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Investigations of Zinc Oxide Nanopowder By XRD, SEM And Antibaterial Activity of *Escherichia Coli* (E.coli)

S. Ranjitha — Post Graduate Student, Department of Physics, Idhaya College For Women, Kumbakonam, Tamilnadu, India.

R. Menaka — Assistant Professor, Department of Physics, Idhaya College For Women, Kumbakonam - 612 001 Thanjavur, Tamilnadu, India

ABSTRACT

Nanoparticles are of great scientific interest and they represent a bridge between the bulk materials and the molecules. The nanoparticles were studied for their physical and chemical properties, now they entered in commercial exploration with huge applications. ZnO nanoparticles was synthesized by direct precipitation method using zinc nitrate hexahydrate and sodium hydroxide in aqueous solution. The precipitated compound was calcined at 1000C. The samples were characterized by XRD and SEM in order to analyze surface morphological size of nanopowder. Nanopowder is increasingly recognized for their utility in biological application. In the present study the antibacterial activity of zinc oxide nanoparticles were investigated using Escherichia coli. Escherichia coli. This study demonstrated that ZnO nanoparticles have wide range of antibacterial activity towards gram negative bacteria E.coli.

KEYWORDS : ZnO, XRD, SEM, Antibacterial Activity.

INTRODUCTION

Nanotechnology ("nanotech") is manipulation of matter on atomic, molecular, and super molecular scale and the earliest, widespread description of nanotechnology ***(Drexler 1986)***. A more generalized description of nanotechnology was subsequently established by the national nanotechnology initiative, which defines nanotechnology as the manipulation of matter with atleast one dimension sized from 1 to 100 nanometers. This definition reflects the fact that quantum mechanical effects are important at this quantum-realm scale, and so the definition shifted from a particular technological goal to a research category inclusive of all types of research and technologies that deal with the special properties of matter that occur below the given size threshold. It is therefore common to see the plural form "nanotechnologies" to refer to the broad range of research and applications whose common trait is size. Because of the variety of potential applications (including industrial and military), governments have invested billions of dollars in nanotechnology research. Until 2012, through its national nanotechnology initiative the USA has invested 3.7 billion dollars. The European Union has invested 1.2 billion and Japan 750 million dollars **(The Daily star Bangladesh 17 April 2012*)***. The assembler is a universal Nanoscaled assembling machine, capable of not only a making nanostructure materials, but also copies of itself as well as other machines ***(*Medina *et al.,* 2007**).

Inorganic nanomaterial's such as quantum dots, nanowires and nanorods.Because of their interesting optical and properties, could be used in optoelectronics ***(*Kerativitayanan *et al.,* 2015**). Nanoparticles or nanocrystal are made up of metals semiconductors or oxides are of particular interest for their mechanical,electrical, magnetic, optical, chemical and other properties. Nanoparticles have been used as quantum dots and as chemical catalysts such as nanomaterial - based catalysts, recently a range of nanoparticles are extensively investigated for biomedical applications including tissue engineering, drug delivery, and biosensor **(Ramsden, 2010*)***.

Zinc oxide is an inorganic compound with the formula ZnO. ZnO is a white powder that is insoluble in water, and it is widely used as an additive in numerous materials and products including rubbers, plastics, ceramics, glass, cement, lubricants, paints, ointments, adhesives, sealants, pigments, foods, batteries, ferrites, fire retardants, and first-aid tapes. It occurs naturally as the mineral incite, but most zinc oxide is produced synthetically.

In material science ZnO is a wide - band gap semiconductor of II - VI semiconductor group .The native doping of the semiconductor is n -type. This semiconductor has several favorable properties, including good transparency, high electron mobility, wide bandgap, and strong room-temperature luminescence. Those properties are used in emerging applications for transparent electrodes in liquid crystal displays and in energy-saving or heat-protecting windows, and electronic applications of ZnO transistors and light-emitting diodes ***(*Sheini *et al.,* 2010*)***.Researchers are developing are customized nanoparticles the size of molecules that can deliver drugs directly to diseased cells in your body. When its perfected, this method should greatly reduce the damage treatment such as chemotherapy does to a patient's healthy cells ***(*Zhang *et al.,* 2007*)***.

