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Preparation of nanohybrid compound from the drugs (naproxen and cephalexin) with zinc oxide and studying biological activities against *Aeromonas* bacteria

Maryam Sabah & Mohammed Abdulla Jabor

Babylon University, College of Science, Department of Biotechnology.
Correspondence to Maryam Sabah (email: maryam.alhasnawy@yahoo.com).
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Objectives This study focus on the preparation of the compound nanoscale layers of Zinc oxide (ZnO) with naproxen and cephalexin.
Methods Ion exchange technique via sol-gel method synthesised under aqueous environment, resulted in the formation of inorganic organic characteristics of nanohybrid by using X-ray diffraction (XRD) method and study antibacterial activity of hybrid and free compound against some bacteria.
Results The powder X-ray diffraction showed new level for nanohybrid compounds differ from free compounds. The antibacterial results show more nanohybrid compounds concentration increased its effectiveness against bacteria, the greater the concentration of the compound greater is the increase in its effectiveness against bacteria inhibitory.
Conclusions The possibility of the bind of drugs (Cephalexin and Naproxen) in the preparation of nanohybrid-ZnO compound and the use of nanocompounds as antibacterial agent in better treatment of pathogenic bacteria. And this nanocompound is more efficient from free compounds.
Keywords nanohybrid, biological activities, zinc oxide, naproxin, cephalexin

Introduction

Metal oxide nanoparticles and composite materials are widely applied in the field of research and development and diverse applications in industries including surface coatings, optoelectronics, bioengineering, biodiagnostics, and agriculture. The emergence of bacterial resistance to antibiotics and its dissemination, however, are major health problems leading to treatment drawbacks for a large number of drugs.[3,4] Consequently there has been increasing interest in the use of inhibitors of antibiotic resistance for combination therapy.[5,6] Investigations have been carried out on the biological activities of zinc nanoparticles; however, the effects of nanoparticles on the activities of antibiotics have not been demonstrated. Their intrinsic properties are mainly determined by size, shape, composition, crystallinity, and morphology. Highly ionic ZnO nanoparticles are unique in that they can be produced with high surface area, unusual crystal structures, and size. The main advantages of using ZnO nanoparticles are its excellent stability or long shelf life with organic antimicrobial agents.[2] In particular, the vigorous antimicrobial properties of nanoscale ZnO particles have been the focus of industrial applications in biocides coating in water treatment, paints, and cosmetic products.[7] The scope of ZnO nanoparticles has been a keen area of interest for biologist due to their distinguished antimicrobial and distinct activity which have opened new frontiers to biological sciences.[8] Especially its nanoscale form has a strong toxicity towards a wide range of microorganisms including bacteria,[9] fungi,[10] fish,[11] algae,[12] and plants.[13]

Materials and Methods

Synthesis of Zinc Nanoparticles

Following the method described by Bashi et al.[14] with some modification in the composite nanoscale hybrid preparation by adding 100 ml of each of the drug (separately) of the prepared ZnO solution (by melting1 g of ZnO in 100 ml of distilled water after removing the ions). The mixture is stirred magnetically at 40°C for 6 hours and then placed in an incubator at room temperature for 18 hours, followed by separation of sludge mediated centrifuge at 3,000 r/min for 20 minutes and then it is washed with distilled water ions removed him several times and then dried sludge at a temperature of 50°C. It was then crushed in a ceramic mortar and finally stored, as explained in Fig. 1.

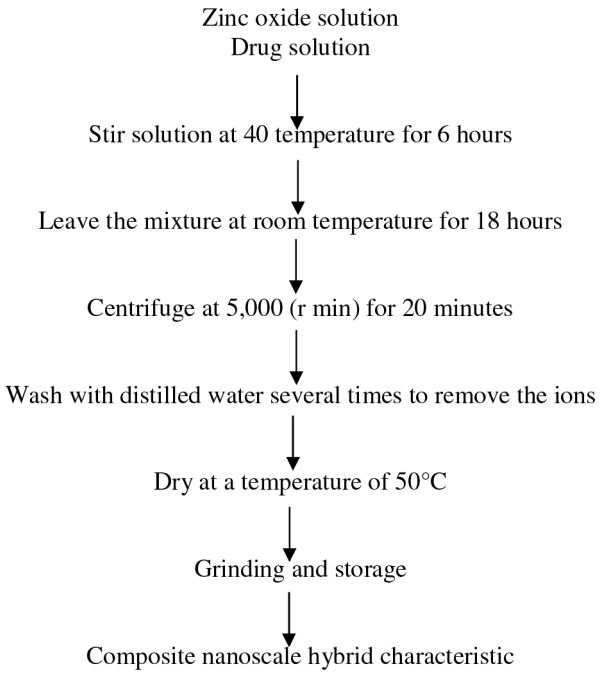

Fig. 1 **Flowchart showing details of synthesis of nano ZnO.**

Characterisation of Nanohybridisation Compounds

The composite nanoscale hybrid characteristic (cephalexin-ZnO and naproxen-ZnO) is found through spectroscopic methods and microscopic examinations scanner (scanning electron microscope, SEM) and atomic force (atomic force microscope, AFM), as follows:

