

Solvent-Free Mechanochemical Synthesis of ZnO Nanoparticles: A Simple Route Toward Efficient Antibacterial Agents

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Abstract

Zinc oxide (ZnO) nanoparticles have garnered considerable interest owing to their superior physicochemical and antibacterial attributes. This study involved the synthesis of ZnO nanoparticles by a straightforward mechanochemical technique, followed by calcination at 500 °C. The synthesized nanoparticles were analyzed by X-ray diffraction (XRD) and scanning electron microscopy (SEM) to verify their structural and morphological characteristics. XRD examination indicated the development of phase-pure ZnO exhibiting a hexagonal wurtzite structure, with an average crystallite size of about 31 nm. SEM scans revealed almost spherical nanoparticles exhibiting little aggregation. The antibacterial efficacy of the synthesized ZnO nanoparticles was assessed using the agar well diffusion method against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). The findings demonstrated modest antibacterial efficacy, with inhibition zones of 14 mm for *E. coli* and 12 mm for *S. aureus*. The enhanced antibacterial efficacy is attributed to the production of reactive oxygen species (ROS) and to the interaction of nanoparticles with bacterial cell membranes. The research indicates that mechanochemically synthesized ZnO nanoparticles can function as efficient antibacterial agents for prospective biomedical uses.

Keywords: ZnO nanoparticles, mechanochemical synthesis, antibacterial activity, SEM, XRD

1. Introduction

Nanotechnology has become a significant field of study owing to its ability to modify materials at the nanoscale, resulting in distinctive physical, chemical, and biological properties. Metal oxide nanoparticles have attracted significant interest due to their extensive use in environmental remediation, catalysis, electronics, and biomedical applications.

Zinc oxide (ZnO) is a prominent semiconductor characterized by a broad band gap (~3.37 eV), substantial exciton binding energy, chemical stability, and non-toxic properties. These characteristics render ZnO nanoparticles exceptionally appropriate for applications including

photocatalysis, sensors, UV-blocking materials, and antibacterial agents [3–5]. ZnO nanoparticles exhibit notable antibacterial efficacy against a wide range of pathogens, encompassing both Gram-positive and Gram-negative bacteria [6–8].

The antibacterial efficacy of ZnO nanoparticles is primarily due to their nanoscale dimensions, extensive surface area, and capacity to produce reactive oxygen species (ROS), including hydroxyl radicals and superoxide ions. These reactive species can induce oxidative stress, compromise cell membranes, and harm intracellular constituents, such as proteins and DNA, ultimately leading to microbial cell death [9–11]. The efficacy of ZnO nanoparticles is significantly influenced by their dimensions, shape, and synthesis technique.

Numerous strategies have been documented for the synthesis of ZnO nanoparticles, including sol-gel, hydrothermal, chemical precipitation, and green synthesis methods [12–14]. Nonetheless, numerous approaches entail complex procedures, prolonged response times, and the use of hazardous substances. Conversely, mechanochemical synthesis has emerged as a straightforward, economical, and environmentally friendly method for the fabrication of nanomaterials. This technique employs mechanical energy to facilitate chemical reactions, thereby obviating the necessity for solvents and mitigating environmental effects [15–17].

The solvent-free mechanochemical approach offers numerous benefits, including simplicity, scalability, reduced processing time, and enhanced control over particle synthesis. Mechanical grinding results in continuous particle breakage and cold welding, producing highly reactive surfaces that promote the creation of nanostructured materials [18–20]. Notwithstanding these benefits, research on the antibacterial efficacy of mechanochemically synthesized ZnO nanoparticles is still scarce.

The current study aims to synthesize pure ZnO nanoparticles via a solvent-free mechanochemical method and to assess their antibacterial efficacy against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). The research examines the structural and morphological characteristics of the synthesized nanoparticles by X-ray diffraction (XRD) and scanning electron microscopy (SEM), and links these attributes with their antibacterial efficacy.

2. Experimental

2.1 Materials

Zinc acetate dihydrate and oxalic acid served as precursors for the creation of ZnO nanoparticles. All compounds were of analytical quality and utilized without additional purification. Deionized water was used for cleaning. All glassware and equipment were meticulously cleaned and dried before use to prevent contamination.

2.2 Mechanochemical Synthesis of ZnO Nanoparticles

ZnO nanoparticles were produced via a solvent-free mechanochemical approach [21–23]. According to the standard protocol, stoichiometric amounts of zinc acetate dihydrate and oxalic acid were precisely weighed and thoroughly blended in an agate mortar and pestle.