Nanostructures of ZnO can be synthesized into a variety of morphologies including nanowires, nanorods, tetrapods, nanobelts, nanoflowers, nanoparticles etc. Nanostructures can be obtained with most above-mentioned techniques, at certain conditions, and also with the vapour-liquid-solid method ***(*Dutta and Barush, 2009*)***. In material science applications, zinc oxides has high refractive index, high thermal conductivity, binding, antibacterial and UV- protection properties ***(*Zak *et al.,* 2011*)***.

In the biomedical field, ZnO nanopowders have been investigated for their strong antibacterial activity. In recent years, nano zinc oxide as antimicrobial agent has received increasing attention, because it is stable under harsh processing conditions and it is generally regarded as safe to humans ***(*Samaele *et al.,* 2010*)***. A mixture of a ZnO with 0.5% Fe_2O_3 is known as calamine and is used in manufacturing calamine lotions. Nanopowder of ZnO have anti microbial and deodorizing qualities and hence they are used for packaging purposes.these properties along with its ability of neutralising acids make it ideal for use in antiseptic creams, healing creams etc., they are also an important component of toothpastes and dental prosthetics (**Ghothi *et al.,* 2010*)***.

MATERIALS AND METHODS

Synthesis of ZnO Nanopowder

The ZnO nanoparticles were synthesized by chemical solution using Zinc nitrate hexahydrate and sodium hydroxide. 7.43M of Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) salt was taken and dissolved in 50 ml distilled water (**Yadav *et al.,* 2006).** 1 M of sodium hydroxide (NaOH) salt was taken and dissolved in 50 ml of distilled water. The above solution was vigorous stirring at 75^0 C for 2 hours. A ($Zn(NO_3)_2.6H_2O$) solution was slowly added dropwise into NaOH solution with stirring. After sometimes the white precipitate powder formed. It was washed thrice thoroughly with distilled water. This procedure was repeated several times until the precipitate was free from any trace impurities. The precipitate powder was calcined at 100^0 C for several hours. After that the coarse molecules were made into nanoform with the aid of pestle and mortar. The product obtained was characterized by X ray diffraction and

Scanning Electron Microscope. In order to analyze the morphological size and magnification of zinc oxide nanoparticle, ZnO nanopowder was tested.

2.2 Characterization of ZnO Nanoparticles

Phase purity and grain size were determined by X-diffraction analysis recorded on a siefert X-ray diffractometer usig the radiation CuK_α (λ = 0.1541nm) at 60 KeV over the range of 2θ = 20-80 θ ***(Mariappan Premanatham, 2010)***.

In XRD, a mono chromatic X-ray beam is focused on sample material to resolve structural information in the crystal lattice. Usually, the materials are composed of repeating uniform atomic planes which make up their crystal. Typically, polychromatic X-rays are produced in a special tube called cathode- ray tube. Filtering polychromatic X-rays through a mono chromate produces monochromatic radiation which hits onto the material atomic planes, separating the diffracted, transmitted and absorbed rays. X-rays are produced within a closed tube under vacuum atmosphere. Application of 15-60 kilovolts current within the tube gives electrons which hit a Cr, Fe, Co, Cu,Mo or Ag anode from which X-ray beams are generated. Thus, produced X-rays are then collimated and directed onto the powder sample having diameter <10 nm. Interactions of incident X-rays with the sample atomic planes create diffracted, transmitted, refracted, scattered and absorbed beams according to Bragg's law ***(Cullity, 1978)***.

2.3 Scanning process and image formation

The surface morphology and topography of Zinc Oxide Nano particles were observed with Scanning Electron Microscope (SEM). In a typical SEM, an electron beam is thermionically emitted from an electron gun fitted with a tungsten filament cathode. Tungsten is normally used in thermionic electron guns because it has the highest melting point and lowest vapour pressure of all metals, thereby allowing it to be electrically heated for electron emission, and because of its low cost. Other types of electron emitters include lanthanum hexaboride (LaB6) cathodes, which can be used in a standard tungsten filament SEM if the vacuum system is upgraded or field emission guns (FEG), which may be of the cold-cathode type using tungsten single crystal emitters or the thermally assisted Schottky type, that use emitters of zirconium oxide. The electron beam, which typically has an energy ranging from 0.2 keV to 40 keV, is focused by one or two condenser lenses to a spot about 0.4 nm to 5 nm in diameter. The beam passes through pairs of scanning coils or pairs of deflector plates in the electron column, typically in the final lens, which deflect the beam in the *x* and *y* axis so that it scans in a raster fashion over a rectangular area of the sample surface.

