

Synthesis of ZnO Nanoparticle and Evaluation of its Potential to Enhance the Activity of Antibiotics

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Abstract

The properties of zinc oxide (ZnO) vary with its crystallite size or particle and often nanocrystalline ZnO is seen to exhibit superior physical and chemical properties due to their higher surface area and modified electron structure. This paper aims at synthesizing nanosized ZnO particles and the evaluation of its potential to enhance the activity of antibiotics. ZnO is prepared by using both hydrothermal and solvothermal methods. The sample was characterized by X-Ray Diffraction (XRD). The nanosize of particles was further confirmed by using TEM studies. The average crystal size of the prepared ZnO nanoparticles was determined by TEM. The synthesized nanoparticle was used to enhance the antibacterial efficacy of antibiotics such as Ampicillin and Streptomycin. It was found effective in both drugs against the clinically important bacterial strains tested. Drug resistance is an emerging crisis and becoming a menace to the public. As the bacterial strains showing antibiotic resistance are escalating day by day, application of these nanoparticles that enhance the antibacterial property will be promising for the development of new drugs.

Keywords: Hydrothermal, ZnO nanoparticles, TEM, Antibacterial, disc diffusion method

1. Introduction

Nanoparticles usually ranging in dimension from 1-100 nanometers (nm) have properties unique from their bulk equivalent. They possess unique physico-chemical, optical and biological properties which can be manipulated suitably for desired applications [1]. Recent advances in the field of nanosciences has lead to the synthesis of nanosized inorganic and organic particles which are finding increasing applications in industrial, medicine and therapeutics, synthetic textiles and food packaging products [2]. With a wide band gap of 3.37eV Zinc Oxide (ZnO) is one of the most important and widely used metal oxides[3,4]. ZnO can be synthesized into a variety of one-dimensional nano structures like nanorods, nanowires, nanorings, nanosheets, etc., which have a wide range of applications [5-7]. The metallic nanoparticles are thoroughly being explored and



extensively investigated as potential antimicrobials. Nanoparticles of silver have been studied as a medium for antibiotic delivery, and to synthesize composites for use as disinfecting filters and coating materials [8-10]. The bactericidal effect of silver nanoparticles typically ranging from 2 to 5 nm has been investigated using green fluorescent protein (GFP)-expressing recombinant *Escherichia coli* [11]. It was reported that the antibacterial activity of gold nanoparticles can be increased by adding antibiotics [12]. There are several such reports on other metal nanoparticles such as Magnesium oxides [13], copper oxide [14], Aluminium oxides [15], titanium dioxides [16-18] and zinc oxides [19]. However, reports also reveal that toxic effect of nanoparticles that may affect the biological behavior at the organ, tissue, cellular, subcellular, and protein levels. [20,21]. Hence it is essential to be alert while selecting metal nanoparticle. In the present study, zinc oxide nanoparticle is considered due to its low toxicity.

2. Experimental Details

2.1 Synthesis of ZnO nanoparticles

Sample 1 was prepared by hydrothermal method. To prepare the aqueous solutions, 5g of Zinc acetate dihydrate [$\text{Zn}(\text{O}_2\text{CCH}_3)_2(\text{H}_2\text{O})_2$] and 2 g of Sodium hydroxide [NaOH] were added in 100ml and 50ml distilled water respectively and stirred well. 5% of Polyethylene glycol (PEG) was added into the Zinc acetate dihydrate solution. The NaOH solution was added drop wise to the Zinc acetate dihydrate $\text{Zn}(\text{O}_2\text{CCH}_3)_2(\text{H}_2\text{O})_2$ solution until a pH of 12 was attained. The resulting white colloidal solution was taken in an autoclave and heated to 100°C for 3 hrs. The residue was washed several times in ethanol and then dried at 50 °C in hot air oven. Sample 2 was prepared by solvothermal method. Instead of water ethylene glycol was used as the solvent. The rest of the preparation method was same as sample 1.

