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Research Article

Synthesis, characterization and evaluation of antibacterial efficacy of zinc oxide nanoparticles

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ABSTRACT

Objective: Objective of the study was to synthesize and characterize Zinc oxide (ZnO) nanoparticles (NP), and to evaluate their application on some bacterial strains.

Methods: ZnO NP was synthesized by chemical methods. Then decomposed by using conventional heating process. The detailed characterization of the nanoparticles was performed using FT-IR, UV-Vis spectroscopy, X-Ray Diffraction analysis and XRF. From the analysis of XRD pattern, UV-VIS spectroscopy and XRF, the formation of nanoparticles was confirmed. Antibacterial assay of synthesized ZnO NP was carried out both in liquid and solid growth medium against a gram positive (*Staphylococcus aureus*) and a gram negative (*Escherichia coli*) bacteria using disc diffusion assay method. Effect of antibacterial activity was observed by zone of inhibition around the antibiotic discs of ZnO NP.

Results: ZnO NP was characterized by the different spectral analysis of the synthesized product. ZnO NPs reveal good antibacterial activity against *S. aureus* and *E. coli*. Kinetic studies were conducted on growth bacteria by loading ZnO NP to *S. aureus* and *E. coli* with this concentration to study the kinetic of growth behavior which showed that NP produced toxicity on both bacteria and therefore the growth was inhibited.

Conclusions: The inhibition of growth of the organisms by ZnO nanoparticles suggests that it could potentially be used as an effective antibacterial agent and as well can be used in the protection of agricultural and food safety. Future studies may be aimed at the further evaluation to establish the nanoparticles as potential antimicrobial agent.

Keywords: Chemical synthesis, Antibacterial effect, *Staphyloccus aureus*, Escherichia coli, Zinc oxide nanoparticles

Introduction

Since many years, people have been preparing the glass windows with tiny colored metal particles especially silver which provide glassy yellow color.[1] The chronicle of nanomaterials is

quite long; however, major developments within nanoscience have taken place during the last two decades. The main important thing in nano dimension is the properties of particles are far different than bulk scale properties. Nanoparticles are being used in different fields including electrical, biological textile and chemistry in which shape and size of colloidal metal particles play crucial role in different application including preparation of magnetic, electronic devices wound healing, anti-microbial gene expression and in the preparation of bio composites. Noble metal colloids have the optical, catalytical electromagnetic properties which are dependent on size and shape of the particles.[2] The synthesis processes for the preparation of colloidal nanoparticles with controlled morphology is crucial.

Once materials are prepared in the form of very small particles, they change significantly their physical and chemical properties. In fact in nano-dimension, percentage of surface molecule compare to bulk molecule is high and this enhances the activity of the particle in nano dimension and therefore, the normal properties of the particle like heat treatment, mass transfer, catalytic activity, etc are all increases. But compare to non-metal nanoparticles, metal nanoparticles have more industrial application. Nanoparticles offer many new developments in the field of biosensors, biomedicine and bio nanotechnology-specifically in the areas- y Drug delivery y as medical diagnostic tools, y as a cancer treatment agent (gold nanoparticles). Nanoparticles and nanostructure are becoming a part in human medical application, including imaging or the delivery of therapeutic drugs to cell, tissues and organs. Drug loaded nanoparticles interact organ and tissues and are taken up by cells. Several studies have shown that the tissue, cell and even cell organelle distribution[3,4] of drugs may be controlled and improved by their entrapment in colloidal nanomaterials, mainly of the micellar structure, such as nanocontainer. Magnetic nanoparticles have been receiving considerable attention because of their wide range of applications, such as the immobilization of the proteins and enzymes, bioseparation, immunoassays, drug delivery, and biosensors.[5] Nanoparticles of ferromagnetic materials are of importance because of their reduced sizes that can support only single magnetic domains. The recent synthesis of arrays of 4 nm diameter Pt nanoparticles with an extremely narrow size distribution has promoted a significant research effort in this area, due to their potential technological application as recording media.[6]

The bulk termination is also subject to high concentrations of dimer vacancies which correspond to fractional occupations in the surface layers.[7] Mechanical properties such as internal stress or adhesion are important in order to guarantee the patterning accuracy and durability for various types of commercial applications.

