RESEARCH ARTICLE

Antibacterial Activity of Magnesium Oxide Nanoparticles Prepared by Calcination Method

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ABSTRACT

Magnesium oxide nanoparticles (MgO NPs) were prepared by a simple wet chemical method using different calcination temperatures. The prepared NPs were characterized by electrostatic discharge (ESD), scanning electron microscope (SEM), and X-ray Diffraction (XRD). It demonstrates a sharp intensive peak with the increase of crystallinity and increase of the size with varying morphologies with respect to increasing of calcination temperature. Antibacterial studies were done on gram-negative bacteria (E.coli) and gram-positive bacteria (S.aureus) by agar disc diffusion method. The zones of inhibitions were found larger for gram-positive bacteria than gram-negative bacteria; this means, antibacterial MgO NPs activity more active on gram-positive bacteria than gram-negative bacteria because of the structural differences. It was found that the antibacterial activity of MgO NPs was found it has directly proportional to their concentration.

Keywords: Antibacterial activity, Calcination method, MgO, Nanoparticles.

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INTRODUCTION

Multi-Drug Resistance (MDR) bacteria are resistant to one or more families of antibiotics, prevention, and controlling of this problem is an international liability.[1] Many techniques fail to overcame MDR, but inorganic nanoparticle antibiotic is promising success alternative to organic antibiotic.[2]

NPs appeared as the forefront of sciences and technologies during the last century.[3] NPs technology plays a serious role in the industrial revolution.[4] Inorganic substance such as metal oxides can resist ruthless processing conditions so they had attracted a lot of concern over the last century.[5-6]

Metal oxides such as magnesium oxide (MgO), zinc oxide (ZnO), titanium oxide (TiO_2) and calcium oxide (CaO) are of particular concern as they are safe materials to human beings and animals yet, selectively stable under harsh processing conditions.[7] Metal oxides can be synthesized into nanoparticles ranging from 1–100 nm. Nanoparticles supply targeted, extended, and strong antimicrobial activity at smaller dosages.[8] However, metal oxide-based nanoparticles physically interact with the cell of bacteria through three major pathways; first: by interaction with phospholipid bilayer, metal oxide NPs can damage the cell membrane potential by binding electrostatically to the bacterial cell wall or/and releasing metallic ions.[9] Second: formation of reactive oxygen species (ROS) or oxygen free radicals (ORS), such as superoxide anions or hydrogen peroxide (H_2O_2).[10] ROS lead to severe oxidative stress and damage to the cell's macromolecules, which overall cause lipid peroxidation, inhibition of enzymes, ribonucleic acid (RNA)/deoxyribo nucleic acid (DNA) damage, and alteration of proteins.[11-12] Third: binding to cytosolic proteins such as enzymes and DNA. This interaction leads to inhibiting ATP production, decreased function, and respiratory and metabolic pathways.[13-14]

The MgO or magnesia is a white hygroscopic solid mineral that occurs naturally as Pericles and is a source of magnesium. MgO is consists of a lattice of Mg^{+2} ions and O_2^{-2} ions held together by ionic bonding.[15] MgO NPs prepared by different methods such as Calcination, Solgel, Hydrothermal, Chemical gas phase, Deposition, and wet precipitation method.[16]

Nanoparticles of MgO have unique properties with antibacterial activity compared to their bulk counterparts. in which NPs size is important in antibacterial effectiveness.[17]

Several tests can use to screen the material such as energy dispersive spectroscopy (EDS), scanning electron microscope (SEM), and XRD. Energy dispersive spectroscopy (EDS) is a swift discharge of electric current between two objects with different charges and different numbers of electrons. This exchange of electrons creates a large electromagnetic field buildup, resulting in EDS.[18] SEM is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals

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that contain information about the surface topography and composition of the sample.[19] The XRD is one of the microstructural analysis methods used for the identification of crystallinity of polymers, recognition of crystalline phases (polymorphism), and orientation of polymers.[20]

MATERIALS AND METHODS

Materials

Magnesium Nitrate $Mg(NO_3)_2.6H_2O$, ethylene glycol solution, Na_2CO_3, and distilled water were purchased locally (Iraq). Dimethylsulfoxide (10%) was a gift from Al-Safwa University (Iraq). Muller-Hinton Agar (OXOID, England)

Methods

Preparation of MgO NPs by Calcination method

A predetermined weight (12.30g) of $Mg(NO_3)_2.6H_2O$ was dissolved in 25 mL solution of ethylene glycol. A magnetic stirrer was used to mix the mixture for 1 hour. A precise weight (2.70g) of Na_2CO_3 was dissolved in 100ml of distilled water, from which 12.5mL was taken and added to the above mixture under sonication for 15 minutes. The last solution was filtered, washed using distilled water, and dried at 50°C. Condition used in the calcination method was (380°C, 6 hours, 1.06 °C/min).[21]

Antibacterial activity test of MgO NPs

The antibacterial effects of MgO NPs were tested against MDR gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria assessed by agar well diffusing method. Dimethylsulfoxide (10%) was used as a solvent, and negative control of two concentrations of MgO NPs (20µg/mL and 80 µg/mL) were used at 37°C for 24hour. Then the inhibitory activity was measured by the diameter of the inhibition zone around the wells.[22]

