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SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES AND ITS ANTIMICROBIAL ACTIVITY AGAINST *BACILLUS SUBTILIS* AND *ESCHERICHIA COLI*

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ABSTRACT

Metal nanoparticles have been intensively studied within the past decade. Nanosized materials have been an important subject in basic and applied sciences. Zinc oxide nanoparticles have received considerable attention due to their unique antibacterial, antifungal, and UV filtering properties, high catalytic and photochemical activity. The objective of this work is to synthesize Zinc oxide nanoparticles using chemical method and characterize zinc oxide nanoparticles using scanning electron microscope and X-ray diffractometer. Further its antimicrobial activity against *Bacillus subtilis* and *Escherichia coli* is studied.

Key words: Nanoparticles, Zinc oxide, *Bacillus subtilis, Escherichia coli*.

INTRODUCTION

Nanotechnology is the production and use of materials at the smallest possible scale[7]. Nanotechnology can be useful in diagnostic techniques, drug delivery, sunscreens, antimicrobial bandages, disinfectant, a friendly manufacturing process that reduce waste products (ultimately leading to atomically precise molecular manufacturing with zero waste), as catalyst for greater efficiency in current manufacturing process by minimizing or eliminating the use of toxic materials, to reduce pollution (e.g. Water and air filters) and an alternative energy production (e.g. Solar and fuel cells)[1].

Bionanotechnology is the integration between biotechnology and nanotechnology for developing biosynthetic and environmental friendly technology for the synthesis of nanomaterials[1]. Nano scale particles have emerged as novel antimicrobial agents owing to the high surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistances against metal ions, antibiotics and the development of resistant strains[3].The recent growth in the field of porous and nanometric materials prepared by non-conventional processes has stimulated the search of new applications of ZnO nanoparticulate[2].

Zinc oxide is an interesting semiconductor material due to its application on solar cells, gas sensors, ceramics, catalysts, cosmetics and varistors[4]. In this work, the precipitation method was used followed by controlled and freezing drying processes[6]. The materials obtained were thermally treated at various temperatures. The influence of temperature on structural, textural, and morphological properties of the materials was studied by powder X-ray diffraction, infrared spectroscopy, scanning electron microscopy, nitrogen adsorption, and thermal analysis.

Certain chemicals can interfere directly with the proliferation of microorganisms at concentrations that can be tolerated by the host. The antimicrobial activity of zinc oxide nanoparticles is well known. Hence we make use of this property to inhibit growth of *Bacillus subtilis, Escherichia coli* using disc diffusion method. These two bacterial strains were selected as they are highly contagious; thence we can evaluate the potential antimicrobial activity of zinc oxide nanoparticles[11].

EXPERIMENTAL

Materials and Methods

In this work, precursor of zinc oxide nanoparticles was synthesized by precipitation method. The chemicals used for synthesis are Zinc acetate 2.1g in 100ml, Ammonium carbonate 0.96g in 100ml, Polyethylene glycol (5%) 5g in 100ml. Instruments used for synthesis are Muffle furnace,Magnetic stirrer,scanning electron microscope (JOEL MODEL 6390)and X-ray diffractometer(SHIMADZU-MODEL XRD 6000).

Synthesis of Zinc Oxide nanoparticles

The zinc oxide nanoparticles were synthesized by precipitation the surfactant Solution (5%PEG) was poured into a three-neck flask, then zinc acetate ,ammonium carbonate were dropped into the flask at same time with vigorous stirring[10] .After the reaction, the suspension was kept under stirring for 2 hours at room temperature, precipitate was filtered washed with ammonia solution and absolute ethanol several times, dried under vacuum for 12 hours, and then calcinated in an oven at 450^{o}C for 3 hours. Then zinc oxide nanoparticles were obtained[6].

Method for Antimicrobial activity

Materials used for antimicrobial activity of zinc oxide nanoparticles are Nutrient broth 1.3g,Nutrient agar 5.6g, Agar-agar 0.5g,petriplates ,antibiotic discs , cotton swabs ,zinc oxide nanoparticle sample ,*bacillus subtilis,Escherichia coli* .Disc diffusion method used for antimicrobial activity of zinc oxide nanoparticles.

Preparation of Inolculum

Nutrient broth (1.3 g in 100 ml D/W[10]) was prepared in 2 conical flasks and sterilized. In one conical flask clinically isolated strain of *Bacillus subtilis,* was inoculated. In the other conical flask clinically isolated strain of *Escherichia coli* was added. These bacterial cultures inoculated in nutrient broth were kept on rotary shaker for 24 hours at 100 r.p.m.