Nanoparticles of oxides like Zinc Oxide nanoparticles (ZnO NP) can be synthesized by various techniques such as chemical vapor method, spray pyrolysis, laser synthesis techniques and vapor condensation method. Among these techniques available, the vapor condensation method have been considered to be the most attractive due to its robust and reliable control of the shape and size of the nanoparticles without requiring the expensive and complex equipments.

ZnO NP is currently being considered as an antibacterial agent in both micro as well as nano scale formulations.[8] Results have specified that ZnO nanoparticles show antibacterial action apparently better than micro particles. While the exact mechanisms of the antibacterial action have not yet been clearly understood, it has been advised that the rule of reactive oxygen species (ROS) produced on the surface of the particles, zinc ion release, membrane dysfunction, and nanoparticles internalization are the main reason of cell swelling.[8]

Metal nanoparticles are highly ionic in nature and can be prepared with very high surface areas and with unfamiliar crystal and morphologies that possess several edge/corner and other reactive surface sites.[8]

Materials and Methods

Pure and analytical grade chemicals were used

in all experiments including synthesis of ZnO and stock cultures, media preparation for growth of bacterial cells. Zinc nitrate hexahydrate, sodium hydroxide (NaOH), absolute ethanol and starch.

Synthesis of ZnO NPs

Wet chemical method was used to prepare ZnO NP using zinc nitrate and sodium hydroxides precursors and soluble starch as stabilizing agent. Soluble starch (0.5%) was dissolved in 500 ml of distilled water. Zinc nitrate, 14.874 g (0.1 mol), was added in the above solution. Then the solution was kept under constant stirring at room temperature using magnetic stirrer for one hour. After complete dissolution of zinc nitrate, 300 ml (0.2 mol), of sodium hydroxide solution was added under constant stirring, drop by drop touching the walls of the container. The reaction was allowed to continue for 2 h after complete addition of sodium hydroxide. After the completion of reaction, the solution was allowed to settle for overnight and the supernatant solution was then discarded carefully. The remaining solution was centrifuged at 10,000 × g for 10 min and the supernatant was discarded. Thus produced nanoparticles were washed three times using distilled water. Washing was carried out to remove the byproducts and the excessive starch that were bound with the nanoparticles. After washing, the nanoparticles were dried at 80°C for overnight. Put it in furnace at 350°C /2 h further completion of conversion of $Zn(OH)_2$ into ZnO NP.

The characterization of the nanoparticles was done using Fourier transform infrared spectroscopy (FT-IR) (Model: SHIMADZU), UV-Vis spectroscopy (Model: Perkin Almer Llambda 25 UV-Vis spectrophotometer), X-Ray diffraction (XRD-Model: Panalytical Empyrean) analysis and XRF (X-ray fluorescence-Model: Rigaku-NEX CG).

Bacterial samples used

The bacterial cultures of *Staphylococcus aureus* and *Escherichia coli* were obtained from General Microbiology laboratory, Koya University. The bacterial stock cultures were maintained on nutrient agar slants at 4°C.

Preparation of antibiotic disc

The stock was prepared from 0.1 gm of ZnO NP dissolved in 10 ml distilled water and filter paper discs. The filter paper discs soaked with stock of ZnO NP, then kept the impregnated discs in incubator at 37°C for 1 h further drying.

Disc diffusion method

The antimicrobial activity of samples was evaluated by disc diffusion method as described by Kirby-Bauer.[9] Loop full growths from bacterial isolate were inoculated into nutrient broth incubated at 37°C for 18 hours. The bacterial suspensions were diluted with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension. Cotton swab was dipped and streak into adjustment suspension the entire Mueller-Hinton agar. Sample pleats or discs were gently pressed on the surface of agar. The plates were incubated over night at 37°C while the antibiotic diffuses from the disc into the agar. After incubation, the plates were observed for presence of zones of inhibition.

