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RESEARCH ARTICLE - PHYSICS

Anti-Serratia marcescens Efficacy of MgO Nanoparticles Prepared by Hydrothermal Methods under Different Calcination Temperature

Farah Noori Ibraheem[1]*, Suzan Saadi Hussain[2], Haitham Talib Hussain [3]

[1] Department of Biology, College of Science, Mustansiriya University, Baghdad, Iraq
[2]Department of Biology, College of Science, Mustansiriya University, Baghdad, Iraq
[3] Department of Applied Physics, College of Applied Science, University of Technology, Baghdad, Iraq
[2]suzansaadialroznamachi@uomustansiriyah.edu.iq , [3]haithamT.hussein@uotechnology.edu.iq
Corresponding author E-mail: fofanoori99@uomustansiriyah.edu.iq

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Abstract

The main aim of this study was to develop a straightforward procedure for synthesis magnesium oxide nanoparticles (MgO NPs) that used as antimicrobial agent against Serratia marcescens, a gram negative bacterium which isolated from post-operative wound infection. A high -purity nanoparticles of MgO has been prepared by applying deferent calcinations temperature (200ºC, 400ºC, 650ºC) using hydrothermal chemical method. The hydrothermal technique is a process used to create a crystalline nanomaterial's from high-temperature aqueous solutions with high vapour pressures, this way can be prepared more shape of nanoparticles that are highly stable. The structural, morphological and optical properties of MgO NPs were conducted using : XRD, FE-SEM and UV-vis analysis respectively. The crystallinity it increases with an increase of calcination temperature that reducing the effect on antibacterial activity were done on pathogenic bacterium as result of increase in particle size with sharp intense peak. The antimicrobial effects of the prepared MgO NPs were examined against multi-drag resistance (MDR) strains using Micro-dilution titer method with various doses of minimum inhibitory concentration (MIC) of MgO NPs (1500, 1000, 500, 250, 125, 62.5, 31.5 µg/ml^{-1}). The higher concentration value which decreased the bacterial growth was recorded at 1500µg/ml^{-1} at 650 ºC, and the lower was recorded as 31.5 µg/ml^{-1} at 200ºC. This preparation procedure yielded higher nanoparticles effectiveness in fast, simple and economical manner that have active role against microbial growth.

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Keywords: magnesium oxide synthesis, crystallite size, structural properties, antibacterial applications, hydrothermal method.

1. Introduction

S. marcescens is an opportunistic pathogenic bacterium, gram negative, non-spore forming, that is commonly spread in the environment. It is one of the most important nosocomial pathogens in patients who have surgical site wound infections [1]. It is occasionally known as a cause of hospital acquired infections, which can result in substantial morbidity and mortality [2]. Post-operative wound infection is a common health care problem due to considered the surgical site represent the primary source of nosocomial infection in surgical patients especially S. marcescens [3]. The wounds signify a susceptible site for opportunistic colonization by organism of endogenous and exogenous derivation, wound patients are public infected by bacteria and are difficult to control [4]. Development resistance of this bacterium towards large number of antimicrobial agents and failure to treat infection lead to increases the prospect that biofilms may form and facilities the spread of genes for antibiotic resistance. Significant concern that has prompted the need for properly though-out alternative treatments [5]. Nano science and technology have gained considerable prominence in the scientific and technological realm in the past ten years[6], especially biological activity (antimicrobial agents) due to small dimension it perfect for antibacterial actions, anti-biofilm, anti-oxidant, anti-cancer, wound healing and others benefits material treatment [7]. Magnesium oxide nanoparticles are a new class of antimicrobial agents[8] have unique several properties such as non-toxic to human cell, high stability, surface properties and chemical composition made it great potential for biological activity[9]. It has been shown to exhibit antibacterial activity against various harmful bacteria including Serratia marcescens. The small size and high surface area of Mgo Nano-particles allowed for major interaction with bacteria, was caused several damages to the cell [9]. Magnesium oxide nanoparticles were successfully

synthesized by hydrothermal technique, It's simple, efficient and economic method, does not need any surfactant and additives, could be used to produce high-quality particles formed of MgO NPs spherical shape.[10] The purpose of this study was to create MgO NPs via hydrothermal technique, characterize them, study the effect of calcined temperature on particle size, and evaluate their antibacterial actions against *Serratia marcescenc*.

