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A Study on the Antibacterial Activity Of Zno Nanoparticles Prepared By Combustion Method against E Coli

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Abstract
Crystalline Zinc Oxide (ZnO) nanoparticles were synthesized by low temperature solution combustion method using Oxalyl dihydrazide (ODH) as fuel, at much lower temperature (300^{o}C). X-ray diffraction (XRD) confirmed the formation of wurtzite-structured pure ZnO No peaks from any else phases of ZnO and no impurity peaks were observed, indicating the high purity of the obtained hexagonal ZnO nanocrystals. The antibacterial activity of the formed nano ZnO were investigated against the pathogenic bacteria namely against E-coli. The bacteriological test is performed in Luria-Bertani and Nutrient agar media on solid agar plates and liquid broth system using different concentration of ZnO by standard microbial method. We have used both colony counting method and disk diffusion method. In both the methods ZnO nanoparticles with 100microg/L showed best antibacterial activity, and further studies on destruction of bacterial genomic DNA was done using PCR and gel electrophoresis revealed the DNA fragment bands, this activity might be due to surface charge interactions between the particles and cells. Free radical scavenging properties of the particles might have helped in cell wall disruption, and drastic antimicrobial action.
Key words: Combustion, Nano-materials, Zinc oxide, X-ray techniques, E Coli

I. Introduction

Nanotechnology is of growing importance in many branches of research because of the opportunity for miniaturization and the interesting properties associated with a small particle size. It is well known that many fundamental properties of nanostructure materials (optical, electrical, mechanical, etc.) can be expressed as a function of their size, composition, and structural order. Meanwhile, nanostructures with different morphologies are nuclear parts of functional nanostructure devices [1, 2]. The preparation of nanoparticles is a complicated process and a wide variety of different variables may affect the properties of the final product. Some important variables have distinct effects on the properties of the final product, while others may have only minor effects or no effect at all. Certain variables can also have an interaction effect on the properties of the prepared nanoparticles. The effects of a large number of variables can be effectively studied with the aid of a statistical experimental design. Their uniqueness arises specifically from higher surface-to-volume ratios and an increased percentage of atoms at the grain boundaries. The ongoing worldwide nanotechnology revolution is predicted to impact several areas of biomedical research and other science and engineering applications. Nanoparticle-assisted drug delivery, cell imaging, and cancer therapy are important biomedical applications of nanotechnology. For semiconductor materials, doping with different elements can adjust their electrical, optical, and magnetic properties effectively [3–8]. Progress in utilizing inorganic nanoparticles for biomedical applications has advanced rapidly as a result of the extensive amount of work done in the synthesis and modification of the nanoparticles. The advantage of using the inorganic oxides for biomedical applications is that they contain mineral elements essential to humans and exhibit strong activity even when administered in small amounts. The synthesis of nanorods, nanowires, and nanotubes has generated much interest in recent years with respect to the advanced nanoscience and nanotechnology in the next generation of electronic and optical Nano devices. Recently, the nanoscience development has been beyond the simple pursuit for single nanoparticle, and the hierarchical assembly of nanoscale of building blocks into complex architectures has attracted much interest due to their special collective properties and wide potential

applications in functional nano devices. Microbial contamination is a serious issue in health care and food industry, so that development of antimicrobial agents and surface coatings has been attracting increasing attention in recent years. Due to the spread of antibiotic resistant infections, interest in alternative antimicrobial agents, such as inorganic materials, has been rising [9]. Antimicrobial properties have been demonstrated for metallic nanoparticles [9, 10] and metal oxide powders and nanoparticles [11]. The inorganic materials can be used in different forms, such as powders [11, 12], coated on cellulose fibers [9], or as a part of organic/inorganic nano composite coating.

Nanomaterials reveal good result than other techniques used in water treatment because of its high surface area (surface/volume ratio). It is suggested that these may be used in future at large scale water purification [13]. Silver is a safe and effective anti-bactericidal metal because it is non-toxic to animal cells and highly toxic to bacteria such as Escherichia coli (E. coli) and Staphylococcus aureus, Staphylococcus epidermidis [14]. Metal nanoparticles with antimicrobial activity when embedded and coated to surfaces can find immense applications in water treatment, synthetic textiles, biomedical and surgical devices, food processing and packaging [15-17].

