NO_3)_2.\ 6H_20$) and potassium hydroxide (KOH) as a precursor. Characterization of the synthesized ZnO samples was analyzed by SEM, XRD, and EDX. Moreover, the synthesized ZnO were evaluated for their antimicrobial activity against Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa) and Gram- positive bacteria (Staphylococcus aureus). Antibacterial activity was performed by agar well diffusion method. In conclusion, the results revealed that ZnO NPs performed better antibacterial activity towards Gram - positive bacteria (higher zone of inhibition) as compared to Gram- negative bacteria (lower zone of inhibition) [53]Vignesh *et al.* synthesized ZnO NPs via sol gel method using zinc nitrate hexahydrate and sodium hydroxide as precursors. The synthesized ZnO NPs were analyzed by using different techniques such as XRD and SEM. The author further investigated the antibacterial action of ZnO NPs against Gram- negative bacteria (Escherichia coli) by well diffusion method. Escherichia coli showed a zone of inhibition (5.4 mm, 6.2 mm, 6.7 mm and 7.2 mm) for the comparable concentrations (25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml). It was concluded that the diameter of the inhibition zone increases as the concentration of ZnO NPs increases [54].

Albuhaty *et al*. used the sol gel method to synthesize ZnO NPs at room temperature. Zinc acetate dihydrate and sodium hydroxide were used in the experiment. Properties of the synthesized ZnO NPs were confirmed using different techniques DLS, SEM and. XRD. The author further investigated the antibacterial activity of ZnO NPs against Staphylococcus aureus and Escherichia coli by agar diffusion method. Results indicate that ZnO NPs at concentration 6.25 µg/ml exhibited the same MIC and MBC results for both bacterial strains [55].

Ficai *et al.* employed a simple – precipitation method to Prepared ZnO NPs using zinc acetate dihydrate and sodium hydroxide as an initial reagent. After synthesis, the particles were characterized by FT-IR, XRD, SEM and TEM. Agar garwell diffusion method was performed to check their antibacterial properties against Gram – positive bacteria (Staphylococcus aureus) and Gram – negative bacteria (Escherichia coli). Among tested bacterial strains Staphylococcus aureus showed high sensitivity towards ZnO NPs with zone of inhibition (19 mm). This was due to greater sensitivity of Gram- positive bacteria versus Gram – negative bacteria [33].

Annadurai *et al.* Prepared ZnO Nanoparticles using zinc acetate and sodium hydroxide as precursors through a wet chemical method. The obtained product was examined by Fourier transform infra-red spectroscopy, X-ray diffraction, and UV-Vi's spectroscopy. Furthermore, the author examined the antibacterial activity of ZnO NPs against Escherichia coli, and Pseudomonas aeruginosa. The antibacterial activity of ZnO Nanoparticles was carried out through broth dilution method at various concentrations (20 µl, 50 µl and 100 µl). Results revealed that the synthesized ZnO NPs show a maximum zone of inhibition (100 µg) at high concentration (100µ/L). Thus, it is concluded that the antibacterial activity of ZnO NPs increases as the concentration of ZnO NPs increases [56].Klink *et al.* ZnO NPs were Prepared through the microwave heating crystallization method using zinc acetate and urea as initial reagents. The synthesized product was analyzed by X-ray diffraction, Fourier transform infra-red spectroscopy, and Transmission electron microscopy. The antibacterial activity of synthesized ZnO NPs were tested against Gram – positive bacteria (Staphylococcus aureus) and Gram – negative bacteria (Escherichia coli, Salmonella enterica, and Shigella sonnei) through agar well diffusion method. Antibiotic neomycin was used as standard. Results indicate that ZnO NPs were more effective against standard neomycin and show high zone of inhibition as compared to Gram - positive and Gram – negative bacteria [43].

Khan *et al.* Synthesized thorn- like ZnO NPs through sol – gel method using zinc acetate dihydrate and sodium hydroxide as precursors. The obtained product was determined by X-ray diffraction, Fourier transform infrared spectroscopy, Transmission electron microscopy, Scanning electron microscopy, Thermal gravimetric analysis, and UV-Vi's spectroscopy. Moreover, the author explored the antibacterial activity of prepared thorn-like ZnO NPs against Gram – positive bacteria (Bacillus subtilis) and Gram – negative bacteria (Escherichia coli). The antibacterial activity was carried out through a disc diffusion method. It was concluded that Bacillus subtilis show maximum zone of inhibition (23mm) as compared to the Gram – negative bacteria (Escherichia coli) due the presence of special cell wall structure [57].

