

Optimisation of Cobalt Oxide Nanoparticles Synthesis as Bactericidal Agents

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Abstract

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AIM: With the increased bacterial resistance and the resulting problems in recent years, it seems necessary to find new biocompatible compounds to confront this problem. This research was conducted to optimise the synthesis of cobalt oxide nanoparticles with the highest antibacterial activity.

METHODS: In the present study, 9 experiments were designed using the Taguchi method. The effect of three factors of cobalt nitrate, KOH and the stirring time in the synthesis of cobalt oxide nanoparticles with the highest antibacterial activity was investigated. The bactericidal effect of synthesised nanoparticles was evaluated using the colony-forming unit (CFU) and disk diffusion methods. The characteristics of nanoparticles were studied using the Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and the scanning electron microscopy (SEM).

RESULTS: The results indicated that all three evaluated factors were effective on the antibacterial properties of the synthesised nanoparticles. The best antibacterial activity of cobalt oxide nanoparticles was observed in experiment 9 (cobalt nitrate 0.6 M, KOH 2M and stirring time 60 min). The study of nanoparticles synthesised by FTIR, XRD, and SEM confirmed the formation of cobalt oxide nanoparticles with size (24 nm) and a proper structure (spinel structure).

CONCLUSION: Due to the optimal antibacterial properties of the synthesised cobalt oxide nanoparticles, they can be used in the fabrication of dental and medical equipment with antibacterial properties.

Introduction

Despite significant advances in the treatment of diseases in recent decades, no proper cure has been still found for some diseases such as cancer [1], [2], cardiovascular diseases [3], chronic pains [4], diabetes [5], autoimmune diseases [6], [7], AIDS [8], [9], and microbial infections [10], [11]. Some common microbial infections with bacterial origin are strongly annoying. Since the discovery of bacteria, the researchers have always been looking for effective ingredients against them. The widespread introduction of antibiotics was made early in the last century. Since

then, the bacterial resistance has grown significantly to the available antibiotics [12]. Over the years, bacteria have achieved effective mechanisms to cope with antibiotics through chromosomal mutations and genetic exchanges [13]. *Escherichia coli* and *Staphylococcus aureus* cause some of the most common infections in a variety of situations in society and hospitals. According to a report by the World Health Organization, the bacteria of *Escherichia coli* and *Staphylococcus aureus* have shown resistance to some common antibiotics in more than 50% of cases [14]. The development of resistance to antibiotics has limited their service life. Finding an antimicrobial agent is not an easy task due to poor penetration of the

compounds into the bacterial cells [15]. The traditional organic compounds used for the disinfection of toxic have side effects such as toxicity for humans and sensitivity to high temperatures and high pressures.

In contrast, inorganic compounds are nontoxic, exhibit high antibacterial properties at low concentrations and are resistant to unfavourable and unbalanced conditions [16]. Due to the increased resistance of bacteria to antibiotics and their adaptability characteristics, finding new solutions to eliminate bacteria has become a priority nowadays. Over the past few decades, the use of nanotechnology and the synthesis and production of nanoparticles have brought new hopes for overcoming the antibacterial resistance.

Cobalt oxide is one of the transition metal oxides, which is in the form of black powder with antimicrobial and magnetic properties. The magnetic nanoparticles are particles with an independent nature and a maximum dimension of 100 nm, which have magnetic properties. Due to its semistable three phases with various crystalline structures, cobalt is one of the most important magnetic metals. The crystalline cobalt oxide structures include the Hexagonal Closed Packed (HCP) phase, Face-Centered Cubic (FCC) phase, and the Epsilon phase [17]. Due to the high capacity for use in various areas, magnetic nanoparticles of iron, cobalt, and nickel have drawn a lot of attention. The cobalt oxide nanoparticles, according to their properties, are used in various fields such as the synthesis of sensors, magnetic materials, electrochemical systems, smart absorbers, catalysts and in the medical area. Considering the use of cobalt oxide nanoparticles in different fields, optimising their synthesis seems to be important in terms of their application. The structures, sizes, morphologies and surface properties of nanoparticles can be improved by controlling the effective factors in the synthesis process [18], [19]. Therefore, changing and targeted determining the properties of nanoparticles can be considered according to their function and activity. Accordingly, this research was designed to optimise the synthesis of cobalt oxide nanoparticles as an antibacterial agent by using the co-precipitation method and evaluate its properties by employing Infrared Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM) devices.

