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Metronidazole-loaded zinc oxide / graphene nanoparticles: synthesis, analysis, drug delivery, and antibacterial efficiency

Asmaa H. Hammadi[1], Saba Abdulmunem Habeeb[2,*], Lena Fadhil Al-Jibouri[3], Samir Azzat Malik[4], Fatema Samer Al bdear[5], Amal Adnan[5]

[1]Department of Pharmaceutics, College of Pharmacy, University of Babylon, Hillah, Iraq
[2]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Babylon, Hillah, Iraq
[3]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Babylon, Hillah, Iraq
[4]College of Pharmacy, Al-Mustaqbal University, Hillah, Iraq
[5]College of Pharmacy, University of Babylon, Hillah, Iraq

***Corresponding author:** Saba Abdulmunem Habeeb, Department of Pharmaceutical Chemistry, College of Pharmacy, University of Babylon, Hillah, Iraq; Tel.: +964-(0)7801733161
E-mail: phar.saba.abd@uobabylon.edu.iq

Abstract

In our study, zinc oxide (ZnO) nanoparticles (NPs) were prepared by precipitation (economically and in high quality) at a temperature range of 60°C to 80°C and at pH 8, and were then adorned with graphene (G) plates. To determine its antimicrobial potential, the ZnO/G complex was loaded with metronidazole. The morphology and diameter of the ZnO nanocomposite before and after the loading were validated by scanning electron microscopy. The average size of the ZnO NPs was found to be 20–40 nm, while X-ray diffraction examined how the physical features of these NPs varied from those of its individual components with an average size of 28.1 nm. The assessment of the ZnO/G complex's antibacterial efficacy against Gram-positive and Gram-negative bacteria was the main aim of our work. The agar well diffusion technique was used in order to assess the antibacterial activity of the ZnO/G complex with and without metronidazole. Our study demonstrates that the ZnO/G complex possesses antibacterial activity and might increase the antibiotic action by inhibiting Gram-positive bacteria (more than Gram-negative ones). It is, therefore, concluded that the ZnO/G NPs could be of use in formulating nano-drug conjugates that could act as antimicrobial agents.

KEYWORDS
ZnO, graphene, nanoparticles, instrumental analysis, drug delivery

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1. INTRODUCTION

Nanoparticles (NPs) are frequently called "the wonders of modern medicine" due to their remarkable abilities in many biological and physical aspects [1]. Microorganisms are omnipresent in the biosphere and always impact their surroundings; controlling their adverse consequences seems

most crucial. Kanamycin, spectinomycin, and penicillin are among the popular drugs used in order to fix this issue. However, their continuous use makes microorganisms resistant to these drugs [2]. Metal and metal oxide NPs have shown significant antibacterial action [3]. Of these, zinc oxide (ZnO; an n-type semiconductor) is used in solar cells, optical and antibacterial coatings, photocatalysts, electric devices, and gas sensors, due to its broad band gap (3.3 eV), its high excitation binding energy (60 mV), and its eco-friendliness [4]. When combined with graphene (G), this hybrid nanomaterial (ZnO/G) can integrate the exceptional electrical and antimicrobial characteristics of graphene, with the optical attributes and lethal efficacy of ZnO NPs against both Gram-positive and Gram-negative bacteria [5].

In our study, we used metronidazole-loaded ZnO/G nanocomposites in order to inhibit pathogens belonging to genera frequently associated to biodeterioration: Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. We also characterized some of the physical properties of these hybrid nanocomposites.

2. MATERIALS AND METHODS

Synthesis of ZnO NPs: ZnO NPs were prepared by direct thermal precipitation. Potassium hydroxide and zinc nitrate (0.4 M and 0.2 M, respectively) were mixed with deionized water at room temperature by slowly adding the aqueous solution while stirring. Controlling the temperature at 60°C for 120 min resulted in a white precipitation. Subsequently, we centrifuged the mixture at 500 rpm for 20 min and washed it three times with deionized water and 100% alcohol. A bespoke tubular muffle furnace facilitated the synthesis of ZnO at 500°C for 2 h [6]. The ZnO/G composites were produced as previously described [3], while the ZnO/G composite loading with metronidazole was performed according to Habeeb *et al.* [1].