SYNTHESIS AND CHARACTERIZATION

Materials

Zinc nitrate and ammonium hydroxide were used in the experiment. All of the chemical compounds were analytical reagent grade, and they didn't need to be further purified before use, additionally, bacteriological grade nutritional agar and broth were acquired in order to achieve an antibacterial activity. Pyrex glassware was used throughout the synthesis process.

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Deionized water was employed in the preparation of all types of solution.

Synthesis of ZnO Nanoparticles

Zinc oxide nanoparticles were synthesized by using zinc nitrate and ammonium hydroxide as precursors using controlled precipitation method. Aqueous solution of ammonium hydroxide (25%) and zinc nitrate (0.10 .2mol/L) were prepared for this purpose. Both the solutions were heated at constant temperature for 5_30 min time intervals. In few cases, aqueous solutions of zinc nitrate were purified with ammonia gas at the flow rate of 110 mL/min under the same reaction conditions. The precipitated powders were then purified by using air-dried, vacuum filtration and stored for further work.

Characterization of ZnO Nanoparticles

X-ray diffractometry (XRD)

XRD was used to identify the phase and crystallinity of the synthesized materials. Monochromatic Cu - kα radiation at a wavelength of 1.54060 Å was used in the X-ray diffraction (XRD) analysis of the dried ZnO NPs using an X-ray diffractometer (JEOLXRD, JDX-3532) with a detector voltage of 40 KV and a current of 30 mA. The recorded range of 2θ was 15°-80° with a scanning speed of 6 °/min. Using the Debye_Scherre formula, the crystallite size of the synthesized ZnO was calculated.

Fourier Transforms Infra-Red Spectroscopy (FT-IR)

An infra–red Fourier transform spectrometer (Shimadzu - 8400S, IR Prestige_21) using KBr pellet method was used to explore the molecular structure of the synthesized product. The FT-IR spectrum of the synthesized powder sample was recorded in the range of 400-4000 cm^{-1} at room temperature. The structural morphology and elemental composition of the Prepared ZnO oxide nanoparticles was studied and measured by scanning electron microscopy (SEM; JEOL, JSM-5910, Japan).

Antibacterial Activity Test

The Antibacterial activity of the Prepared ZnO NPs was determined by agar well diffusion method against the two strains of Gram – positive bacteria (S. aureus and S. mutans) and the two strains of Gram–negative bacteria (E. coli and Citrobacter). Each culture of test microorganisms at the concentration of 7.5× 106 CFU/50 µL spread uniformly on nutrient agar plates. The agar wells are established using a sterile cork – borer with a diameter of 8mm. About 20 µL of the synthesized ZnO NPs were then added to each well, and bacterial plates were cultured at 37°C for 24 hours using a series of various concentrations (0.25, 0.50, and 0.75 µg/µL). Antibiotic Ciprofloxacin was used as a standard. The antibacterial activity of prepared ZnO NPs was resolved by the formation of an inhibition zone. The diameter of the zone of inhibition was calculated in mm

RESULT AND DISCUSSION

Synthesis of Zinc Oxide Nanoparticles

Zinc nitrate and ammonium hydroxide aqueous solution was heated at a consistent temperature to produce uniform and innovative ZnO particles. In every instance, the preparation processes that primarily manifested in the aqueous medium led to the formation of ZnO particles in the reaction mixture. The following equations serve as a summary of the process by which ZnO is produced when dissolved Zn^{2+} reacts with ammonia.

$NH_3 + H_2O \rightleftharpoons NH^+ + OH^-$ (1) $Zn^{+2} + 4NH_3 \rightleftharpoons [Zn$

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$(NH_3)_4]^{2+} \rightarrow ZnO + 4NH_3 + 2H_2O$ (2) $[Zn\ (NH_3)_4]^{2+}]$
$+4OH^- \rightarrow [Zn\ (OH)_4]^{2-} + 4NH_3$ (3) $Zn^{+2} + 4OH \rightleftharpoons [Zn$
$(OH)_4]^{2-} \rightleftharpoons ZnO + H_2O + 2OH^-$ (4)

Scanning Electron Microscope (SEM)

The morphological investigation of ZnO NPs was carried out by SEM, according to the results of the SEM analysis, the majority of the precipitated solids was primarily composed of irregularly shaped particles. It was commonly acknowledged that the particle's morphological properties were also influenced by the reactant solution ph. The particle size increases as the pH of the reaction medium increases which is also followed by a change in particle shape. The SEM image made it clear that the produced powders were composed of colloidal nanospheres with magnificent uniformity in particle size and shape. The particle took on the shape of monodispersed ellipsoidal nanorods when the pH of the reactant mixture was raised.