The amalgamation underwent incessant grinding for almost 50 minutes. The combination first formed a paste-like substance upon contact between the reactants. With further grinding, the

paste progressively transformed into a fine powder, signifying the development of a homogenous precursor by mechanochemical activation [24,25].

The grinding process generates mechanical energy that causes particle deformation, fracture, and molecular-level mixing, leading to the creation of highly reactive intermediate species [21,26]. The acquired precursor powder was subsequently placed in a ceramic crucible and calcined in a muffle furnace at 500 °C for a specified time to promote thermal breakdown and the formation of crystalline ZnO nanoparticles [22,27].

Following calcination, the sample was permitted to cool naturally to ambient temperature within the furnace. The resultant white ZnO nanopowder was collected and stored in sealed containers for subsequent characterization and antibacterial testing.

2.3 Characterization Techniques

The structural and morphological characteristics of the synthesized ZnO nanoparticles were examined by X-ray diffraction (XRD) and scanning electron microscopy (SEM).

XRD examination was performed utilizing Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) across a 2θ range of 20°–80° to ascertain the crystal structure, phase purity, and crystallite size of the nanoparticles [28,29]. The diffraction data were used to verify the formation of ZnO and assess the crystallinity of the synthesized material.

The surface morphology and particle size distribution were examined using SEM. This technique provides comprehensive data on particle morphology, dimensions, surface characteristics, and the extent of agglomeration, which are critical factors affecting the physicochemical and antibacterial properties of ZnO nanoparticles [30].

2.4 Antibacterial Activity

The antibacterial efficacy of the synthesized ZnO nanoparticles was assessed using the agar well diffusion method against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive).

New bacterial cultures were cultivated and evenly distributed on sterilized nutrient agar plates in aseptic conditions. Wells of consistent diameter were formed utilizing a sterile cork borer, and a predetermined volume of ZnO nanoparticle suspension was added to each well.

Ampicillin functioned as the standard antibacterial drug for comparison, whereas dimethyl sulfoxide (DMSO) acted as a negative control to confirm the absence of inhibition in the absence of nanoparticles. The inoculation plates were incubated at 37 °C for 24 hours, after which the antibacterial activity was assessed by measuring the diameter of the inhibitory zone surrounding each well in millimeters [31–33].

3. Results and Discussion

3.1 X-ray Diffraction (XRD) Analysis

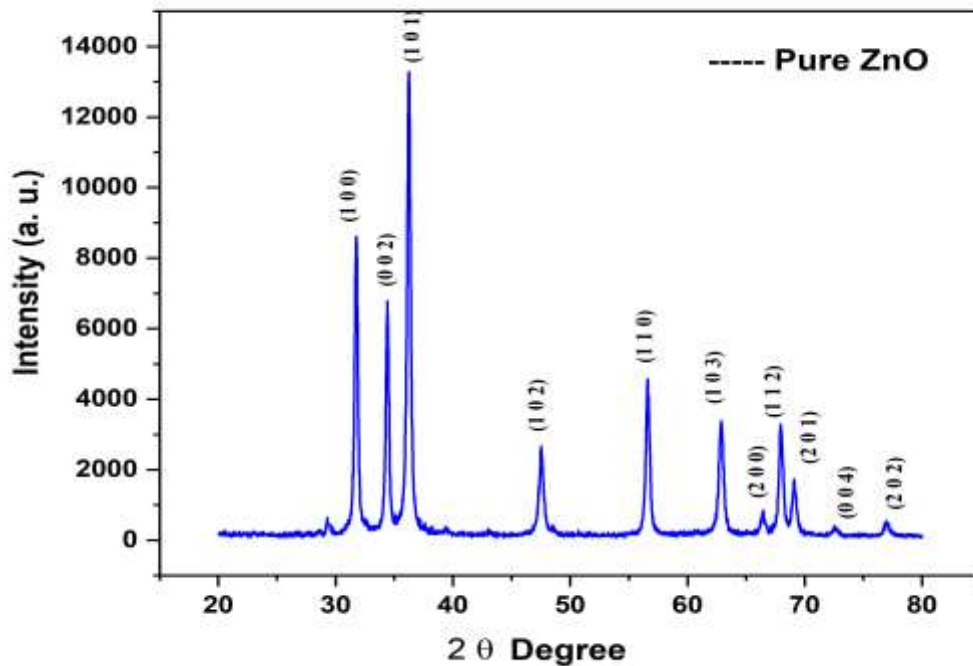


Figure 1: XRD of Pure ZnO synthesised by Mechanochemical method at 500 °c

The XRD pattern of the synthesized ZnO nanoparticles confirms the formation of a phase-pure, hexagonal wurtzite crystalline structure. The notable diffraction peaks at 2θ values of 31.7° , 34.4° , and 36.2° correspond to the (100), (002), and (101) crystallographic planes of ZnO, respectively, and align well with the standard JCPDS data (Card No. 36-1451) [28,29].