2. Experimental work

2.1 Preparation of Nano-MgO

MgO nanostructure was prepared by using hydrothermal method. Firstly, prepared (0.5 mol) magnesium chloride and (0.5 mol) sodium carbonate each of them was dissolved in 100 ml of deionized water separately. The 100 ml of Na_2CO_3 solution was added gradually to the $MgCl_2$ solution when was stirring in a magnetic stirrer under room temperature to gain $MgCO_3.3H_2O$, the solution become white color. After the powerful stirring for 30 minutes, the product was transmitted inside a stainless steel autoclave lined with Teflon, locked and put it in oven heat-treated under a fixed temperature of 60ºC for 4h to obtain the product. Then, the precipitated particles after filtration washed with distilled water repeatedly until near to pH 7. After that placed the product in tube furnace, were calcined for 2 hours at various temperature 200ºC, 400ºC, 650ºC to get final output white powder MgO NPs.[11]

2.2 Specimens collection

150 samples were taken from postoperative wound infection patients at two hospitals in Baghdad. These samples were all obtained using a swab, stored in a transport medium, and then imparted to a lab for examination.

2.3 Isolation and Identification of *S. marcescens*

Collected specimens were streaked on MacConky agar as a selective cultural medium and incubated for 24h at 37ºC. VITEK-2Compact system was used to recognize the cultured isolates.

2.4 Testing of Antibiotic Susceptibility

The VITEK-2 compact system used antimicrobial drugs to assess the isolate's susceptibility. It performs a sensitive test and determines the degree of antibiotics' effect against bacteria by using a card containing sixteen types of antibiotics. This system needs 18 hours of analysis time to reveal the result.

2.5 Antibacterial activity of MgO-NPs

By using the micro-dilution assay [12], MgO NPs was examined for the minimum inhibitory concentration (MIC) against clinical isolates of S. marcescens isolated from post-operative wound infection. The MIC [13] was calculated using a micro-titer plate dilution 96-well. Initially, each well containing 100µL of Muller-Hinton Broth, then add 100µL from concentralized concentration (inhibition concentration) of MgO NPs to the first well. Preparation the inoculum by direct colony suspension method that means chosen colonies from 24-h agar plate (fresh culture) and placed in tube contain saline. The suspension was then adjusted to reach a turbidity that was equal to the 0.5 McFarland standard. After that taken 20µl from suspension tube of each isolate and inoculum in the wells. Incubation the plate at 37ºC then, assessed the bacterial growth inhibition by measuring the optical density at 630 nm using an ELX 808 micro-plate reader (Biotek Instruments, Winooski VT, USA). The control was a blank control without MgO-NPs [14].

2.6 Characterization of MgO NPs

For study the physical properties determination the orientation and crystallinity of magnesium oxide nanoparticles using XRD analysis, field emission scanning electron microscopy (FE-SEM) is used to observe the morphology and shape of the synthesis nanoparticles in high microscopic magnification. The optical property of MgO NPs synthesized was analyzed by UV spectrophotometer.

3 Results and Dissection

3.1 Identification of bacterial isolates

Depending on these results, 24 (16.0%) of isolate among 150 samples were identified as S. marcescens using various examinations and conformed using VITEK2 compact system.

3.2 Antibiotic Susceptibility test for *S. marcescens*

Sixteen types of antibiotics used by the VITEK-2compact system to test the sensitivity of isolates from diverse classes of antibiotics were used, the results exhibited : all 24 isolate (100%) were resistance to each Ampicillin, Tetracycline, Doxycycline, 3 isolates(12.5%) resistance to each Piperacillin, Cefotaxime, Aztreonam, 23 isolate (95.8%) resistance to Cefoxitin, 1 isolate (4.1%) resistance to Ceftazidime, 2 isolate (8.3%) resistance to Ceftolozane and Tobramycin, 5 isolate (20.8%) resistance to Minocycline and Chloramphenicol, while all 24 isolate (100%) sensitive to each Tigecycline, Doripenem, Meropenem, 22 isolate (91.6%) sensitive to Ceftolozane and Tobramycin, 21 isolate (87.5%) sensitive to each Piperacillin, Aztreonam, Cefotaxime, 2 isolate (8.3%) sensitive to Chloramphenicol, 23 isolate (95.8%) sensitive to Ceftazidime, 1 isolate (4.1%) sensitive to Cefoxitin, 19 isolate (79.1%) sensitive to Minocycline, finally 17 isolate (70.8%) intermediate to Chloramphenicol shown in fig 1.