Antibacterial Activity

For evaluation of antibacterial activity, two samples of ZnO (ZnO-1, ZnO-2) were examined against two Gram - positive bacterial strains such as (S. aureus and S. mutans) as well as two Gram-negative bacterial strains (E. coli and Citrobacter). The antibacterial activity of ZnO NPs was performed by agar diffusion method. Three different concentrations (0.25, 0.50, 0.75 µg/µL) of the prepared ZnO samples were used. As standard, ciprofloxacin was also used. The antibacterial activities were calculated by knowing the diameter of the inhibition zone throughout the wells. The inhibition zone produced by the prepared ZnO samples against both Gram - positive and Gram - negative bacterial strains are demonstrated on the figure 4,5 respectively. Anyhow, further studies proved that ZnO toxicity depends on several conditions like size, morphology, porosity, and concentration of the particles but the present study focuses on the concentration of ZnO SNPs. Thus, it means that the concentration of ZnO.

NPs have an impact on the antibacterial activities. Higher the concentration of ZnO NPs, high will be their antibacterial activity. The diameter of the zone of inhibition increases with the increase in the concentration of ZnO NPs.

From table 2, it is illustrated that the zone of inhibition observed in the present study was higher for the S. aureus (33 mm) at higher concentration 0.75 µg /µL. One of the main reasons for this higher bacterial activity is due to the structural difference in the bacterial membrane. Moreover, some researchers understood that the structure of the bacterial cell also affected the inhibitory action of the nanoparticles. A membrane encloses Gram- positive bacteria cells, which are primarily formed of peptidoglycan layers. Gram- negative bacteria have more convoluted cell walls because they have an exterior membrane consisting of lipopolysaccharides and a thin peptidoglycan layer. Thus, the outer membrane of Gram – negative bacteria act as permeability barrier, so that absorption of ROS in the cell is reduced. Thus, Gram negative – bacteria show increased resistance to ZnO NPs as compared to the Gram – positive bacteria

The exact mechanism of antibacterial activity of ZnO NPs has not settled, but there have been multitudinous proposed procedures including (1) generation of reactive oxygen species (ROS), (2) release of antimicrobial metal ions, (3) direct interconnection of ZnO particles into the bacteria membrane, leading to the physical damage of the cell wall and eventually to the microorganism death.

It is deliberate that the antibacterial activity of ZnO through the well diffusion method was probably due to the disturbance of the cell membrane by direct interconnection of ZnO with

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the bacterial membrane. The effect of ZnO on the bacteria cell is trigger by electrostatic forces