The absence of supplementary peaks associated with impurity phases such as $\text{Zn}(\text{OH})_2$ or ZnCO_3 indicates the successful synthesis of pure ZnO nanoparticles. The distinct, well-defined peaks indicate high crystallinity, attributable to the calcination at 500°C . The average crystallite size was calculated using the Debye–Scherrer equation and determined to be approximately 31 nm, thereby affirming the nanocrystalline characteristics of the synthesized ZnO.

3.2 Scanning Electron Microscopy (SEM) Analysis

SEM research indicates that ZnO nanoparticles exhibit shapes ranging from almost spherical to quasi-spherical, with nanoscale dimensions. The particles exhibit relative uniformity in size and are dispersed over the examined area.

A modest level of agglomeration is observed, typically associated with metal oxide nanoparticles due to their elevated surface energy and robust interparticle interactions. The particle size observed in SEM pictures is marginally greater than the crystallite size derived from XRD analysis, as each particle may comprise many aggregated crystallites.

The nanoparticle surface exhibits a rough, granular texture, indicating the formation of interconnected nanostructures during calcination. This nanoscale morphology provides a large surface area, increasing contact between nanoparticles and bacterial cells and thereby enhancing antibacterial efficacy [30].

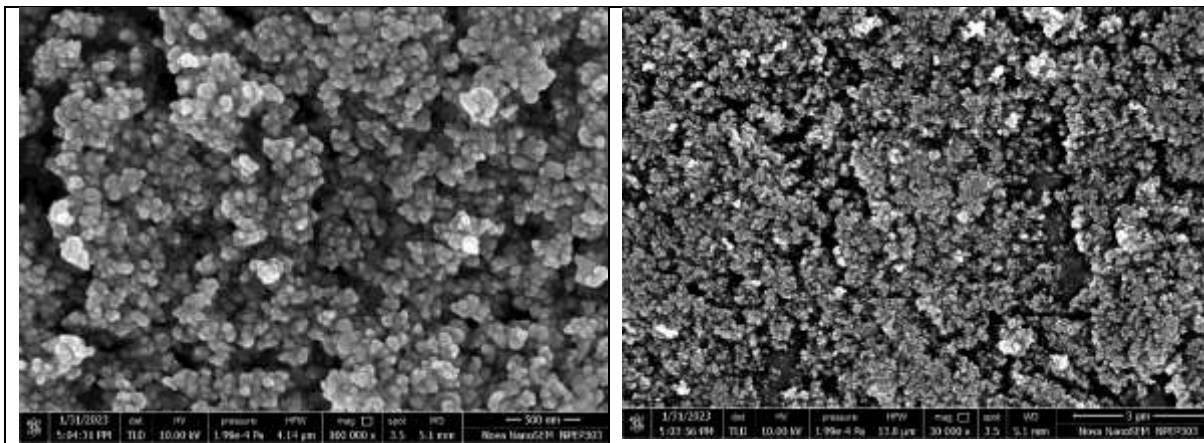


Figure 1: SEM of Pure ZnO synthesised by Mechanochemical method at 500 °c

3.3 Antibacterial Activity

The antibacterial efficacy of the synthesised ZnO nanoparticles was assessed against *Escherichia coli* and *Staphylococcus aureus*, with the findings summarised in Table 1.

Table 1: Antibacterial activity of ZnO nanoparticles

Microorganism	Zone of Inhibition (mm)
<i>Escherichia coli</i>	14
<i>Staphylococcus aureus</i>	12

The findings indicate that ZnO nanoparticles possess significant antibacterial efficacy against both Gram-negative and Gram-positive bacteria. A larger inhibition zone was observed for *E. coli* (14 mm) than for *S. aureus* (12 mm), indicating greater efficacy against Gram-negative bacteria.

This disparity can be ascribed to differences in cell wall architecture. Gram-negative bacteria have a thinner peptidoglycan layer, facilitating the penetration of nanoparticles and reactive

species, whereas Gram-positive bacteria have a thicker cell wall, which confers greater resistance.