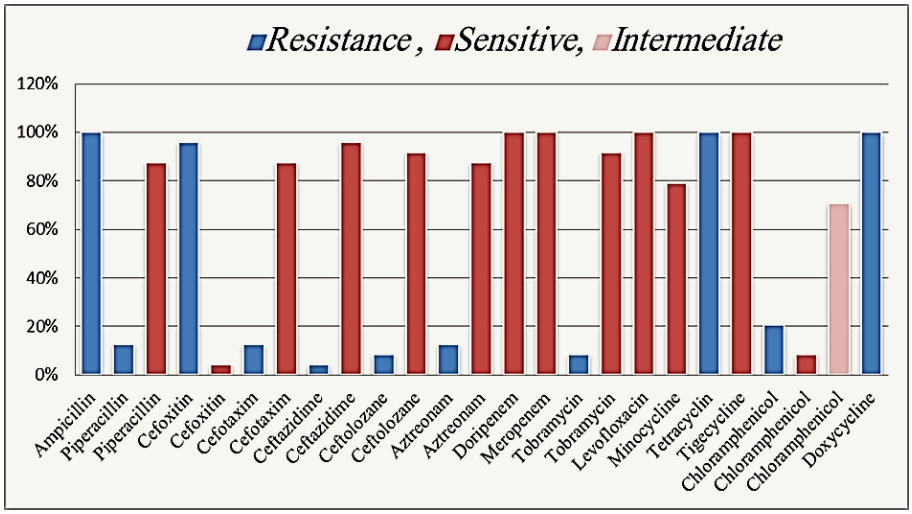


Figure 1. display the isolates of *S. marcesens* and numbers of antibiotics resistant to them.

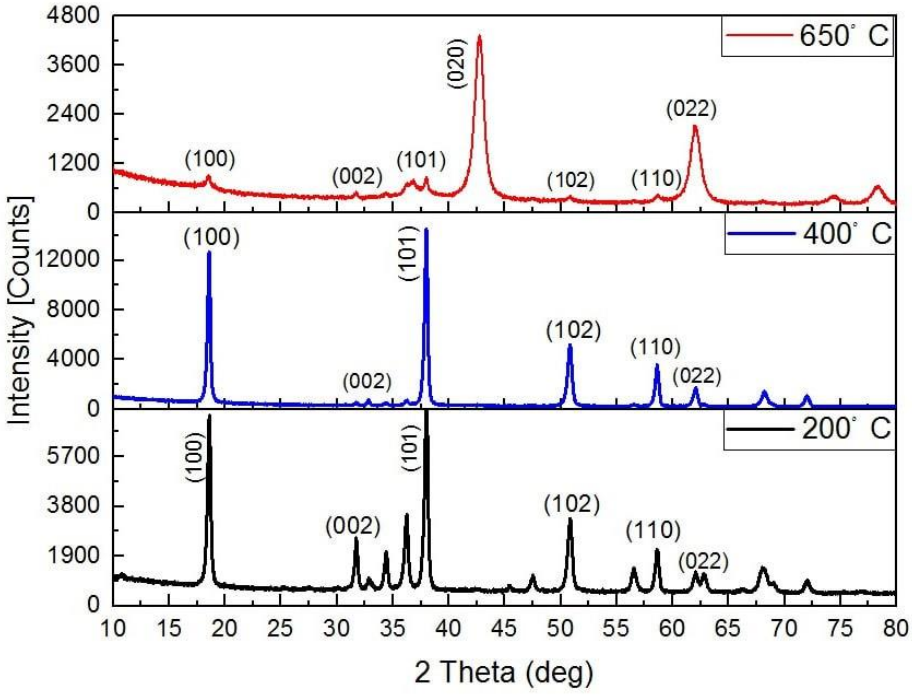
3.3 X-Ray analysis of MgO-NPs

X-ray diffraction was used to determine the crystalline structure of MgO NPs , it important technique to investigate the structure properties and crystalline nature. Fig,2 clarify the x-ray patterns of chemically synthesis of magnesium oxide nanoparticles at different calcination temperature. The diffraction patterns at 200ºC was indicates distinct and high intensity peaks at 18.1º, 31.7º, 37.31°, 51.11°, 58.11º and 62.18° corresponding to miller indexes (hkl) values (100), (002), (101), (102) , (110) ,(002) respectively. While at 400ºC was indicate 2θ= 18.58º, 32.80º, 37.96º, 50.80º, 57.95ºand