Antibacterial activity: The agar well diffusion approach was used in order to cultivate *S. aureus* and *E. coli* in a Mueller-Hinton agar and to test their antibacterial properties. We swabbed 100 µL of old mature cultured media with the L-shaped rod after 24 h. The wells were created with a sterile 6-mm cork tool. The zone of inhibition was measured in mm. Each Petri dish included three wells with 50 µL each [1].

3. RESULTS

The scanning electron microscopy (SEM) images in Figures 1A–C present the morphologies of ZnO, of G, and of the ZnO/G-drug complex, respectively. Figure 1A shows the ZnO NPs' shape and formation, with a size ranging from 20 to 40 nm (as assessed with the use of the Image J software).

In order to gain information regarding the crystal structure of ZnO, of G, of ZnO/G, and of the ZnO/G-drug complex, we used X-ray diffraction (XRD) technique (Figure 1D). The following characteristic peaks at 2θ were observed: 31.7°, 34.4°, 36.2°, 47.5°, 56.7°, 63.0°, 66.4°, 68.1°, and 69.3°; corresponding to the planes 100, 002, 101, 102, 110, 103, 200, 112, and 201, respectively. The XRD patterns indicated the existence of ZnO NPs, and excluded the existence of other phases or impurities, thereby indicating the high purity of the herein assessed catalysts [1,5].

The antibacterial activity of ZnO/G NPs was tested against two bacterial strains at one concentration, with metronidazole as a standard. The highest antibacterial activity of ZnO/G with the drug was noted against *S. aureus* at 5 mm, while *E. coli* was not affected, as seen in (Figures 1E and F).

4. DISCUSSION

Figures 1A–C display the SEM images at a high magnification, and demonstrate the formation of NPs. They also provide a clearer idea about the particles' separation, without being highly affected by agglomeration. Figure 1A clearly shows that ZnO NPs appear as granule-like nanostructures, while Figure 1C exhibits the SEM imaging of the metronidazole-loaded ZnO/G NPs. The surface shape of metronidazole has been altered after being loaded on the ZnO/G complex. As one can see, the NPs manifested as granules with a light colour, and are evenly spread over the surface of metronidazole. When we compare Figures 1A and 1C, one can detect a considerable difference in the shape of metronidazole-loaded ZnO/G NPs.

The absence of the characteristic G diffraction peak at 2θ=24.6° in the XRD pattern of the ZnO/G complex is noteworthy. This is likely due to the fact that the ZnO crystals were obscured by a negligible amount of G, which has altered their structure [7]. Based on previous studies, the presence of grain boundaries in ZnO and ZnO/G nanocomposition is due to the existence of amorphous superficial and intergranular layers between ZnO and ZnO/G [8].

ZnO has an innate advantage of exerting broad antibacterial activities against bacteria, fungi, and viruses. The US Food and Drug Administration has recognized ZnO as a safe material. The release of Zn ions from ZnO has been suggested as one of the primary antibacterial mechanisms. Moreover the penetration of a bacterial membrane upon contact with ZnO can also contribute to the antibacterial ability of ZnO NPs [9].