2.4 Antibacterial Activity

Preparation of culture inoculums

The stock cultures of bacteria purchased Doctor Diagnostic Center, Trichy and maintained on nutrient agar slants at 4°C. Inoculums was prepared by suspending a loop full of bacterial cultures into 10 ml of nutrient broth and was incubated at 37°C ± 2°C for 24 to 48 hours .

Agar well-diffusion method was followed to determine the antibacterial activity. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 24 hours cultures of *E.coli* bacteria. Agar wells (5mm diameter) were made in each of these plates using sterile cork borer. About different concentration (0.1, 0.2 and 0.3 µl) of zinc oxide powder insert into the respective wells and plates were left for 1 hour to allow a period of pre incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions. The plates were incubated in an upright position at 37°C ± 2°C for 24 hrs. The diameters of the zones were measured using diameter measurement scale. Triplicates were maintained and the average values were recorded for antibacterial activity **(Perez *et al.*, 1990)**.

RESULTS AND DISCUSSION

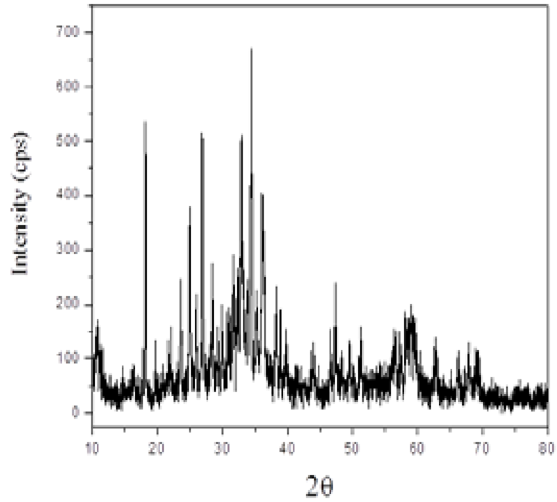

Figure 1: XRD of Zinc Oxide Nanopowder

2θ	FWHM	D value
34.380	0.094	2.6063

Table 1: Variation of peak height and FWHM (Peak 17)

The average grain size of the ZnO Nanopowder is D=92 particle size determined by using Debyes-scherres (DS) equation. With increasing calcination temperature. The peak height in increased and FWHM (Full Width Half Maximum) decreased as diffraction peaks become stronger and sharper.

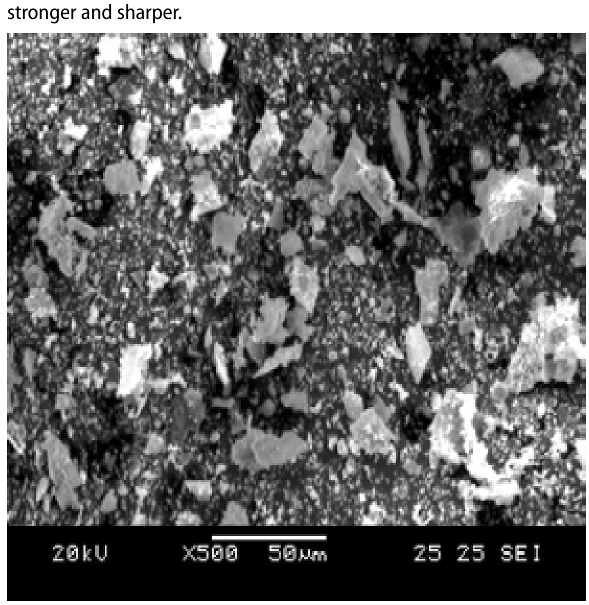

Figure 2: SEM Image of Zinc oxide Nanopowder in 50µm

S.No	Organism	Zone of inhibition (mm)		
		0.1	0.2	0.3
1	E.coli	15	17	5

Table2: Zone Inhibition of Antibacterial activity of ZnO NP.

Figure 3: Antibacterial activity of disc for different concentration of Zinc oxide Nanopowder (Escherichia coli).

In this study, the ZnO nanoparticles were successfully synthesized by direct precipitation method using zinc nitrate as zinc source and NaOH as precipitating agent in aqueous solution. The size range of the generated ZnO powder was approximately 92 nm. Present study successfully designed a facile and fast synthesis route to produce ZnO nanoparticles and finally ZnO nanoparticles were characterized by XRD and SEM. And also Zinc oxide nanopowder exhibited impressive antibacterial properties against an important food borne pathogen, E. coli and the inhibitory effects decreased as the concentrations of ZnO NP increased.

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