Therefore, development of nanostructured coatings with antimicrobial properties is of considerable interest. In this work, we investigated antimicrobial properties of ZnO nanoparticles prepared by low temperature solution combustion method. It has been demonstrated that ZnO powders and nanoparticles exhibit antimicrobial activity against Escherichia coli [12]. ZnO nanoparticles arrays fabricated by combustion method have an advantage of low growth temperature (300 °C). A range of (1 – 100 micro M) concentration of ZnO was used on an environmentally relevant gram-negative model microorganism, E.Coli to study the antibacterial activity with standard microbiological test.

II. Materials and methods:

2.1 Materials and instruments:

All chemicals were by Merck chemicals. ATCC strains of *E. coli* (ATCC 25922) and the antimicrobial susceptibility test discs were from Hi Media Pvt. Ltd. The PXRD patterns were obtained using a Philips PW/1050/70/76 X-ray diffract meter. The morphology of powders was examined using JEOL (JSM-840A) scanning electron microscopy (SEM). FTIR spectra were recorded using Nicollet IMPACT 400 D FTIR spectrometer, as KBr pellet. The absorption spectra were recorded with a UV-visible spectrophotometer (Elico-159).DNA fragment studies using Genotype PCR and Genei electrophoresis instrument

2.2 Preparation of nano ZnO:

- Nanosized ZnO powders were prepared using the Solution combustion method. In Solution combustion method, the heating and evaporation of metal nitrate solution (oxidizer) with fuel results in self-firing, thus generating intense heat by exothermic reaction. This intense heat can be utilized to synthesize the powders. The stoichiometry of the redox mixture for combustion is calculated on the total oxidizing and reducing valencies of the oxidizer and the fuel using the concept of propellant chemistry
- Nano-crystalline ZnO has been prepared by using Zinc nitrate and Oxalyl Di hydrazide (ODH) as fuel, at much lower temperature (300^oC).

$$Zn(NO_3)_2(aq) + C_2H_6N_4O_2\ (aq) \longrightarrow ZnO\ (s) + 2CO_2 + 3H_2O + 3N_2$$

2.3 Antibacterial Assay

ATCC strains of Escherichia Coli (ATCC 25922) bacteria grew in Luria–Bertani (LB) medium containing 4.0 g peptone, 2.0 g yeast extract, 5.0 g NaCl and 400 mL H_2O of which pH value was adjusted to 7.2–7.5 with 1 mol L^{-1}NaOH before autoclaving. We then added 6.8 g agar to 1 L of LB medium, producing LB agar. The bacteria were inoculated in the LB medium in a self-regulating thermostat for 6 h at 37^0C. One milliliter original bacterial inoculum was added into 9 mL 0.9% normal saline and they were diluted to 10^6cfumL^{-1} (colony forming unit, cfu), then inoculated into LB broth for 12 hour at 37^0C. Once the standard culture were prepared 2 methods were used to study the antibacterial activity

Colony counting method: This method is simple and very efficient method in determining the effect of any agent used to test the antibacterial activity. The method involves firstly adding of a bacterial strain on the media. Bacteria were grown at 37 °C. The optical density of bacteria cell at 600 nm wavelength used for all testing was 0.3–0.4, in which cells are growing rapidly in the mid-log phase. Then different concentration of ZnO nano particles(100µg/L, 75µg/L, 50µg/L, 25µg/L) along with bacteria were used, One milliliter of bacteria cell in culture broth containing different concentrations of ZnO nanoparticles was incubated at appropriate temperature on a shaking platform at 250 rpm. After 24 h, 10 µl cell suspensions were collected from each sample tube, spread onto culture agar plate and incubated overnight (for 24h) and then the total number of colonies are counted by using a colony counter.

Disc diffusion method was used to determine the inhibition zones, sterile molten Mueller Hilton agar (Himedia) cooled at 45 °C was used with disks containing ZnO nanoparticles. Then plates were incubated at 37 ^{0}C for 24 h with different concentration of ZnO nanoparticles, The zone of inhibition was measured using a zone reader.

