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Research Article

Optimized Synthesis of Magnesium Oxide Nanoparticles as Bactericidal Agents

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Increased antibiotic resistance of microorganisms as well as the need to reduce health-care costs necessitates the production of new antimicrobials at lower costs. For this reason, this study was aimed to optimize the synthesis of magnesium oxide nanoparticles with the greatest antibacterial activity. In this study, 9 experiments containing different proportions of the factors (magnesium nitrate, NaOH, and stirring time) effective in the synthesis of magnesium oxide nanoparticles were designed using the Taguchi method. Magnesium oxide nanoparticles were synthesized using the coprecipitation method, and their antibacterial activity was evaluated using colony-forming unit (CFU) and disk diffusion. Morphology, crystalline structure, and size of synthesized nanoparticles were investigated using Fourier transform infrared (FTIR), X-ray diffraction (XRD), and scanning electron microscope (SEM). The optimum conditions (0.2 M magnesium nitrate, 2 M NaOH, and 90 min stirring time) for the synthesis of magnesium oxide nanoparticles with the greatest antibacterial activity were determined using the Taguchi method. The results of colony-forming unit and disk diffusion revealed the optimal antibacterial activity of synthesized nanoparticles against *Staphylococcus aureus and Escherichia coli* bacteria. The results obtained from FTIR and XRD analyses confirmed the synthesis of nanoparticles with favorable conditions. Also, according to the SEM image, the average size of synthesized nanoparticles was determined to be about 21 nm. According to the results, magnesium oxide nanoparticles can significantly reduce the number of Gram-positive and Gram-negative bacteria and can be used as an appropriate alternative to commonly used antibacterial compounds in order to tackle drug resistance among pathogens.

1. Introduction

Despite significant advances in the production of drugs for the treatment of various diseases, there is still no effective treatment for some diseases, such as autoimmune diseases [1, 2], chronic pain [3], cancer [4, 5], and microbial infections [6]. The incidence of various types of microbial infections worldwide is increasing. On the contrary, overuse of antibiotics and self-medication with antibiotics are common in many regions, and the use of medicines in many places is higher than global standards which have led to antimicrobial resistance among bacterial pathogens [7]. Therefore, finding new solutions such as the use of modern medicine to combat the spread of antimicrobial resistance can be helpful in industry and medicine.

Today, nanotechnology is mentioned as a factor which influences science and industry. According to experts, nanotechnology influences the economies of countries and their positions in future. Over the past decades, the use of nanotechnology and the production of nanoparticles have created new hope for solving human problems [8, 9]. The use of nanomaterial has expanded rapidly in various fields including pharmaceutical and medical industries, which has led to the introduction of applied and novel products into the market. Today, many problems have been confronted in dealing with pathogenic bacteria due to emergence and spread of drug resistance among them. The increasing rates

of drug resistance in bacteria threaten human ability to treat common infectious diseases [10, 11]. Nanotechnology contributes to reduced use of antibiotics. Nanoparticles containing compounds can inhibit bacterial growth without increasing drug resistance. The use of nanoparticles increases the ratio of surface area to volume which increases nanoparticle penetration and their interactions with cells and tissues. The very small size of these particles results in their deep tissue penetration and increased cell uptake [12, 13]. Magnesium oxide nanoparticles are odorless and nontoxic white powder which possesses a high melting point and high hardness. These nanoparticles are widely used in industries due to their biocompatibility, biodegradability, and relatively low cost. In medicine, magnesium oxide is used to relieve heartburn and sour stomach, improve bone regeneration and also as an antimicrobial and antitumor agent [14]. Magnesium oxide nanoparticles can be used as effective antimicrobials alone or in combination with other antimicrobial agents [15]. Previous studies have demonstrated that the bactericidal efficacy of MgO NPs increases with decreasing of their size and increasing of their concentration [16]. To our knowledge, optimized synthesis of magnesium oxides nanoparticles as bactericidal agents has not been studied. In this study, the optimum conditions for synthesis of MgO NPs were determined using the Taguchi method. Then, the properties of nanoparticles were investigated using Fourier transform infrared (FTIR), X-ray diffraction (XRD), and scanning electron microscope (SEM). The antibacterial activity of different concentrations of synthesized nanoparticles against Gram-negative bacteria *Escherichia coli* and Gram-positive bacteria *Staphylococcus aureus* was studied using colony-forming unit (CFU) and disk diffusion tests.