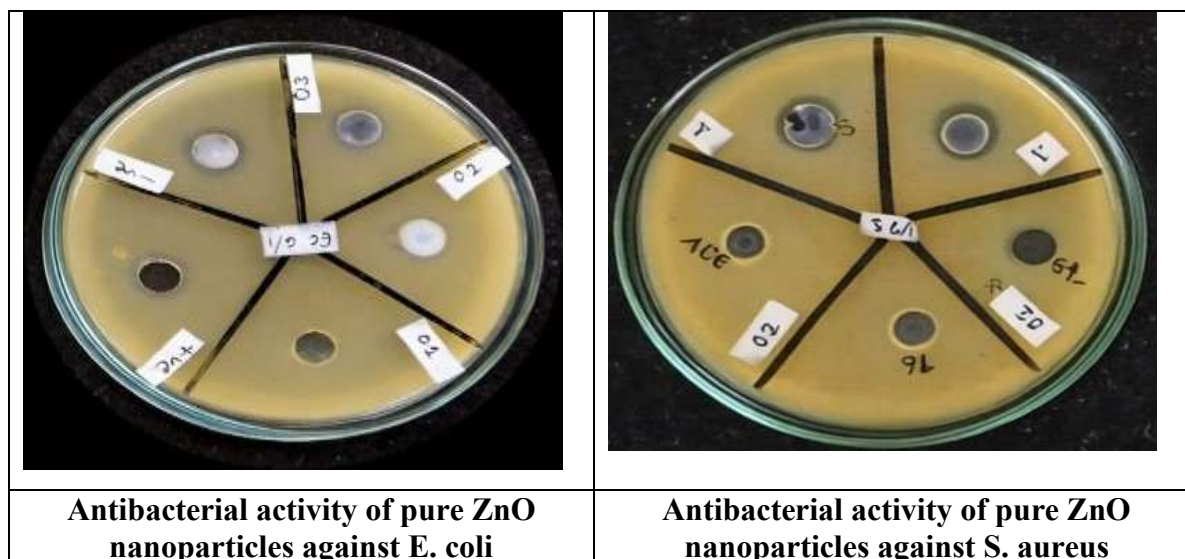


Figure 2 : Antibacterial activity of pure ZnO nanoparticles against *E. coli* and *S. aureus*

The antibacterial action of ZnO nanoparticles is chiefly linked to the production of reactive oxygen species (ROS), including hydroxyl radicals ($\bullet\text{OH}$) and superoxide ions ($\text{O}_2^{\bullet-}$). These reactive species induce oxidative stress, disrupt cell membranes, and damage intracellular biomolecules, ultimately leading to bacterial cell death.

Moreover, the nanoscale dimensions and elevated surface area of ZnO nanoparticles increase their contact with microbial cells, thereby enhancing their antibacterial efficacy. The absence of an inhibitory zone in the negative control (DMSO) confirms that the observed antibacterial activity is attributable solely to the ZnO nanoparticles.

4. Conclusion

ZnO nanoparticles were effectively synthesized via a solvent-free mechanochemical approach and subsequently calcined at 500 °C. Structural investigation confirmed the presence of phase-pure, nanocrystalline ZnO with a hexagonal wurtzite structure, whilst SEM revealed almost spherical nanoparticles with minimal agglomeration.

The synthesized ZnO nanoparticles showed moderate antibacterial activity against both Gram-positive and Gram-negative bacteria, with greater effectiveness against *Escherichia coli*. The antibacterial efficacy is primarily ascribed to the formation of reactive oxygen species and robust interactions between nanoparticles and cells.

References

1. Kumar S, Karthikeyan S. Recent advances in ZnO nanostructures for photocatalytic applications. *Mater Sci Semicond Process.* 2020;107:104832.