Antibacterial susceptibility test

The antibiotic susceptibility of bacteria is shown in Table 1 which is assessed by Disc-Diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) and bacteria were classified as resistant (R), intermediate (I) or sensitive (S), according to the zone table.[23]

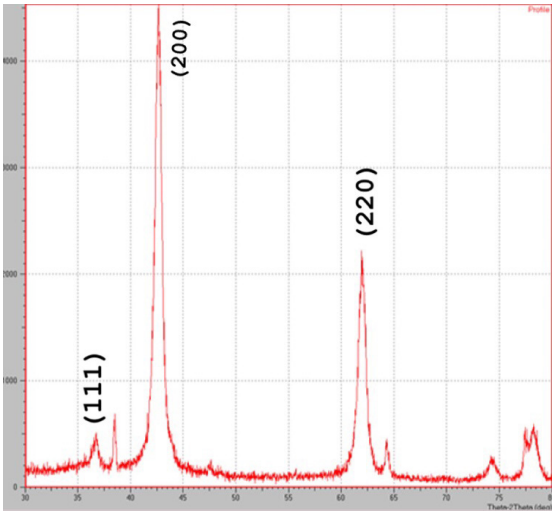

Figure 1: XRD pattern for the prepared MgO NPs

RESULT AND DISCUSSION

X-Ray Diffraction (XRD)

XRD peaks of MgO NPs in the powder form are shown in Figure 1. Shape Peaks at 2Θ values of (36.65)◦, (42.64)◦ and (62.14)◦ corresponding to (111), (200) and (220) planes of MgO, are in good agreement with the reported JCPDS powder diffraction card of MgO which indicates the purity of prepared MgO NPs.[24]

Scanning Electron Microscope Analysis

Figure 2 shows the SEM image for prepared MgO NPs in the powder form. It shows small particles spherical in shape, with crystalline structure distributed within a big mass of equalizer aggregate.

The above figure is consistent with the antibacterial activity, which depends on the surface area. The large surface area of the MgO NPs enables them to act as an antibacterial agent.

Energy Dispersive Spectroscopy

The EDS spectrum of prepared MgO NPs Figure 3 shows shape peaks corresponding to magnesium and oxygen, which indicate the purity of the prepared sample.

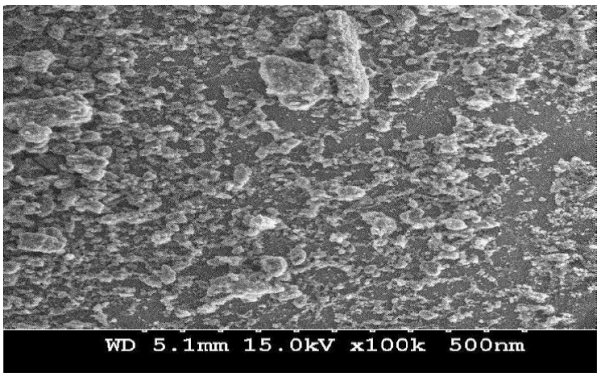

Figure 2: SEM for the prepared MgO NPs

Table 1: Antibiotic used in the susceptibility test

Antibiotic	*Dose (mcg)*	*Symbol*
Erythromycin	10	E
Trimethoprim	10	TMP
Amikacin	10	AK
Gentamicin	10	CN
Penicillin G	10	P
Clindamycin	10	DA
Nitrofurantoin	300	F
Ciprofloxacin	10	CIP
Vancomycin	10	VA
Oxacillin	5	OX
Tetracycline	10	TE
Norfloxacin	10	NOR
Cephalothin	30	KF