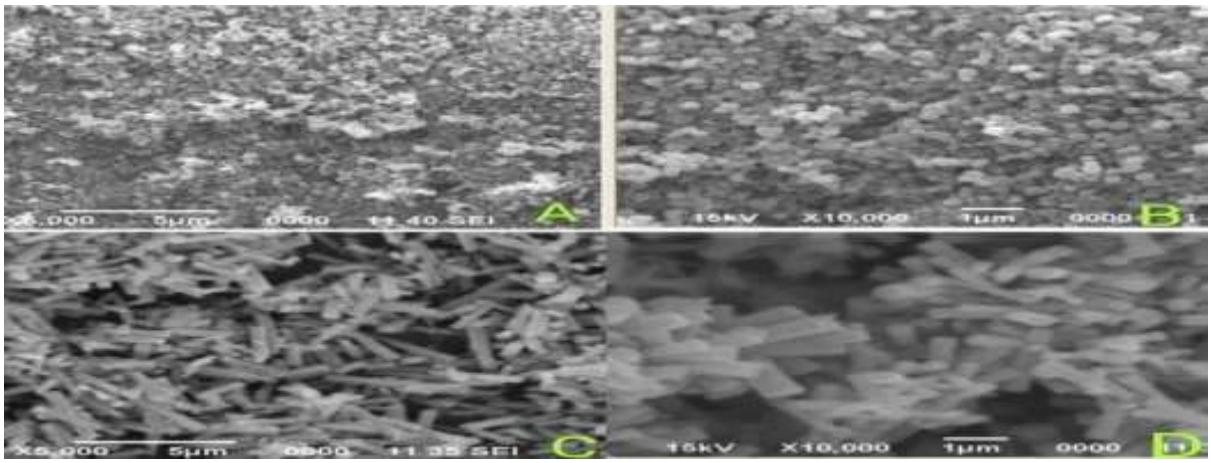

Figure 3: SEM Micrographs of ZnO-1 and ZnO-2

Bacterial cells have a negative charge at biological pH as a result of an excess of carboxylic groups that detach and cause the cell surface to have a negative charge. Contrarily, with a zeta potential of +24 mV, ZnO are positively charged. Due to electrostatic forces established by the opposite charges between the nanoparticles and bacteria, the bacteria surface and the nanoparticles attach firmly, inevitably causing cell membrane damage.

FT-IR Analysis of Zinc Oxide Nanoparticles

The composition and purity of synthesized ZnO samples (ZnO –1 and ZnO –2) were determined by FTIR spectroscopy. The FT-IR spectrum of ZnO NPs was recorded in the range of 400-4000 cm^{-1}.The FT-IR spectrum was made up of a number of absorption bands that occurred as a result of distinct chemical groups present on the surfaces of test particles. The peaks at 1518-1323 cm^{-1} were due to the bending vibration of OH. Furthermore, the peaks at 3800-3100 cm^{-1} are due to the stretching vibrations of OH. Additionally, the characteristic absorption peak of Zn – 0 stretching vibrations was in the range of 527-430 cm^{-1}.

XRD Analysis of Zinc Oxide Nanoparticles

The White powder ZnO NPs from the preparation step of the material was subjected by XRD analysis. Through XRD technique, the composition, phase and crystallinity of ZnO NPs were analysed. XRD patterns of the powder ZnO-1 and ZnO-2 were recorded over the 2 θ range of 15°- 80°. The strong and narrow diffraction peaks describe that the product has a well crystalline nature of particles. All the diffraction peaks are in good agreement with standard data of hexagonal phase of ZnO ICDD NO. 50664. The crystalline size of the samples (ZnO-1 and ZnO–2) were estimated from major peaks of obtained XRD pattern with characteristic reflection lines identified as (100), (002), (101), (102), (110), (103), (200), (112) and (201) respectively. The presence of similar diffraction peaks for both samples (ZnO-1 and ZnO-2) with different intensities is due to different crystal arrangement of atoms in both morphologies. No other peaks are observed in the patterns which indicate the absence of impurities.

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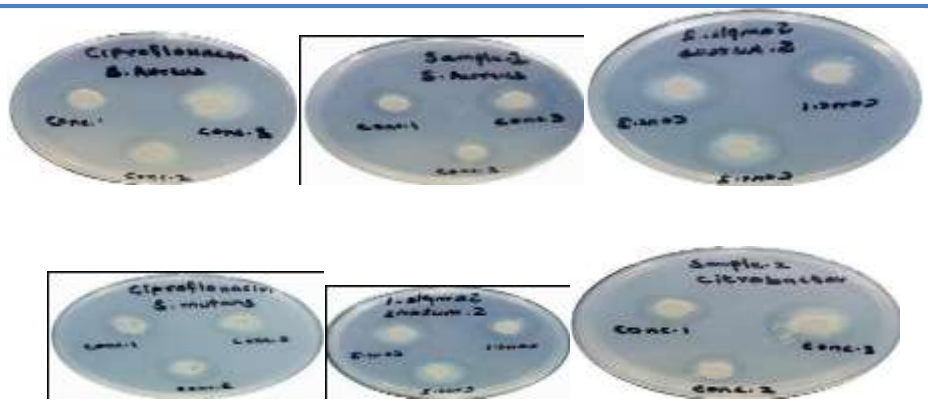

Figure 4: Comparative Antibacterial Activity of ZnO and Positive Control against Various Gram-Positive Bacterial Strains (Conc. 1 - 0.25 µ g / µ L; Conc. 2 – 0.50 µg/µL; Conc. 3 – 0.75 µg/µL)