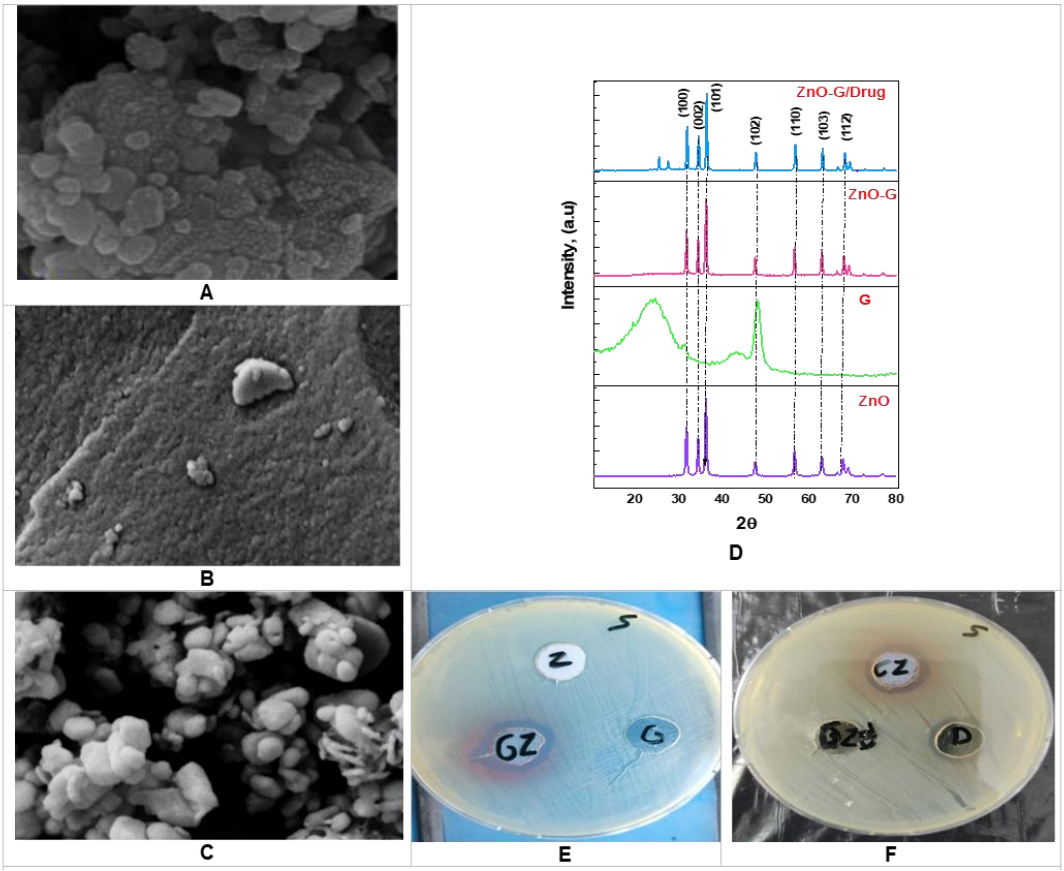

Figure 1. Instrumental analysis and antibacterial activity of zinc oxide (ZnO) / graphene (G) nanoparticles (NPs). **A–C:** Scanning electron microscopy images of ZnO, G, and the ZnO/G-drug complex, respectively. **D:** X-Ray diffraction patterns before and after decoration with graphene and loading with metronidazole. **E** and **F:** Zone of inhibition as a measure to establish the antibacterial potential of ZnO NPs and of ZnO-G-metronidazole NPs, respectively.

Several mechanisms have been proposed for the bactericidal activities of ZnO NPs, including the direct contact with cell membrane, the release of metallic ions, the generation of reactive oxygen species (ROS), and the internalization of ZnO NPs. For the direct contact killing mechanism, ZnO NPs tend to disrupt the cell membrane function and interfere the electron transport chain upon attachment on the cell wall, thereby leading to the production of ROS. Positively charged ZnO NPs can attach readily on the cell membranes of Gram-positive and Gram-negative bacteria due to the electrostatic interaction. This interaction disrupts the membrane structure and damages the cell integrity, leading to the leakage of intracellular contents. In particular, ZnO NPs with very small sizes (≤10 nm) can easily enter the cytoplasm, thereby inducing DNA damage. Due to their cell wall differences, Gram-positive and Gram-negative bacteria react differently to ZnO NPs. Gram-positive bacteria contain 20–80-nm peptidoglycan layers attached to the cytoplasmic membrane by lipoteichoic acid (LTA). As the peptidoglycan layers are porous and do not block tiny substrates, the cell walls of Gram-positive bacteria are highly charged anionic polymers with teichoic acid and LTA phosphate groups that facilitate the electrostatic attraction of positively-charged NPs. This is why ZnO NPs prefer Gram-positive bacteria over Gram-negative ones. However, the Gram-negative peptidoglycan is thinner (<10 nm) and wrapped with an outer membrane of lipopolysaccharide (LPS). LPS is a complex macromolecule that hydrophobic antibiotics cannot penetrate. Hydrophobic antibiotics and many hazardous chemicals are blocked by this outer membrane. This is why Gram-negative bacteria with thinner peptidoglycan layers and outer LPS membranes can withstand ZnO NPs

better than Gram-positive bacteria [10].