1. Characteristic using X-ray diffraction (XRD)

The nanohybrid compound (naproxen-ZnO and cephalexin-ZnO) characteristic by use XRD. The XRD between ZnO and cephalexin shows diffraction level at (111) at 2θ = 9.466 with crystalline distance (d) = 0.933 and another diffraction level (222) at 2θ = 18.9285 with crystalline distance (d) = 0.469 and (333) at 2θ = 28.3907 with crystalline distance (d) = 0.314.

The XRD between ZnO and Naproxen shows diffraction level at (111) at 2θ = 4.7661 with crystalline distance (d) = 1.90651 and another diffraction level (200) at 2θ = 56.7084 with crystalline distance (d) = 1.62195 this result provide the squeeze drugs in the layers of ZnO.

2. Antibacterial activity

The study of the effectiveness of inhibitory monohybrid compounds (ZnO-Ceph and ZnO-Napro) and free compounds (Napro, Ceph and ZnO) against *Aeromonas* spp by using diffusion method.[16] The concentrations using in the study range is between 0.1 and 15 mg/ml.

Results and Discussion

Spectrum XRD

XRD pattern of the prepared ZnO nanoparticles is shown in Fig. 2. The observed diffraction peaks of ZnO at 2-theta = 31.72°, 34.38°, and 36.26° are associated with 100, 002, and 110; all the reflections can be assigned to the standard powder pattern of ZnO.[14]

Spectrum XRD for Cephalexin-ZnO

XRD pattern of the prepared Ceph-ZnO nanohybridisation is shown in Fig. 3. The XRD between ZnO and cephalexin shows diffraction level 111 at 2θ = 9.466 with crystalline distance (*d*) = 0.933 and another diffraction level 222 at 2θ = 18.9285 with crystalline distance (*d*) = 0.469 and (333) at 2θ = 28.3907 with crystalline distance (*d*) = 0.314. In addition, the presence of the diffraction level related to ZnO indicates that the level of ZnO still keep of their natural structure and the cephalexin may squeeze in the layers of ZnO.

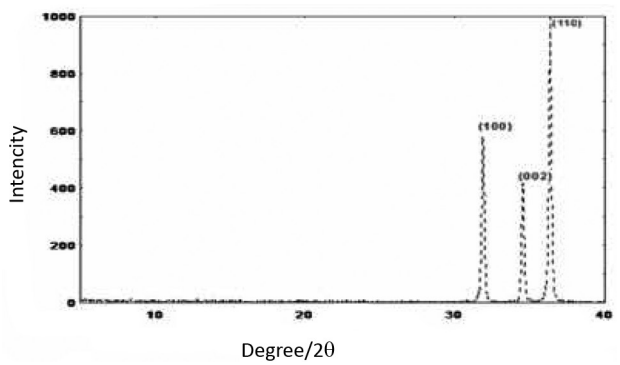

Fig. 2 **Spectrum XRD for ZnO.**

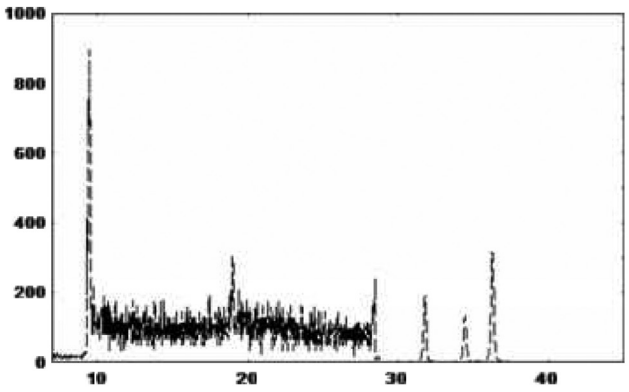

Fig. 3 **XRD for Cephalexin-ZnO nanohybridisation.**

Effectiveness of the Two Nanocompounds Inhibitory (Zno-Ceph and Zno-Napro) and Free Compounds (Napro, Ceph, and ZnO) against Bacteria

Results of statistical analysis in Table 1 shows that there are significant differences $P < 0.05$ between the tested nanohybrid compounds and free compounds on one hand and between the concentrations used for the same compound on the other hand.