62.56º corresponding to miller indexes (hkl) values (100), (002), (101), (102), (110), (002) respectively. Finally the patterns of 650ºC was indicates at 18.9 º, 32.74º, 37.86º, 42.68º, 52.11º, 57.96º, 62.95º corresponding to miller indexes (hkl) values (100), (002), (101), (020), (102), (110) and (022) respectively. All diffraction of MgO NPs could be indexes as the hexagonal structure only diffraction peaks at 650ºC calcination temperature would be indexed as cubic structure confirmed by JCPDS card No. 96-900-6331. The crystalline size of MgO NPs were calculated using the equation of Debye-Scherrer's [15]:

$$D = k\lambda/\beta \cos\theta$$

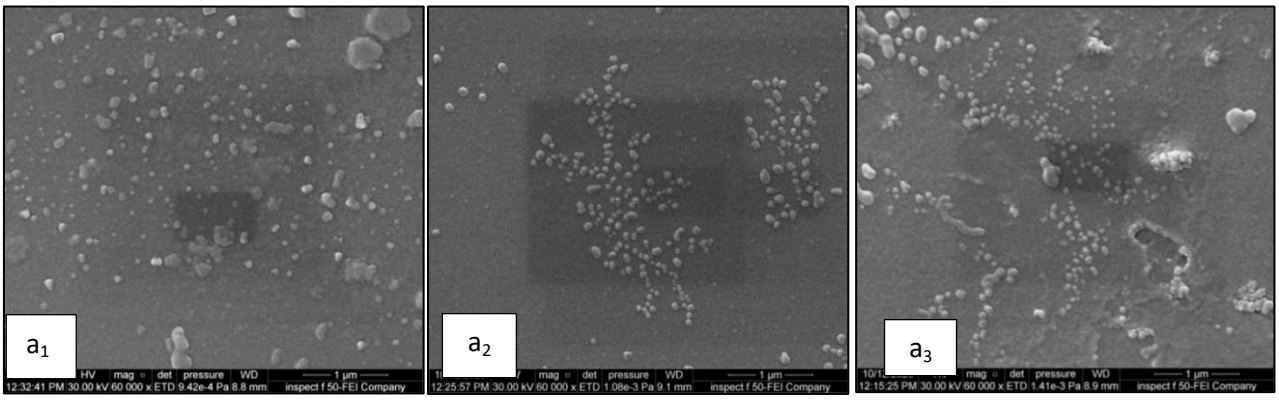
Where 'k' is the shape factor equal to 0.94, β is the full width half maximum, θ is the diffraction angle and λ is the wavelength of Cu. Kα radiation (0.154nm).from this result the peaks of the XRD patterns where lightly shifting with increase of the calcinations [16], this due to stress in crystals that related to size of particles. This improved that the prepared nanoparticles it is a highly pure and has crystal nature.



Figuer.2 show the X-ray analysis of MgO-NPs at 200ºC, 400ºC and 650ºC

3.4 FE-SEM analysis of MgO NPs

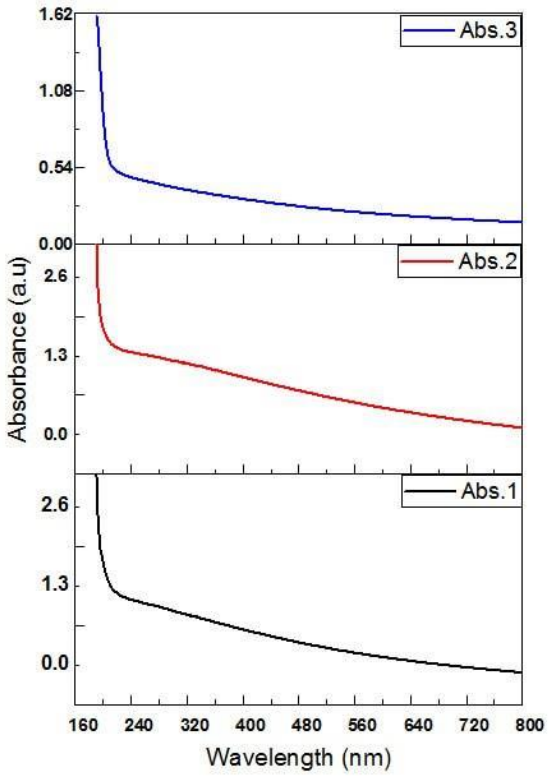
The morphology and shaped of the synthesized MgO NPs are observed via field-emission scanning electron microscopy. [Fig 3 (a1, a2, a3)] evidently displays the creation of spherical shaped nanoparticles in 1µ magnification at 200ºC, 400ºC and 650ºC. The size distribution of the produced nanoparticles was measured, and it was found to be average diameter 34 nm at 200ºC, 40 nm at 400ºC and 52 nm at 650º. It's possible that the smaller NPs aggregated to form the large ones [17]. The increasing size of nanoparticles with increase temperature is attributed to the capacity of nanoparticles to aggregates at higher temperature caused by inter-particle electrostatic forces.