2. Materials and Methods

2.1. Synthesis of Magnesium Oxide Nanoparticles. The coprecipitation method was used for synthesis of magnesium oxide nanoparticles. Nine experiments were designed to determine the optimum conditions for synthesis of magnesium oxide nanoparticles using the Taguchi method. For this purpose, 0.05, 0.1, and 0.2 M of $Mg(NO_3)_2$ as well as 0.5, 0.1, and 0.2 M of NaOH was prepared. Then, based on the 9 suggested experiments using the Taguchi method, different concentrations of $Mg(NO_3)_2$ were added to NaOH solutions and stirred for 30, 60, and 90 minutes. The resulting components were separated by centrifugation and dried in the oven after being washed to remove the impurities. Then, magnesium hydroxide powder was calcined at 450°C in a furnace, and a white powder made up of magnesium oxide nanoparticles was obtained.

2.2. Characterization of Magnesium Oxide Nanoparticles. The synthesis of magnesium oxide nanoparticles was confirmed by an X-ray diffraction (XRD) using a Philips X'Pert machine; therefore, the crystalline structure of the synthesized samples were examined. The FTIR spectrum of the synthesized nanoparticles was prepared in the range of 400–4000 cm^{-1} using an alpha FTIR spectrometer (Bruker, Germany). Microscopic images of magnesium oxide nanoparticles were obtained by TESCAN scanning electron microscopy (Czech Republic) to investigate morphology and size of the nanoparticles.

2.3. Antibacterial Activity. Colony-forming unit was used to determine the optimum conditions for synthesis of magnesium oxide nanoparticles with the greatest antibacterial activity against Gram-positive *S. aureus* (ATCC 43300) and Gram-negative *E. coli* (ATCC 25922). To isolate bacteria, each bacterium was cultured on a nutrient agar plate. After preparing isolated strains, a solution containing each bacterium (10^8 CFU/ml) and synthesized magnesium oxide nanoparticles in various experimental conditions was stirred for 6 h. Finally, in order to calculate the growth rate of colonies, the bacteria suspensions were diluted using a ten-fold serial dilution. The solutions were transferred to the culture medium and cultured for incubation (at 37°C for 24 h). Then, the rate of growth colonies on each plate was calculated by counting viable colonies. After determining the optimum conditions for the synthesis of nanoparticles using Qulitek-4 software, nanoparticles were synthesized using the proposed conditions, and their antibacterial activity was studied using colony-forming unit and disk diffusion methods. Colony-forming unit was done like before, and gentamicin was used as a positive control. Bacteria were cultured on a nutrient agar medium for disk diffusion test. Disks containing magnesium oxide nanoparticles and gentamicin (positive control) were placed on the medium, and after 24 h incubation at 37°C, the diameter of the zone of inhibition was measured for each disk. All experiments were performed three times with three replicates, and their averaging results were presented [17].

3. Results and Discussion

Given the increased resistance of pathogens to almost all of the easily available antibiotics, it is important to find new and biocompatible antibacterial compounds. For this purpose, this study aimed at optimizing the synthesis of magnesium oxide nanoparticles with the greatest antibacterial activity. Table 1 shows Taguchi design and the effects of magnesium nitrate, NaOH, and stirring time on antibacterial activity of synthesized magnesium oxide nanoparticles in the 9 different experiments. The results showed that magnesium oxide nanoparticles synthesized by 0.2 M magnesium nitrate, 2 M NaOH, and 60 min stirring time (experiment 9) had the greatest antibacterial activity against Gram-positive and Gram-negative bacteria.

The results of the antibacterial tests of synthesized magnesium oxide nanoparticles indicated a significant reduction in the number of Gram-positive and Gram-negative bacteria. Consistent with the results of this study, previous studies reported the antibacterial activity of magnesium oxide nanoparticles alone or in combination with other antimicrobials agents [18–20]. In line with the results of this

Table 1: Taguchi design of experiments and antibacterial effect of manufactured MgO nanoparticles.