Material and Methods

Synthesis of cobalt oxide nanoparticles

The cobalt oxide nanoparticles were synthesised using the coprecipitation method. The Qualitek-4 software and Taguchi method were used to optimise the synthesis of cobalt oxide nanoparticles

with the highest antibacterial activity. To do so, 9 experiments were designed and the effect of 0.2, 0.4 and 0.6 M levels of $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and the levels of 1, 2 and 3 M of KOH at the stirring times of 30, 60, and 90 min were evaluated on the antibacterial activity of the synthesized nanoparticles (Table 1).

Table 1: Taguchi design of experiments and antibacterial effect of manufactured cobalt oxide nanoparticles on gram-positive and gram-negative bacteria

Experiment	Cobalt nitrate (M)			KOH (M)			Stirring time (min)			Bacterial survival (Log_{10} CFU/ml)	
	0.2	0.4	0.6	1	2	3	30	60	90	Gram-positive	Gram-negative
1	0.2			1			30			6.42	6.76
2	0.2			2			60			5.84	6.13
3	0.2			3			90			5.10	5.32
4	0.4			1			60			5.32	5.51
5	0.4			2			90			5.60	5.71
6	0.4			3			30			4.94	5.06
7	0.6			1			90			4.40	4.75
8	0.6			2			30			4.76	4.89
9	0.6			3			60			3.72	3.97

A volume of 100 ml of KOH solution at concentrations of 1, 2 and 3 M and cobalt nitrates with the concentrations of 0.2, 0.4, and 0.6 M were prepared in separate containers. Then, according to the nine tests suggested by the Taguchi method, the solutions of KOH at different concentrations were added drop-by-drop to the containers containing cobalt nitrate solution while continuously stirring, which were mixed and combined at 30, 60 and 90 min, respectively. Initially, pink sediment was formed, which was easily oxidised in contact with the air, and dark sediment was obtained. The dark sediment was separated by centrifugation and washed out with deionised water several times. It was then dried in an oven at 100°C for 24 h. The cobalt hydroxide powder was calcined in air at 400°C furnace for four hours to obtain the cobalt oxide nanoparticles.

Antibacterial activity

The antibacterial activity level of synthesised cobalt oxide nanoparticles according to 9 experiments suggested by Taguchi method against *Staphylococcus aureus* and *Escherichia coli* bacteria was studied using the colony-forming unit method. Bacterial suspensions were made from both bacteria with an approximate concentration of 10^8 CFU/ml, which were shaken along with the synthesised nanoparticles for 6 h. The solutions containing bacterial suspensions and nanoparticles were incubated for 24 h at 37°C on a nutrient agar culture medium. After incubation, the colonies growth rate was calculated. Then, cobalt oxide nanoparticles were produced using the suggested conditions by Taguchi method, and their bactericidal effect was surveyed using CFU and disk diffusion methods. CFU method was done similar before and for disk diffusion test homogenous suspensions of *Staphylococcus aureus* and *Escherichia coli* bacteria were cultured by a swab on a nutrient agar medium. The discs containing cobalt oxide nanoparticles and Gentamycin (positive

control) were then placed on the media and incubated for 24 h at 37°C. Afterwards, the diameter of the zone of inhibition was measured for each disk [10].

Characteristics

After determining the optimal conditions based on the Taguchi method, the nanoparticles of cobalt oxide were synthesised under the proposed optimum conditions (cobalt nitrate 0.6 M, KOH 3M and stirring time 60 min) and their characteristics were evaluated. The FTIR spectrum of cobalt oxide nanoparticles was provided by the alpha spectrometer (Bruker, Germany) after the preparation of KBr tablets from the specimens. The X-ray diffraction test was performed by Philips X 'Pert (40 kV, 30 mA) to analyse and evaluate the crystalline structure formed in the synthesised nanoparticles. The microscopic images were taken of the cobalt oxide nanoparticles by a high-resolution scanning electron microscopy (TESCAN, Czech Republic) to examine the morphology and determine the size of the synthesised nanoparticles.