5. CONCLUSION

ZnO/G nanostructures with positive surface charge are able to adhere and attach on negatively-charged membranes *via* electrostatic interaction when they come in contact with bacteria. This effect disrupts the bacterial cell membrane function, interferes electron transport chain, and deactivates bacterial enzymes, thereby leading to cell death. Apart from the "contact" killing effect, other mechanisms such as the ROS production and the release of Zn ions have also been reported to be responsible for the bactericidal activity of ZnO-containing NPs. The antimicrobial activity of ZnO NPs is size-, shape-, and concentration-dependent.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

REFERENCES

1. Habeeb S.A., Hammadi A.H., Abed D., Al-Jibouri L.F.: Green synthesis of metronidazole or clindamycin-loaded hexagonal zinc oxide nanoparticles from *Ziziphus* extracts and its antibacterial activity. *Pharmacia* 69(3): 855-864 (2022).
DOI: **10.3897/pharmacia.69.e91057**

2. Tegos G.P., Demidova T.N., Arcila-Lopez D., Lee H., Wharton T., Gali H., *et al.*: Cationic fullerenes are effective and selective antimicrobial photosensitizers. *Chem. Biol.* 12(10): 1127-1135 (2005).
DOI: **10.1016/j.chembiol.2005.08.014**
PMID: **16242655**

3. Lin Y., Hong R., Chen H., Zhang D., Xu J.: Green synthesis of ZnO-GO composites for the photocatalytic degradation of methylene blue. *J. Nanomater.* 2020: 4147357 (2020).
DOI: **10.1155/2020/4147357**

4. Habeeb S.A., Zinatizadeh A.A., Zangeneh H.: Photocatalytic decolorization of direct red 16 from an aqueous solution using B-ZnO/TiO_2 nano photocatalyst: synthesis, characterization, process modeling, and optimization. *Water* 15(6): 1203 (2023).
DOI: **10.3390/w15061203**

5. Zanni E., Bruni E., Chandraiahgari C.R., De Bellis G., Santangelo M.G., Leone M., *et al.* Evaluation of the antibacterial power and biocompatibility of zinc oxide nanorods decorated graphene nanoplatelets: new perspectives for antibiodeteriorative approaches. *J. Nanobiotechnology* 15(1): 57 (2017).
DOI: **10.1186/s12951-017-0291-4**
PMID: **28764786**

6. Hammadi A.H., Habeeb S.A., Al-Jibouri L.F., Hussien F.H.: Synthesis, characterization and biological activity of zinc oxide nanoparticles (ZnO NPs). *Syst. Rev. Pharm.* 11(5): 431-439 (2020).
DOI: **10.31838/srp.2020.5.59**

7. Wu Y., Zhang L., Zhou Y., Zhang L., Li Y., Liu Q., *et al.*: Light-induced ZnO/Ag/rGO bactericidal photocatalyst with synergistic effect of sustained release of silver ions and enhanced reactive oxygen species. *Chin. J. Catal.* 40(5): 691-702 (2019).
DOI: **10.1016/s1872-2067(18)63193-6**

8. Straumal B., Mazilkin A., Protasova S., Myatiev A., Straumal P., Goering E., *et al.*: Influence of texture on the ferromagnetic properties of nanograined ZnO films," *Phys. Status Solidi* (B) 248(7): 1581-1586 (2011).
DOI: **10.1002/pssb.201001182**

9. Bykkam S., Narsingam S., Ahmadipour M., Dayakar T., Rao K.V., Chakra C.S., *et al.*: Few layered graphene sheet decorated by ZnO nanoparticles for anti-bacterial application. *Superlattices Microstruct.* 83: 776-784 (2015).
DOI: **10.1016/j.spmi.2015.03.063**

10. Li Y., Liao C., Tjong S.C.: Recent advances in zinc oxide nanostructures with antimicrobial activities. *Int. J. Mol. Sci.* 21(22): 8836 (2020).
DOI: **10.3390/ijms21228836**
PMID: **33266476**