Inoculation of test plate

Nutrient agar is prepared(5.6gnutrient agar0.5g Agar Agar in100ml distilled water)[9]and sterilized.The agar suspension within 15 min is used to inoculate plates by dipping a sterile cotton-wool swab into the suspension and remove the excess by turning the swab against the side of the container. Then we spread the inoculum evenly over the entire surface of the plate by swabbing in three directions. Allow the plate to dry before applying antibiotic to discs.

Preparation of Antibiotic discs

Discs used for antimicrobial activity are nitrofuration, tetracycline, nalidixicacid, Vancomycin, amoxyclav, gentamycin, ciprofloxarin, erythromycin, ceftazdine and Methicillin. Discs should be firmly applied to the surface of an agar plate that has previously

been dried. The contact with the agar should be even. A 60 mm plate will accommodate two discs and ZnO nanoparticles without unacceptable overlapping of zones. Agar plate is divided into 3 sections antibiotic disc, zinc oxide nanoparticles sample, and both antibiotic disc and zinc oxide nanoparticle sample.

Disc diffusion method for Antimicrobial activity

Antibacterial tests were carried out by the disc diffusion method using the suspension of bacteria spread on nutrient agar[11]. Dip the swab into the broth culture of the organism. Gently squeeze the swab against the inside of the tube to remove excess fluid. Use the swab to streak agar plate or a nutrient agar plate for a lawn of growth. This is best accomplished by streaking the plate in one direction, then streaking at right angles to the first streaking, and finally streaking diagonally. We end by using the swab to streak the outside diameter of the agar.The inoculated plates were incubated at appropriate temperature for 24hours.Antibiotic discs can be placed on the surface of the agar using a dispenser that dispenses multiple discs at the correct distance apart, or by obtaining individual discs and placing them on the surface of the agar using flame sterilized forceps. The antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms[11].

Finally we measure (mm) diameters of zones of inhibition of the control strain and test with a ruler, calipers.

RESULTS AND DISCUSSION

Zinc oxide nanoparticles prepared from the solution of zinc acetate, PEG and ammonium carbonate. Here we get a new line of high purity zinc oxide nanoparticles primarily targeted for antimicrobial activity against *Bacillus subtilis* and *Escherichia coli* .as shown in figure 1.Zinc oxide nanoparticles prepared were characterized by scanning electron microscope and X-ray diffraction. Figures 2(a) and (b) shows SEM images Zinc oxide nanoparticles. Scanning electron microscope was used to decide size, location and shape of the Zinc oxide nanoparticles. These images demonstrated that zinc oxide nanoparticles are spherical in shape and their sizes are about 30-63nm.

X-ray diffraction studies reveal the characterization through X-ray diffraction graph as shown in Figure 3.Here 12 peaks are noticed in accordance with zincite phase of ZnO.No peaks due to impurity were observed, which suggest that high purity zinc oxide was obtained. In addition the peak was widened implying that the particle size is very small.

The average crystallite size D was calculated by the Debye Sherrer formula($D = K\lambda/\beta\cos\theta$) where K is the sherrer constant,λ Is the X-ray wavelength,β is the peak width at half-maximum,θ is the bragg diffraction angle .On substituting the values K =0.90 λ =1.5418A ,β = 0.05, $\cos\theta$ =0.94; in the debye-sherrer formula, (0.90×1.5418)÷ (0.05×0.94) = 30nm; the crystallite diameter 30 nm was obtained.

Disc diffusion method was used for the assessment of antibacterial activity. Antimicrobial activity of ZnO nanoparticles against *Bacillus subtilis* is shown on basis of the Inhibition zone (mm) size in Table 1.Here the zone of inhibition is more for both Zno nanoparticles and antibiotics like nitrofurantoin, tetracycline, nalidixicacid, gentamicin, methicillin. Erythromycin has no zone of inhibition as *Bacillus subtilis* is not susceptible to Erythromycin. In table 2 Antimicrobial activity of ZnO nanoparticles against *Escherichia coli* is shown,here zone of inhibition is more for vancomycin, nalidixicacid as *Escherichia coli* is susceptible to these Antibiotics. Erythromycin and Amoxyclav has no zone of inhibition as *Escherichia coli* is not susceptible to these two antibiotics.