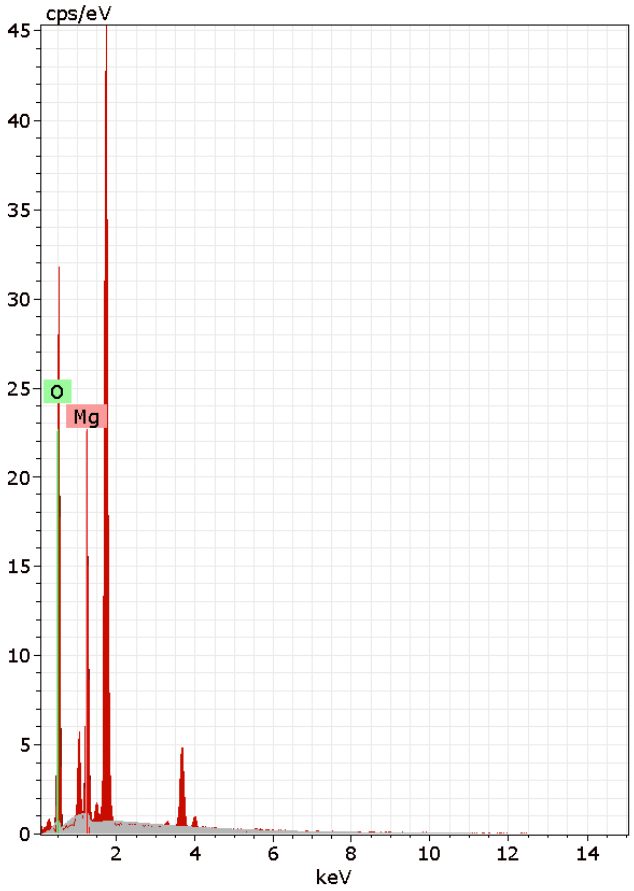

Figure 3: The EDS of prepared MgO NPs

Table 2: Antibiotic susceptibility test

	Diameter of inhibition zone (mm)	
Antibiotic	*E. coli*	*S. aureus*
E	31	30
TMP	R*	R
AK	R	R
CN	R	R
P	R	R
DA	32	R
F	R	R
CIP	40	40
VA	20	R
OX	R	R
TE	R	R
NOR	34	40
KF	R	R

*R = resistance bacteria

Table 3: Antibacterial activity of the prepared MgONPs

	MgONPs inhibition zone (mm)		
Bacterial species	*20 µg/mL*	*80 µg/mL*	*Negative control*
MDR *S. aureus*	30		± 0.5
			40 ± 0.5
			5 ± 0.5
MRD *E. coli*	27		± 0.5
			30 ± 0.5
			5 ± 0.5

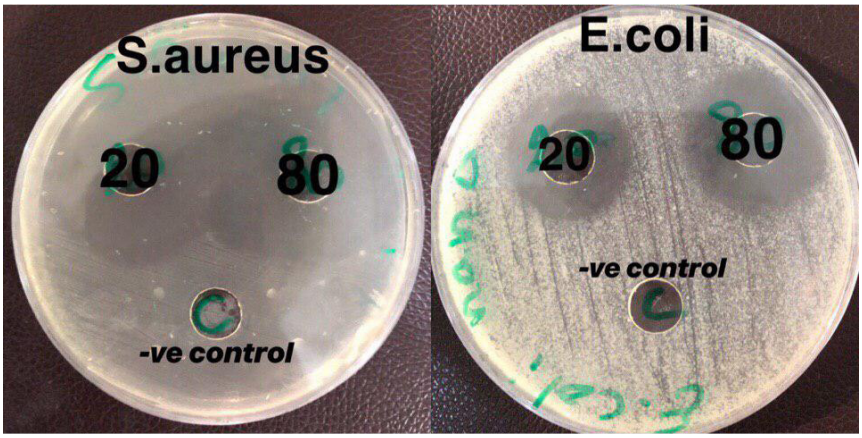

Figure 4: antibacterial activity of the prepared MgO NPs against *S.aureus* and *E. coli* .

Antibacterial activity

Antibacterial susceptibility of different antibiotics was tested against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria by the Disc-Diffusion method.

The result shows that both bacteria were MDR. The *S. aureus* was resistance to Trimethoprim, Amikacin, Gentamicin, Penicillin G, Clindamycin, Nitrofurantoin, Vancomycin, Oxacillin, Tetracycline, and Cephalothin, but it was sensitive to Erythromycin, Ciprofloxacin, and Norfloxacin with a diameter of inhibition zone (30,40,40 mm) respectively. As shown in Table 2.

Regarding *E. coli,* it was resistance to Trimethoprim, Amikacin, Gentamicin, Penicillin G, Nitrofurantoin, Oxacillin, Tetracycline, and Cephalothin, but it was sensitive to Erythromycin, Clindamycin, Ciprofloxacin, Vancomycin and Norfloxacin with diameter of inhibition zone[31,32,40,20 ,34] respectively, as shown in Table 2.

Antibacterial activity of different antibiotic and MgO NPs were evaluated against MDR (*E. coli* and *S. aureus*) by agar well diffusion method and dimethylsulfoxide as a negative control, as shown in Table 3 and Figure 4.

At the same sample size, the prepared MgONPs antibacterial activity was very good at low concentration (20, 80 µg/ml) as compared to other literature in which they found that MIC of MgONPs of *E. coli* and *S. aureus* were 125µg/mL.[25]

At 20 µg/ml, 30mm inhibition zone was observed around MgONPs in MDR *S. aureus*, but slightly less diameter was found in MRD *E. coli* Higher inhibition zone was recorded at 80 µg/mL in MDR *S. aureus* and MRD *E. coli.* as (40, 30 mm), respectively, which may be considered as a significant difference between the different types of bacteria. Ibrahim et al., Palanisamy et al. also found that MgONPs were more active on gram-positive than gram-negative bacteria.[26,27]

MRD *E. coli* showed stronger resistance to MgONPs than MDR *S. aureus* because of the difference in the cell wall structure. Espitia et al. explain that to the presence of a lipopolysaccharide layer in the cell wall of gram-negative bacteria in addition to peptidoglycan in contrast to gram-positive which had peptidoglycan only.[28]

CONCLUSIONS

The prepared MgO NPs was effective against MDR bacteria which can be used as an alternative for currently used antibiotics to treat infections caused by MDR *E. coli* and *S. aureus.*

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