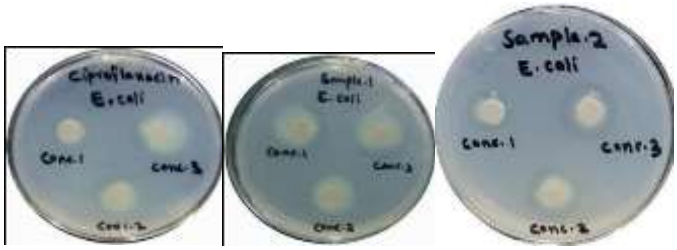

Figure 5: Comparative antibacterial activity of ZnO and positive control against various Gram-negative bacterial strains (Conc 1 – 0.25 µg/µL; Conc. 2 – 0.50 µg/µL; Conc. 3– 0.75 µg/µL) 0.25 µg/ µL 0.50µL 0.75µg/ µ 0.25 µL/ µL 0.50 µg/ µL 0.75 µg/ µL 0.25 µg/ µL 0.50 µg/ µL 0.75 µg/ µL

Table 2: Antibacterial activities of ZnO against Test Bacterial Strains Bacterial Zone of Inhibition/mm

Strain	Sample 1			Sample 2			positive control		
S.aurs	23	24	29	27	28	30	23	24	30
S.mut ans	28	31	33	20	21	31	27	28	29
E. coli	28	30	32	18	21	24	20	25	29
Citrob actor	24	25	28	24	24	28	22	25	27

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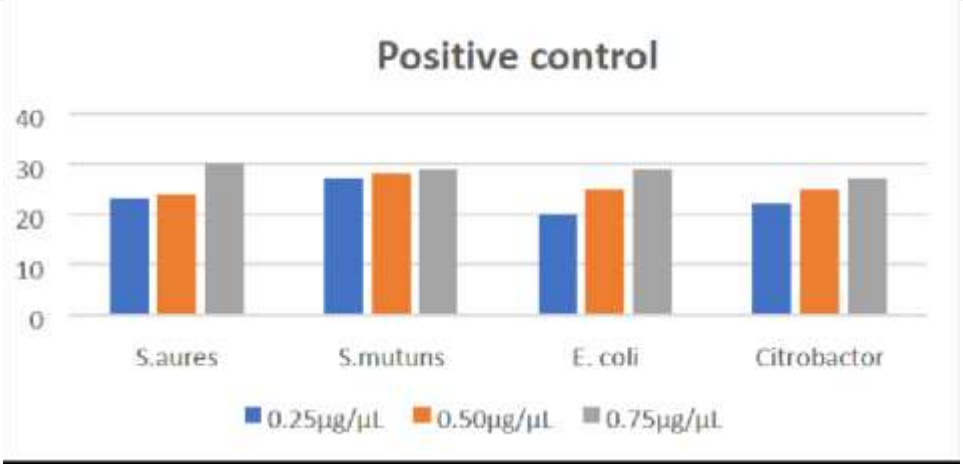
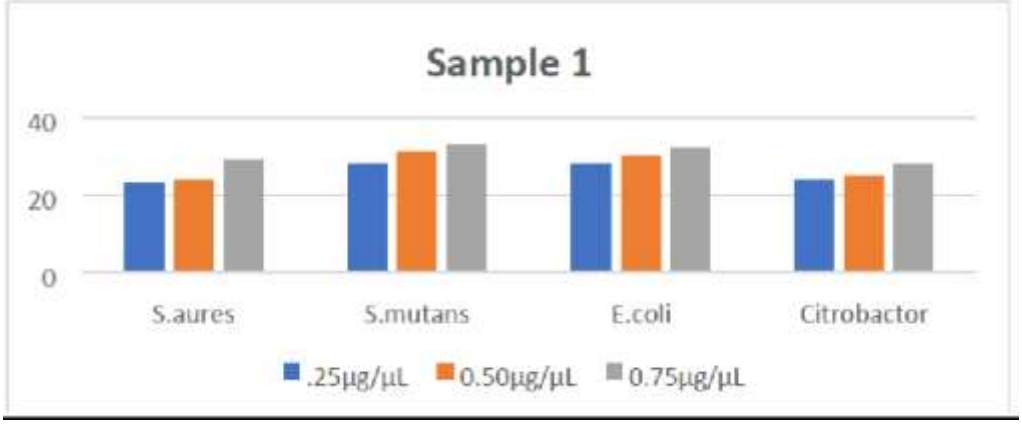
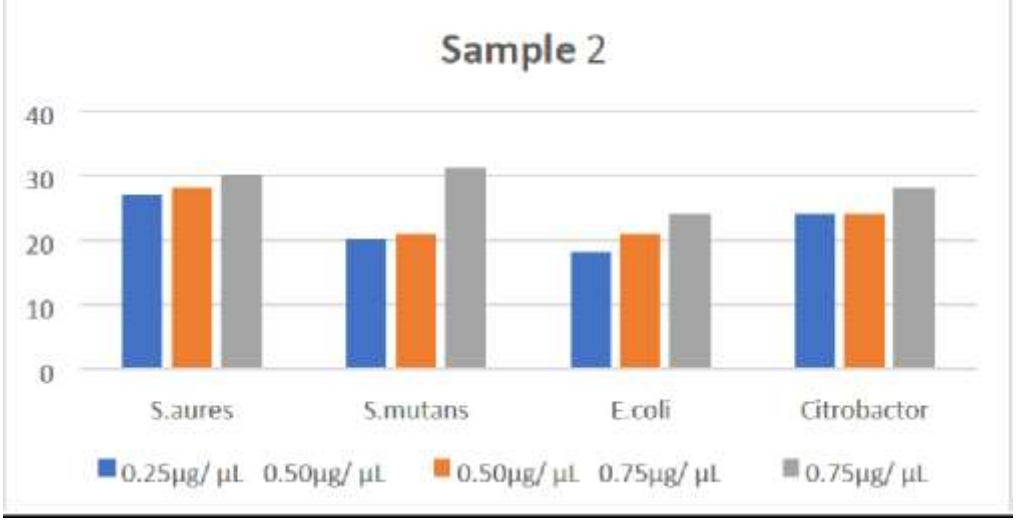