Results and Discussion

Characterization of nanoparticles

UV-visible spectrophotometer was used to prove the existence of nanoparticles. For analytical study of the prepared sample, the amount of absorption within wave length of 300–550 nm was observed by UV-Vis spectrophotometer. It is known that an absorption band at about 370 nm due to surface plasmon resonance in ZnO NP. Fig.1 showed that the UV-Vis spectra of ZnO NP recorded between 300 and 550 nm. As illustrated the SPR band cantered 372 nm confirms the formation of ZnO nanoparticles in the solution.

Band gap of ZnO nanoparticles based UV-Vis spectroscopy

The determination of direct band gap energy (Eg) for the ZnO nanoparticles by fitting the reflection data to the direct transition equation as below:

$\alpha h\tilde{o}=E_D (h\tilde{o}- Eg)^{½}$

Where α is the optical absorption coefficient, hõ is the photon energy, Eg is the direct band gap and E_D is a constant.

Plotting $(\alpha h\tilde{o})^2$ as a function of photon energy and extrapolating the linear portion of the curve to absorption equal to zero gives the value of the direct band gap to be 3.4eV. This value is higher than that of 3.3498eV reported in the literature.[14] Band gap energy increases with decreasing particle size due to quantum size effects.

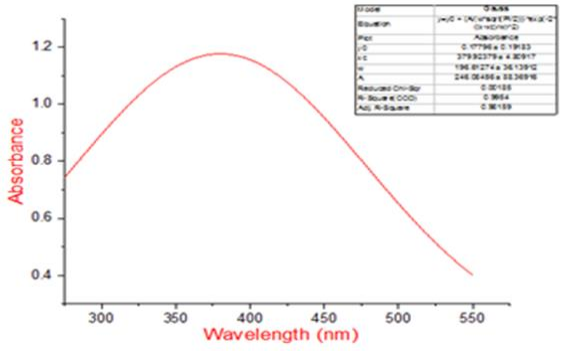

Figure 1: UV-Vis spectrum of ZnO NP.

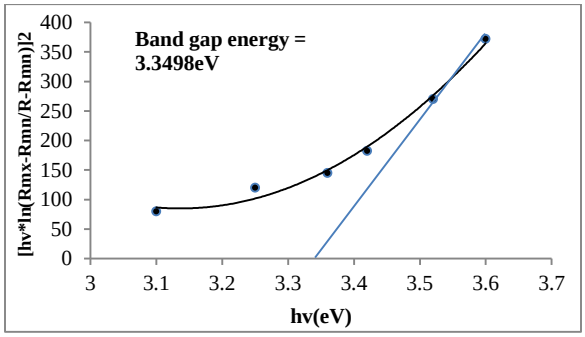

Figure 2: Band gap energy of ZnO nanoparticles.

Characterization of FT-IR

Usually most of the metals and its oxide, give the FT-IR peaks at lower wave number ranging from 400 to 800 cm^{-1}. ZnO stretching was found to be at 436.2 cm^{-1}. FT-IR spectra of samples of ZnO nanoparticles are generally influenced by the particle size and morphology. Fig.3 shows the FT-IR spectra of the synthesized ZnO particles. The peak at v = 670 cm^{-1} is related to the stretching vibrations of Zn-O bond. The peak at 3450.6 cm^{-1} indicates the presence of –OH residue, probably due to atmospheric moisture.

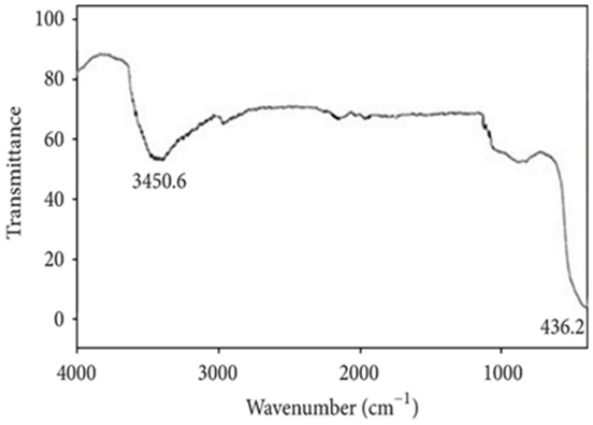

Figure 3: FT-IR spectrum of ZnO NP.