Effect on the DNA of bacteria

To determine ZnO effect on the DNA damage of the treated E Coli bacterial isolates, The reaction mixture containing 0.5 mL LB broth medium. ZnO 100mg/l and bacteria in 20 mM potassium phosphate buffer (pH 7.4) was pre-incubated for 24 h at 37°C [18]. The amount of DNA from normal bacteria cells and treated bacteria cells by ZnO was evaluated by agarose gel electrophoresis. 1×10^6 cells were lysed in 250 µL cell lysis buffer containing 50 mM Tris HCl, pH 8.0, 10 mM ethylene diamine tetraacetic acid, 0.1 M NaCl, and 0.5% sodium dodecyl sulfate. The lysate was incubated with 0.5 mg/mL RNase A at 37°C for one hour, and then with 0.2 mg/mL proteinase K at 50°C overnight. Phenol extraction of this mixture was carried out, and DNA in the aqueous phase was precipitated by 25 µL (1/10 volume) of 7.5 M ammonium acetate and 250 µL (1/1 volume) isopropanol. DNA electrophoresis was performed in a 1% agarose gel containing 1µg/mL ethidium bromide at 70 V, and the DNA fragments were visualized by exposing the gel to ultraviolet light, followed by photography [19]

III. Results and discussion

3.1 Characterization of ZnO nanoparticles

The powder X-ray diffraction pattern (XRD) patterns **(Figure 1)** of the combustion synthesized ZnO nanoparticles demonstrated that the ZnO is crystalline in nature, and the diffraction peaks matched very well with hexagonal wurtzite phase of ZnO (JCPDS No. 36-1451). The diffraction pattern and inter-planar spacing closely matched to those in the standard diffraction pattern of ZnO [20]. No characteristic peaks of any impurities were detected, suggesting that high-purity ZnO was obtained. In addition, the peak was widened, implying that the particles size is small according to the DS formula $D = k\lambda/(\beta\cos\theta)$, where k is the Scherrer constant, λ the x-ray wavelength, β the peak width at half maximum, and θ the Bragg diffraction angle. The average crystallite size estimated by the DS equation was 30-40nm.The UV-Vis spectrum of ZnO showed a sharp absorption band at 362 nm **(Figure 2)**. The energy calculated according to the Planck's theory was 3·443 eV.21 **(Figure 3)** presents the FTIR spectra of the ZnO nanoparticles synthesized by combustion method, which showed the composition and quality of the product. The band at 434 cm^{-1} was correlated to the stretching vibration of ZnO [20, 21].The band

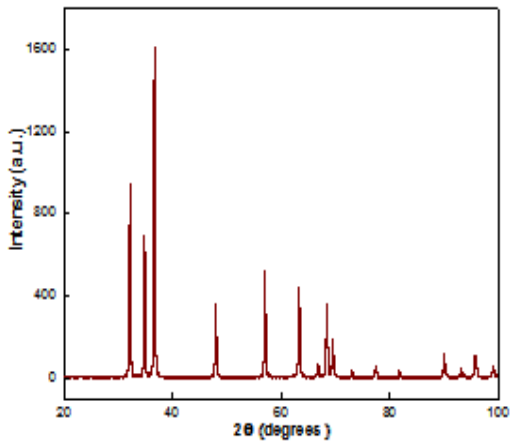

Figure 1: The powder X-ray diffraction pattern (XRD) patterns of the combustion synthesized ZnO nanoparticles