When comparing the three free compounds first used (Napro, Ceph, ZnO) with concentrations of these compounds, it did not show any inhibitory effect against bacteria at any of the concentrations studied. This indicates that there was no significant difference $P > 0.05$. As for the nanohybrids compounds Ceph and Napro showed no effective inhibitory at three concentrations 0.05, 0.1, 0.25 mg/ml while the nanohybrid compounds (Napro-ZnO) 0.05 if given the inhibition rate of 20 mm followed by nano hybrid composite (Ceph-ZnO) at a rate of inhibition of 16.66 mm at the same concentration and thus differed in terms of efficiency Free compounds did not show any effect against bacteria. But when comparing all compounds at the first three concentrations 0.05, 0.1, 0.25 mg/ml found that there was no significant difference $P > 0.05$.

While the results showed significant differences clear of nanohybrid compounds and superiority on free compounds at the same concentrations as superiority nanohybrids (Napro-ZnO) if given the inhibition rate 20 mm followed by Ceph-ZnO at a rate of 16 mm inhibitory 16.66 mm. This indicates the efficiency of nanohybrid compounds compared to free compounds. The result show the nanoparticle compound affected the bacteria for several reasons. Many studies provide nanoparticles have larger surface area available for interactions, which enhances bactericidal effect than the large-sized particles; hence they impart cytotoxicity to the microorganisms.[17] The mechanism by which the nanoparticles are able to penetrate the bacteria is not understood completely, but studies suggest that when bacteria were treated with ZnO nanoparticles, changes took place in its membrane morphology that produced a significant increase in its permeability affecting proper transport through the plasma membrane[18] leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting in cell death.[19] Experimental observations of this study have

Table 1. **Inhibition zone (mm) of Naproxen and Cephalexin and its nanocompounds against *Aeromona***

Concentration (mg/ml)	ZnO	Naproxen	Cephalexin	Cephalexin-ZnO	Naproxen-ZnO	$LSD_{0.05}$ compound
0.05	A 0.0 ± 0.0 a	A 0.0 ± 0.0 a	A 0.0 ± 0.0 a	D 0.0 ± 0.0 a	E 0.0 ± 0.0 a	
0.1	A 0.0 ± 0.0 a	A 0.0 ± 0.0 a	A 0.0 ± 0.0 a	D 0.0 ± 0.0 a	E 0.0 ± 0.0 a	
0.25	A 0.0 ± 0.0 a	A 0.0 ± 0.0 a	A 0.0 ± 0.0 a	D 0.0 ± 0.0 a	E 0.0 ± 0.0 a	
0.5	A 0.0 ± 0.0 b	A 0.0 ± 0.0 b	A 0.0 ± 0.0 b	D 0.0 ± 0.0 b	D 8.0 ± 0.57 a	0.322
1	A 0.0 ± 0.0 c	A 0.0 ± 0.0 c	A 0.0 ± 0.0 c	C 6.33 ± 0.33 b	D 8.0 ± 0.52 a	
5	A 0.0 ± 0.0 c	A 0.0 ± 0.0 c	A 0.0 ± 0.0 c	B 10.0 ± 0.57 a	C 9.0 ± 0.55 b	
10	A 0.0 ± 0.0 c	A 0.0 ± 0.0 c	A 0.0 ± 0.0 c	B 10.0 ± 1.15 b	B 11.0 ± 0.59 a	
15	A 0.0 ± 0.0 c	A 0.0 ± 0.0 c	A 0.0 ± 0.0 c	A 16.66 ± 0.88 b	A 20.0 ± 1.15 a	
$LSD_{0.05}$ Concentration			0.373			$LSD_{0.05}$ Interference 0.843

The numbers refer to mean ± standard error; capital letters indicate significant differences ($P < 0.05$) between the concentrations of each compound; small letters indicate significant differences ($P < 0.05$) between compounds of each concentration.

explained significantly the antibacterial behaviour of ZnO nanoparticles. It is clear that ZnO nanoparticles have high preventive effect on life of *Listeria monocytogenes*. Nanoparticles have a relatively large surface area and have more contact with bacteria.[20] As a final result the nonspecific mode of action of nanoparticles against bacteria makes them ideal candidates as antimicrobial agents with less risk of development of bacterial resistance. The results have shown that the particles possess antibacterial properties against bacterial pathogens.[21]

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