Characterization of XRD patterns

The XRD pattern of prepared ZnO nanoparticles was taken. All the XRD peaks were indexed by hexagonal wurtzite phase of ZnO (PIXcel 1D) as shown in Fig.4. XRD pattern indicates the formation of hexagonal wurtzite phase of ZnO which is in agreement with the electron diffraction results. The peak broadening in the XRD pattern clearly indicates that small nanocrystals are present in the samples. There is no evidence of bulk remnant materials and impurity. The sharp diffraction peaks indicate the good crystallinity of the prepared particles. As ZnO crystallizes in the wurtzite structure in which the oxygen atoms are arranged in a hexagonal close packed type with zinc atoms occupying half the tetrahedral sites. Zn and O atoms are tetrahedrally coordinated to each other and have, therefore, an equivalent position. The zinc structure is open with all the octahedral and half the tetrahedral sites empty. According to Bragg's law[10],

$$n\lambda = 2dSin\theta$$

Where n is the order of diffraction (usually n = 1), λ is the X-ray wavelength, *d* is distance between adjacent planes in the lattice and θ is the incident angle of the X-ray beam.

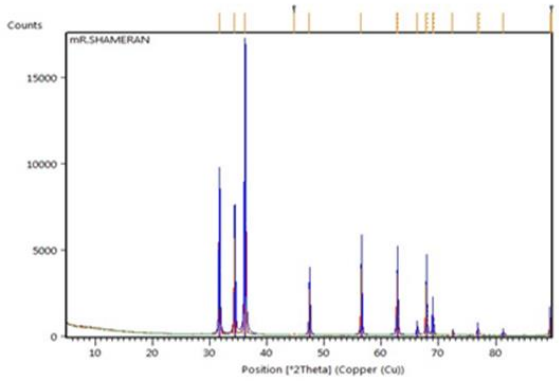

Figure 4: XRD pattern of ZnO nanoparticles.

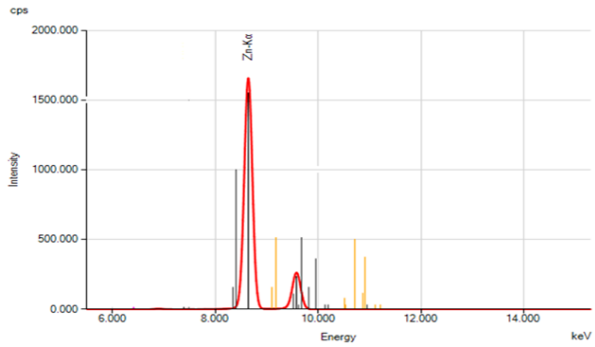

Figure 5: Intensity versus energy for Zn NPs using XRF technique.

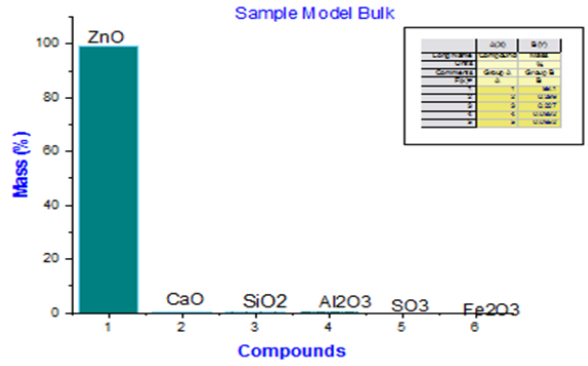

Figure 6: Mass % of ZnO NPs analyzed by X-ray fluorescence.

Characterization of X-Ray fluorescence

XRF analysis is the phenomenon where a material is exposed to X-rays of high energy, and as the X-ray or photon strikes an atom or a molecule in the sample, energy is absorbed by the atom. The peaks in the spectrum correspond to the elements in the sample.[11] The number of X-ray in each peak is proportional to the number of atoms. Figure 5 shows the frequency or intensity of appearance measured in counts per second, on the vertical axis and energy of the fluorescent signal, measured in keV, across the horizontal axis.

Table 1: Data proved by XRF technique.