Statistical analysis

The data were analysed using Qualitek-4 software (Nutek Inc., MI, USA). All tests were carried out three times with three replicated, and their averaging results were reported.

Results

One of the most important features of metal nanoparticles received considerable attention in recent years is their use as antibacterial compounds. Aimed at this, we used the Taguchi method to assess the effect of three factors of cobalt nitrate, KOH, and stirring time on the antibacterial activity of cobalt oxide nanoparticles (Table 1). The results indicated that the synthesised nanoparticles in experiment 9 (cobalt nitrate 0.6 M, KOH 2M, and stirring time of 60 min) had the highest antibacterial activity against gram-positive bacteria (3.72) and gram-negative bacteria (3.97).

The effect of each factor on the antibacterial properties of the synthesised cobalt oxide nanoparticles is displayed in Table 2.

Table 2: The main effects of factors of cobalt nitrate, KOH and stirring times at different levels on the bacterial survival

Factors	Gram positive bacteria (<i>S. aureus</i>)			Gram-negative bacteria (<i>E. coli</i>)		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Cobalt nitrate	5.79	5.29	4.29	6.07	5.43	4.54
KOH	5.38	5.40	4.59	5.67	5.58	4.78
Stirring time	5.37	4.96	5.03	5.57	5.20	5.26

Cobalt nitrate, KOH, and the stirring time showed the highest performance in reducing the growth of *Staphylococcus aureus* in the third level (4.29), in the third level (4.59), and the second level (4.96), respectively. The studied factors had similar effects on the *Escherichia coli* as well. The optimal performance of cobalt nitrate (4.54) and KOH (4.78) was seen at the third level, while the stirring time (5.20) showed its bests at the second level.

Table 3 shows the interaction effect between the concentrations of the cobalt salt, KOH and the stirring time. The interaction effect rate of the studied factors varied from 15.92 to 40.37 in the gram-positive bacteria and showed changes from 14.87 to 44.62 on the gram-negative bacteria. The highest interaction effect rate was seen in the case of KOH × Stirring time, while the lowest interaction rate was related to cobalt nitrate × KOH on both gram-positive and gram-negative bacteria.

Table 3: The interactions among studied factors on the survival rate of bacteria

	Interacting factor pairs	Column	Severity Index	Optimum
			(%)	conditions
Gram-positive bacteria (<i>S. aureus</i>)	KOH × Stirring time	2×3	40.37	[3,2]
	Cobalt nitrate × Stirring time	1×3	17.77	[3,2]
	Cobalt nitrate × KOH	1×2	15.92	[3,3]
Gram-negative bacteria (<i>E. coli</i>)	KOH × Stirring time	2×3	44.62	[3,2]
	Cobalt nitrate × Stirring time	1×3	19.35	[3,2]
	Cobalt nitrate × KOH	1×2	14.87	[3,3]

The analysis of variance of the examined factors (cobalt nitrate, KOH, and stirring time) is presented in Table 4. According to the results, the factors of cobalt nitrate, KOH and the stirring time respectively indicated the optimal effect on the synthesis of nanoparticles with the highest antibacterial activity against gram-positive and gram-negative bacteria.

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 (<i>S. aureus</i>)	Cobalt nitrate	2	3.47	1.73	49.74	3.40	66.35
	KOH	2	1.29	0.64	18.53	1.22	23.86
	Stirring time	2	0.29	0.14	4.19	0.22	4.34
Gram negative bacteria (<i>E. coli</i>)	Cobalt nitrate	2	3.56	1.78	39.18	3.47	65.25
	KOH	2	1.43	0.71	15.76	1.34	25.22
	Stirring time	2	0.23	0.12	2.57	0.14	2.69

DOF, degree of freedom.

The optimum conditions for the synthesis of cobalt oxide nanoparticles with the most favourable antibacterial activity are reported in Table 5. The results suggested that the use of cobalt nitrate and KOH at the third level and the stirring time at the second level can provide the optimal antibacterial performance for the synthesis of cobalt oxide nanoparticles. The antibacterial performance rate of synthesised nanoparticles in the optimal conditions improved compared to the average performance of the synthesised nanoparticles in the 9 experiments.