CONCLUSION

Synthesis of zinc oxide nanoparticles was achieved by using zinc acetate, polyethylene glycol and ammonium carbonate by precipitation method. Detailed structural characterizations demonstrate that the synthesized products are spherical and crystalline in structure and their diameter was about 30nm. These structures clearly evident from SEM and XRD.SEM result were in accordance with X-ray diffraction. Due to the large specific surface Area and high surface energy, some nanoparticles aggregated. The aggregation occurred Probably during the process of drying.XRD Patterns of zinc oxide nanoparticles calcinated at 450^0 C. the average particle size increased with the increase of calcinations temperature X-ray diffraction (XRD) with Cu-Kα radiation was used for checking the formation and identification of present compounds in the obtained particles. The average crystallite size D was calculated by Debye-sherrer formula.

Microorganisms used for antimicrobial activity are *Bacillus subtilis* and *Escherichia coli.* The antibacterial activity performance of ZnO nanoparticles was done by using disc diffusion method. The disc diffusion method for antibiotic susceptibility testing is the Kirby-Bauer method. The agar used is Meuller-Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be Considered in the disc diffusion method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. There is also a zone of intermediate resistance indicating that some Inhibition occurs using this antimicrobial but it may not be sufficient inhibition to eradicate the organism from the body. The zone of inhibition increases with the increase in Zinc oxide nanoparticle concentration and decrease in particle size.

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Fig.-1: Picture of synthesized Zinc Oxide nanoparticles