Experiment	$Mg(NO_3)_2$ (M)			NaOH (M)			Stirring time (min)			Bacterial survival (log_{10} CFU/ml)	
	0.05	0.1	0.2	0.5	1	2	30	60	90	Gram-positive	Gram-negative
1		0.05			0.5			30		6.51	6.84
2		0.05			1			60		5.42	6.11
3		0.05			2			90		5.36	5.43
4		0.1			0.5			60		5.38	5.68
5		0.1			1			90		3.66	3.98
6		0.1			2			30		4.05	4.42
7		0.2			0.5			90		4.38	4.76
8		0.2			1			30		3.97	5.17
9		0.2			2			60		3.62	3.79

study, the researchers suggested that magnesium oxide nanoparticles have a better antibacterial activity against Gram-positive bacteria compared to that against Gram-negative bacteria [14]. The precise mechanism of bactericidal action of magnesium oxide nanoparticles is still unclear; however, various mechanisms have been proposed. The mechanism of action of nanoparticles depends on their binding with bacterial surface as well as metabolism in the organism [21, 22]. Leung et al. [23] reported that oxidative stress and lipid peroxidation do not play any role in cell death in Gram-negative bacteria (*Escherichia coli*) in the presence of magnesium oxide nanoparticles, and cell membrane damage is the main cause of death in these cells. This difference can be due to different membrane structures of Gram-positive and Gram-negative bacteria. Gram-negative bacteria possess a complex outer membrane structure that act as a major barrier to the penetration of ROS into the cell. It has also been reported that increase in concentration and size of magnesium oxide nanoparticles improves antibacterial activity of these nanoparticles [24, 25].

Table 2 shows the effects of magnesium nitrate, NaOH, and stirring time on antibacterial activity of magnesium oxide nanoparticles at different levels. All three factors showed the best performance against *Staphylococcus aureus* and *Escherichia coli* bacteria at the third level.

The interactions between the factors studied are displayed in Table 3. The effects of magnesium nitrate, NaOH, and stirring time on Gram-positive bacteria varied from 10.89 to 44.63, and the effects on Gram-negative bacteria varied from 15.90 to 34.42. The highest NaOH × stirring time interaction was observed on Gram-positive and Gram-negative bacteria at 44.63 and 34.42, respectively. Also, $Mg(NO_3)_2 \times$ NaOH showed the least interaction on both *Staphylococcus aureus* and *Escherichia coli* bacteria.

Table 4 shows the analysis of variance (ANOVA) of the studied factors. All three factors affected the antibacterial activity of magnesium nanoparticles. Magnesium nitrate, NaOH, and stirring time were the most effective factors in the synthesis of magnesium nanoparticles with the greatest antibacterial activity, respectively.

Table 5 shows the proposed conditions for the synthesis of magnesium oxide nanoparticles with the greatest antibacterial activity. The results showed that at the third level, all three factors had the best performance at the synthesis of magnesium oxide nanoparticles with the greatest antibacterial activity. Magnesium nitrate, NaOH, and stirring time were the most effective factors in improving antibacterial activities of synthesized nanoparticles, respectively.

Table 2: The main effects of studied factors at different levels on the survival of bacteria.

Factors	Gram-positive bacteria (*Staphylococcus aureus*)			Gram-negative bacteria (*Escherichia coli*)		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
$Mg(NO_3)_2$	5.76	4.36	3.99	6.13	4.69	4.57
NaOH	5.42	4.35	4.34	5.76	5.09	4.55
Stirring time	4.84	4.81	4.47	5.48	5.19	4.72

The level of antibacterial activity of magnesium oxide nanoparticles (synthesized under the proposed conditions by the Taguchi method), against *Escherichia coli* and *Staphylococcus aureus* bacteria is shown in Table 6. In colony-forming unit, the growth rate of *Escherichia coli* and *Staphylococcus aureus* bacteria reduced to 3.41 and 3.56 in the presence of magnesium oxide nanoparticles, respectively. According to the results of disk diffusion test, the diameters of the zones of inhibition for *Escherichia coli* and *Staphylococcus aureus* bacteria were 15.66 and 15, respectively. Slight difference was perceived in the antibacterial activity level of magnesium oxide nanoparticles and gentamicin antibiotic against Gram-positive and Gram-negative bacteria that indicating the desired antibacterial activity of synthesized magnesium oxide nanoparticles. The significant increase in the use of nanoparticles in the current world has made it necessary to target their synthesis. The Taguchi method enabled savings of cost and time and identifying the optimum conditions for synthesis of nanoparticles with desirable antibacterial activity.