Table 5: Predicted the optimal conditions for producing cobalt oxide nanoparticles with the highest antibacterial activity

Factors	Gram-positive bacteria (<i>S. aureus</i>)		Gram-negative bacteria (<i>E. coli</i>)	
	Level	Contribution	Level	Contribution
Cobalt nitrate	3	0.83	3	0.81
KOH	3	0.54	3	0.56
Stirring time	2	0.16	2	0.14
Total contribution from all factors		1.53		1.51
Current grand average of the performance		5.12		5.34
Bacterial survival at optimum condition		3.59		3.83

Table 6 shows the level of antibacterial activity of the cobalt oxide nanoparticles synthesised under the proposed optimum conditions against *Staphylococcus aureus* and *Escherichia coli* bacteria. Evaluating the bactericidal effect of synthesised cobalt oxide nanoparticles by CFU method demonstrated that the growth rate of gram-positive and gram-negative bacteria reduced to 3.60 and 3.78, respectively. In the disc diffusion method, inhibition zone diameters for gram-positive and gram-negative bacteria were 15 and 14.66, respectively. The slight difference was observed in the bactericidal activity of cobalt oxide nanoparticles and antibiotic (gentamicin) against *Staphylococcus aureus* and *Escherichia coli* bacteria that denoting the favourable bactericidal effect of synthesised cobalt oxide nanoparticles.

Table 6: The bactericidal activity of synthesised cobalt oxide nanoparticles in optimal conditions

Type of essay	Type of bacteria	Bactericidal activity	
		Cobalt Oxide	Gentamycin
Bacterial survival (Log ₁₀ CFU/ml)	Gram positive bacteria (<i>S. aureus</i>)	3.60	2.56
	Gram negative bacteria (<i>E. coli</i>)	3.78	2.84
Zone of inhibition (mm)	Gram-positive bacteria (<i>S. aureus</i>)	15	17
	Gram negative bacteria (<i>E. coli</i>)	14.66	15.66

Examining the properties of nanoparticles showed that the synthesised nanoparticles have a suitable structure and size for biological applications. Figure 1 shows the FTIR spectrum of cobalt oxide nanoparticles in the range of 400-4000 cm⁻¹ wavelengths. Some peaks were found in the range of 667 cm⁻¹ and 574 cm⁻¹ in the FTIR spectrum of cobalt oxide nanoparticles, which represented the synthesis of cobalt oxide nanoparticles. The peaks observed in the FTIR spectrum of the synthesised nanoparticles confirm the spinel structure of the cobalt oxide nanoparticles.

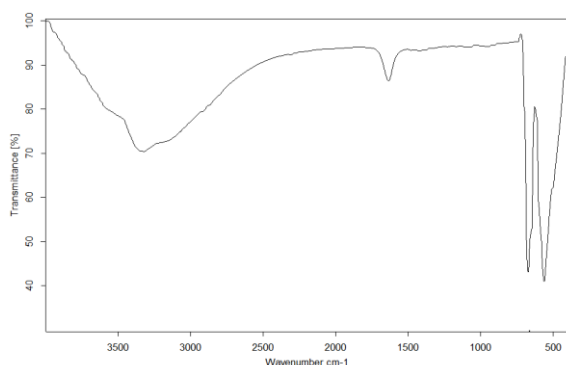


Figure 1: FTIR spectra synthesised cobalt oxide nanoparticles

The phase formation and crystallography of cobalt oxide nanoparticles were investigated using the X-ray diffraction (Figure 2). The nature of peaks in the XRD pattern of cobalt oxide nanoparticles was by the JCPDS 74-2120, suggesting the cubic structure of the synthesised nanoparticles. Considering the magnetic properties of these nanoparticles, they are suitable for use in various fields, including biological and medical applications.