No	Component	Result (mass %)	Statistical error	Detection limit	Quantitation limit
1	ZnO	99.1			
2	CaO	0.296	0.0054	0.0018	0.0054
3	SiO_2	0.227	0.0052	0.0064	0.0192
4	Al_2O_3	0.0932	0.0075	0.0167	0.0502
5	SO_3	0.0652	0.0013	0.0016	0.0049

Antibacterial activity of ZnO NPs

The antibacterial activity of ZnO NPs was studied against a gram positive (*S. aureus*) and a gram negative (*E. coli*) bacterial pathogen. The selection of the organisms was based on their roles for causing infections such as diarrhea in both children and early-weaned piglets. ZnO NPs at a concentration of 10 mg/ml showed inhibitory effect against the growth of both *S. aureus* and *E. coli*. The clear zone of inhibition around the discs were the evidence of antibacterial activity, which is presented in Figure 7 and 8. Results showed that ZnO NPs had good antibacterial activity against *S. aurous* and *E. coli*.

Kinetic study on growth bacteria

Kinetic studies were conducted on growth bacteria by loading ZnO NP to *Staphylococcus* and *E. coli* with various concentrations to study the kinetic of growth behavior. From the Figure 10. It was observed when the concentration of ZnO NP increased the growth was reduced.[12] This clearly indicates that NP produced toxicity

on both bacteria and therefore the growth was inhibited. 15 mM ZnO NP concentration was found to have highest toxicity to the bacteria.

Figure 7: Antibacterial activity *S. aureus* treated by ZnO NPs.

Figure 8: Antibacterial activity *E. coli* treated by ZnO NPs.

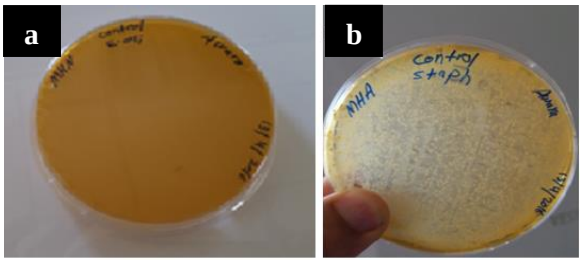

Figure 9: Bacterial control (a) untreated *E. coli* and (b) untreated *S. aureus*.

The antibacterial action resulted in the study is to be dependent on the activity of ZnO nanoparticles. Some workers reported that the damage of the bacterial cell membrane and extrusion of the cytoplasmic contents may be due to ZnO which causing in the death of the bacterium.[13] Some researchers have also indicated the disruption of bacterial membranes may be triggered by the production of ROS.[8]

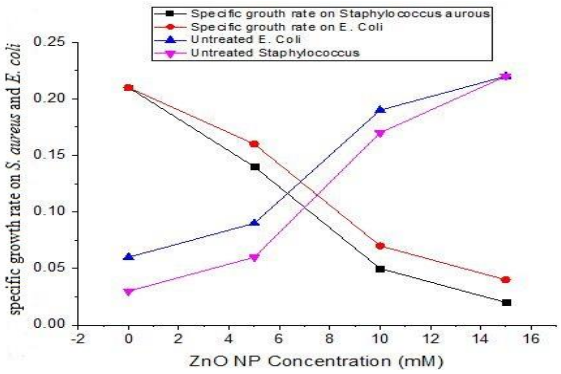

Figure 10: Kinetic study of ZnO NP on treated and untreated bacteria.

Conclusions

Zinc oxide nanoparticles was synthesized successfully by chemical reduction. The detail characterization of the nanoparticles was carried out using FTIR, UV-Vis spectroscopy, X-Ray Diffraction (XRD) analysis, X-ray fluorescence (XRF). Antibacterial potential of ZnO nanoparticles was investigated against two different bacteria viz. *S. aureus* and *E. coli* by disc diffusion assay. Clear zone of inhibition of growth of the organisms around the discs were the indication of antibacterial effect against *S. aureus* and *E. coli.* The observation from the kinetic study i.e., the growth of the organisms decreased with increased concentrations of ZnO NP established the influence. From this study it can be concluded that ZnO NP could potentially be used as an effective antibacterial reagent to treat diseases caused by above organisms. The future study may be aimed at the further screening of ZnO NP against various organisms to establish the drug as potential antimicrobial agent.

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Conflict of interest: None declared

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