2. Al-Gaashani R, Radiman S, Daud AR, Tabet N, Al-Douri Y. XRD and optical characterization of ZnO nanoparticles. *Ceram Int.* 2020;46:19592–19599.
3. Saravanan R, Khan MM, Gupta VK, et al. ZnO nanostructures for photocatalytic applications. *J Photochem Photobiol C.* 2020;44:100339.
4. Zhang J, Wang Y, Jin J, et al. ZnO-based photocatalysts for environmental remediation. *Catalysts.* 2020;10:784.
5. Rahman MM, Khan SB, Asiri AM. ZnO nanostructures for environmental applications. *Nanomaterials.* 2020;10:2216.
6. Jones N, Ray B, Ranjit KT, Manna AC. Antibacterial activity of ZnO nanoparticles. *FEMS Microbiol Lett.* 2008;279:71–76.
7. Raghupathi KR, Koodali RT, Manna AC. Size-dependent antibacterial activity of ZnO nanoparticles. *Langmuir.* 2011;27:4020–4028.
8. Sirelkhatim A, Mahmud S, Seeni A, et al. Review on ZnO nanoparticles antibacterial activity. *Nano-Micro Lett.* 2015;7:219–242.
9. Applerot G, Lipovsky A, Dror R, et al. Enhanced antibacterial activity of ZnO nanoparticles due to increased ROS generation. *Adv Funct Mater.* 2009;19:842–852.
10. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. *Langmuir.* 2002;18:6679–6686.
11. Lipovsky A, Nitzan Y, Gedanken A, Lubart R. Antifungal activity of ZnO nanoparticles—the role of ROS mediated cell injury. *Nanotechnology.* 2011;22:105101.
12. Li X, Chen G, Yang L, Jin Z. ZnO nanoparticles for photocatalytic degradation. *J Alloys Compd.* 2020;828:154411.
13. Sharma S, Kumar S, Ghosh P. Structural and optical properties of ZnO nanoparticles. *Opt Mater.* 2021;117:111201.
14. Zhang Y, Wang L, Huang Z. Green synthesis of ZnO nanoparticles using plant extracts. *Mater Chem Phys.* 2021;259:124028.
15. Bansal P, Singh J, Kumar A. Mechanochemical synthesis of metal oxide nanoparticles. *Mater Today Chem.* 2021;19:100378.
16. Liu H, Wang J, Zhao Y. Mechanochemical synthesis of oxide nanomaterials. *Mater Today Chem.* 2020;17:100314.
17. Wang L, Zhao Y, Chen H. Mechanochemical synthesis of nanomaterials: A review. *Mater Today Chem.* 2023;29:101407.
18. James SL, Adams CJ, Bolm C, et al. Mechanochemistry: opportunities for new and cleaner synthesis. *Chem Soc Rev.* 2012;41:413–447.
19. Takacs L. The historical development of mechanochemistry. *Chem Soc Rev.* 2013;42:7649–7659.
20. Baláž P. *Mechanochemistry in Nanoscience and Minerals Engineering.* Springer; 2008.
21. Zhang H, Chen G, Bahnemann DW. Photoelectrocatalytic materials for environmental applications. *J Mater Chem.* 2009;19:5089–5121.
22. Wang ZL. Zinc oxide nanostructures: growth, properties and applications. *J Phys Condens Matter.* 2004;16:R829–R858.



23. Ozgur U, Alivov YI, Liu C, et al. A comprehensive review of ZnO materials and devices. *J Appl Phys.* 2005;98:041301.
24. Kumar SG, Rao KSRK. Zinc oxide based photocatalysis: tailoring surface-bulk structure. *Appl Surf Sci.* 2017;391:124–148.
25. Janotti A, Van de Walle CG. Fundamentals of zinc oxide as a semiconductor. *Rep Prog Phys.* 2009;72:126501.
26. Kołodziejczak-Radzimska A, Jesionowski T. Zinc oxide—from synthesis to application: a review. *Materials.* 2014;7:2833–2881.
27. Bhosale RR, Pujari SR, Muley GG. Synthesis and characterization of ZnO nanoparticles by mechanochemical method. *Mater Sci Appl.* 2015;6:590–595.
28. Cullity BD, Stock SR. *Elements of X-ray Diffraction.* 3rd ed. Prentice Hall; 2001.
29. Kumar R, Sharma P, Singh J. Structural characterization of ZnO nanostructures using XRD. *Mater Res Express.* 2022;9:055011.
30. Kumar R, Umar A, Kumar G, Kim SH. ZnO nanostructures for photocatalysis and sensing. *Mater Lett.* 2021;283:128778.
31. Padmavathy N, Vijayaraghavan R. Enhanced bioactivity of ZnO nanoparticles—antimicrobial study. *Sci Technol Adv Mater.* 2008;9:035004.
32. Premanathan M, Karthikeyan K, Jeyasubramanian K, Manivannan G. Selective toxicity of ZnO nanoparticles toward bacteria. *Appl Biochem Biotechnol.* 2011;165:1364–1374.
33. Azam A, Ahmed AS, Oves M, et al. Antimicrobial activity of metal oxide nanoparticles. *Int J Nanomedicine.* 2012;7:6003–6009.