After determining the optimum conditions for the synthesis of nanoparticles, they were synthesized using the proposed conditions, and their characterization was studied. Phase formation and crystallography of synthesized magnesium oxide nanoparticles by X-ray diffraction were investigated (Figure 1). The peaks in the XRD pattern of magnesium oxide nanoparticles were in accordance with JCDS 75–1525 which expressed the cubic structure of synthesized nanoparticles [26]. The existence of sharp peaks in the XRD spectrum of magnesium oxide samples

Table 3: The interactions among studied factors at different levels on the survival rate of bacteria.

	Interacting factor pairs	Column	Severity index (%)	Optimum conditions
Gram-positive bacteria (*Staphylococcus aureus*)	NaOH × stirring time	2 × 3	44.63	[2, 3]
	Mg $(NO_3)_2$ × stirring time	1 × 3	41.86	[2, 3]
	Mg $(NO_3)_2$ × NaOH	1 × 2	10.89	[3, 3]
Gram-negative bacteria (*Escherichia coli*)	NaOH × stirring time	2 × 3	34.42	[2, 3]
	Mg $(NO_3)_2$ × stirring time	1 × 3	32.62	[2, 3]
	Mg $(NO_3)_2$ × NaOH	1 × 2	15.90	[3, 3]

Table 4: The ANOVA test for studied factors on reducing the growth of bacteria.

Type of bacteria	Factors	DOF	Sum of squares	Variance	F-ratio (F)	Pure sum	Percent (%)
Gram-positive bacteria (*Staphylococcus aureus*)	Mg $(NO_3)_2$	2	5.24	2.62	29.69	5.07	63.36
	NaOH	2	2.32	1.16	13.13	2.14	26.78
	Stirring time	2	0.26	0.13	1.46	008	1.03
Gram-negative bacteria (*Escherichia coli*)	Mg $(NO_3)_2$	2	4.48	2.24	9.53	4.01	49.91
	NaOH	2	2.22	1.11	4.71	1.75	21.73
	Stirring time	2	0.87	0.43	1.85	0.40	4.96

DOF, degree of freedom.

Table 5: Predicted the optimal conditions for producing MgO nanoparticles with the greatest antibacterial activity.

Factors	Gram-positive bacteria (*Staphylococcus aureus*)		Gram-negative bacteria (*Escherichia coli*)	
	Level	Contribution	Level	Contribution
Mg $(NO_3)_2$	3	0.716	3	0.56
NaOH	3	0.363	3	0.58
Stirring time	3	0.239	3	0.41
Total contribution from all factors		1.32		1.55
Current grand average of performance		4.71		5.13
Bacterial survival at optimum condition		3.39		3.58

Table 6: The antibacterial activity of synthesized MgO nanoparticles in optimal conditions proposed by Taguchi method.

Type of assay	Type of bacteria	Antibacterial activity	
		MgO	Gentamicin
Bacterial survival ($\log_{10}$ CFU/ml)	Gram-positive bacteria (*Staphylococcus aureus*)	3.41	2.52
	Gram-negative bacteria (*Escherichia coli*)	3.56	2.88
Zone of inhibition (mm)	Gram-positive bacteria (*Staphylococcus aureus*)	15.66	17.33
	Gram-negative bacteria (*Escherichia coli*)	15.00	15.66

confirmed the formation of nanoparticles, and increase in the peak width represented a decrease in the size of nanoparticles. Also, the absence of extra peaks in the synthesized nanoparticles confirmed their high purity.

Figure 2 shows the FTIR spectrum of magnesium oxide nanoparticles at the wavenumber ranges of 400–4000 cm^{-1}. The bands observed at the wavenumber ranges of 3420 cm^{-1} and 1099–1484 cm^{-1} indicated the adsorption of the O-H group and vibrations of carbonate ion (C-O), respectively. The peaks observed below 800 cm^{-1} confirmed the bond between magnesium and oxygen [27, 28].