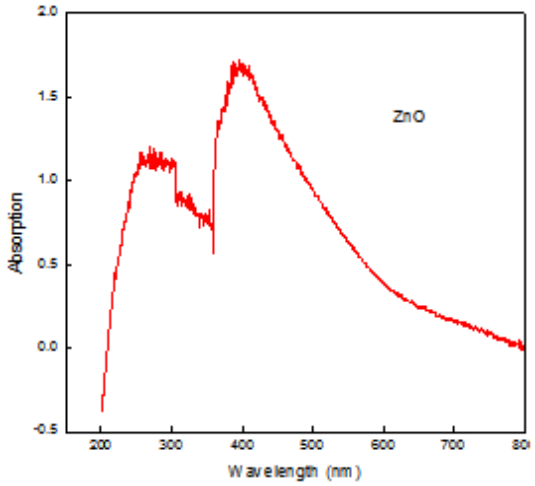

Figure 2: The UV-Vis spectrum of combustion synthesized ZnO nanoparticles

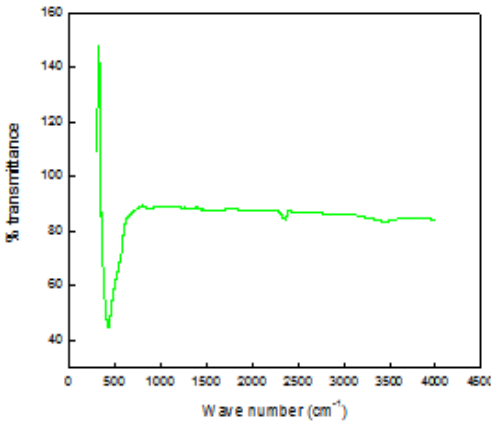

Figure 3: The FTIR spectra of combustion synthesized ZnO nanoparticles

observed at the region of 3452 cm^{-1} corresponded to O-H stretching and bending modes of vibration. The other bands at 593 cm^{-1} and 786 cm^{-1} were probably

due to the carbonate moieties that are generally observed when FTIR samples are measured in air [22].The SEM analyses also revealed the presence of agglomerates of the nanoparticles. SEM image of ZnO **(Figure 4)** depicts that, ZnO nano particles are connected to each other to make large network

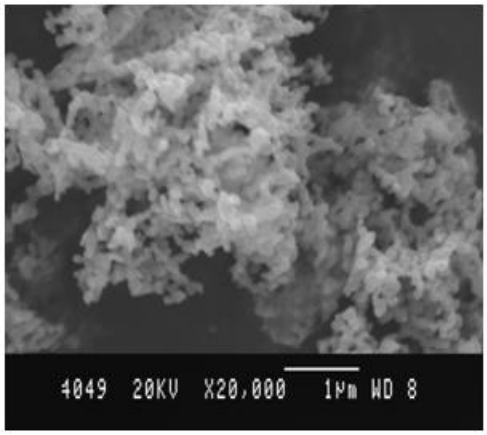

Figure 4: SEM of combustion synthesized ZnO nanoparticles

systems with irregular pore sizes and shapes. Pore formation in the combustion derived products is due to large number of escaping gases which results in high surface area of the nano particles. Fig. 4 shows the FTIR spectrum of the synthesized material which was acquired in the range of 300–4000 cm^{-1}. The spectrum contains one strong absorption band at 418cm^{-1} which confirms the stretching vibration of ZnO (ν -ZnO) bands. The broad absorption bands at 3410cm^{-1} encompass the O-H stretching vibrations of adsorbed water on the ZnO surface. No other absorption peaks of any impurities were observed which confirms the purity of ZnO nanoparticles.

Antibacterial assay:

Colony counting method: According to standard reduction of bacteria criterion, less than 0–20% reductions indicates no bactericidal effect; between 20–50% reduction indicates a low bactericidal effect; between 50–70% reduction indicates an expressive bactericide; greater than 70% reductions is considered a powerful bactericidal effect. According to this criterion,**(Figure 5)** 25µg/L ZnO has no bactericidal effect. But, the plates containing concentration of 50µg/L, 75µg/L shows 30% and 50% denote expressive bactericidal effect and plates with 100µg/L concentrations has a powerful bactericidal effect. Based on these results, ZnO nanoparticles have an expressive antibacterial effect for the gram negative E. coli strains.

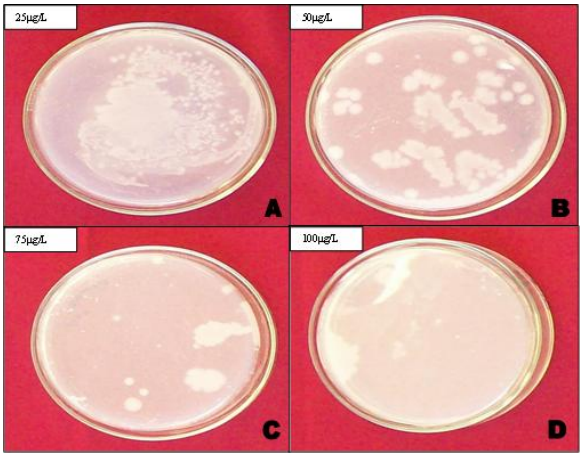

Figure 5: Antibacterial activity of different concentration of ZnO nano particles