Figure 6: Comparative Bar Graph Showing Zone of Inhibition Introduced by Different Concentration of ZnO NPs





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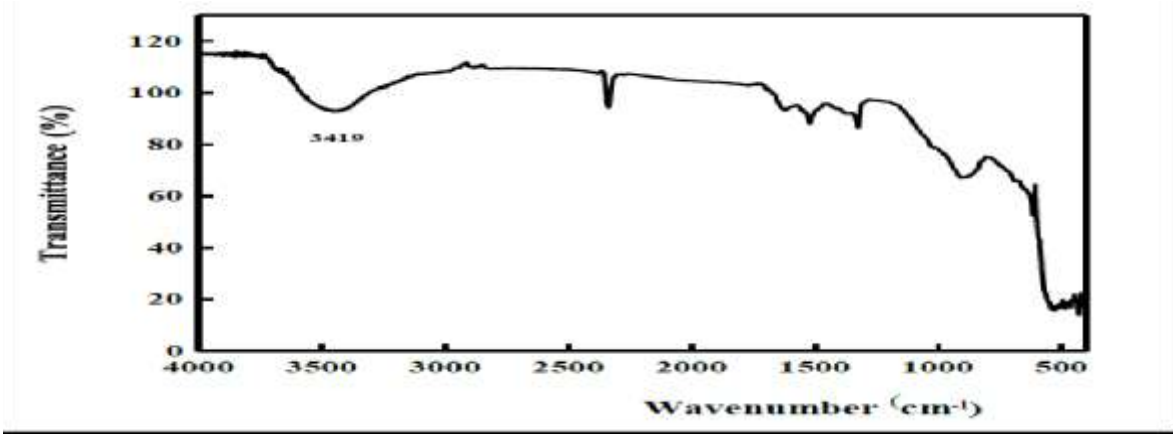