2.2 Antibacterial assay

The potential of ZnO nanoparticles was evaluated on antibiotics- Ampicillin and Streptomycin and were applied against the bacterial strains viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Citrobacter freundii*. The experiment was carried out using Kirby-Bauer disc diffusion technique [22]. Antibiotic discs coated with ZnO nanoparticles (test- T), were placed on Muller-Hinton agar medium and kept for overnight incubation. Antibiotic without nanoparticle coating was kept in the same plate as control (C). Agar plates were examined for zone of clearance. The diameter of the clear zone was noted

and compared with the control. Sample 1 is used in combination with Ampicillin and sample 2 with Streptomycin.

3. Results and Discussion

3.1 XRD analysis

The XRD pattern of ZnO nanoparticles synthesized by hydrothermal (Sample 1) and solvothermal method (Sample 2) are shown in figures 1 and 2 respectively. The prominent peaks corresponding to the diffraction planes (100), (002), (101), (102), (110), (103) and (112) agree well with JCPDS card 36-1461, confirming the hexagonal wurtzite structure of ZnO nano particles.

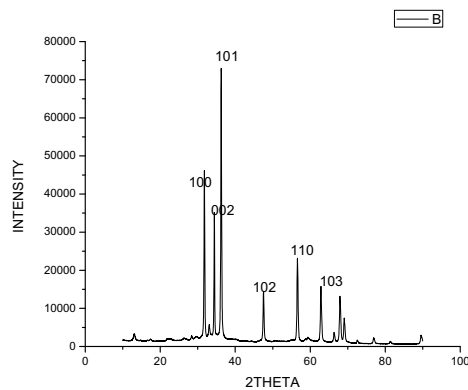


Fig. 1. XRD pattern of Sample 1

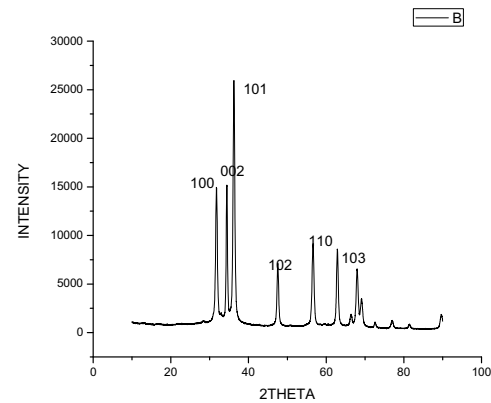


Fig. 2. XRD pattern of Sample 2

3.2 TEM Studies

TEM studies were carried out to find out exact particle size of synthesized ZnO. Figures 3 and 4 show the TEM images of the synthesized ZnO nanoparticles. From the TEM images the average particle size of sample 1 was found to be 62.15nm and that of sample 2 was 9.83nm.

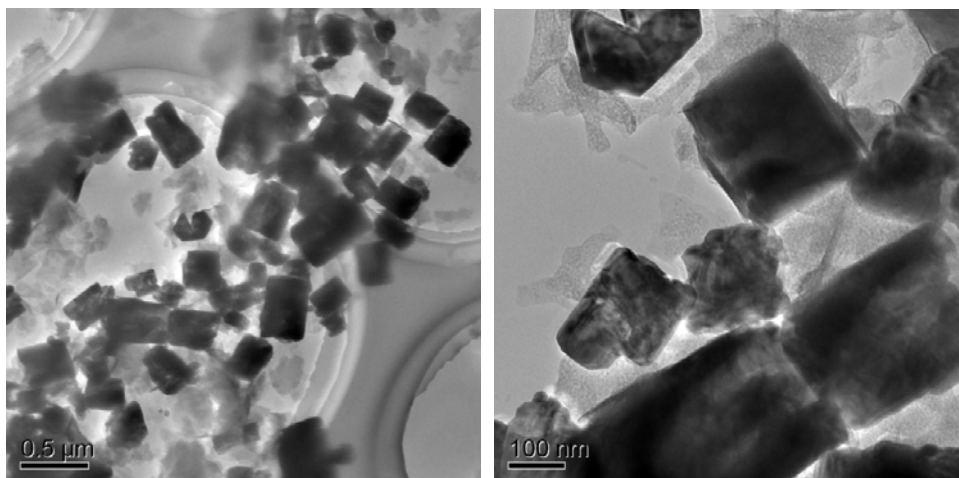


Figure 3. TEM image of synthesized ZnO nanoparticles (sample 1)