Disk diffusion method: The antibacterial effect of the prepared ZnO nanoparticle at different concentrations was studied on E Coli isolates,**(Table 1 and Figure 6)** showed the inhibition zone of different concentrations of metals nanoparticles. Results showed that, ZnO exhibited inhibition zone (mm) of about 0, 18, 20 and 24 mm in diameter for 100µg/L 75µg/L, 50µg/L, and 25µg/L of zinc nanoparticles concentrations, respectively.

Table 1: Zone of inhibition (mm) of ZnO nanoparticle against E Coli.

ZnO Nanoparticle µg/L	Zone of Inhibition (mm)
0	0
25	0
50	18
75	20
100	24

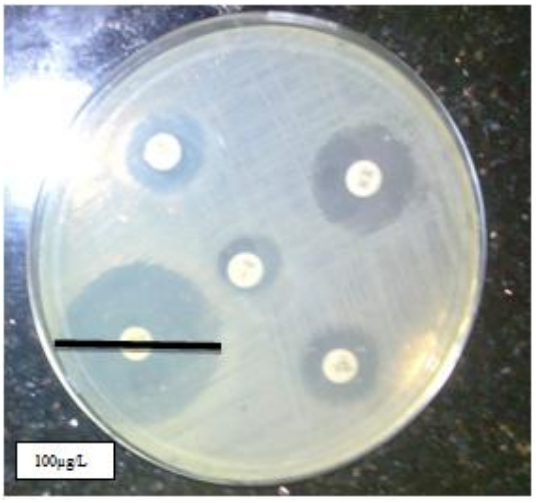

Figure 6: Zone of inhibition of E Coli against ZnO nanoparticle

ZnO nanoparticles affect on the DNA of bacterial isolates

The amount of DNA from normal bacterial cells and ZnO treated bacterial cells was evaluated by agarose gel electrophoresis. Agar gel electrophoresis images as shown in **(Figure 7)** revealed that there are destructive effects of ZnO on pathogenic bacteria genome. The results showed that, there was single band for normal E Coli cell detected at a distance 1.5 cm of about molecular weight 2300 bp, while DNA of ZnO-treated E Coli cells was fragmented showing the evidence of action of nano ZnO particle effect in DNA damage there by increasing antibacterial activity.

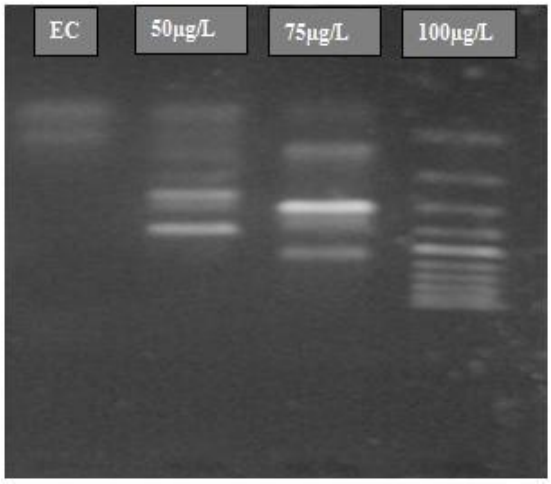

Figure 7: The amount of DNA from normal E Coli cells (EC) and bacterial cells treated by nano ZnO on agarose gel

IV. Conclusion

The antibacterial activity of ZnO nanoparticle on E Coli sp. was investigated. The study with the colony counting and disk diffusion method showed best antibacterial activity at 100mg/l concentration and the morphological and DNA structures of the bacterial cells following treatment with potentially effective ZnO were detected. The experimental results indicated that 100 mg/l of ZnO was MIC at which bacterial cells were inhibited and the cellular components became disorganized and scattered from their original ordered. Also ZnO caused a destructive effect on DNA, resulting in a loss of replication and degradation of DNA, thereby inhibiting bacterial growth. Thus use of nano particles may help in better treatment of pathogenic bacteria and can be further used has a potential agent for treating against bacteria

V. Acknowledgement

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