Figure 7: FTIR Spectra 0f ZnO-1 Particle

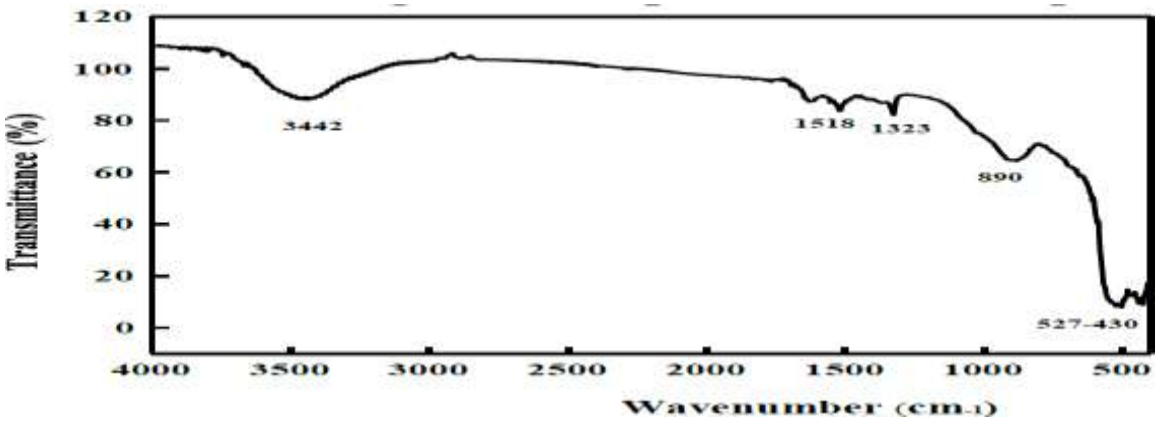

Figure 8: FTIR Spectra of ZnO-2 Particle

The crystallite size of ZnO-1 and ZnO-2 NPs were measured from FWHM of the most intense peak using Debye – Scherrer formula given below.

$D=K\lambda/\beta . \cos\theta$

Where k is Scherrer constant, λ is the wavelength of X-ray, B is the peak width at half of maximum and θ is the diffraction angle. The average crystallite size of the ZnO-1 and ZnO-2 was 13.76 and 9.95 nm respectively.

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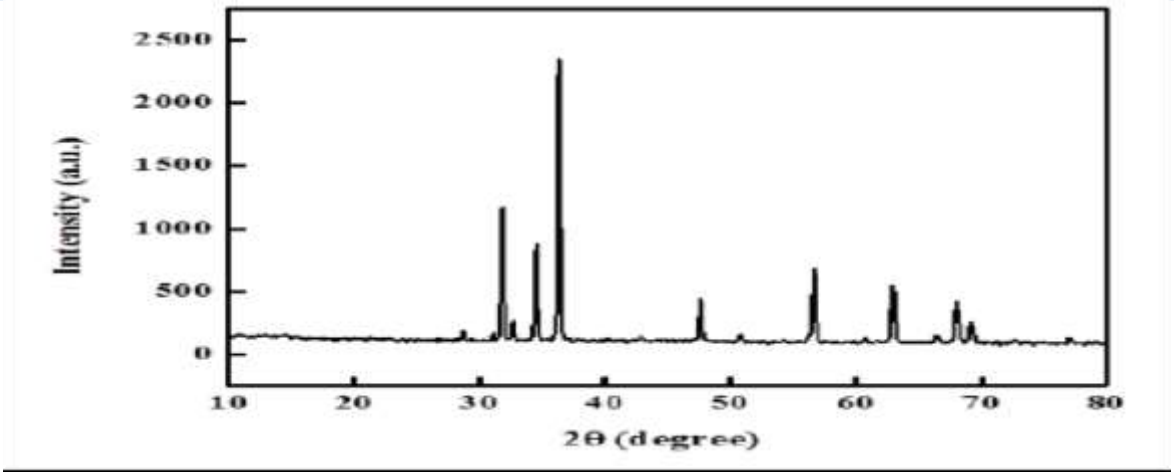