The size and morphology of the synthesized magnesium oxide nanoparticles was studied through SEM micrograph (Figure 3). The scanning electron micrograph demonstrated agglomeration of some magnesium oxide nanoparticles. It is

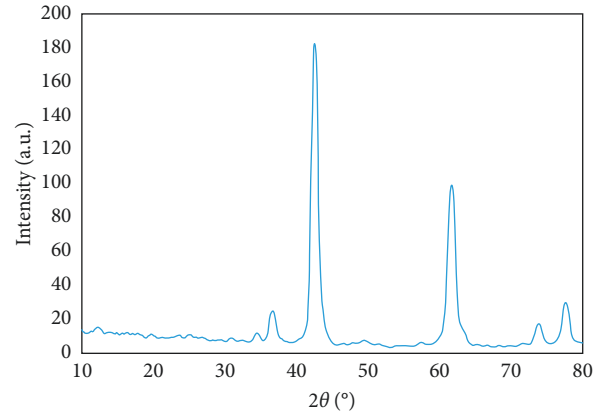

Figure 1: XRD pattern of magnesium oxide nanoparticles.

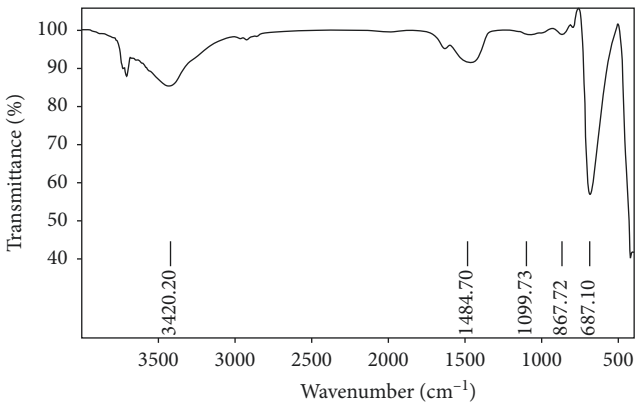

Figure 2: FTIR spectra of magnesium oxide nanoparticles.

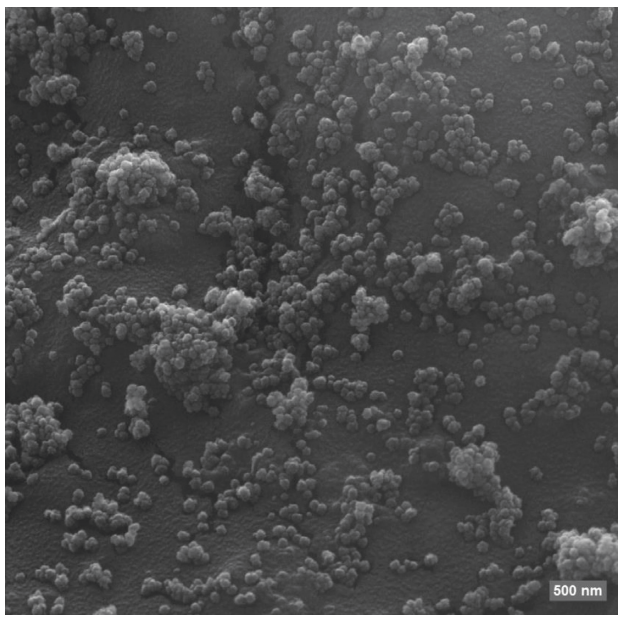

Figure 3: SEM image of magnesium oxide nanoparticles.

also clear that the synthesized nanoparticles have a spherical shape. The scanning electron microscope image showed that the average size of the synthesized nanoparticles was about 21 nm.

4. Conclusions

Based on the results of this study, the optimum conditions (0.2 M magnesium nitrate, 2 M NaOH, and 90 min stirring time) for the synthesis of magnesium oxide nanoparticles with the greatest antibacterial activity were determined using the Taguchi method. Antibacterial activity of synthesized magnesium oxide nanoparticles against *Escherichia coli* and *Staphylococcus aureus* bacteria was improved at proposed optimum conditions. X-ray diffraction, scanning electron microscopy, and Fourier transform infrared spectroscopy of synthesized nanoparticles indicated formation of nanoparticles with desirable characteristics and morphology. Magnesium oxide nanoparticles could serve as an alternative to the most commonly used antibiotics due to their good antibacterial properties.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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