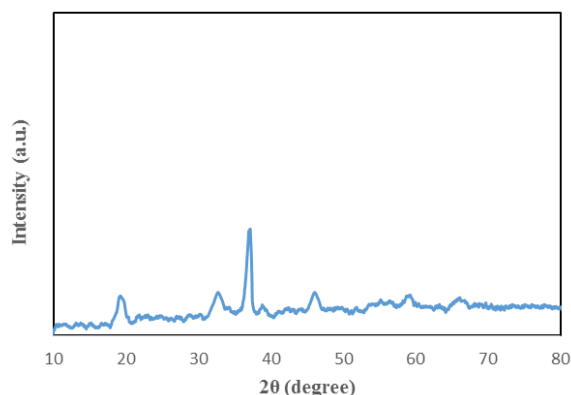


Figure 2: XRD pattern of synthesised cobalt oxide nanoparticles

The scanning electron microscopy images were used to study the structure and morphology of the synthesised cobalt oxide nanoparticles (Figure 3). The analysis of the scanning electron micrographs revealed the agglomeration of some cobalt oxide nanoparticles. According to the SEM image, the approximate average size of the synthesised nanoparticles was about 24 nm. The nanoparticles had a relatively rugged surface and a spherical shape, resulting in higher adsorption sites and their better interactions.

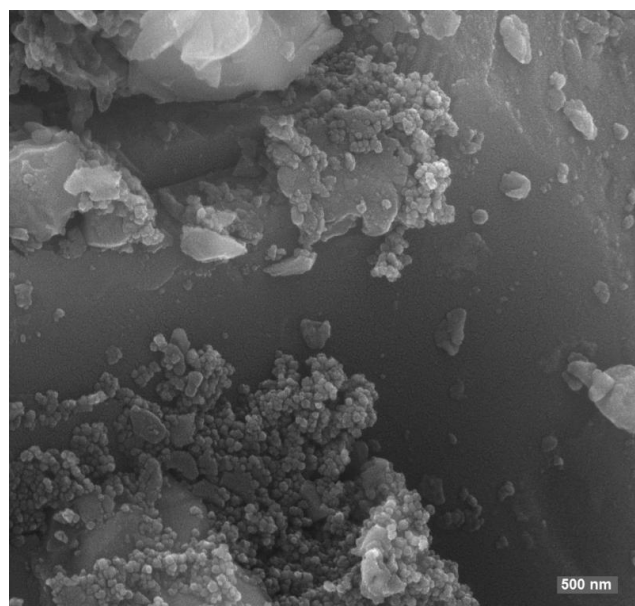


Figure 3: SEM image of synthesised cobalt oxide nanoparticles

Discussion

Regarding the use of metal oxide nanoparticles in various fields, optimising their synthesis can be important in terms of their application. Previous studies have shown that the factors effective in the synthesis of nanoparticles can affect their shape, size, and properties [10], [20].

The evaluation of the antibacterial properties of the synthesised cobalt oxide nanoparticles indicated a significant reduction in the colony formation of studied gram-positive and gram-negative bacteria. Consistent with the results of this study, previous studies have reported the antibacterial activity of cobalt oxide nanoparticles alone or in combination with other materials [21], [22], [23]. In research, Alsharaeh et al. [24] evaluated the synthesis of graphene-cobalt oxide nanocomposite and its antibacterial properties. According to them, a compound contacting cobalt oxide nanoparticles shows a desirable antibacterial effect against gram-negative bacteria. The precise mechanism of action of the cobalt oxide nanoparticles, which kills the bacteria, is still unclear and no specific mechanism has been suggested in this regard. However, due to mechanisms of action of other nanoparticles, probably processes such as the induction of oxidative stress, the release of toxic metal ions and damage to the cell membrane and interfering its activities can generate the antimicrobial properties of cobalt oxide nanoparticles [25], [26].

Due to the rapidly growing use of metal nanoparticles in the therapeutic applications, the targeted optimisation of their synthesis seems to be important regarding their application. Applying the Taguchi method, we determined the optimum conditions for the synthesis of cobalt oxide nanoparticles with the highest antibacterial activity (experiment 9). The results on antibacterial activity of factors effective in the synthesis of cobalt oxide nanoparticles showed that the cobalt nitrate and KOH at the third level and the stirring time at the second level had a greater effect on the antibacterial properties of the synthesized nanoparticles against the gram-positive bacteria of *Staphylococcus aureus* and the gram-negative bacteria of *Escherichia coli*. The results of FTIR, XRD, and SEM analyses revealed that the cobalt oxide particles were synthesised in nanoscale with a spherical form, a relatively uniform size and a high crystallisation degree. Due to the optimal antibacterial activity and proper stability of the cobalt oxide nanoparticles, they can be used as an alternative to conventional antibacterial compounds to combat the pathogenic bacteria.

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