Figure 9: XRD Pattern of Prepared ZnO-1 Particle

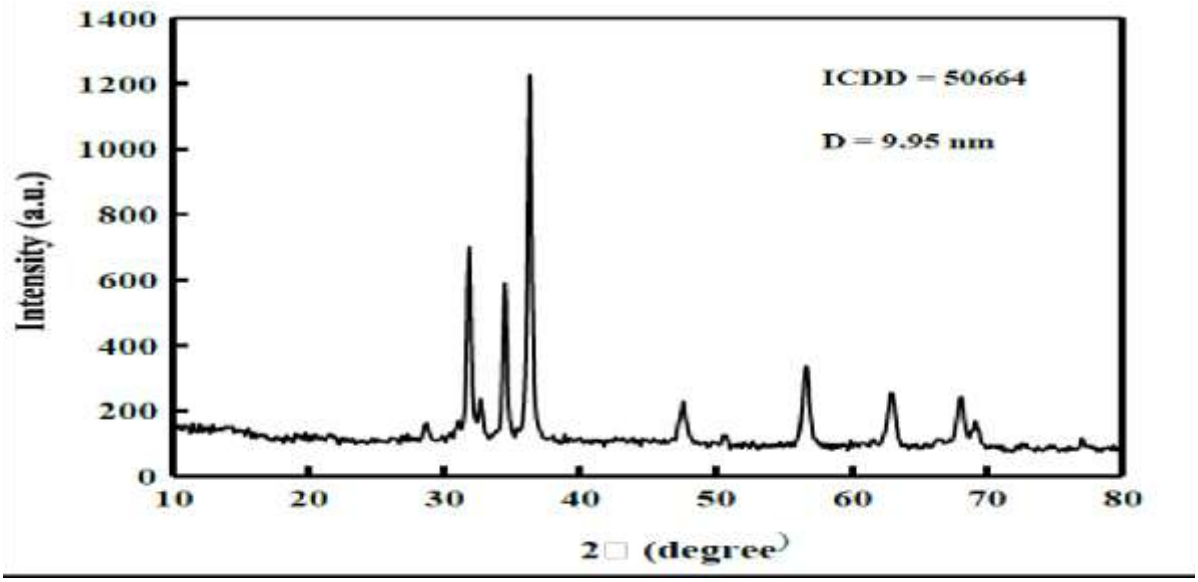

Figure 10: XRD Pattern of Prepared ZnO-2 Particle

Table 3: Crystallite Size With Respective Diffraction Peaks for Synthesized ZnO Samples

Sample	XRD peak	θ /rad	FWHM/rad	Crystallite size/nm	Average Crystallite size/nm
ZnO-1	(100)	0.277	0.0097	14.22	13.76
ZnO-1	(002)	0.300	0.0104	13.39	
ZnO-1	(101)	0.316	0.0102	13.66	
ZnO-2	(100)	0.277	0.0162	8.6	9.95
ZnO-2	(002)	0.300	0.0150	9.3	
ZnO-2	(101)	0.316	0.0117	11.95	

CONCLUSION AND RECOMMENDATIONS

Conclusion

Following closures may be made based on this study:

1. ZnO NPs were synthesized by controlled precipitation method.

2. The hexagonal wurtzite and crystalline nature of prepared ZnO – 1 and ZnO – 2 were determined by XRD analysis. The average crystallite size of the ZnO – 1 and ZnO–2 was 13.76 nm and 9.95 nm respectively.
3. Through FT-IR, the peaks of Zn –O stretching vibrations at 527 - 430 cm^{-1} were identified.
4. SEM images shows the nanorod and nano sphere morphology of ZnO -1 and ZnO-2 nanoparticles.
5. The antibacterial activity unveils that the ZnO nanostructure with high concentration has a strong effect on inhibiting the growth of bacteria. Moreover, the antibacterial activity of the prepared powder was more towards Gram – positive bacteria. This study proved that the ZnO nanoparticles have an implicit to be developed as antibacterial agents

Recommendations

Optimization of Synthesis Conditions

- To produce ZnO NPs with the appropriate properties, research and optimise the synthesis parameters, including temperature, pH, precursor concentration, and reaction time. XRD research showed that better crystallinity and size uniformity could result from fine-tuning the synthesis conditions.
- Carry out a methodical investigation to comprehend the connection between synthesis parameters and the XRD-determined structural properties of ZnO NPs. By doing this, you will be able to create a reliable synthesis protocol that will allow you to regularly produce high-quality nanoparticles.

Comprehensive Characterization

- Go beyond FT-IR, SEM, and XRD characterization techniques to incorporate additional analytical techniques such energy-dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM), and UV-Vis spectroscopy. These extra methods can offer a more thorough comprehension of ZnO NPs' dimensions, composition, form, and optical characteristics.
- Conduct a thorough examination of FT-IR spectra in order to pinpoint particular functional groups or chemical bonds connected to the stabilizing agents or surface modifications applied during the synthesis. This will assist in clarifying the nanoparticles' surface chemistry and chemical makeup.

Enhancement of Antibacterial Activity

- Examine ZnO NPs' antibacterial capabilities against a wider variety of bacterial strains to gauge their potential as antimicrobial agents. To find the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) for various microorganisms, carry out thorough dose-response investigations.
- Investigate methods to improve ZnO NPs' antibacterial activity, such as creating ZnO nanocomposites or altering their surface with bioactive chemicals. These changes may increase the nanoparticles' ability to combat different infections while decreasing

cytotoxicity to human cells

- Examine the antibacterial action mechanism by sophisticated methods such as molecular biology assays or electron microscopy to comprehend how ZnO NPs interact with bacterial cells and inhibit their growth.

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