Figuer 3. Exibited the FE-SEM analysis of MgO NPs at (a_1 200ºC, a_2 400ºC, a_3 650ºC).

3.5 UV-Visible spectroscopy

UV-vis spectrophotometer is used to record UV-visible spectra of the prepared MgO NPs in the absorbance mode, and in the wavelength range between 200-1000nm at different calcination temperature that used to created nanoparticles. Fig.4 shows the UV-Vis spectrum at 200ºC, 400ºC and 650ºC, in which the absorption peak is located in the area of 220-800 nm, confirmed the formation of MgO particles nanosized. This change in absorption peak is a result of the change in granular size; the smaller the granular size leads the direction of absorption towards UV-region [18-19].



Figuer.4 shown the UV-vis. of MgO NPs absorbance at 200ºC (Abs.1), 400ºC(Abs.2), 650º(Abs.3).

3.6 Antibacterial studies

The antimicrobial effectiveness of chemically created MgO NPs was estimated against six MDR bacterial strains of S. marcescens (S4, S8, S13, S18, S19, S20). Bacterial isolates were treated with various doses (31.5, 62.5, 125, 250, 500, 1000, 1500µg/ml-1) of MgO NPs. According to the results in table (2) MIC of MgO NPs contrast with different isolates and diverse calcination temperature,

31.5 µg/ml the less inhibition concentration of magnesium oxide viewed at 200ºC, confirmed that better-quality method to synthesis magnesium oxide, its hydrothermal method at 200ºC given highly effectiveness antimicrobial agents against S. marcescens that isolated from post-operative wound infections.

MgO NPs exhibited antimicrobial activity using electrochemical way of action to enter and upset their cell wall. When the cell wall penetrates, outflow of metabolites happens that it makes the cell lose its function, there by hindering the organism from reproduction [20]. The anti-bacterial property of prepared nanoparticles decreases with increases in calcination temperature due to higher temperatures can cause nanoparticles to agglomerate or clump together, reducing their surface area that interaction with bacteria, acceding to the Table (1) below.

Table 1: Effect of methods/ Calcination temperature ºc and particle size in MIC conc.

Calcination temperature ºC	**Morphology**	**Particles size(nm)**	**MIC conc. (µg/ml)**	**Structure**
200ºC	Spherical	34-38 (39.00)	62.50 ±12.50	Hexagnol
400ºC	Spherical	40-46 (43.00)	250.00 ±37.00	Hexagnol
650ºC	Spherical	50-76 (63.00)	1250.00 ±50.75	Cubic
L.S.D.	--	7.238 **	102.84 **	--
P-value	--	0.0001	0.0001	--
** (P≤0.01).				

Table 2: Effect of methods/ Calcination temperature ºc in MIC conc. with difference isolates

Calcination temperature	**MIC conc. (µg/ml)**					
	S4	**S8**	**S13**	**S18**	**S19**	**S20**
200ºC	31.5 ±7.50	31.5 ±7.50	125 ±18.54	62.5 ±12.5	62.5 ±12.5	125 ±18.54
400ºC	125 ±18.54	125 ±18.54	500 ±40.5	250 ±37.0	250 ±37.0	500 ±40.5
650ºC	1000 ±50.00	1000 ±50.00	1500 ±75.50	1500 ±75.50	1500 ±75.50	1500 ±75.50
L.S.D.	42.78 **	42.78 **	107.62 **	81.57 **	81.57 **	107.62 **
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
** (P≤0.01).						

4. Conclusions

MgO NPs created through hydrothermal rout. The prepared MgO powder was calcined at 200ºC, 400ºC and 650ºC and analyzed by XRD, SEM and UV-vis. technique. The particle size was measured as 34 nm at 200ºC, 40 nm at 400ºC and 52 nm with respect to 650 °C using Debye Scherer's equations by X-ray diffraction analysis. The nanoparticles image under various temperatures depicts looking like dense spherical shaped examined by scanning electron microscopy. Antibacterial studies done against six MDR isolates of *S.marcescens* by micro-dilution titer assay, the size effect of nanoparticles on the antibacterial efficacy has been inspected under various temperatures. This process for preparation of magnesium oxide will be beneficial in cost effective manner and biotechnological applications.

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