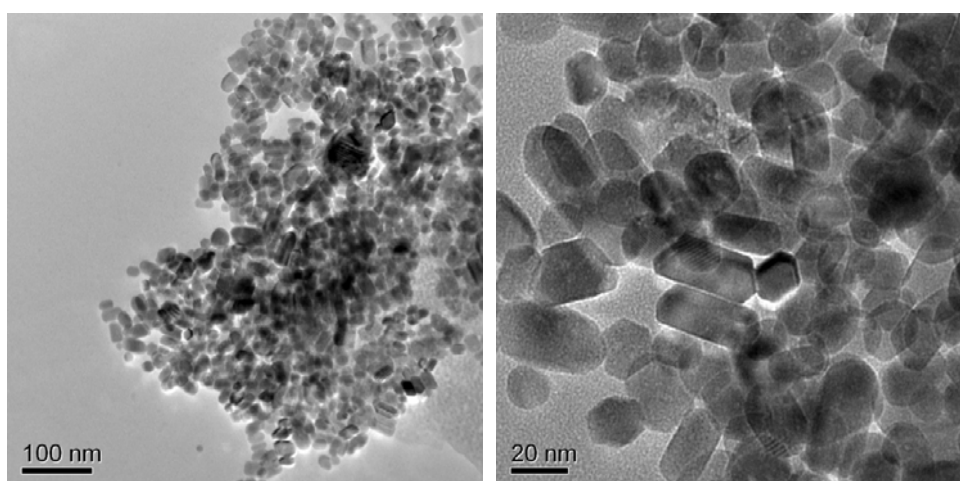


Figure 4. TEM image of synthesized ZnO nanoparticles (sample 2)

3.3 Antibacterial activity

Addition of ZnO nanoparticles to Ampicillin have resulted in slight enhancement of its activity it and maximum inhibition was produced against *C. freundii*. Streptomycin showed better activity against the tested bacterial strains and it was further enhanced by the activity of ZnO nanoparticles. A remarkable enhancement was noticed on *S. aureus* and *Citrobacter freundii* (Table 1). Combination of nanoparticle with Streptomycin was not much effective against *P.aeruginosa* where the diameter of clear zone remained same in both test and control. Earlier reports has showed the susceptibility of *S.aureus* towards

silver and gold nanoparticles [23, 24]. There were very few reports on the susceptibility of *C. freundii*. Inhibitory effect is significant when the emergence of drug resistant varieties of these strains is considered.

Table 1. Clear zone obtained for each bacterial stain

S. No.	Bacteria	Ampicillin		Streptomycin	
		(clear zone in mm)		(clear zone in mm)	
		T	C	T	C
1	<i>S. aureus</i>	8.5	7	26	19
2	<i>P. aeruginosa</i>	9	7	19	19
3	<i>E. coli</i>	10	7	19	16
4	<i>C. freundii</i>	14	9	20	8

*T- Test, C- Control

Ampicillin and Streptomycin are broad spectrum antibiotics that are used against both gram positive and negative strains. *S. aureus* is the only gram positive strain used in this study while the other three belongs to gram negative group. *S. aureus* and *C. freundii* were the two bacterial strains that were found to be highly susceptible to the synergic effect of antibiotics with ZnO nanoparticles. Hence the potential of ZnO nanoparticles was found to be equally effective in gram positive and gram negative strains. Comparing the particle size of the nanoparticles used sample 2 with smaller particle size have exhibited better performance than the large sized sample 1. The size of the particle plays a central role in antimicrobial activity [25] The colloidal silver particles, with variable sizes (44, 50, 35, and 25 nm), synthesized by the reduction of $[\text{Ag}(\text{NH}_3)_2]^+$ complexes with carbohydrates were tested for antimicrobial activity [26].

4. Conclusion

ZnO nanoparticles were synthesized using hydrothermal and solvothermal methods. The prepared ZnO nanoparticles were characterized by powder XRD and TEM studies. It was found that the ZnO particles prepared using Ethylene glycol as solvent had an average

particle size of 9.83nm and the nanoparticles prepared using water as solvent had an average particle size of 62.15nm. ZnO nanoparticles when combined with antibiotics, an enhancement in the activity of antibiotics was observed and was found effective in all the bacterial strains tested. Further research may help in the formulation of new drugs that may inhibit drug resistant bacterial strains.

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