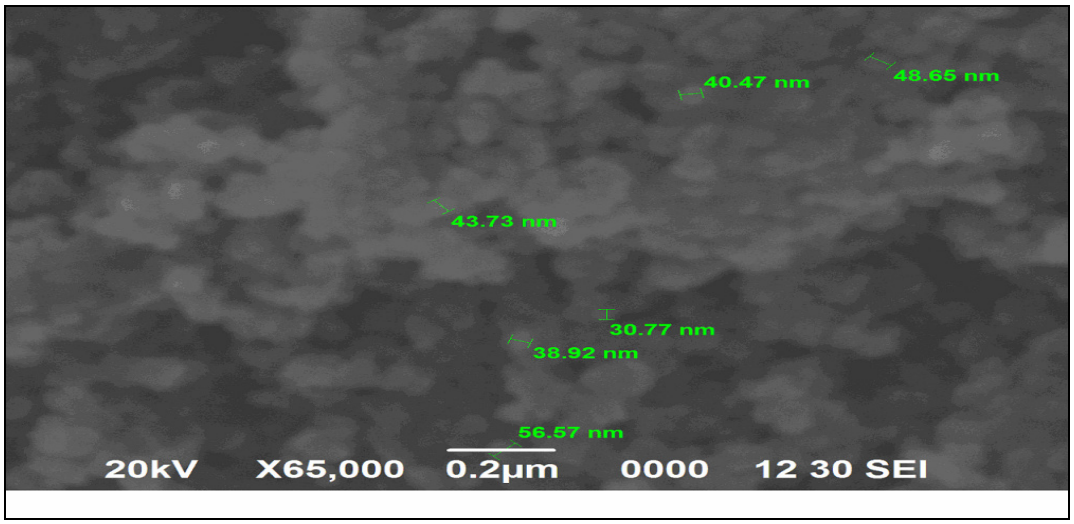

Fig.-2(a): SEM images of ZnO nanoparticles

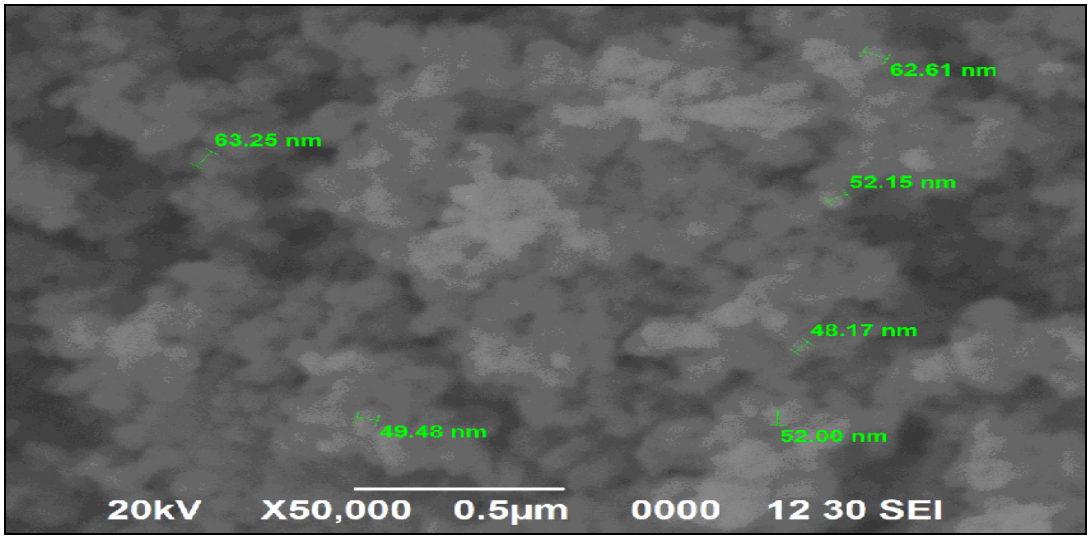

Fig.-2(b): SEM images of ZnO nanoparticles

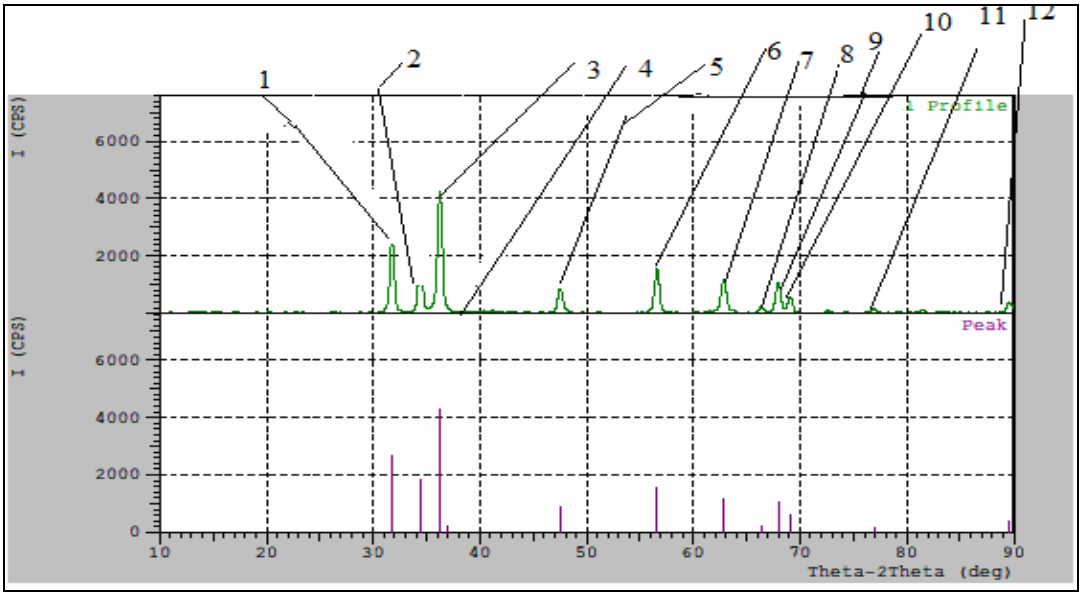

Fig.-3: X-ray diffraction graph

Table-1: Inhibition zone(mm) size against *Bacillus subtilis* by various antibiotics

Organism	Antibiotics	ZnO Nanoparticle and antibiotic	Only antibiotic	Only ZnO nanoparticle
Bacillus subtilis	Nitrofurantoin	22	17	7
	Tetracycline	24	20	12
	Nalidixicacid	20	18	10
	Vancomycin	17	25	6

	Amoxyclav	15	16	14
	Gentamicin	23	21	9
	Ciprofloxacin	20	11	8
	Erythromycin	-	-	7
	Ceftazidime	19	17	11
	Methicillin	22	-	5

Table 2: Inhibition zone(mm) size against *Escherichia coli* by various antibiotics

Organism	Antibiotics	ZnO Nanoparticles And antibiotic	Only antibiotic	Only ZnO Nanoparticles
Escherichia coli	Gentamycin	17	16	9
	Erythromycin	-	-	6
	Ceftazidime	11	9	5
	Nitrofurantoin	17	16	12
	Vancomycin	20	23	14
	Methicillin	12	9	15
	Nalidixicacid	23	20	11
	Ciprofloxacin	9	8	7
	Amoxyclav	-	-	8
	Tetracycline	15	13	6

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